Aryadeep Roychoudhury *Editor*

Rice Research for Quality Improvement: Genomics and Genetic Engineering

Volume 1: Breeding Techniques and Abiotic Stress Tolerance

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Foreword

"Rice is life," feeding more than 50% of the global population today, but was known since 8000–10,000 years back. Rice, a crop originated from Indo-China regions, spread around the world along with the domestication, cultural revolution, improvement of the crop with scientific tools (conventional art of breeding, mutation breeding, genomic breeding, transgenesis, and genome editing), adaptation, and need-based policy decisions of different countries. Abiotic stress is a major environmental constraint to crop productivity, particularly rice. Breeding techniques, particularly genetic engineering, envisage at cross-talk between different stress-signaling pathways and are expected to find the road map of the metabolic pathways leading to stress tolerance. Osmotic adjustment (OA) is a powerful, effective component of abiotic stress (salinity and drought) tolerance in rice. Several stress-related genes, including transcription factor genes (*DREB*), and stress-induced promoters, including *rd29*, have now been cloned and transferred in rice to enhance osmolyte levels, and Genetically Modified (GM) rice transformed with different other genes has also shown increased tolerance to osmotic stress.

Rice (*Oryza sativa* L.) belongs to the family Poaceae with more than 40,000 cultivated varieties. It is the second largest produced cereal and will be the main daily staple food of about 4.6 billion people by 2025. Increasing population and economic development have been posing a growing pressure for increase in food production. According to FAO forecast estimate, the food production must be doubled by 2050. So increasing the rice production appears crucial for human life.

The major abiotic stresses worldwide causing risks to food security are high salinity, drought, submergence, and cold. Among these stresses, drought is the primary environmental stress responsible for decrease in agricultural production worldwide, and to fulfill the demand, efforts are being applied to improve crop yield. Drought affects plants in countless ways like it affects plant growth, yield, membrane integrity, pigment content, osmotic adjustments, water relations, and photosynthetic activity. Salinity is the second most prevalent soil problem in rice-growing countries after drought, and rice is considered as a salt-sensitive crop in early seedling stages, which limits its productivity. Due to global warming, rise in sea levels, surplus irrigation without appropriate drainage in lands, and underlying rocks rich in detrimental salts, the areas under salt stress are increasing. It is expected that if the present scenario persists, and for other social requirements, 50% of the current cultivated land will be lost for agriculture by 2050. To improve the yield under drought and salt stress conditions, several breeding programs have been initiated. Although conventional breeding programs such as hybridization, hybrid breeding, wide hybridization, mutation breeding, and ideotype breeding have resulted in the development of some salt- and drought-tolerant rice varieties and several lines have been released worldwide and in India, they have their limitations and take long time to achieve the goals. Drought tolerance in rice is a complex trait, and it is determined by various component traits. These traits are governed by many genes with huge environmental interaction, with low heritability, and thus are difficult to generate desirable results. Salinity stress tolerance is a quantitative trait which is controlled by multiple genes. So there is a need to develop rice varieties that can withstand high levels of drought and salt and at the same time uphold optimal yield levels. The development of plant transformation techniques during the past decades has made it possible to develop improved crop plants by the introduction of cloned genes. The two critical steps accountable for transformation of plants are transfer of foreign DNA into the plant cell and regeneration of plants from transformed cells. The callus induction and regeneration of rice via tissue culture depend upon different factors like genotype, type of explants, and media supplements like basal salts, organic component, and growth regulators. Among them, the most crucial factor is the genotype. Rice transformation is a major goal in cereal biotechnology, because rice is the world's most important food crop, and it is also known as the model of cereal genomics. Genetic engineering has been used as a prominent tool for rice improvement. Although gene transformation in japonica rice is performed routinely in several laboratories, it is noteworthy to mention that the first *japonica* rice transformation was reported by Ko Shimamoto's laboratory in 1989. The system of genetic engineering in *indica* rice is comparatively complicated. However, the breakthrough of developing genetically engineered homozygous indica rice plants using embryogenic protoplast systems came from ETH-Zurich by Swapan K Datta and Karabi Datta along with Ingo Potrykus (Bio/Technology/Nature Biotechnology, 1990). It is revealed that since the number of copies of gene (s) inserted and chromosomal locations of the integrated genes are not controllable, the expression of the introduced genes varies among individual transformants. Therefore, relatively large number of transgenic plants must be developed in order to select desirable transformants as well as to study the expression of introduced genes. Since the last two decades, a large number of salinity tolerance genes were isolated and cloned, which are involved in signal transduction, transcription regulation, ion transporters, and metabolic pathways. A lot of work has been done by introducing these genes into rice. Still, there is ample scope for this research because till now none of the rice varieties is released which has magical tolerance to abiotic stress. In conclusion, to feed the ever growing population, we need to solve the abiotic stress problem in rice, and this is the principal challenge for plant biotechnologists. Despite the discovery of lots of genes, still it is a bigger challenge to meet the demand.

This book has around 31 chapters prepared by specialized researchers and scientists working in different avenues of environmental stress in rice and the range of topics are quite broad. The initial chapters highlight the major constraints in rice production, especially with respect to diverse environmental stresses, the potentiality of wild rice in improving domesticated rice species, and the varietal differences between the sensitive and tolerant rice cultivars. A few chapters are focused on tissue culture and breeding techniques required to develop improved rice, including C4 rice, and the role of genome sequencing in rice biotechnology. The succeeding chapters highlight the diverse environmental stresses like salinity, drought, heavy metal/metalloid, arsenic, temperature, ozone, UV, anoxia, submergence, and nutrient deficiency and the genetic engineering approaches to develop tolerance against such diverse stresses. There are chapters that emphasize how the overall metabolic pathways or phytohormone levels could be manipulated to develop stress tolerance. Finally, the challenges faced in maintaining rice grain quality due to abiotic stresses have been discussed in the chapters towards the end of this volume.

I am grateful to Dr. Aryadeep Roychoudhury, the editor of this book, whom I know for many years since he was associated with me to work on abiotic stress tolerance in rice at the University of Calcutta, for asking me to write the Foreword note. I understand that the chapters are well selected and written and carefully edited by the experts and viewed by the editor. I am sure the book will be of great value to scholars, teachers, and students involved in modern rice breeding and particularly on abiotic stress physiology of rice.

Biography

Swapan K. Datta, Ph.D. is currently holding the DBT Distinguished Biotechnology Research Professor position at the Department of Botany, University of Calcutta, where he also served as Rashbehari Ghosh Chair Professor. Dr. Datta received B.Sc. (Hons.) from Presidency College (1972) and M.Sc. (1974) and Ph.D. (1980) from the University of Calcutta. He received DAAD Fellowship while working at Visva-Bharati University (1985–1986) and worked in Germany with Prof. G. Wenzel on resistance genetics of wheat and barley. He was awarded the Friedrich Miescher Institute (FMI) Fellowship attached to CIBA-GEIGY at Basel, Switzerland (1987). He took the senior scientist position at Swiss Federal Institute of Technology (ETH), Zurich, Switzerland (1987–1993) and worked on gene technology for crop improvement with Prof. Ingo Potrykus, being associated with international collaborators. Dr. Datta was awarded Rockefeller Foundation-supported Senior Visiting Faculty at UC Davis, USA (1989) before joining at the International Rice Research Institute (IRRI), Manila, Philippines, where he contributed significantly in rice improvement with Dr. Gurdev Khush and with many international collaborators (1993–2005). Dr. Datta is a recipient of many national and international fellowships/awards including TATA Innovation Fellowship (2007–2009), CGIAR Best Science Research Paper, and Paul Johnnes Brouhl Memorial Medal (2009). Other **professional affiliations:** Elected Fellow of the Indian National Science Academy in 2014 (**FNA**); Elected Fellow of the Indian Academy of Science in 2017 (**FASc**); Elected Fellow of the National Academy of Agricultural Science in 2005 (**FNAAS**); Elected Fellow of National Academy of Sciences in 2006 (**FNASc**);

Elected Fellow of the World Academy of Science in 2014 (**TWAS**); Dr. Datta contributed significantly in **rice research and traits improvement** over 40 years of research in agriculture and plant biology, particularly on genetic transformation system in rice, jute, and chickpea, which has been established with landmark crop improvement. He has published over 150 research papers in reputed journals including ***Nature*****,** ***Science*****,** ***Nature Biotechnology*****, and** ***Nature Genetics*** with contributions from 38 Ph.D. students and postdoctoral fellows and a large number of international collaborations.

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Preface

Rice (*Oryza sativa* L.), belonging to the Poaceae family, is the staple food crop for more than 60% of the world population and is of special importance in Southeast Asian countries. The global land area for rice cultivation is about 158 million hectares. India and China are the leading rice producers with 640 million tons of rice coming from Asia, which is almost 90% of the world rice. More than 3.5 billion inhabitants of the world depend on rice extensively for obtaining 20% of their daily calorie intake. The global population is projected to increase from six to eight billions by 2025, for which an additional 40% rice production will be necessary by 2025. Moreover, the global requirement of rice as food is expected to increase from 439 Mt in 2010 to 555 Mt in 2035. Therefore, it clearly shows how vital it is to not only enhance global rice production but also protect rice plants growing in the agricultural field from getting affected by different atrocities of nature.

Abiotic stress is the main factor detrimentally affecting crop growth and productivity worldwide. The global climate change that involves increase in temperature and atmospheric CO_2 concentration, greenhouse gases and ultraviolet rays (UV), rampant disposal of heavy metals or metalloids as industrial effluents, salinity, drought, cold, submergence, nutrient or mineral deficiencies due to leaching of soil nutrients, and reduction in safe unpolluted water for agriculture has emerged as major environmental issues. All these stressors, either singly or in combination, severely influence the yield and productivity of rice, so that development of climate-resilient rice should be emphasized in order to safeguard global rice production to fulfill the demand of population explosion. In addition to protection against environmental stresses, focus should also be given towards milling traits, augmenting head rice yield, increasing chalkiness due to reduced starch accretions, and improving grain quality. All these together make rice production under changing climatic conditions a tremendous challenge in the forthcoming years. Rice exhibits genotypic differences in their sensitivity or tolerance to different stresses, with some varieties showing higher degree of tolerance to a particular stress. In addition, there are some wild varieties of rice which are also capable of surviving under hostile conditions and provide a valuable source of novel genes that could be exploited for generating tolerance in the susceptible varieties, e.g., *Porteresia coarctata* (Roxb.) Tateoka, a halophytic wild rice, growing abundantly in the coastal regions, exhibits salt tolerance property and has evolved genes and proteins that can function under saline environment. Isolation and characterization of these genes definitely seem to be valuable to generate salt-tolerant rice. The same approach holds true for developing rice plants resilient against other environmental stresses as well.

Rice quality improvement and development of new varieties at one point of time were solely dependent on several breeding techniques which involve pedigree, bulk, modified bulk, single seed descent, and doubled haploids, of which the pedigree method has been the most popular one. Rice, harboring relatively small genome size (400–430 Mb), was the first sequenced crop genome so that it could be used as a model for sequencing other cereals with larger and more complicated genomes. Not only that, the greater aspect of the rice reference genome has been in advancement of rice improvement through development of molecular markers, understanding their physical order and proximity to annotated genes for deciphering the gene-trait associations. With the development of recombinant DNA technology, tissue culture methods, and gene transfer protocols like *Agrobacterium tumefaciens*-mediated transformation, particle bombardment, electroporation, and “in planta transformation,” it has been possible to introgress either alien genes or overexpress endogenous genes to develop abiotic stress-tolerant transgenic rice. The doubled haploid rice development also requires a tissue culture-based strategy in order to make genetically homozygous lines in a single step. The modern “omics” approaches and tools like next-generation sequencing are also enabling identification and bioprospecting of novel genes and proteins from tolerant cultivars to be introgressed in susceptible varieties for tolerance against single or combined stresses.

This book has around 31 chapters prepared by specialized researchers and scientists working in different avenues of environmental stress in rice, and the range of topics is quite broad. The chapters at the beginning highlight the major constraints in rice production, especially with respect to diverse environmental stresses, the potentiality of wild rice in improving domesticated rice species, and the varietal differences between the sensitive and tolerant rice cultivars. Few chapters are focused on tissue culture and breeding techniques required to develop improved rice and the role of genome sequencing in rice biotechnology. The succeeding chapters highlight the diverse environmental stresses like salinity, drought, heavy metal/metalloid, arsenic, temperature, ozone, UV, anoxia, submergence, and nutrient deficiency and the genetic engineering approaches to develop tolerance against such diverse stresses. There are chapters which emphasize how the overall metabolic pathways or phytohormone levels could be manipulated to develop stress tolerance. Finally, the challenges faced in maintaining rice grain quality due to abiotic stresses have been discussed in chapters towards the end of this volume.

I strongly feel that this volume will be of great interest to students, research scholars, teachers, and scientists working in the area of abiotic stress in rice at research institutes, universities, and colleges all over the world. It will also be helpful to the multinational companies involved in agricultural development or seed production.

I am highly grateful to all the contributors and academicians working in this field who have been the major pillars in making this volume a success. I pay respect to all my teachers who taught me the basics of plant science and created my interest in the field of rice research. I acknowledge the immense support and encouragement of the Principal, St. Xavier's College (Autonomous), Kolkata, where I am currently working. I thank all my family members for their continued support and patience in my academic pursuits. Finally, I appreciate the co-operation and support of Springer International Publishing House, which largely helped me to handle this voluminous editorial work single handedly.

Kolkata, West Bengal, India Aryadeep Roychoudhury

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About the Editor

Aryadeep Roychoudhury is working as Assistant Professor at the Department of Biotechnology, St. Xavier's College (Autonomous), Kolkata, West Bengal, India. He received his B.Sc. (Hons.) in Botany from Presidency College, Kolkata, and M.Sc. in Biophysics and Molecular Biology, University of Calcutta, West Bengal, India. He did his Ph.D. from Bose Institute, Kolkata, under Jadavpur University, Kolkata. His Ph.D. thesis was mostly based on characterization of different salt-inducible genes in rice. Following his Ph.D. work, he joined as Research Associate (Postdoctorate) at the University of Calcutta, continuing with rice as model system. Dr. Roychoudhury is currently handling several government-funded projects on abiotic stress responses in rice and supervising five (05) Ph.D. students as Principal Investigator. To date, he has published over 110 articles in peer-reviewed journals and chapters in books of international and national repute. He is a regular reviewer of articles in high-impact, international journals, life member of different scientific associations and societies, and the recipient of the Young Scientist Award 2019, conferred upon him by International Foundation for Environment and Ecology, at the University of Allahabad, Prayagraj, Uttar Pradesh.

Origin and Evolution of Rice as Domesticated Food Crop

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and Saher Islam

Abstract

Rice forms the staple diet of about half of the population all over the world and is the world's largest food crop. The domestication of rice began centuries ago, and currently *Oryza glaberrima* and *O. sativa* are the two species grown globally. The history of the domestication of rice can be traced back to 10,000 years ago when the *Oryza* sp. was a mere wild grass and artificial continuous selection by humans has led to the development of a stable amenable cultivar. Several studies show that Asian rice or *O. sativa* has *O. rufipogon* as its progenitor, from which it was domesticated. *O. sativa* is further comprised of five subpopulations, out of which *indica* and *japonica* show distinct genetic variation. Since phenotypes of domesticated rice variety differ significantly from their ancestor which is of the wild variety, organisms that interact with domesticated variety experience selective pressure to adapt to these novel phenotypes. Modern genetic tools have paved the way for new insights into such genetic variation, population structure and identification of domestication loci in species of rice. Thus domestication is a

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complex evolutionary process. Studies on molecular genetics behind the dynamics of domestication will help us breed better varieties for future generations.

Keywords

Domestication · Origin · *Oryza glaberrima* · *Oryza sativa* · Rice Domestication syndrome · Wild relatives

1 Introduction

Rice is a major staple food that about 50% of the human population depend on. Rice has many species but only *Oryza glaberrima* and *O. sativa* are widely cultivated in the world. There are 21 wild relatives of domesticated rice (Vaughan et al. 2003). Like every other food crops, both originated from the wild. Rice probably originated about 130 million years ago as a wild grass in Gondwanaland (before the break up) and from there spread to Asia, Africa, the Americas, Australia and Antarctica after the break up (Kole 2006). The domestication of Asian rice and African rice was believed to occur about 10,000 years and 3000 years ago, respectively (Khush 1997) from different wild grasses through continuous human selection. However, the precise location of domestication of rice is difficult to determine as the debate on its origin remains contentious. Domestication is a complex evolutionary process and the origin of cultivated rice is the best example of directional evolution among crop plants (Kole 2006) (Fig. 1).

2 Single vs. Multiple Origin of Rice

There are a lot of controversies whether rice has single or multiple origins (Meyer et al. 2012). It generally accepted that domestication of rice occurred at least twice, one in Asia and another one in Africa. Several authors believed that the Asian rice (*O. sativa*) was domesticated from wild Asian species belonging to the A-genome group of the genus *Oryza* (Chang 1976; Oka 1988; Wang et al. 1992; Khush 1997; Ge et al. 1999). The wild progenitor of Asian rice is *O. rufipogon*, which occurs as either annual or perennial. The annual *O. nivara* was domesticated to *O. sativa* 9000 years ago whereas the African rice was domesticated independently in West Africa from *O. barthii* about 3000 years ago (Vaughan et al. 2008; Khush 1997) (Table 1).

However, controversy has persisted on the issue of the origin of Asian rice either as a single origin or multiple origins. Some authors suggested the two major subspecies (*O. indica* and *O. japonica*) of Asian rice have single domestication (Sang and Ge 2007; Kovach et al. 2009; Huang et al. 2012; Gross and Zhao 2014) while others differed and claimed they were domesticated differently of single and multiple origins especially two major issues (Civáň et al. 2015). According to the former, *japonica* was domesticated first, whereas *indica* subsequently emerged by

Fig. 1 The domestication transformation of rice from *O. rufipogon* to *O. sativa*. (**a**) Panicle from *O. rufipogon*; (**b**) seeds from *O. rufipogon*; (**c**) panicle from *O. sativa*; (**d**) seeds from *O. sativa* (Reproduced with permission (Kovach et al. 2007), Copyrights reserved to Elsevier Inc., 2007)

adopting domestication alleles from *japonica*. According to Kole (2006), "domestication of rice in Asia could have occurred independently and concurrently at several sites within or bordering a broad belt that extends from the plains below the eastern foothills of the Himalayas in India through upper Myanmar, northern Thailand, Laos, and Vietnam to south western or southern China".

Contradictory genetic and archaeological studies are available. Some genetic evidence shows that both *indica* and *japonica* come from a single domestication event that occurred in the Pearl River valley region of China about 8200–13,500 years (Molina et al. 2011; Huang et al. 2012). In another scenario, two studies analysing the same data arrived at two different domestication conclusions: Huang et al. (2012) supporting the single domestication with introgression model with Civáň et al. (2015) supporting the multiple domestication model.

Table 1 Genome-wise classification and distribution of genus *Oryza*

Genome	Taxa	Geographical distribution	References
AA	*O. sativa*	Worldwide	Li and Rutger (2000)
	O. glaberrima	West Africa, Central Africa	Agnoun et al. (2012)
	O. nivara	Tropical Asia	Banaticla-Hilario et al. (2013)
	O. rufipogon	Tropical Asia to Northern Australia	Banaticla-Hilario et al. (2013)
	O. meridionalis	Indonesia, Northern Australia	Juliano et al. (2005)
	O. barthii	Africa	Semon et al. (2005) and Choi et al. (2019)
	O. longistaminata	Africa	Agnoun et al. (2012)
	O. glumaepatula	Latin America, Cuba, Brazil	Karasawa et al. (2007)
BB	*O. punctata*	Africa	Wambugu et al. (2013) and Zou et al. (2015)
BBCC	*O. minuta*	Philippines, Papua New Guinea, Thailand	Elgamal and Elshenawy (2018)
	O. schweinfurthiana	Africa	Zou et al. (2015)
CC	*O. officinalis*	Southern Asia, Papua New Guinea	Bautista et al. (2006) and Zou et al. (2015)
	O. rhizomatis	Sri Lanka	Zou et al. (2015)
	O. eichingeri	West Africa, Central Africa, East Africa, Sri Lanka	Zou et al. (2015)
CCDD	*O. malampuzhaensis*	India	Zou et al. (2015)
	O. alta	Latin America	De-Bin et al. (2010)
	O. grandiglumis	South America	Sanchez et al. (2006)
	O. latifolia	Latin America	De-Bin et al. (2010)
EE	*O. australiensis*	Northern Australia	Henry et al. (2010)
FF	*O. brachyantha*	Africa	Ricachenevsky et al. (2018)
GG	*O. granulata*	South Asia, Southeast Asia	Qian et al. (2006)
	O. meyeriana	South Asia, Southeast Asia	Qian et al. (2006) and Molla et al. (2018a)
	O. neocaledonica	New Caledonia	Molla et al. (2018b)
HHJJ	*O. longiglumis*	Indonesia, Papua New Guinea	Naredo et al. (2003)
	O. ridleyi	Southeast Asia, Indonesia, Papua New Guinea	Naredo et al. (2003)
HHKK	*O. schlechteri*	Papua New Guinea	Khanna et al. (2018)
KKLL	*O. coarctata*	South Asia	Lu and Ge (2005) and Mondal et al. (2017)

However, it is clear that the two most cultivated rice, African rice and Asian rice, were independently domesticated at different region and period (Fig. 2).

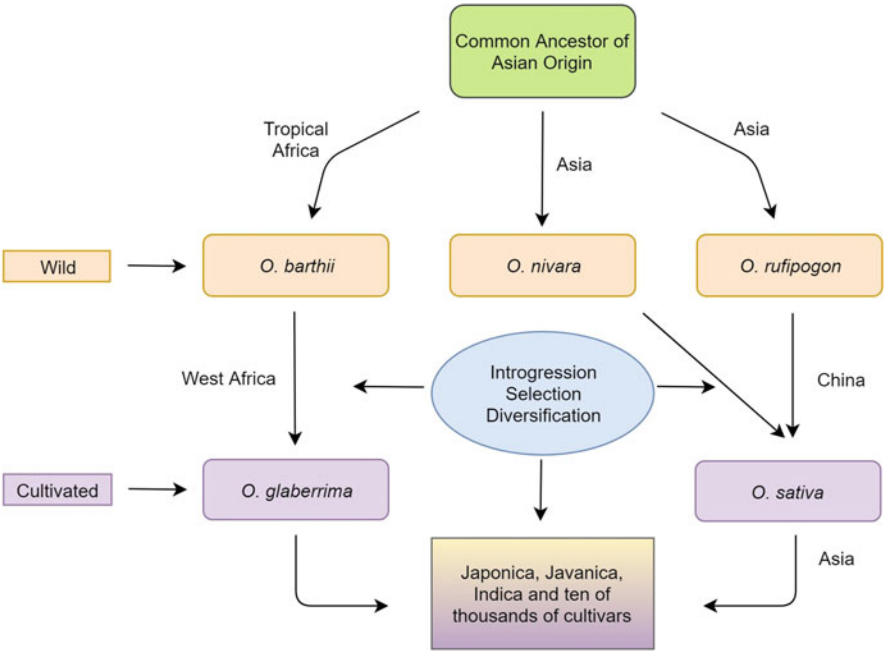

Fig. 2 The evolutionary history of cultivated rice (Reproduced and modified with permission from Vaughan et al. (2008), Copyrights reserved to Elsevier Inc., 2008 and modified from Gross and Zhao (2014))

3 Domestication of Rice in Asia

From time immemorial rice has been a major crop that is well associated with the Asians. Rice supplies more than half of the calories consumed in Asia. Asia is the largest producer of rice, with a production of 668 million tonnes representing almost 90% of the global production (International Rice Research Institute 2016). The pronounced association of rice with the Asians is probably due to the fact that rice was believed to be first domesticated in the region (Garris et al. 2005; Zhao et al. 2011; Civáň et al. 2015). Several areas in Asia were suggested as the first place of domestication including India and South China (Matsuo et al. 1997; Zhao 1998). There are two types now referred to as *indica* and *japonica* subspecies having distinct morphology in leaf and seed traits that made them to be classified as subspecies (Kato et al. 1928; Oka 1988). A third subpopulation was identified as *javanica* based on morphological, cytological and genetic analyses (Matsuo 1952; Engle et al. 1969; Morishima and Oka 1970; Glaszmann 1987; Garris et al. 2005). The domestication of rice in Asia particularly in India and China has started about 9000 years ago (Kato et al. 1928; Andrus and Mohammed 1958; Solheim 1972; Whyte 1972; Chang 1976; Sharma and Manda 1980; Khush 1997).

Rice cultivation appears to have diversified throughout the whole rice-growing area. It is further believed that the rice-growing culture is responsible for the emergence of the Yangtze River civilizations. Some of the derivatives of rice originated in the homeland may have reached the Yellow River Basin by 6000 BP, becoming the staple food of the civilizations of this region. Following this, however, the conflict between various struggling factions led to a dramatic decline in the early civilizations of the Yangtze River region in around 3000 BP. During this period, it is believed that many people left this region in search of new homelands. Some of these people reached the present-day southwestern provinces of China with japonica rice and their associated cultures. Others arrived in western Japan crossing the East China Sea, or by way of the Korean peninsula. Thailand received domesticated rice from China – archaeological data indicate that until about 300 BCE, the dominant type was *O. japonica* – relationship with India around 300 BCE, makes the establishment of rice regime that completely depend on wetland agrosystems using *O. indica* (Crawford and Shen 1998; Higham 1995; Fuller 2011).

4 Domestication of Rice in Africa

Rice is a major staple food in many countries of Africa as it constitutes most of the diet of the people. It is important in the continent's food security planning policies. Presently, Africa consumes a total of 11.6 million tonnes of milled rice per year (FAOSTAT 2017). In Africa, Nigeria is the largest producer and consumer of rice, recently overtaking Egypt in production (FAOSTAT 2017; International Rice Research Institute 2018). Before the introduction of Asia rice (*O. sativa*), African rice (*O. glaberrima* Steud.) existed. Africa rice was domesticated 3000 years ago independently from *O. barthii* (Portéres 1976; Sweeney and McCouch 2007; Vaughan et al. 2008). African rice is well adapted by having several potentially beneficial traits for extensive cultivation in West Africa (Brar and Khush 1997; Sanchez et al. 2013). Western Africa is the main producing sub-region, accounting for more than 58.8% of African production in 2017. In individual countries, the leading producers of paddy (2017) are Nigeria (9.9 million tonnes), Egypt (6.4 million tonnes) and Madagascar (3.1 million tonnes) (FAOSTAT 2017).

According to Portéres (1962), African rice was first domesticated in the inland delta of the upper Niger River and later diffused to two secondary centres of diversity, the first was one along the coasts of Senegal and the Gambia and the second in the interior highlands in Guinea (Dresch 1949; Portéres 1956, 1962, 1970; Second 1982; McIntosh 1995; Klee et al. 2000; Semon et al. 2005; Blench 2006). Plant breeders have crossed Asian rice and African rice, combining the stress-tolerance traits of African rice with the yield potential of Asian rice (Jones et al. 1997; Gridley et al. 2002), which have become popular among West African farmers as NERICAs (New Rice for Africa). It combined the high-yielding traits of Asian rice with the adaptive traits of West Africa rice (Jones et al. 1997).

5 Domestication of Rice in Other Region

Outside Asia and Africa, rice is also cultivated on large scales in America, Europe and Australia. In addition, there are histories of rice domestication in these other regions. In America, there are rice types that belong to the genus *Zizania*. Their genus is made up of three species: Northern wild rice (*Zizania palustris*), Wild rice (*Z. aquatica*) and Texas wild rice (*Z. texana*) which are important in the diet of Native American tribes. They served as essential seasonal food of the indigenous people of America long before the introduction of Asian and African rice. The rice is still gathered and consumed in various modern localities along the Guapore River in Brazil (Littlefield 1991). African rice was likely the first rice to be cultivated in America, but higher yielding Asian rice varieties (*O. sativa*) subsequently displaced it (Carney 2001).

Rice is not native to Europe. It was introduced through trade and military expedition from Asia (Sallare 1993). The Muslims played a vital role in the introduction of rice to Europe. In Europe, Italy is the largest rice production country, with about 250,000 hectares of cultivation area and 1.6 million tons of total grain production (FAOSTAT 2017). Almost all rice varieties cultivated in Italy are temperate *O. japonica* subspecies (Faivre-Rampant et al. 2010). Results based on SSR fingerprints of a large set of rice germplasm from Italy and Asia demonstrated a close genetic relationship between Italian and Chinese rice varieties, suggesting the possible origin of Italian traditional rice from northern China (Cai et al. 2013). Wild *Oryza* species are widespread in northern Australia (Henry et al. 2010). This is an area without a long history of rice cultivation, implying that the wild populations have remained largely isolated from the impacts of gene flow from domesticated crops that has apparently been widespread in Asia.

6 Rice Domestication Syndrome

Rice is an important food crop that is cultivated under different agro-climatic conditions all over the world (Jena 2010). *O. sativa* (Asian rice) grown in the Asian region and *O. glaberrima* (African rice) grown in certain parts of Africa are two of the domesticated varieties of rice. Genetic studies show that *O. sativa* is related to the wild variety *O. rufipogon* and *O. glaberrima* is related to the wild variety *O. barthii*. Domestication of rice involves the selection process that is complex in nature and the modification of the genetic makeup of the wild variety of rice to produce a new variety which meets human requirements (Izawa 2008). During the domestication process, the agronomic traits are subjected to artificial selection which results in the change of seed size, changes in the growth of the plant and flowering and all these changes are useful for humans. As a result of domestication, rice undergoes certain modifications from the wild ancestors which include seed dormancy and seed shattering, increase in seed size and inflorescence which is termed as rice domestication syndrome (Li et al. 2005; Asano et al. 2011). Domestication syndrome varies among different varieties of rice crops and is dependent on how the crop is produced (Smýkal et al. 2018). Among the domesticated rice varieties, diversity of Asian variety is remarkable. The selection associated with the domestication of rice resulted in the reduction of recombination and a great degree of inbreeding which means both harmful and useful changes have happened (Vaughan et al. 2008).

One of the major problems related to the high diversity of rice is that certain traits related to domestication are related to the cultivated variety of rice except for two traits. These two traits, loss in the secondary dormancy and degree of shattering of spikelet, are key features to the rice domestication. Both these traits vary depending on the rice varieties. In different varieties of rice, ease of shattering is different. *Japonica* variety lacks the presence of abscission layer at the base of the seed, in the *Indica* species abscission layer is present partially and the abscission layer is present completely in weedy rice. The complexity of dormancy is more and it consists of hull and seed dormancy. Secondary dormancy is important in the *Indica* variety because seeds remain viable during humid and hot climate between the planting seasons. It has been seen that in the domesticated varieties of certain crops the size of the organs that can be consumed has increased but in the case of rice, the size of the seed of most of the cultivated varieties are slightly different from those that of the wild varieties. Thus, seed size cannot be used as a parameter to differentiate between domesticated and wild varieties. Synchronous maturity and the seeds present per panicle are other two traits that are very important during the domestication process. The domestication syndrome studies carried out in rice have identified the presence of quantitative trait loci (QTL) belonging to the parent varieties of the hybrids. Diversification after domestication has resulted in the variation of traits related to domestication (Vaughan et al. 2008). A study was conducted by Li et al. (2005) on *O. nivara* to understand the genetic basis of the rice domestication syndrome. They found that for planting and harvesting, QTL which had larger phenotypic effects were chosen for domestication selection which included reduced seed dormancy and seed shattering and seed maturation synchronization. Shattering of seeds was found in *O. nivara* and not in *O. sativa*.

7 Impact of Domestication of Rice on Human Civilization

Before the dawn of widespread cultivation of rice as a major food crop, it served as a source of food to people who earned their livelihood from fishing, hunting and gathering. In many areas, where the environmental conditions are not quite favourable, even today, rice farming acts as a source of sustenance. In other areas, where there was proper management of water resources, soil fertility, weeding and tillage, rice was cultivated and yields rose steadily. The food surpluses supported the development of the human population. Increase in areas under rice cultivation and enhancement in rice yields have helped in the flourishing of human civilizations.

As far as the human diet is considered, both rice and wheat have equal importance. However more than wheat, rice is being consumed by man. Eighty percent of the calorie intake of the Asian population is contributed by rice. For people in Africa, rice makes up 33% of the calorie intake (Portéres 1976; Chang 1987).

Mainly, domesticated rice is of two types, *O. sativa* (Asian rice) and *O. glaberrima* (African rice). The domestication history differs for both. *O. rufipogon* and *O. nivara* are known as the progenitors of *O. sativa* or the Asian rice whereas the African rice originated from *O. barthii*. *O. sativa* is cultivated globally with higher emphasis in Asia. *O. glaberrima* is however restricted to West Africa.

Several phenotypic differences have been observed between the domesticated species and wild relatives. Wild varieties have long awn and high shattering of seeds whereas domesticated rice has short awns and less shattering to facilitate maximum harvest. Wild rice has higher dormancy levels. Viable seeds can remain in a state of dormancy for years before germination. In cultivars, dormancy level is relatively low so that these can yield uniform germination. African cultivars retain the red colour of the seed coat seen in wild grains but Asian cultivars lack the pigmentation. Wild rice has dark-coloured hulls and in the domesticated ones, hulls are straw-coloured (Sweeney and McCouch 2007).

8 Impact on Selected Civilizations

The masses should be adequately fed for any civilization to flourish. Rice has played an important role in the flourishing of many Asian civilizations. It forms an integral part of many Asian civilizations such as Japanese, Thai, Sri Lankan, Indian and Sichuanese (Fuller 2011).

8.1 China

The excavations at Ho-mu-tu site, China has revealed huge amounts of carbonized rice grains belonging to the *indica* type which can be dated back to around 5008 BC. Husks and straws were also found. Pots made out of clay for cooking rice, tillage tools and animal bones were discovered. In another nearby site, *Sinica* rice grains dated back to 7000 BC was encountered. These remains provide an insight that 7000 years ago, a well-developed culture was supported by combined practice of rice cultivation, hunting and food gathering (Chang 1987).

8.2 India

The oldest rice sample was found in northern India and was dated back to 4530 BC. Later, excavations at Mahagara, Uttar Pradesh have changed the date to around 6570 BC. From the remains excavated from the site, it seemed that rice grains were the cultivated race. The Tamil kingdoms were earlier situated on river deltas.

Transplantation of rice seedlings was a common practice on Godavari and Krishna deltas (Chang 1987).

Latest archaeological research has revealed that Indus population are the earliest known people to employ multiple cropping strategies during summer and winter with rice being cultivated in the summer. A group of regional farmers sold the yield to the civilizations' ancient markets.

Lahuradewa, in the Ganges basin, has been reported to harbour the earliest use of rice. Wetland rice was introduced from China and this being more productive, was widely taken up for cultivation in India. But studies reveal that even before the introduction of rice from China, there was already a well-established rice production in India during Indus civilization as a summer addition to winter farming. It is believed that Indus, being located in an area that received rain during both summer and winter, would have substantiated the seasonal crop rotation (Bates et al. 2016).

8.3 Japan

It was in about 3000 BC that rice was introduced from China and Korea to Kyushu Island. It gradually replaced practices of hunting and vegetable and nut gathering. The advent of iron tools and irrigation facilities further aided to flourish rice as a cultivated crop. Within around 600 years, rice cultivation spread to Shikoku and Honshu Islands. Some *indica* rice cultivars were replaced by *sinica* or *japonica* type that produced better yield. This enhanced the cultural development rate (Chang 1987).

9 Conclusion

Availability of fertile lands for farming, adequate irrigation facilities, method of transplantation of seedlings and multiple cropping together favoured the increase in settlement of human population and thus, population increase. However, the present scenario is that the rice cultivation is unable to keep up to par with the booming rate of population. Prospects for the addition of more area under rice cultivation are almost nil. Thus the supply of the adequate amount of rice in the coming decades is going to be one of the great challenges. Feeding of the rapidly increasing population requires improved farming systems. Moreover, there is an urgent need for the development of rice cultivars that are capable of producing high yields and are resistant to different types of biotic and abiotic stresses.

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Major Constraints for Global Rice Production: Changing Climate, Abiotic and Biotic Stresses

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Abstract

Rice (*Oryza sativa* L.) is among major cereals that show significant susceptibility to a number of abiotic stresses. Environmental constraints inhibit relevant physiological and biochemical indices that, in turn, determine grain yield in rice. Heat stress induces significant changes in metabolic profiling in flag leaves and developing seeds that reduces grain yield and quality in rice. The grain yield in rice is also impeded as a result of salinity, drought, and heavy metal stress. The literature shows that rice plants growing in the environment with combined heat and drought stress show lesser spikelet fertility. The decrease in plant growth and productivity is the consequence of inhibited ions homeostasis, photosynthesis, the efficiency of both photosystems alongside the enhanced generation of reactive oxygen species, and loss of membrane integrity measured in the form of accumulation of malondialdehyde contents. Moreover, changes in secondary metabolites accumulation and disturbed ions homeostasis under stressful conditions also reduce growth and productivity of rice. The defense mechanisms manifested by rice entail osmotic adjustment, stimulated activities of antioxidant enzymes along with enhanced production of non-enzymatic antioxidant compounds. In this chapter, we have discussed the negative impact of abiotic and biotic stresses on key physiochemical processes. The defense responses of rice to different environmental constraints are discussed at length.

Keywords

Osmotic adjustment · Oxidative defense system · Reactive oxygen species · Ions homeostasis · Photosynthesis · Plant water relations · Chlorophyll fluorescence

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1 Introduction

Plant growth and development is hampered at physiological and biochemical levels due to biotic and abiotic constraints resulting in significant yield losses in agriculture crops (Kazan 2015). Several environmental constraints such as heavy metals, cold, heat, salinity, and drought stress noticeably inhibit growth and productivity in rice. The response of rice plants to abiotic stresses is determined by the developmental growth stage (Feller and Vaseva 2014; Paul and Roychoudhury 2019). A number of crops indicate greater sensitivity to abiotic stresses during early growth stages, while these plants show enhanced tolerance to environmental hazards at later growth stages. Among different abiotic stresses, drought is considered as the most severe constraint responsible for a substantial reduction in growth and yield throughout the globe (Nasim et al. 2018). Salinity stress is considered as the second most severe abiotic stress for plant productivity after a drought that reduces crop efficiency (Roychoudhury and Chakraborty 2013; Mariani and Ferrante 2017). The food insecurity is an emerging problem, mainly resulting from significant yield reductions in plants under abiotic stresses (Thakur et al. 2010). Plant growth, development, and grain yield in rice are markedly reduced in rice due to biotic and abiotic environmental stresses. Abiotic stresses induce a number of alterations in morphology and physiochemical processes of plants that, in turn, negatively affects plant productivity (Pandey et al. 2017). The reduction in plant productivity due to these stresses is estimated to be around 50% that results in a significant financial loss (Ishaq and Memon 2017). The supply of food to the ever-increasing human population is undermined due to significant yield losses in plants facing environmental constraints (Krishnan et al. 2011). The schematic representation of the inhibitory effects of abiotic stresses on plant growth and productivity is given in Fig. 1.

2 Salinity Stress in Rice

Soil salinization is the main obstacle in improving the growth and grain yield of rice (Golldack et al. 2014). Salinity is the second most severe environmental constraint after a drought for rice production (Wani and Sah 2014). The soil salinity is cumulative of inland salinity that arises due to poor agricultural practices and coastal salinity, which is the consequence of high tides in coastal regions (Ganie et al. 2016). The ionic and osmotic components of salinity disrupt the crucial processes involved in plant growth, development, and productivity (Roychoudhury et al. 2008). The seedling and reproductive stages of rice are highly sensitive to salinity (Waziri et al. 2016). The drastic effects of salinity on rice production are evident in the form of delayed flowering and panicle emergence alongside the reduction in panicle length and number (Kaur et al. 2019). Plants display noticeable alterations in morphology, physiochemical, and molecular responses under salinity. The reduction in growth and production in rice is ascribed to low tillering, chlorosis, leaf burning, and leaf rolling under salinity. The integrity of membranes and activities of antioxidant enzymes is abated due to the enhanced generation of reactive oxygen species

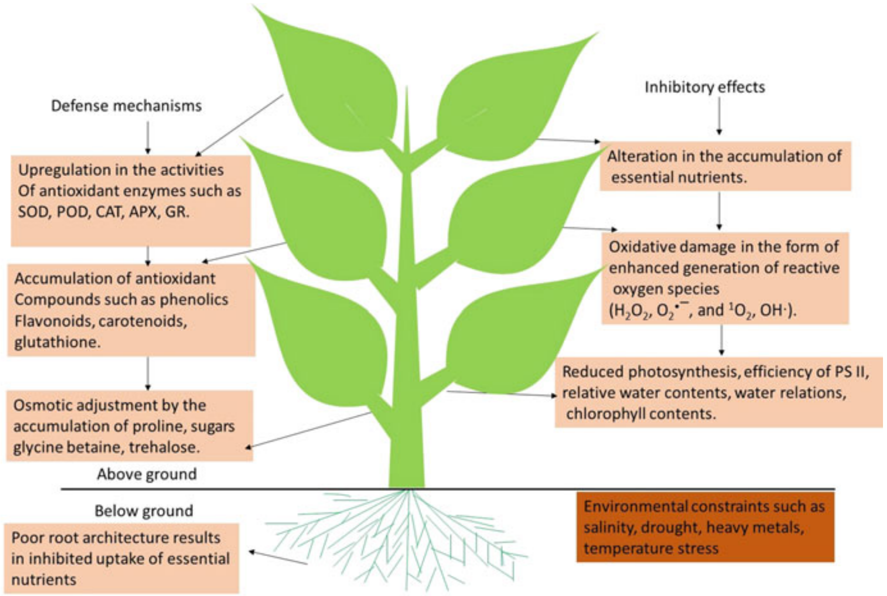

Fig. 1 Schematic representation of abiotic stress-induced inhibitory effects on plants and defense mechanisms employed by plants to overcome the environmental constraints

(ROS) under salinity (Ganie et al. 2016). The physiology of essential processes such as photosynthesis, respiration, and nutrient acquisition is impeded in rice under salinity (Gupta and Huang 2014). The delicate balance in the water content of plants is disturbed by salinity-mediated osmotic stress that induces stomatal closure and limited water content. In addition, salinity also causes significant changes in the expression pattern of proteins and genes that determine the growth and development of rice (Gharsallah et al. 2016). The requirement to increase rice production in saline areas is of prime importance to meet the food demands of increasing human population (Kaur et al. 2019). There are several sources of salinity that inhibit growth and productivity of crops. The type of salt that causes salinity is of significant importance. However, many other factors such as crop and soil management, climatic conditions, and soil types further add to soil salt concentration. For instance, coarse-textured soil in a humid climate would manifest higher infiltration rate and rainfall to leach down salt. However, fine-textured soils under arid and semiarid conditions do not exhibit salt leaching (Riaz et al. 2019). Salinity is a widespread problem that impedes germination, plant growth, and productivity (Munns and Tester 2008). The adverse effects of salinity on morphological and biochemical attributes can be ascribed to a decrease in cell division and expansion, loss of membrane integrity, alterations in metabolite functions, oxidative stress, nutritional imbalance, specific ion toxicity, and water stress (Hasegawa et al. 2000).

According to a report of Economics of Salt-Induced Land Degradation and Restoration, every year agricultural land from 75 countries is affected by salinity, which is estimated as the average of 2000 hm^2 (unu.edu/mediarelations/releases). A major cause of salinity is linked with elevated sea level which causes the saline water flow to the inland which further results in the rice fields to face the saline conditions (irri.org/news/hot-topics/rice-and-climatechange). In *Oryza sativa* crop, yield loss up to 1 t/hm^2 is shown due to the electric conductivity, which is almost more than 2 dS/m resulted by flooding water (Asch and Wopereis 2001). Favorable areas for rice cultivation such as South and Southeast Asia, millions of hectares of agricultural land are left without cultivation or have a significant reduction in yield due to drastic effects of salt stress (knowledgebank.irri). Different geological and agro-climatic zones have significant factors that lead to the accumulation of salts in the soil (Reynolds 2001). Regarding the effects of salt stress, plants can be classified into two groups. One group involved the plants which are susceptible to saline conditions known as glycophytes, while, on the other hand, salt-tolerant or plants growing in the salty condition named as halophytes (Tuteja et al. 2011). Plants may react to different stresses at the cell level and ultimately at a whole organism level. At the time of salt treatment or after some days or weeks of treatment with salt, a significant reduction in growth and development can be visible (Roy et al. 2014). The rise in leaf temperature and minimal shoot elongation are caused by the impact of salinity on stomatal closure (Rajendran et al. 2009; Sirault et al. 2009), and not dependent on salt uptake in the shoot region. This phenomenon was reported as “osmotic Phase” by Munns and Tester (2008) while the “shoot salt accumulation independent effect” by Roy et al. (2014). As a result of increased salinity duration, retardation in growth over a time span and before time senescence in older leaves can be seen which was named as “ionic phase” by Munns and Tester (2008). Differences based on the genotype in growth and development of upland rice crop due to saline effects have been impaired with osmotic adjustment. Maintaining the water level in shoot and complete plant, extrusion of Na^+, regulation of K^+ in readily growing leaves and tissues lead to salinity tolerance in rice cultivars (Yeo et al. 1987). Plant yield production is ensured by its potential to tolerate salinity (Momayezi et al. 2009). Salinity has severe impacts on rice growth and development, adjustment of plant and its responses to salt-containing conditions. Salinity results in rice sterility if plants exposed at the stage of pollination and fertilization. The panicle sterility was noticed in a few cultivars of rice grown under salinity stress (Khatun and Flowers 1995). There is a need to develop new cultivars with a potential of high yield and tolerance under stressful environments across the different agro-climatic zones. This could be possible only by readily discovering different genes and their alleles through marker-assisted selection and genetic engineering in crops.

3 Salinity Stress-Responsive Mechanism in Rice

It is essential to know about the exact mechanism behind molecular regulation of plant grown under saline conditions to produce salt-tolerant and high-yielding varieties. Salt tolerance is considered as a complex and quantifiable trait that is regulated by several genes (Chinnusamy et al. 2005). Rice plant is classified as the salt-sensitive cereal, particularly at the initial stage (Lutts et al. 1995); however, saline conditions also affect the potential of productivity at the mature stage (Todaka et al. 2012). At the seedling stage, the effect of salinity is removed through the transplantation method while stress cannot be mitigated at the flowering stage. The flowering stage is salt-sensitive stage which results in a reduction in yield (Singh et al. 2004). Under the extended duration of salinity, salt tolerance can be estimated by the comparison of biomass percentage under salinity to the control conditions (Munns 2002).

Salinity tolerance in plants relies on ion extrusion and osmotic adjustment (Munns and Tester 2008). These processes are also grouped as ion extrusion, osmotic tolerance, and tissue tolerance (Roy et al. 2014). To avoid uptake of Na^+ and Cl^- in leaves, Na^+ and Cl^- transportation mechanisms are used. In this process, Na^+ ions are retrieved from xylem and then released back to the rhizosphere. Osmotic tolerance in *Oryza sativa* is synchronized through the systemic signal molecules which results in the reduction of shoot development and this happens prior to the accumulation of Na^+. The osmotic tolerance increases the potential of plants against salinity-induced water-deficit conditions, promotes leaf expansion and conductance through stomata (Rajendran et al. 2009). Tissue tolerance promotes the storage of Na^+ ion in vacuole achieved through the biosynthesis of several suitable solutes as well as different enzymes having the scavenging ability to detoxify ROS.

4 Effects of Salinity on Physiochemical Attributes in Rice

The inhibitory effects of salinity on plants are due to osmotic and ionic components of salinity. The osmotic component of salinity is the condition where salt concentration in the growth medium becomes greater than the threshold potential of the plant (Munns and Tester 2008). Ionic stress of salinity represents the exceeding higher concentration of Na^+ in the vacuole and cytoplasm of the leaf impeding essential metabolic reactions that result in cell death (Munns and Tester 2008). The decrease in Ca^{2+} and K^+ contents is ascribed to the antagonistic nutrient uptake effect of salinity in rice (Dobermann and Fairhurst 2000). The primary reason of injury to rice under saline conditions is due to specific ion toxicity compared with osmotic stress though water content is reduced (Korres et al. 2019). The deficiency in K^+ and Ca^{2+} uptake is the result of antagonistic nutrient uptake. For example, the competition of Na^+ with K^+ uptake leads to high Na^+/K^+ and limited K^+ uptake. Likewise, Na^+-induced reduction in Ca^{2+} uptake is associated with lower growth rate. Salinity-induced reduction in photosynthetic pigments is the typical morpho-physiological response of plants (Cha-Um et al. 2007). The adverse effects of salinity on leaf ultrastructure vary with the degree of salinity tolerance in plants (Korres et al. 2019). The salinity-induced damage to chloroplast integrity was higher in sensitive rice cultivar Amber than that of tolerant cultivar IR2153 (Flowers et al. 1985). Salinity decreased photochemical quenching attributes and electron transport rate in sensitive rice cultivar, whereas minimal reduction in these variables was present in salt-tolerant cultivar (Moradi and Ismail 2007). Likewise, the adverse effects of salinity on Na^+/K^+, chlorophyll fluorescence, and gas exchanges parameters were studied in two rice cultivars with differential salinity tolerance. The exposure of plants to salinity resulted in a significant increase in Na^+/K^+ ratio in sensitive cultivars Bankat, IR28, and Hitomebore, whereas salinity-mediated increase in Na^+/K^+ was not substantial in salinity-tolerant cultivar Pokkali. There existed a strong reduction in the net photosynthesis rate and stomatal conductance in all rice cultivars due to salinity stress. Salinity did not influence the efficiency of PS II as reflected in the form of no change in Fv/Fm. The overall efficiency of photochemical energy conversion (ΔF/FM) increased in all sensitive cultivars under saline conditions. Similarly, sensitive cultivars showed enhancement in total non-photochemical quenching under salinity. The results of the study suggested that salinity sensitivity in rice relied on a decrease in PS II photochemical efficiency alongside the improvement in non-photochemical quenching and shoot Na^+ contents (Dionisio-Sese and Tobita 2000). Similarly, NaCl salinity resulted in a substantial decrease in chlorophyll a and b contents. The efficiency of PS II, non-photochemical quenching, and photon yield of PS II were inhibited alongside the significant reduction in the photosynthesis of salinity-stressed rice plants (Amirjani 2011; Banerjee and Roychoudhury 2018).

In another study, rice plants were exposed to varying NaCl levels (0, 25, 50, 100, and 200 mM NaCl) at the reproductive stage. The decrease in photosynthesis was the result of the limited availability of CO_2 due to stomatal closure. The salinity-mediated decrease in relative water contents, osmotic and water potentials, transpiration rate, and biochemical indices such as protein, soluble carbohydrates, and photosynthetic pigments was not responsible for the reduction in net photosynthetic rate. Salinity reduced grain dry matter and this reduction was ascribed to a substantial decline in photosynthesis. The results of the study suggested that plants had the potential to overcome the inhibitory effects of salinity when it was imposed for a short period of time (Sultana et al. 1999). Likewise, a study was conducted to appraise the changes in physiological and biochemical attributes in CSR-13 (salt-tolerant) and MI-48 (salt-sensitive) under salinity. The rice seedlings (25-day) were transplanted in earthen pots with two 6.17 and 11.68 dS m^{-1} salinity treatments. The reduction in photosynthesis, RuBPC activity, and chlorophyll content was maximal in sensitive rice cultivar MI-48 while the tolerant cultivar CSR-13 displayed a marginal decrease in these indices (Pal et al. 2004). Similarly, a marked reduction in photosynthesis, transpiration, and stomatal conductance was recorded in sensitive rice cultivar IR-29 compared with tolerant cultivars IR-632 and IR-651 under saline conditions. Salinity resulted in a significant increase in non-photochemical quenching alongside the decrease in electron transport rate (Moradi and Ismail 2007). In another study, salinity-mediated changes in physiological parameters were appraised in rice plants. Rice seedlings (cv. IR-20) were transplanted in saline (100 mM) or non-saline hydroponics culture. Salinity induced a substantial decrease in photosynthesis, stomatal conductance, relative water content, and chlorophyll content. The rice plants grown in saline hydroponics solution depicted a strong deterioration in the efficiency of PS II alongside the reduction in chlorophyll a/b. It was also revealed in the results that the treatment of rice plants with a sublethal dose

(50 mM) of NaCl might have protected plants from the inhibitory effects of lethal dose (100 mM) of salinity in the nutrient solution (Djanaguiraman et al. 2006). The inhibitory effects of salinity on the efficiency of PSII and Na^+ and K^+ contents were measured in the old and young leaves of two Mexican rice varieties namely Cotaxtla and Tres Ríos. Besides, K^+/Na^+ ratio was also measured in stem and root. Rice plants were exposed to salinity in hydroponics solution for 2 weeks. The efficiency of PSII was significantly decreased in both rice cultivars. The endogenous K^+ contents were lower in plants treated with salinity which had a significant increase in endogenous Na^+. The rice cultivar Tres Ríos had a maximal decline in maximal quantum yield of PSII, K^+ contents, and K^+/Na^+ ratio that indicated its salinity sensitivity over Cotaxtla rice cultivar (García Morales et al. 2012). Salinity imposition in rice resulted in chlorophyll loss and decrease in photochemical reactions in photosynthesis (Chattopadhayay et al. 2002). The higher photosynthesis rate in tolerant rice cultivar is due to greater water oxidation in the light reaction of photosynthesis reflected as improvement in quantum efficiency of PSII and quantum yield of PSII alongside the reduced non-photochemical quenching (Cha-Um et al. 2009a). In another study, a decrease in the photosynthesis of rice plants was positively correlated with the chlorophyll and carotenoid contents under saline conditions (Chaum and Kirdmanee 2010). Similarly, a salinity-mediated decrease in photosynthesis was related to stomatal factors in rice plants (Khan et al. 1997).

Four rice cultivars namely Pokkali, Rashpanjor, IR42, and FR13A were subjected to salinity. The cultivars FR13A and IR42 were salt-sensitive. The accelerated absorption of Na^+ along with lower K^+ absorption resulted in higher $Na^+:K^+$ ratio in sensitive rice cultivars, whereas tolerant rice cultivars did not show a marked increase in $Na^+:K^+$. Salinity induced a significant decrease in chlorophyll and photosynthesis in rice. There were also marked differences in the efficiency of PSII between tolerant and sensitive rice cultivars (Sarkar et al. 2013). The chlorophyll a, b, and carotenoids significantly decreased in sensitive and tolerant rice cultivars in response to salinity under acidic and alkaline pH. However, the decrease in photosynthetic pigments was higher in salinity-sensitive cultivars. Similarly, maximum quantum yield of PSII, photon yield of PSII, non-photochemical quenching, and photosynthesis were abated in rice under salinity and extreme pH conditions. The inhibition in crucial physiological processes was more significant in salinity-sensitive cultivars compared with salt-tolerant rice cultivars (Cha-Um et al. 2009b). Similarly, chlorophyll contents, the efficiency of PSII, and photosynthesis decreased markedly in salt-tolerant (HJ) and sensitive (PT1) rice cultivar (Siringam et al. 2009). Salinity reduced photosynthesis, stomatal conductance, intercellular CO_2 levels, osmotic and water potentials, and relative water contents in rice plants. The inhibitory effects of salinity on the physiological indices were mitigated by the exogenous application of nutrients (Sultana et al. 2001). In another study, salinity resulted in a significant decrease in chlorophyll, photosynthesis, transpiration, stomatal conductance, and water use efficiency in two coarse and two fine rice cultivars. The efficiency of PSII (Fv/m) was substantially lower alongside the higher non-photochemical quenching (NPQ) in four rice cultivars. The foliar application of nitric oxide mitigated the salinity-mediated inhibition in the physiological attributes in rice plants (Habib et al. 2013). Salinity significantly decreased the efficiency of PSII, and this decrease was higher in sensitive rice IR28 compared with salt-tolerant rice cultivar Pokkali (Demiral and Türkan 2006). Salinity led to a marked decrease in photosynthesis and water potential in tolerant (SR26B) and sensitive (IR28) rice cultivars (Nakamura et al. 2002).

4.1 Oxidative Defense System in Rice Under Salinity

Elevated levels of NaCl in the growth medium impaired growth due to enhanced generation of ROS such as $O_2^{\bullet -}$, H_2O_2, hydroxyl, and singlet oxygen species (Bezrukova et al. 2008). ROS, being highly reactive in nature, interacts with cellular components resulting in oxidative damage reflected in the form of DNA fragmentation, protein oxidation, and lipid peroxidation. There is a need to actively scavenge ROS to minimize the oxidative injury due to salinity. Plants contain oxidative defense system in the form of enzymatic antioxidants and non-enzymatic antioxidant compounds. The enzymatic antioxidants include superoxide dismutase (SOD), peroxidase (POD), catalase (CAT), and ascorbate peroxidase (APX). The non-enzymatic antioxidant compounds include α-tocopherols, glutathione, ascorbic acid, carotenoids, phenolics, and flavonoids (Das and Roychoudhury 2014; Rasheed et al. 2018). For example, the role of antioxidant system to mitigate oxidative injury due to salinity was examined in rice (Mishra et al. 2013). The rice seedlings when subjected to saline conditions resulted in a marked increase in the generation of $O_2^{\bullet -}$ and H_2O_2 that caused lipid peroxidation measured in the form of thiobarbituric acid reactive substances. The results also indicated strong protein oxidation as evident in the form of reduced glutathione and ascorbate peroxidase. There was a significant increase in the activities of antioxidant enzymes (SOD, APX, GPX, CAT, GR) (Banerjee and Roychoudhury 2017). The authors suggested that efficient detoxification of ROS was carried out by both enzymatic and non-enzymatic antioxidant systems in a coordinated manner. Higher salinity tolerance was associated with a better oxidative defense system in rice (Mishra et al. 2013). Likewise, salinity significantly increased H_2O_2 and $O_2^{\bullet -}$ radicals in rice cultivars. However, salinity-tolerant rice cultivars did not show prominent accumulation of ROS, whereas salt-sensitive cultivars accumulated a substantial amount of ROS. The lower ROS levels are attributed to higher activities of antioxidant enzymes (SOD, POD, CAT, POD, APX) in tolerant rice cultivars compared with sensitive cultivars. The degree of oxidative injury was lower in rice cultivars with an efficient ROS detoxification mechanism (Chawla et al. 2013).

The exposure of salt-sensitive (BRRI dhan49) and salt-tolerant (BRRI dhan54) rice cultivars to saline conditions resulted in marked increase in H_2O_2 and lipid peroxidation. Salt stress markedly enhanced the activities of glutathione (GSH), ascorbate (AsA), GSH/GSSG, APX, CAT, GR, DHAR, MDHAR, and GPX in salt-tolerant rice cultivar, while the activities of these enzymes decreased in sensitive cultivar under saline conditions. These authors suggested the positive association between salinity tolerance and antioxidant defense system in rice. Roychoudhury et al. (2008) also showed that the aromatic rice cultivar Gobindobhog was highly sensitive to salt stress, and this susceptibility was mitigated by the exogenous application of higher polyamines like spermidine and spermine (Roychoudhury et al. 2011). The exogenous application of proline and glycine betaine resulted in an enhancement in antioxidant enzyme activities that reduced oxidative injury in both rice cultivars (Hasanuzzaman et al. 2014; Roychoudhury and Banerjee 2016). Similarly, salinity resulted in oxidative injury in the form of an enhanced generation of MDA and H_2O_2. The histochemical studies indicated the higher production of H_2O_2 and $O_2^{\cdot -}$ was responsible for oxidative injury in rice plants. Salt stress increased the levels of ascorbic acid and glutathione alongside the stimulated activities of antioxidant enzymes (SOD, POD, APX, GR, MDHAR, and DHAR). However, there was a reduction in the activities of CAT under saline conditions. However, the exogenous application of trehalose significantly mitigated the oxidative injury in rice plants by the upregulation of the oxidative defense system. It was suggested that rice plants overcome ROS-induced oxidative injury by increasing the concentrations of non-enzymatic antioxidants and activities of antioxidant enzymes (Mostofa et al. 2015a, b). The effect of UV-B radiations on the membrane integrity, ROS production, and lipid peroxidation was studied in two UV-B-sensitive rice cultivars (IR74 and Dular). The rice seedlings (10-day) were subjected to UV-B radiations for 28 days. The degree of oxidative injury was estimated by measuring the production of MDA, H_2O_2, and relative membrane permeability in two rice cultivars. The activities of antioxidant enzymes and the concentration of non-enzymatic antioxidants markedly increased and thereby protected rice plants from ROS-mediated oxidative injury (Dai et al. 1997).

In another study, the degree of oxidative damage due to Na^+ and Cl^- toxicities was measured in two rice genotypes. The equimolar concentration of Na^+ and Cl^- (100 mM) enhanced the production of H_2O_2 and $O_2^{\bullet -}$ radicals. Salt-sensitive rice cultivar manifested higher level of oxidative injury compared with tolerant cultivar. The activities of antioxidant enzymes (SOD, POD, CAT, APX, and GR) significantly increased in plants under salinity. The stimulated activities of antioxidant enzymes are correlated with higher salt tolerance reflected in the form of lower endogenous levels of H_2O_2 (Khare et al. 2015). Likewise, two rice cultivars grown under salinity depicted a significant increase in oxidative injury alongside the enhanced antioxidant system. Salinity produced higher endogenous levels of H_2O_2 that induced a marked damage to cell membranes measured in the form of MDA. Higher salt tolerance in cv. Lunishree was associated with better ROS detoxification ability in terms of antioxidant defense system compared with sensitive rice cultivar Begunbitchi. The accumulation of Na^+ was maximal in sensitive rice cultivar than that in the tolerant cultivar and otherwise was true for K^+ contents (Khan and Panda 2008). Salt-mediated changes in the activities of antioxidant enzymes such as CAT, POD, SOD, APX, and GR alongside lipid peroxidation was measured in two rice cultivars with differential salinity tolerance. The higher tolerance in salt-tolerant cv. Pokkali was associated with a better antioxidant defense system that decreased lipid peroxidation reflected in the form of lower MDA content, whereas otherwise was true for salt-sensitive rice cultivar IR28 (Demiral and Türkan 2005). The imposition of salinity in two rice cultivars namely Giza 178 and Giza 177 enhanced the activities of antioxidant enzymes (SOD, POD, and CAT). The exogenous application of trehalose showed marked alleviation of inhibitory effects of salinity in two rice cultivars. The cv. Giza 178 displayed higher salt tolerance in terms of the stimulated activities of antioxidant enzymes compared with cv. Giza 177 (Abdallah et al. 2016). In another study, the efficacy of two osmoprotectants (trehalose and proline) was investigated in salt-sensitive (KDML 105) and salt-tolerant cultivar (Pokkali). Salinity enhanced the levels of H_2O_2, and activities of antioxidant enzymes (SOD, POD, CAT, and APX) in two rice cultivars. The limited accumulation of toxic H_2O_2 was observed in plants with an improved antioxidant defense system. The exogenous application of osmoprotectants improved salinity tolerance by enhancing the oxidative defense system in two rice cultivars. The higher salinity tolerance in tolerant cultivar relied on the antioxidant defense system (Nounjan and Theerakulpisut 2012).

4.2 Drought Stress

Drought is one of the major environmental concerns for agricultural production across the globe. The inhibited growth and reduction in yield is the result of drought impact on photosynthetic pigments, nutrient balance, and photosynthesis (Chen et al. 2011). Rice crop is sensitive to the environmental conditions like water limiting conditions as compared to the other crops (Showler 2016). The exposure of plants to drought conditions resulted in a significant increase in the production of $O_2^{\bullet -}$, lipid peroxidation alongside the decrease in thiols, and total soluble proteins. Drought-induced oxidative injury was alleviated by ROS scavenging antioxidant system and enzymes of the ascorbate-glutathione cycle appear to play an essential part in defense response (Sharma and Dubey 2005). Plant face drought when evapotranspiration is very high or when the supply of water to roots is limited. The growth and productivity of rice is severely impeded under drought conditions. The growth suppression is associated with the poor root architecture alongside the impaired leaf traits such as leaf color, pubescence, composition of cuticular wax, form and shape of leaf which ultimately inhibit plant growth (Pandey and Shukla 2015). Imposition of rice to water-deficit conditions at the anthesis stage led to spikelet sterility and grain reduction. The spikelet sterility happens due to the direct influence of water stress on spikelet desiccation, spikelet opening, and panicle expansion. The inhibitory effects of drought at the flowering stage were studied in upland IRAT 13 and lowland IR20 cultivars. The sensitivity of lowland cultivar IR20 was greater than that of upland cultivar IRAT 13. The number of opened spikelets was significantly affected in response to low panicle water potentials. The panicles with water potential lower than -1.8 MPa and -2.3 showed inhibition in spikelet opening (Ekanayake et al. 1989). Likewise, Peleg et al. (2011) identified pre- and post-anthesis stages as the sensitive stages of rice under drought conditions. PEG-mediated drought conditions caused a conspicuous reduction in the germination of seed (Kaya et al. 2006). Furthermore, PEG-induced water scarcity improved the root architecture alongside the significant decline in the biomass of root and shoot, germination, and hypocotyls elongation (Zeid and Shedeed 2006). The impact of PEG-mediated drought stress in rice was also observed by Basu et al. (2010a, b). A significant reduction in vegetative growth in rice was recorded due to the application of drought conditions at early growth stages (Manickavelu et al. 2006). The usual parameters used for the estimation of growth and vigor of rice plants under water-deficit conditions are root/shoot fresh biomasses and soil-plant water potentials which are highly affected by moisture limitations (Jaleel et al. 2009). Rice lines w-14 (drought-sensitive) and w-20 (drought-tolerant) were subjected to drought stress and changes in physiological indices and plant mineral nutrition were studied. There existed a strong reduction in plant biomass, gas exchange attributes, water potential, and the efficiency of PSII in rice under water scarcity. The exogenous spray of Si improved drought tolerance in rice reflected as the higher photosynthesis, transpiration, and efficiency of PSII. The endogenous levels of Fe, Mg, Ca, Na, and K were higher in plants under drought conditions and the application of Si reduced the nutrient contents. The authors suggested that improved drought tolerance due to Si was the result of improvement in photochemical efficiency and regulation of the ions homeostasis in rice (Chen et al. 2011). When rice plants were subjected to drought, a significant decrease in plant fresh and dry biomass was recorded alongside the significant decline in leaf water status, water use efficiency, and photosynthesis. H_2O_2-mediated loss of membrane integrity was evident in rice plants under drought conditions. However, the foliar application of polyamines improved drought tolerance reflected in the form of improved photosynthesis and antioxidant defense system. The impact of drought on chlorophyll fluorescence indices, chlorophyll contents, photosynthetic light curve, lipid peroxidation, and the metabolism of ROS was studied in rice plants. The imposition of drought stress significantly decreased photosynthesis, chlorophyll contents, and the efficiency of PSII along with the notable rise in MDA contents. The activities of antioxidant enzymes including SOD, POD, and CAT were significantly higher that effectively diminished ROS and protected plants from oxidative injury under drought conditions (Yang et al. 2014). There was a significant reduction in fresh and dry biomass, plant water relations, and photosynthesis in rice under drought conditions. The exogenous application of brassinosteriods led to the improvement in the plant fresh and dry biomass that was associated with a higher rate of photosynthesis alongside the significant decrease in H_2O_2 generation and lipid peroxidation (Farooq et al. 2009). A review of the literature on drought-mediated alterations in growth and physiochemical indices is given in Table 1.

4.3 Heavy Metals and Temperature Stress

Some of the heavy metals are found necessary in rice plants for their role as micronutrients, while several others have detrimental effects on rice even in trace amounts. The abundance in the uptake of these metal causes plasma layer disturbance in crops. Based on the cell, it is reported that metal ions interfere with the

Table 1 The inhibitory effects of drought on growth and key physiochemical attributes in rice

Growth regulator/ mycorrhizal association	Medium of growth	Plant responses	References
Calcium silicate	Soil	Drought inhibited plant growth and photosynthetic capacity. The accumulation of proline was higher alongside the minimal accumulation of sugar content. Application of calcium silicate improved root and shoot biomass, photosynthesis, transpiration rate, and reduced the accumulation of proline content. The limited accumulation of proline was related to drought-ameliorative potential of calcium silicate	Yang et al. (2019)
Corantine (COR)	Hydroponic culture	Presoaking treatment of rice (*Oryza sativa* L.) cultivar Nipponbare (japonica) seeds with COR improved DPPH activity, relative water content, proline content and decreased electrolytic (EC) leakage, and malondialdehyde content. In this study, the expression of gene related to down- and upregulation due to COR was studied with the help of real-time PCR. COR induced some genes which may improve metabolism of rice and improve defensive mechanism against drought stress	Gao et al. (2019)
Glycine betaine (GB)	Soil	Drought stress caused decrease in biomass, chlorophyll, net photosynthesis as well as yield and increase proline content in rice. Foliar application of glycine betaine was done in two rice cultivars SPR1 (high amylose) and RD43 (low amylose). Application of GB showed improvement of physiobiochemical and yield attributed in severe water stress condition in SSR1 rice cultivar. The authors suggested that tolerance to drought stress was induced by the application of glycine betaine	Tisarum et al. (2019)
No growth regulator was used	Soil	Water stress caused a substantial decrease in growth and yield of plant by disturbing different metabolic pathways, antioxidant enzyme activities, and produced oxidative stress in rice (*Oryza sativa* L.). furthermore, drought stress also inhibited photosynthetic efficiency by disturbing stomatal conductance and production of ROS. Scientists suggested that the use of breeding techniques and tolerant rice varieties is the best solution to reduce drought stress	Xiong et al. (2019)

(continued)

Table 1 (continued)

Growth regulator/ mycorrhizal association	Medium of growth	Plant responses	References
Arbuscular mycorrhizal fungi (AMF)	Liquid MS media	In this investigation, AMF was used to reduce the effect of drought. The endogenous levels of ABA and proline were minimal in plants treated with AMF. These authors reported that AMF treated plants experienced less drought compared with control plants	Kavitha Mary et al. (2018)

SH-group of proteins phospholipid's OH-group in the same manner, Ca ion is supplanted at the cell level. Trace metals can displace the Ca from different cellular compartments. Metal stress results in the perturbation of the lipid bilayer integrity and changes in ions homeostasis (Rasheed et al. 2018). Cadmium (Cd) causes several health problems in humans and the animals feeding on plants which are contaminated by the accumulation of toxic metal ions in the plant tissues. Among trace metals, cadmium (Cd) is thought to be the most toxic that arrests photosynthesis, inhibits the uptake of essential ions, reduces yield, and declines the root/shoot ratio of development (Rasheed et al. 2018). The prolonged exposure and elevated Cd content in the soil casts the detrimental effects on plants in terms of growth suppression and poor root development that impedes the uptake of essential nutrients thereby resulting in significant yield losses in rice (Moya et al. 1993). Lead (Pb) is thought to be one of the toxic elements abundantly found in soil. The toxic effects of Pb are seen even when present in small concentrations in agricultural soil in the form of inhibited photosynthesis, enzymatic activities, germination, cell division, uptake of nutrients, and water contents (Patra et al. 2004). Several studies have been reported that heavy metals such as Ni, Cu, Cd, and Al have a negative impact on the enzymatic activity, secondary metabolism, and ion homeostasis. Heavy metal stress affects the H^+-ATPase flow which varies on the basis of plant type, heavy metal exposure duration, and heavy metal concentration (Kabała et al. 2008). The stress of these heavy metals also has a huge negative effect on emergence, growth, and grain yield in rice plants (Arif et al. 2019). The reports on the effects of heavy metals on rice growth and grain yield are summarized in Table 2.

The frequency of heat stress occurrence has become more frequent due to recent global warming that incurs adverse effects on grain yield and quality in rice. For example, two japonica varieties, Wuyunjing 24 and Nanjing 41, were given four different temperature regimes (i.e., 27, 31, 35, and 39 °C) for 2, 4, and 6 days. Plants subjected to 35 and 39 °C for 4 and 6 days manifested a significant decline in different yield attributes, viz. chalky grain rate, grain size, seed-setting rate, panicle size, amylose contents, and milling characteristics along with an increase in protein contents. Heat stress induced a substantial decline in peak viscosity and breakdown but enhanced pasting temperature. There existed differential sensitivity concerning grain yield characteristics between two cultivars. It was suggested that grain yield of

Table 2 Effect of heavy metals on growth and physiochemical attributes in rice

Growth regulator	Type of heavy metal	Plant response	References
Sodium nitroprusside and glutathione	Copper (Cu)	Under Cu stress (100 μM) ROS and MDA increased that reduced plant growth and yield. GSH, phytochelatin, and increased proline contents improved plant growth under metal stress. SNP (200 μM) and GSH (200 μM) treatment reduced MDA, ROS, and proline content. Both treatments alleviated the toxic effect of Cu and reducedmetal transportation and accumulation in rice seedling	Mostofa et al. (2014)
Hydrogen sulfide	Cadmium (Cd)	Cd stress reduced photosynthesis, chlorophyll content, RWC, non-enzymatic antioxidants, and induced oxidative stress. Cd toxicity (250, 500, and 1000 μM) alleviated by application of hydrogen sulfide. Hydrogen sulfide enhanced photosynthesis, total protein content, and maintained homeostasis of mineral ions	Mostofa et al. (2015a, b)
Nitric oxide	Arsenic	Arsenic toxicity induced oxidative stress, suppression in plant growth, chlorophyll degradation alongside limited relative water contents. Arsenic reduced GSH/GSSG. Nitric oxide treatment reduced the impact of heavy metals on rice by modulating the transportation and accumulation of arsenic in plant parts. Besides, enhanced antioxidant enzyme activities and proline content in nitric oxide-treated plants resulted in minimal oxidative injury	Singh et al. (2016)
Silicon (Si)	Chromium (Cr)	Two rice cultivars (Dan K5 and Xiushui 113) under Cr stress (100 μM/L) manifested a significant reduction in plant biomass, seedling vigor, plant height, protein content alongside enhanced production of ROS that deteriorated chlorophyll content. Silicon alleviation of Cr stress depends upon plant species. Silicon inhibited the Cr uptake and translocation that reduced MDA and ROS content. Increased RWC, chlorophyll, and photosynthesis efficiency was seen in plants treated with silicon	Zeng et al. (2011)
Nitric oxide (NO)	Cd	Rice seedling growth reduced under Cd stress but its toxicity was alleviated by calcium. Cd reduced root and shoot growth and chlorophyll content. Cd toxicity (100 μm/L) was minimal in plants treated with calcium (10 mmol/L) and nitric oxide supplementation. The exogenous application of nitric oxide also enhanced cell wall pectin, cellulose, and hemicellulose	Zhang et al. (2012)

(continued)

Table 2 (continued)

Growth regulator	Type of heavy metal	Plant response	References
Cerium	Cd	Seedling growth of rice was inhibited by the Cd toxicity (100 μm/L) reflected in the form of decreased root and shoot dry weight, and chlorophyll content. Cd stress significantly altered antioxidant enzyme activity alongside the enhanced production of ROS species. Cerium (10 μm) application increased photosynthetic activity and showed lower deterioration of chloroplast ultrastructure. Cerium improved antioxidant enzyme activity, reduced ROS, and thereby increased plant growth	Wu et al. (2014)
Salicylic acid (SA)	Cu	Cu stress (75 and 150 μM) reduced chlorophyll content, RWC, GSH, phytochelatin along with enhanced generation of ROS, MDA, and proline content. Improvement in endogenous contents of proline was related to better plant growth and yield under Cu stress. Salicylic acid (100 μM) treatment reduced MDA, ROS, and proline content. Both treatments alleviated the toxic effect of copper and reduced their transportation and accumulation in rice seedling. The results advocated that SA improvement in growth relied on antioxidant activity	Mostofa and Fujita (2013)
Glutathione (GSH)	Chromium (Cr)	Cr stress reduced plant growth, protein, and chlorophyll content but carotenoids, proline, and MDA level increased. Treatment with GSH (100 μM) reduced the impact of chromium stress (100 μM) by increasing protein, chlorophyll content, and antioxidant enzymes activity. Besides, GSH application decreased carotenoids, proline, and MDA level. GSH reduced the aerial translocation of Cr in rice	Zeng et al. (2012)
Silicon (Si)	Cd	Cd toxicity (10 μM) decreased chlorophyll content, relative water content, osmotic potential, proline content, but increased antioxidant enzymes activity Si (0.6 mM) decreased hydrogen peroxide content and ROS detoxification through stimulated antioxidant defense system. Si enhanced ascorbate, glutathione, and stress associated proteins that show strong defense against Cr	Farooq et al. (2016)
Selenium (Se)	Cd	*Three rice cultivars (Oryza sativa, Japonica unwaxy, Xiushui 63) subjected to Cd stress (50 μM) showed stunted growth along with*	Lin et al. (2012)

(continued)

Table 2 (continued)

Growth regulator	Type of heavy metal	Plant response	References
		enhanced MDA accumulation. Cd decreased the uptake of nutrients (K, S, Fe, Mn, Zn, and Cu). Application of Se (3 μM) enhanced antioxidant enzymes except CAT activity. Se interaction with Cd ameliorated the uptake of nutrients	
Silicon (Si)	Zinc (Zn)	Two rice cultivars (TY-167 and cv. FYY-326) under Zn stress (2 mM) showed stunted growth, reduced antioxidant enzyme activities, whereas MDA and hydrogen peroxide concentration increased. Under Zn stress, roots growth was inhibited. Treatment with Si (1.5 mM) increased antioxidant enzymes activities (SOD, POD, APX) and decreased MDA and hydrogen peroxide level under Zn toxicity. Si mediated Zn uptake and transportation in plants	Song et al. (2011)
Nitric oxide	Cd	Cd toxicity decreased plant growth and development in rice. Cd stress (100 μM) inhibited photosynthetic and other gas exchange parameters as well as increased SOD, POD, CAT, and APX activities. Increased MDA, electrolyte leakage in rice plants were evident under Cd stress. SNP (sodium nitroprusside) decreased Cd accumulation, alleviated Cd toxicity in terms of increase in protein, antioxidants, and photosynthetic pigments	He et al. (2010, 2014)
Salicylic acid (SA)	Cd	Rice seedling under Cd toxicity (1–200 μM) manifested a significant reduction in root length, plant height, alpha-amylase activity, and vigor index. SA application (0.1 mM) alleviated the toxic effects of Cd by enhancing cell division and antioxidant enzyme activities	He et al. (2010)

rice could withstand mild heat stress, but exposure to severe heat stress at booting stage reduces and deteriorates grain yield quality of rice. The inhibitory impact on grain yield could be estimated from the intensity and duration of heat at the boot stage (Zhen et al. 2019). In another study, two rice cultivars were given heat stress treatment of 40 °C for 2 h after flowering. Heat stress treatments caused a significant decline in spikelet fertility that was noticeably mitigated by the application of 1-naphthaleneacetic acid (Zhang et al. 2018). Likewise, three rice cultivars were given heat stress and drought treatments under field conditions. The stress treatments applied at either early grain filling or flowering stage led to limited and hampered grain yield. The metabolic profile of flowering spikelets, developing seeds, and flag leaf displayed noticeable differences among three rice cultivars (Lawas et al. 2019). A literature review on the growth and productivity of rice is given in Table 3.

4.4 Biotic Stresses

Rice production faces numerous biotic stresses such as blast, brown planthopper, bacterial blight, sheath blight, leaf blight, false smut, and stem borers (Wang et al. 2011). Biotic infection stresses are brought about by the living life entities, for example, viruses, bacteria, fungi, and bugs (Fan et al. 2011). Fungal pathogens like *Magnaporthe oryzae* resulted in the rice blast ailment, one of the most genuine fungal sicknesses in rice, and have been known to cause a significant yield reduction in rice. *Rhizoctonia solani* contradictory to *M. oryzae*, produces rice sheath blight sickness for the most part in the sheath tissue (Lee et al. 2006). Blast is a disease of rice which is induced by fungus *Pyricularia grisea*. As in the case of other diseases such as leaf smut, eye spot, root rot, brown spot, and stem rot, rice blast resulted in the significant injury which commonly infects seedlings. Rice blast is a significant risk to rice as it decreases the rice yield by 30%. The pathogen damages all parts of the plant including panicle.

Symptoms of Rice Blast

- Pin-shaped spots on foliage with grey inside.
- Nodes rotting becomes visible and black in color leads to the breaks.
- Panicle neck starts decaying.
- Quality of grain affected by turning chaffy.

Management

- Seeds are treated with carbendazim, soaking for 12 h.
- Fertilizer such as nitrogen should not be given as a heavy dose.
- Carbendazim solution is used to dip the plants in when transplantation proceeds.
- Tolerant cultivars should be selected.

4.4.1 Brown Spot

Fungi are also the principal cause of this ailment. The character of this infection becomes visible as oviform brown spots on the foliage. The fungus has the damaging effects on all stages of plant life such as seedling as well as the mature stage. The ailment occurs due to the infection of *Bipolarisoryzae*, *Cochilobolusmiya beans*. Generally, seedlings are affected in this disease; however, the negative effects on the panicle are also reported. This infection causes the death of the seedlings up to 50% due to the use of diseased seeds.

Table 3 The effect of temperature stress on growth, physiochemical indices, and grain yield in rice

Growth regulators/ mycorrhizal association	Temperature stress	Plant response	References
–	Temperature range (19 to 21 °C)	Rice plants showed a reduction in growth and yield attributes under low temperature and drought conditions. Low temperature caused a reduction in all growth attributes, disturbed antioxidant enzyme system, and gas exchange attributes (photosynthetic rate, transpiration rate, and water use efficiency). Low temperature stress also reduced yield, i.e., seed percentage, 1000 grain weight, grain yield, and effective panicles of rice in both cultivars viz. Guinongzhanand Yueza 763. Low temperature stress caused oxidative damage measured in terms of MDA. The oxidative injury was efficiently mitigated by stimulated antioxidant enzyme activity (CAT, POD, and SOD)	Rao et al. (2019)
–	Chilling	Chilling stress caused adverse effects on the growth and yield of rice plants. Chilling stress reduced photosynthesis, transpiration, stomatal conductance, and quantum efficiency of photosystems. Two rice genotypes were viz. Nipponbare (tolerant) and M202 (sensitive), and about 21 genes were studied in this investigation which was related to the physiology of the rice plants. Genes of tolerant variety showed better expression with respect to photosynthesis, transpiration rate, and other photosynthesis-related parameters as compared to sensitive variety	Moraes de Freitas et al. (2019)

(continued)

Table 3 (continued)

Growth regulators/ mycorrhizal association	Temperature stress	Plant response	References
–	Heat stress	Heat stress caused the most adverse effects at the flowering stage as compared to grain filling stage and reduced grain yield. Rice under temperature stress showed a reduction in photosynthesis and the dry matter of panicle, culm, leaf sheath, and leaf area	Lu et al. (2013)
Salicylic acid, citric acid, ascorbic acid, and hydropriming	Heat stress	Temperature stress and elevated level of CO_2 caused reduction in germination rate. Germination stage was the most sensitive stage for rice. Application of salicylic acid, citric acid, ascorbic acid, and hydropriming improved germination by improving its antioxidant enzyme activities, i.e., POD, CAT, SOD, and reduced oxidative stress as well. The authors suggested that the application of these growth regulators may improve germination, seed quality, and seedling growth	Nedunchezhiyan et al. (2019)
Brassinolide (BR)	Chilling stress	Chilling stress caused a substantial decrease in growth, chlorophyll, and yield of rice plants. Chilling stress disturbed antioxidant enzyme activity and induced oxidative damage. Brassinolide (BR) improved the antioxidant system of rice plants and reduced oxidative stress. Improvement was much better in temperature tolerant cultivar (Kongyu 131) as compared to sensitive cultivar (Kenjiandao 6). BR treatment on leaves showed significantly mitigated oxidative injury reflected as greater activities of	Wu et al. (2014)

(continued)

Table 3 (continued)

Growth regulators/ mycorrhizal association	Temperature stress	Plant response	References
		antioxidant enzymes alongside the higher P, K, and chlorophyll in Kenjiandao 6 as compared to Kongyu 131. Yield was higher in Kenjiandao 6 as compared to other	
γ-Aminobutyric acid (GABA)	Heat stress	Rice under high temperature caused a decrease in root and shoot length, and reduced the survival of rice plants. But exogenous applied GABA improved growth attributes and survival of the plant. GABA increased the accumulation of different osmolytes such as trehalose and proline. It reduced the oxidative damage reflected in the form of lower H_2O_2 and MDA in leaves. It also improved the activities of enzymes, i.e., SOD, POD, APX, CAT, glutathione reductase as well as non-enzymatic antioxidants such as glutathione and ascorbate	Nayyar et al. (2014)
–	Different temperature ranges (33/27 °C, 27/21 °C, 21/15 °C)	In this study, various levels of temperature were used to assess the effects on growth, physiological, and biochemical changes in rice. Increase in temperature improved photosynthesis in Xiangyaxiangzhan as compared to Basmati385. Different temperature ranges affect antioxidant enzyme activities, i.e., SOD, POD, CAT, and protein synthesis in both cultivars. Furthermore, the authors suggested that the most suitable temperature for rice yield is 27 °C/21 °C at the grain filling stage as compared to other ranges	He et al. (2019)

(continued)

Table 3 (continued)

Growth regulators/ mycorrhizal association	Temperature stress	Plant response	References
Spermidine	Heat stress	Temperature stress-induced substantial decrease in growth attributes and inhibited photosynthesis as well as antioxidant activity. MDA accumulation was higher in temperature-treated rice seeds. Application of spermidine improved photosynthesis, transpiration rate, stomatal conductance, antioxidant enzyme activity, and reduced MDA accumulation. Spermidine also increased soluble sugar. The authors suggested that spermidine improved growth and biochemical attributes and hence effectively alleviated the adverse effects of high temperature	Tang et al. (2018)
Brassinosteroid mimics (7,8-dihydro-8a-20-hydroxyecdysone (αDHECD))	Heat stress	Brassinosteroid mimics (αDHECD) shown to have improved photosynthesis capacity under heat stress. Heat stress substantially decreased growth, photosynthesis, and grain yield attributes. Foliar application of αDHECD improved growth, dry matter, grain yield, photosynthesis, and gas exchange quality. αDHECD also increased leaf starch and soluble sugar contents. Researchers suggested that αDHECD increased tolerance in rice plants against heat stress	Sonjaroon et al. (2018)
–	Heat stress	Heat stress reduced crop yield and growth of rice plants. It reduced antioxidant enzyme activities (SOD, POD, APX, and CAT), and increased oxidative stress in terms of H_2O_2 and MDA. K-95 showed maximum	Ali et al. (2016)

(continued)

Table 3 (continued)

Growth regulators/ mycorrhizal association	Temperature stress	Plant response	References
		accumulation of proline, MDA, and H_2O_2 content as compared to IR-6, IR-8, and Sada Hayat. K-95 showed better antioxidant enzyme activities (CAT and APX) as compared to IR-8 and Sada Hayat. The authors suggested that K-95 rice variety is temperature-tolerant. Tolerance was due to a better antioxidant system	
H_2O_2	Chilling	Chilling stress could substantially reduce growth, biochemical, and yield attributes of rice (*Oryza sativa* L.). chilling stress reduced mean germination time, germination index, germination percentage, and germination coefficient. Chilling reduced growth parameters such as shoot and root length and its fresh weight and chlorophyll contents. It also disturbed the antioxidant system. Priming with H_2O_2 showed improvement in growth attributes, chlorophyll, and antioxidant enzyme activities	Afrin et al. (2019)

Symptoms

- Oviform spots of dark brown color are visible on foliage and stem.
- This disease often takes place in nutrient deficit soil.
- This disease attacks on nursery or in the field.

Management

- Nutrients and fertilizers should be timely applied to avoid this disease.
- Tolerant cultivars should be selected in nutrient-deficit soil.

- For the control of this disease, seeds are treated with Agrosan.

4.4.2 Bacterial Blight

Bacterial blight is generally found to be caused by bacteria. This disease is caused by *Xanthomonasoryzaepv*. oryzae (Xoo). The infestations of bacterial blight can be visible at the seedling level as grayish-green rolled leaves which turn yellow as the blight advances. When the disease is progressing, the wilting of leaves takes place which further changes from yellow to straw color and then leads to the death of the entire seedling.

Symptoms

- Entire leaves are infected along the edges and white lesions appear on leaves.
- Disease expands by the action of air current, rain, and temperature.
- Transplantation time is more susceptible to the infection.

Management

- The abundant supply of nitrogen should be avoided.
- Presoaking seed treatment should be conducted in Streptocycline for 12 h.
- Agromycin 100 should be sprayed which is effective against the disease.

4.4.3 Udbatta Disease

Fungus causes the Udbatta disease. This disease is caused due to the sowing of infected seeds, hence, it is a seed born disease. As the panicle emerges, the infection becomes visible at the top of the panicle. If the untreated seeds are sown then the more infected seedling is produced. To avoid this disease, seeds must be soaked in hot water prior to sowing (Kato et al. 1988).

Control Measures

- Uninfected and healthy seeds should be selected.
- High dose of nitrogen fertilizer should not be applied.
- Carbendazim should be applied to seeds before the sowing.
- At the stage of seedling formation, seedlings can be sprayed to avoid the disease.

4.4.4 Sheath Blight

Sheath blight is a parasitic ailment brought about by *Rhizoctoniasolani*. The side effects of sheath blight are visible in temperate, tropical, and subtropical areas on rice crops from tiller formation to grain milk stages. As indicated by the IRRI report, sheath blight resulted in a production loss of about 6% across the areas under rice cultivation in tropical Asia.

Symptoms

- Sheath of foliage is damaged.
- The influenced regions of the plants are infected.

Control Measures

- Since it is soil-borne sickness, plant to plant space should be maintained.
- Substantial nitrogen fertilizing should not be practiced.
- Foliar application with carbendazim should be carried out on crops which is infected with this disease.

4.4.5 Rice Yellow Mottle Virus

A significant and common disease in rice plant is rice yellow mottle virus (RYMV) caused by a viral infection. RYMV brings about yellow leaves with rotating yellow to green stripes formation and also results in the reduction of tiller formation, mottling of yellow color on the leaves, stunted panicle, reduced height, and formation of sterile spikelet. RYMV is moved unexpectedly by creepy crawlies (entomophily) from a pervaded plant to a healthy plant. Disease invasion becomes visible in the form of yellow specks.

4.4.6 Rice Tungro Disease (RTD)

It is a viral disease of rice, predominant in the South and Southeast Asia. RTD results in yearly yield losses of more than 109 US dollars in the vulnerable countries (Herdt 1991). In this context, in India, 2% yield losses have been recorded (Muralidharan et al. 2013).

5 Conclusion

It can be inferred from the above-presented review of literature that the growth and productivity of rice is prone to the environmental constraints in the form of salinity, drought, temperature, and heavy metal stress. Besides, biotic factors are also major determinants of rice growth and grain production. Researchers around the globe performed several experiments which detailed different defense mechanisms in rice under biotic and abiotic conditions. The reduction in grain yield due to environmental constraints would lead to food insecurity as rice is consumed as major cereals in different regions of the world. There are fewer reports on drought-mediated alterations in physiochemical indices in rice. Drought is the emerging problem especially in Asia. Therefore, there must be more research on understanding the physiological response of rice plants in drought-prone regions.

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Germplasm and Genetic Diversity Studies in Rice for Stress Response and Quality Traits

Reza Mohammad Emon and Golam Jalal Ahammed

Abstract

Understanding germplasm diversity and genetic dissection of complex traits is the mainstay of crop improvement. However, breeding for stress tolerance and quality traits of rice germplasms has been limited by the complex and polygenic nature of desired traits. Rice quality traits depend on the grain shape as specified by grain length, grain width, length-width ratio, translucency of the endosperm, amylose content, gelatinization temperature, and gel consistency. From theoretical and practical points of view, research on the inheritance of grain shape and grain weight for breeding of rice variety with high yield and good quality is of great importance. Nonetheless, the choice of germplasm is critical to the success of breeding for stress tolerance and quality traits. It has been important for rice improvement, is known for its genetic and molecular characterization of agronomical important genes, such as resistance genes, and genes driving quality. Genetic diversity analysis is an excellent way to local germplasm adaptation, selection, and breeding in many crop species including rice. Germplasm collection and genetic diversity analysis of rice are also necessary for targeted breeding such as high yield, superior grain quality, and strong environmental adaptability. Identification of the genetic basis of diverse germplasms provides important insights into the breeding elite varieties for sustainable agriculture. This chapter focuses on the above-said traits along with the potential benefits for researchers who are involved in rice, for farmer's food security and livelihoods, and also provides a thorough understanding of genetic diversity, population structure, and familial relatedness necessary for rice breeding program.

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Keywords

Stress response · Quality traits · Gene · Rice · Breeding · Crop improvement

1 Introduction

Breeding for stress responses and quality traits using germplasms and understanding the genetic diversity have been limited by the complex and polygenic nature in rice genotypes. The genetic knowledge on germplasm that can be potentially used as a parent is a prerequisite of any breeding program. Rice (*Oryza sativa* L.) is a very important cereal crop worldwide. It is the staple food for more than half of the world's population. The development of stress tolerance and quality rice varieties has been the principal objective throughout the history of plant breeding. A priority issue in many rice-producing areas of the world along with improvements in the standard of living, the demand for biotic and abiotic stress tolerance, and superior grain quality is increasing day by day. Among the different stresses, drought, salinity, water logging/flooding, and temperature stress (high and low) are the main factors reducing crop production worldwide and accounts for more than 50% yield loses (Boyer 1982; Bray et al. 2000). When plants are subjected to abiotic stresses, they activate different mechanisms to survive and sustain growth and yield. Thus, it is important to know what mechanisms exist to help plants surviving under severe stress. Rice is classified as a salt, submergence, drought-sensitive crop, especially in the early growth stages. Cultivated germplasm has been a valuable genetic resource in international plant breeding. Landraces endemic to different crops are a genetic resource that is distinct from different germplasm. Patterns of genetic diversity among cultivated accessions and relationship to other germplasm pools are unknown, despite the proven value and potential novelty. Local germplasm has been shown to be both genetically and morphologically unique, as well as a valuable genetic resource in plant improvement for stress response and quality traits.

2 Germplasm and Genetic Diversity for Stress Responses in Rice

Genetic diversity is commonly measured by genetic distance or genetic similarity, both of which imply that there are either differences or similarities at the genetic level (Weber 1990). Assessment of genetic diversity is an essential component in germplasm characterization and conservation. Genetic diversity plays an important role in plant breeding and is a key component of any agricultural production system. This genetic diversity or similarity may be measured through genetic markers. These have been used to determine evolutionary relationship within and between species, genera, or higher taxonomic categories. The stress responses and the quality traits of rice depend on the utilization of the broad diversity of rice germplasm, and also on knowledge of its genetics. The genetic diversity in the world rice germplasms is

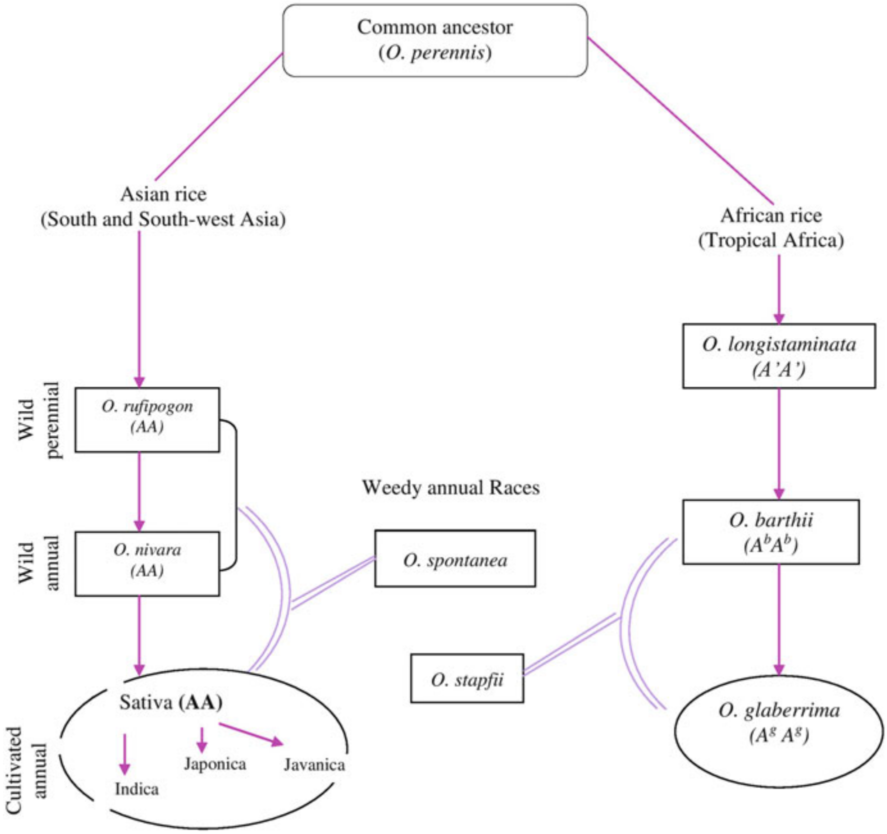

Fig. 1 Schematic representation of the evolutionary pathways of Asian and African cultivated rice. Arrows with solid line indicate direct descent. Double lines indicate introgressive hybridization (Sources: Randhawa et al. 2006)

quite large than other crops (Yang et al. 1994; Aggarwal et al. 1999; Yu et al. 2003). In case of rice, *Oryza sativa* is considered to have been domesticated between 9000 and 7000 BC. *Oryza* is a modest-sized genus consisting of 20 well recognized wild species and two advanced cultigens, *Oryza sativa* which is grown worldwide and *Oryza glaberrima* grown in parts of West Africa (Chang 2002). The primary center of origin of *Oryza sativa* was the foothills of the Himalayas in the north and hills in the north-east of India to the mountain ranges of south-east Asia and south-west China as, and the delta of river Niger in Africa for that of *Oryza glaberrima*, called African rice. Another species *Oryza perennis* is a common ancestor for both *Oryza sativa* and *Oryza glaberrima*. The wild progenitors of *Oryza sativa* are the Asian common wild rice named *Oryza rufipogon* which shows wide variation ranging in their habit from perennial to annuals. Schematic representation of evolutionary pathways of the Asian and African cultivated rice is shown in Fig. 1. The wild progenitor of the African cultivar *Oryza glaberrima* is *Oryza longistaminata* Chev.et Roehr endemic to West Africa.

The primary center of diversity for *Oryza glaberrima* is the swampy basin of Upper Niger (Randhawa et al. 2006). Genetic diversity is required for populations to evolve and cope up with environmental change, new disease, and pest epidemics. Loss of genetic diversity is often associated with reduced reproductive fitness (Frankham et al. 2002). The continuing trend toward the erosion of genetic diversity is worrisome in the face of pest epidemics under intensive cultivation and global climate change (Chang 2002). The green revolution in rice was centered largely on the *sd-1* gene found in the Indonesian landrace Dee-eoo-woo-gen and the cytoplasm of the cultivar cina (China), the genetic base of improved *Oryza sativa* cultivars all over the world became greatly narrowed. Meanwhile, numerous traditional types were displaced and lost from farmers' fields. Concurrently, disturbance or destruction of many natural habitats has reduced the populations of its wild relatives. The centers of origin of rice species are characterized by topological heterogeneity and are considered to be the center of rice diversity. The diversity in these centers is being lost rapidly with many rice growers shifting to modern cultivars (Randhawa et al. 2006). In many areas, high-yielding modern varieties were adopted by farmers and the cultivation of the landrace varieties declined. The wild species are threatened with extinction through changes in land use, extension of agriculture into marginal areas and deforestation (Jackson 1997). International Rice Research Institute reported that there is still a tremendous amount of unexploited genetic diversity in the primary gene pool of rice, can be used for enhancing the diversity in local germplasms and their performance under diverse agro-ecological conditions (Guimaraes 2000; Ali et al. 2006; Lafitte et al. 2006). Wild species of *Oryza* also represent a potential source of new alleles for improving yield, quality, and stress tolerance in rice cultivars (Xiao et al. 1998; Moncada et al. 2001; Ahn et al. 2002; Thomson et al. 2003; McCouch 2004; Kovach and McCouch 2008). Improvement of the performances of the introgression of valuable genes from wild germplasm into elite rice cultivars is very much important. Lines derived from crossing the wild species *Oryza rufipogon* with *Oryza sativa* cultivars showed higher yields than their progenitors and are tolerant to several abiotic stresses (Moncada et al. 2001; Nguyen et al. 2003; Tian et al. 2006; Xie et al. 2006; McCouch et al. 2007). For example, low temperature is one of the most important abiotic stresses affecting early vegetative stages in rice. Temperature below 15 °C inhibits or delays seedling emergence and early vegetative growth, compromising rice field establishment (Yoshida 1981). Study on genetic variation of different species is a common phenomenon among many researchers in various parts of the world. Almost all diversity analyses identify clusters for the two rice subspecies, *indica* and *japonica*, and often subsequently delineate both temperate and tropical *japonica* sub-clusters (Thomas et al. 2007). Chakravarthi and Naravaneni (2006) observed the robust nature of molecular markers in revealing polymorphism and grouped the rice genotypes into 10 classes after cluster analysis in which *japonica* types DH-1 (Azucena) and Moroborekan clustered separately from *indica* types. It was revealed that five different varietal groups of accessions, *aromatic*, *aus*, *indica*, *temperate japonica* and *tropical* *japonica* and if a given accession was an admixture, having more than one group present in its ancestry (Pritchard et al. 2000; Garris et al. 2005). Agrama et al. (2007) identified *indica*, *temperate japonica*, and *tropical japonica* sub-species in rice cultivars from eight different regions of the world, subsequently it was determined that there were probably seven different breeding backgrounds present in this collection. More recently, a collection of 72 *O. sativa* accessions representing the five aforementioned varietal groups and 21 accessions of the *O. sativa* ancestral species, *O. rufipogon* Griff., was genotyped with SNP markers (Caicedo et al. 2007). Data analysis revealed the *O. sativa* accessions had a very small amount of shared ancestry with *O. rufipogon*; whereas all *O. rufipogon* accessions had some shared ancestry with at least one of the five varietal groups. High-density genetic map (saturated with molecular markers), tightly linked QTLs/genes with markers, and confirmed QTLs/genes across the environments and in different genetic backgrounds are the most important criteria for effective selection of germplasm for knowing the genetic diversity. Selection of germplasm for abiotic stress-related traits like drought, salinity, and submergence tolerance has been initiated but not to the extent of abiotic/biotic stresses. Molecular markers have been used widely for rice germplasm evaluation, interpreting population structure and genetic dissection of quantitative trait loci (QTL) for important agronomic traits (Singh et al. 2009; Venuprasad et al. 2009; Li et al. 2009; Agrama et al. 2007). Multi-allelic DNA markers have been used to identify the genetic diversity in several rice collections which represented many of the world's rice-growing regions (Ni et al. 2002; Xu et al. 2004; Garris et al. 2005; Eizenga et al. 2006), whereas others contain accessions from a given region like Southeast Asia (Garris et al. 2003), USA (Lu et al. 2005), Argentina (Giarrocco et al. 2007), Indonesia (Thomas et al. 2007), and Portugal (Jayamani et al. 2007). In a plant germplasm collection, analysis of the relationship between the phenotypic variation for a given trait and genetic polymorphisms identified with multi-allelic markers can be used to ascertain marker-trait associations by locating regions of the genome that are in linkage disequilibrium (Breseghello and Sorrells 2006). If markers are well dispersed throughout the genome and the population subgroups identified to decrease the rate of false positives, especially in self-pollinated plants like rice, association mapping can be used to map the given phenotypic trait, complementing the more common method of linkage analysis typically conducted on plants in a population derived from a bi-parental cross (Yu and Buckler 2006). High polymorphisms of rice microsatellite loci and their great resolving power will be particularly helpful for germplasm evaluation and evolutionary studies for better strengthening the conservation and utilization of genetic diversity of wild rice in the field gene banks (Gao et al. 2006). This is a brief description. Still a lot of research is undergoing on genetic diversity of rice for the exploitation of valuable genes and allele mining. SNP (DNA chip and sequencing-based DNA markers and based on microarray technologies) can identify even in single nucleotide variation between the genome. Genetic diversity is essential to meet the diverse goals of plant breeding such as producing cultivars with increased yield, wider adaptation, desirable quality, pests, and disease resistance. Published reports on molecular marker-based genetic diversity analysis (MMGDA) in rice are enormous and reflect potential applications in rice breeding. In most cases, genetic divergence was studied to identify specific parents for realizing heterosis and recombination in the breeding program. It is fundamental that the number of molecular markers for the molecular characterization of rice is high and that they are regularly spaced and representative of all chromosomes, independent of their degree of information content. Genetic diversity reveals the differences in the hereditary constitutions of the individuals of a species and it is important in maintaining and distributing genetic resources in nature.

3 Germplasm and Genetic Diversity in Rice for Quality Traits

The development of quality rice varieties has been the principal objective throughout the history of plant breeding. A priority issue in many rice-producing areas of the world along with improvements in the standard of living, the demand for superior grain quality is increasing day by day (Juliano et al. 1990; Hao et al. 2009). Grain quality is an important consideration in rice production. Preferences for rice grain performance and cooking quality vary among rice consumers living in different parts of the world or even among people living in different regions of the same country. Grain quality is determined by many factors, including milling ratio, head rice recovery, grain shape and size, grain appearance, and cooking and eating quality. After milling, rough rice yield is usually made up of 20–22% (by weight) hulls, 8–10% bran and embryos, and 70% milled rice. Milled rice is separated into broken rice and whole grains with a sieving device, with the proportion of whole grains defined as head rice recovery. Grain appearance is mostly determined by grain shape and the translucent or opaque appearance of the grain, known as the amount of chalkiness. The density of starch granules is lower in chalky grains compared to translucent ones (Del Rosario et al. 1968), and because chalky grains are not as hard as translucent ones, they are more prone to breakage during milling (Septiningsih et al. 2003). Rice quality is an important character that influences its consumption rate, thus enhancing its production. Therefore, the improvement of grain quality becomes a second major concern in rice breeding program after yield. Rice grain quality includes the milling, appearance, cooking, and nutritional qualities. Among these, people pay more attention to the appearance and cooking quality (Huang et al. 1998). In rice, it has been reported to reduce grain seed size and yield (Jing et al. 2009) while also affecting endosperm quality (Duan et al. 2012). As rice grain quality is an endosperm trait, its inheritance can be more complicated because the genetic expression of an endosperm trait in cereal seeds is conditioned not only by the triploid endosperm genotype, but also by the diploid maternal genotype and any additional possible cytoplasmic differences. The appearance quality is often judged by the percentage of grain with a white core and a square of white core. The cooking quality is judged by the amylose content, alkali spreading core, and gel consistency (Pooni et al. 1992; Zhu and Weir 1994; Mo 1995). Genetic and environmental factors have been reported to influence physical-biochemical properties of grain endosperm Chandusingh et al. (2013) as illustrated in this review (Fig. 2). Among

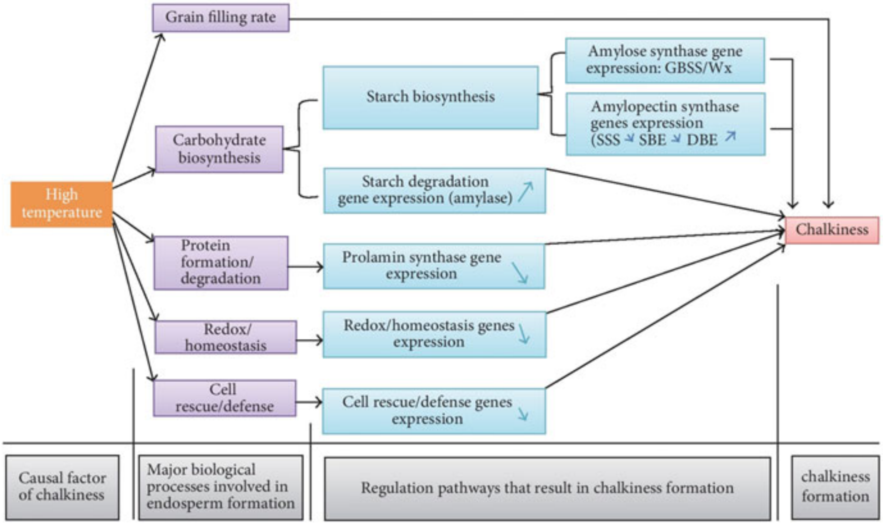

Fig. 2 Principal environmental causal factors and the mechanism pathways of the chalkiness formation in rice. Up arrows designate upregulation of the starch synthase genes and down arrows designate downregulation of synthase starch genes. Yellow box constituted the causal factor of the chalkiness formation, the purple boxes are the major physiological processes involved in endosperm formation, the light blue represents the regulatory pathways resulting in chalkiness formation, and red box is chalkiness formation. (Source: Nevame et al. 2018)

the grain quality traits, endosperm appearance (chalkiness) remained a principal factor that determines the market value of the rice grain. These quality traits are interrelated in that any change in appearance is likely to affect the milling and cooking properties. Rice grain quality is a complex characteristic including many components such as appearance, cooking qualities, and eating qualities. Among these properties, consumers often pay most attention to the appearance (Rabiei et al. 2004; Guo et al. 2011). Chalkiness directly affects the market value of rice. Normally consumers and producers are not interested in the rice variety with high rate of chalky grain. Several genetic and environmental factors were shown to influence rice grain appearance, which is usually evaluated as percentage of grains with chalkiness (PGWC) (Tan et al. 2000). Furthermore, quality traits were polygenic phenomenon and many quantitative trait loci (QTLs) associated with their phenotypic expression which is generally affected by pleiotropic effects of genes for non-target traits (Li et al. 2003; Jiang et al. 2005; Bian et al. 2014; Xie et al. 2006; Woo et al. 2008). Currently, more than 100 QTLs were reported to be associated with different types of chalkiness in rice (Li et al. 2003; Wan et al. 2005; Kobayashi et al. 2007; Zhou et al. 2009; Xiao et al. 2012; Chandusingh et al. 2013; Bian et al. 2014). QTLs for grain quality might facilitate the development of strategies for the improvement of milling and cooking of various sources of rice germplasm by providing new genetic sources of enhanced grain quality characteristics and this suggest that the local germplasm might be a valuable new source for introgression and improvement of several traits that affect quality traits demanded by the different rice export markets. Different types of chalkiness such as white core, white back, and white belly were reported to be present in the endosperm of rice grain (Chandusingh et al. 2013). Percentage chalkiness in rice grain is an index that determines the appearance quality. So for improving the milling and cooking quality, the rice endosperm should be free of chalkiness. Starch characteristics determine the quality of various products of rice, e.g., eating, cooking, and processing qualities. Starch content and protein storage were also attributed to chalkiness because chalky grains were indicated to contain a lower density of starch granules compared to vitreous ones (Del Rosario et al. 1968).

On the other hand, *OsPPDKB* and *starch synthase IIIa* (*SSIIIa*) genes were implicated to have pleiotropic effects on white-core endosperm (Kang et al. 2005; Fujita et al. 2007; Ryoo et al. 2007) which indicates that these genes may have quantitative effects in the endosperm formation in rice. The gene *OsPPDKB* controls the carbon flow into starch and lipid biosynthesis during grain filling (Kang et al. 2005), while the gene *SSIIIa* interfere in the amylopectin chains elongation (Fujita et al. 2007). Therefore, it is quite obvious that the rice endosperm appearance depends upon the complex genetic mechanism regulation of genes involved in endosperm formation could produce a dysfunction in plant metabolic activity and further cause chalkiness formation in rice. Molecular breeding via marker-assisted selection and gene pyramiding was proposed for this achievement. For example, *Chalk5* was found as a major QTL encodes a vacuolar H^+-translocating pyrophosphatase which causes chalkiness formation in rice (Li et al. 2014). The reduction of chalkiness percentage could provide a rice grain with good quality to consumers. However, this requires the collection and screening of different rice germplasm without neglecting the available stress-tolerant varieties. Therefore, a pragmatic genetic approach like the assembly of germplasm with considerable stress tolerance ability into adaptable rice varieties is very important (Fig. 3). Consumer's preference for cooking, processing, and eating properties of rice varies around the world. Hence, it is critical that breeders develop rice cultivars with diverse grain qualities to meet the consumer's demand. Amylose content has been recognized as one of the pivotal determinants of eating and cooking qualities of rice.

Low amylose content is usually associated with tender, cohesive, and glossy cooked rice; while high amylose content results in dry, fluffy, and granular cooked rice (Juliano 1971). The apparent amylose level is also an important factor for the market value of rice (Larkin et al. 2003). Although amylose content is a key-determining factor of rice quality, there are minor differences in the texture of cooked rice among cultivars with similar amylose contents (Juliano 1985). It has been verified that both structure and characteristics of amylopectin are also determinants of rice texture (Takeda et al. 1987; Ong and Blanshard 1995). Gelatinization temperature and apparent amylose content are key parameters used to describe the eating and cooking qualities of rice. Sequence variants of SSIIa and Waxy genes are important determinants of gelatinization temperature and apparent amylose content, respectively (Caffagni et al. 2013). Key parameters most frequently used to describe eating and cooking quality of rice are directly related to rice grain

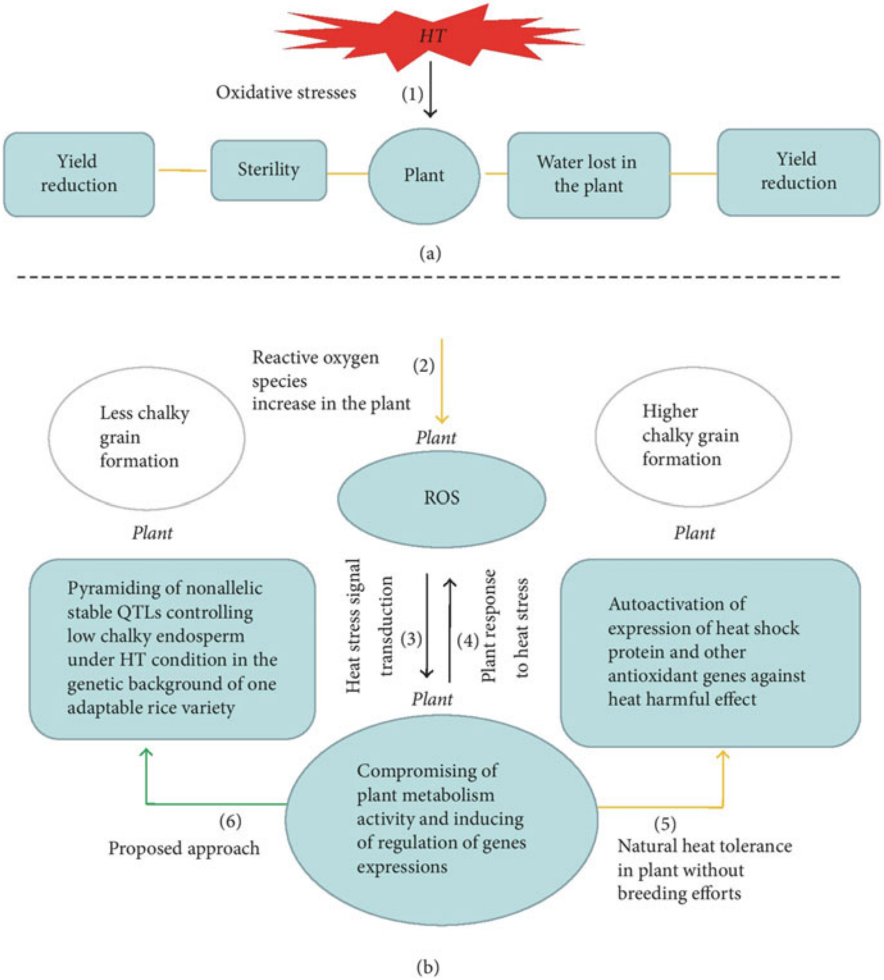

Fig. 3 Proposed approach for mitigating heat stress damage in plant. (1) Oxidative stresses from higher temperature. (2) Reactive oxygen species production. (3) Heat stress signal transduction in the plant and change in metabolic activity and genes expression. (4) Plant response to heat stress. (5) Heat stress tolerance via the natural metabolic defense. (6) Defect in HS tolerance and chalkiness formation. Heat stress tolerance via heat tolerance genes pyramiding. Stress tolerance and less chalky grain formation. Heat stress effect on plant fertility and yield reduction. Heat stress on plant water content. Water lost and grain filling rate (GFR) reduction. Effect of GFR on grain yield. Effect of GFR on chalkiness formation in rice. Yellow lines indicated the detrimental effect of HS on the rice plant with weak tolerance. Green lines showed tolerance for the HS from strengthened metabolic defense. Black lines indicate the detrimental action of ROS and transcriptional responsiveness of the plant. (Source: Nevame et al. 2018)

starch: apparent amylose content (AAC) (Juliano 1985), gel consistency (GC) (Cagampang et al. 1973), gelatinization temperature (GT) (Little et al. 1958), and the Rapid Visco Analyser (RVA) profile (Bason and Blakeney 2007). Rice starch, constituting 90% of the endosperm weight, is composed of two types of glucose polymers, linear amylose, and highly branched amylopectin. The functional pasting properties of starch are affected by the branch chain length, and the amylose-to-amylopectin ratio (Jane et al. 1999), thus both the amylose content and the fine structure of amylopectin are key determinants of processing and eating quality of rice (Ong and Blanshard 1995). The inheritance of grain quality is more complicated than that of other agronomic traits in cereals due to epistasis, maternal and cytoplasmic effects, and the triploid nature of endosperm (He et al. 1999). Rice eating and cooking quality are mainly influenced by the physical properties of its starch. Starch is composed of amylose and amylopectin, and the apparent amylose content is recognized as one of the most important determinants of the eating and cooking quality of rice. Rice eating quality still differs among varieties with a similar apparent amylose content, which can be explained by differences in amylopectin structure (Reddy et al. 1993; Ong and Blanshard 1995). Therefore, other testing methods, such as gel consistency and pasting viscosity, have been established in order to differentiate quality among different genotypes with similar apparent amylose content. Germplasm plays a major role in determining the cooking and eating quality of rice. Although grain quality is a complex trait controlled by polygenes and largely influenced by the environment, the fine molecular mechanisms underlying these traits await further elucidation for the improvement of eating and cooking quality of rice using collections of germplasm from different sources and also genetic diversity analysis.

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Potentiality of Wild Rice in Quality Improvement of Cultivated Rice Varieties

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Abstract

Rice serves as a major food crop to the majority of the world's population. *O. rufipogan* and *O. nivara* are the two common wild varieties of rice found in Asia. *O. sativa* and *O. glaberrina* are two of the cultivated varieties of rice found in Asia. *O. rufipogan* has disease resistance and drought tolerance. Domestication of rice started when people thought to cultivate rice in different geographical regions to where they migrated. The cultivated rice varieties started to adapt to new climatic and environmental conditions in which they are cultivated, resulting in domesticated varieties of rice. Genetic diversity of rice is exploited to breed new varieties of rice. Rice blast, a disease caused by *Xanthomonas oryzae* in rice, can be controlled by breeding disease-resistant varieties through the exploitation of genetic diversity. Techniques like molecular markers and other next-generation sequencing technologies have been used to identify the genetic variation among different varieties of rice. For broadening the gene pool of the rice varieties, a technique known as wild hybridization has been widely used. It involves the hybridization of rice with related species of wild varieties. Marker-assisted selection method of breeding increases the efficiency of breeding rather than conventional methods. Marker-assisted pyramiding involves combining many genes together to form one genotype. This method is useful in providing pest resistance and tolerance to abiotic stress. Gene introgression involves the movement of alleles of a species to the gene pool of other species. Gene introgression

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can result in plant evolution because it introduces variations among genes for the plant to adapt changes in the environment. Nevertheless, gene introgression provides short-term genetic diversity; the original genetic diversity of rice species is usually lost.

Keywords

Bacterial resistance · Gene introgression · Genetic diversity · Hybridization · Molecular markers · *Oryza* · Pre-breeding · Virus resistance · Wild rice

1 Introduction

1.1 Origin of Rice

Most probably first rice cultivation began in Ancient India and Southeast Asia. Puddling and transplanting cultural techniques were originally urbanized in the North and Central China and later passed on to Southeast Asia (Chang 1976). Polyphyletic origins of rice were both indica and japonica. Indica was perhaps cultivated in Himalayan foothills in India while japonica was found cultivated in China. From South China, japonica rice stimulated northward and turn into tropical ecotype. Japonica rice also stimulated Southeast Asia and from Southeast Asia to Brazil and West Africa and finally turn into tropical ecotype (Khush 1997).

In the Gondwana land nearly 130 million years ago, genus *Orzya* possibly invented, when Gondwana land breaks into different continents diverse species of rice are scattered in the continents (Chang 1976; Khush 1997). After breaking supercontinent, turn into broadly scattered in the moist tropics of South America, Africa, Oceania, and South and Southeast Asia (Chang 1976). Genus *Oryza* has 21 wild species and 2 cultivated species. Asian *O. sativa* produced everywhere in the world whereas African *O. glaberrima* produced only in some areas of West Africa (Khush 1997). In the remote past, two domesticated species had a general progenitor. Wild perennial ~ wild annual ~ cultivated annual is the sequence and after these sequences independent evolutionary and parallel processes occurred in Africa and Asia (Chang 1976). A common ancestor with the AA genome is the source of origin of cultivated species of rice. *O. glaberrima* almost certainly cultivated in the delta of Niger River (Khush 1997).

In the last glacial age, annuals differentiation and diversification in South Asia were increased by climate change. Some other effects by climate change were human selection, over altitude or latitude, manipulation of the cultural environment, and dispersal of plants. Wetland culture was first used in China before the lowland and dryland culture. After the huge spread of Asian rice cultivation, there were formed three eco-geographic races between japonica, indica, and javanica and developed three distinct cultures such as upland culture, lowland culture, and deep water culture in monsoon Asia. Cultivator preference, socioreligious traditions and population pressure are the root cause for varietal development in rice over a millennium. Parallel to ecological diversification, genetic differentiation also developed. African farmers developed after Asian farmers so that they passed through less diversification. African rice and Asian rice are still passing through evolutionary changes at habitats (Chang 1976).

The diversity on the basis of the genetic affinity of *O. sativa* is categorized into six groups. Broadly famous indica rice match up with the group I. *Japonica* rice correspond with group VI; similarly, *Javanica* rice is also gone in group VI. *Javanica* is selected as tropical japonicas because of japonicas developed in the temperate climate. Rice cultivation is occurring now in among 55° N and 36° S latitudes. It is being cultivated in various growing conditions like flood-prone ecosystems, irrigated, rain-fed upland, and rain-fed lowland. It is assumed that approximately 120,000 types of rice varieties are available in the world. In 1960 International Rice Research Institute was established and it contributed to increased quality of rice varieties and also developed high-yielding varieties of rice. About 70% of the world's rice land area is covered with these varieties of rice. Because of the large-scale implementation of these developed varieties, production of rice increased and reached to double production during 1966–1990 (Khush 1997).

1.2 Regional Development of Rice Cultivation

1.2.1 Asia

Oldest domesticated rice was discovered in China 12,000 years ago in the Yangtze Valley and believed on archaeological evidence that first-time rice cultivation occurred in this region. Diaotonghuan archaeological site's rice phytoliths analysed morphologically and showed the transition from the collection of wild rice to the cultivation of domesticated rice. Large numbers of wild rice phytoliths were dating from 12,000–11,000 BP (years before the present) at the Diaotonghuan, which indicates wild rice collection was the fulfilment of daily needs of life. At the time of 1000–8000 BP, morphological changes were observed at Diaotonghuan phytoliths and this has also witnessed the domestication of wild rice. After that two major types of indica and japonica rice started growing in central China. At the time of the third millennium BC, there was a rapid development of rice farming into mainland Southeast Asia and westwards across India and Nepal (Wang et al. 2010).

Oldest grains were seen first time in India from 7000–6000 BC, while cultivated rice is placed at around 3000–2500 BC (Indus Valley Civilization). Perennial wild rice has been seen now in Assam and Nepal in any season. After that, before 1400 BC rice was seen in Southern India and Northern Plains. Later, rice spread to all the fertile alluvial plains watered by rivers. Methods of cooking and cultivating of rice were developed and spread to the West. Europe introduced rice as a hearty grain (Patra et al. 2016). Rice production is majorly received from China, India, Indonesia, Pakistan, Bangladesh, Vietnam, Thailand, Myanmar, Philippines and Japan. From the World's total production of rice, 92% of production is done by Asia only (Mutert and Fairhurst 2002).

1.2.2 Africa

About 3500 years ago rice cultivation started in Africa. During 1500–800 BC, *O. glaberrima* spread from the Niger River Delta to Senegal. African rice was first introduced in East Africa then it reached to Westward. African rice played an important role in saving people during Africa conquers famine of 1203 (Patra et al. 2016).

1.2.3 America

Colonial South Carolina and Georgia from the USA increased their wealth by slavery labours from the Senegambia (West Africa) and from coastal Sierra Leone. These African slavery labours get the maximum price because of their prior knowledge of rice culture. Rice culture increased and well developed by African slavery labour's work and efforts at the Georgetown, Charleston and Savannah. Owners learned from the enslaved Africans to how to flood the fields and dyke the marshes. Milling of rice was firstly done by hand with wooden paddles and then slaves from Africa developed a skill of winnowed in sweet-grass baskets. Profit of crop increased with the invention of rice mill in 1787. Because of American civil war, the Southern USA lost slavery labours and that resulted in less profit in rice culture. Rice culture stopped totally in the Southern USA at the end of the twentieth century and Southern Carolina is the only place today which can be visited for rice culture (Carney 1998).

2 *Oryza*: Wild and the Cultivated

Rice serves as an important and nutritive food for nearly 70% of the global population. Rice is an ancient crop that appears to be cultivated in Indochina regions as early as 6200–7100 B.C. (Chen 1989; Pearsall et al. 1995). Rice is cultivated in all continents except Antarctica in the World (Londo et al. 2006). The *Oryza* genus is a significant crop for human civilization for food security with two independently domesticated species: *O. sativa*, the Asian rice cultivated from last 10,000 years and *O. glaberrima*, the African rice cultivated from 3000 years. Asian and African rice species are the two major domesticated rice cultivars in the World. Varieties of Asian rice species are cultivated in China, India, Korea and Japan. Where and how rice was originated, domesticated and developed as a staple food is still a vast research area. Asian rice *O. sativa* is the world's oldest rice species with a record of domestication from nearly 8000–9000 years ago (Higham and Lu 1998; Liu et al. 2007). *O. sativa japonica* and *O. sativa indica* are two subspecies of *O. sativa*. According to the researches from genetics and archaeology 8000 BP ago *O. sativa japonica* was domesticated in the Yangtze River valley of southern China. *O. sativa indica* was domesticated in India 5000 BP ago as a product of favourable allele introgression with *japonica*. These findings have added new things to our understanding of domestication and the complex evolutionary process of rice. Macrobotanical researches indicated that rice has been diffused from China to Korean Peninsula 3400–2800 BP ago (Rounsely et al. 2009; Jacquemin et al. 2011). Also archaeological researches revealed that rice agriculture was diffused from the Korean peninsula to Japan nearly 2800 BP ago (Ma et al. 2004). Rice research in India unveils the prehistory of *indica* and *japonica*, their domestication, spreading and interactions of cultivars of the same genus. The *O. rufipogon* and *O. nivara* are the two close wild relatives of cultivated rice *O. sativa* are native to India and well distributed since 9000 BP (Vitte et al. 2007; Schneeberger et al. 2011). The genetic level and phylogeographic studies reveal that India and Indochina areas were the centres of wild rice *O. rufipogon* diversity. Further, these studies convey that cultivated rice was domesticated from different *O. rufipogon* wild populations in two steps to produce two most cultivated varieties, *O. sativa indica* and *O. sativa japonica*. The *O. sativa indica* was domesticated in India, Thailand and Myanmar and *O. sativa japonica* was domesticated in Southern China (Atwell et al. 2014).

2.1 Wild Relatives of Cultivated Rice

The genus *Oryza* includes 2 cultivated and 21 wild species: *O. glaberrima* and *O. sativa*. The *O. glaberrima* is an African cultivated rice species and *O. sativa* is an Asian cultivated rice species. But today *O. sativa* is cultivated globally, as Asian cultivated rice has been diffused all over the world with migrating populations (Rounsely et al. 2009). As *O. rufipogon* is perennial wild, studies suggest that cultivated rice *O. sativa* is the annual form of *O. rufipogon* and *O. nivara* species (Jacquemin et al. 2009; Rounsely et al. 2009). As the world population is estimated to increase by almost three billion by 2050, rice cultivators are in immediate need to develop new high-yielding, adaptive and biotic and abiotic stress-tolerant varieties of rice. As wild relatives of rice are adapted to a vast biogeographic ranges, they are stress-tolerant and serve as an important reservoir for crop improvement (Iwamatsu et al. 2008; Mizuta et al. 2010; Giuliani et al. 2013; Atwell et al. 2014; Garg et al. 2014; He et al. 2014). Wild relatives of cultivated crop plants are considered as potential reservoirs of plant defence genes. All the progenitors of cultivated rice *O. barthii*, *O. rufipogon*, *O. nivara*, *O. longistaminata*, and *O. glumaepatula* are the potential reservoirs of NBS-encoding genes (Rawal et al. 2018). Wild relatives of cultivated rice have always remained as rich source of unexploited genes and allelic variations (Sanchez et al. 2014). As rice is one of the world's major staple food crops, the advantage of allele mining from wild relatives of rice may be a potential approach to get superior genes and alleles. The number of bacterial blight resistance and blast resistance genes has been taken from wild species of rice like *O. nivara*, *O. rufipogon*, *O. longistaminata*, *O. officinalis*, *O. minuta*, *O. rhizomatis*, and *O. australiensis* (Das et al. 2012; Devanna et al. 2014; Sanchez et al. 2014; Rawal et al. 2018). Genetic researches have suggested that *O. rufipogon* is the wild progenitor of domesticated Asian rice (Oka 1988; Molina et al. 2011) (Table 1).

The wild species of *Oryza* are with enormous potential that can be significantly used to improve the productivity of cultivated *O. sativa* and *O. glaberrima*. According to 'Oryza Map Alignment Project' (OMAP) and International Rice Genome Sequencing Project, 11 different wild rice species including six diploid genomes (AA, BB, CC, EE, FF and GG) and four tetraploid genomes (BBCC,

Table 1 Wild relatives of cultivated rice with their agronomically useful traits[a]

Oryza species	Agronomically useful traits	References
O. nivara	Resistance to grassy stunt virus, blast, sheath blight, stemborer and whorl maggot; drought avoidance, cytoplasmic male sterility, hybrid breakdown locus, pollen sterility locus	Heinrichs et al. (1985), Brar and Khush (1997, 2003), Hoan et al. (1997), Khush and Brar (2002), Miura et al. (2008), Prasad and Eizenga (2008) and Win et al. (2009)
O. rufipogon	Resistance to bacterial blight, stem rot, tungro virus, blast, stem borer and white-backed planthopper; elongation ability, tolerance to aluminium and soil acidity, source of cytoplasmic male sterility, improved yield, salinity tolerance, fertility restoration ability	Chaudhary and Khush (1990), Kobayashi et al. (1993, 1994), Brar and Khush (1997, 2003), Hoan et al. (1997), Khush and Brar (2002), Li et al. (2002), Ram et al. (2005), Chen et al. (2006, 2008, 2010), McCouch et al. (2007) and Fu et al. (2010)
O. barthii	Resistance to bacterial blight, bacterial leaf streak, blast, brown spot, sheath blight, and green leafhopper; drought avoidance, source of cytoplasmic male sterility	Nayar (1968), Chu (1970), Chu and Oka (1970), Devadath (1983) and Brar and Khush (1997, 2003)
O. glumaepatula	Elongation ability, source of cytoplasmic male sterility	Brar and Khush (1997, 2003) and Khush and Brar (2002)
O. longistaminata	Resistance to bacterial blight, blast, brown planthopper, nematodes and yellow stemborer; drought avoidance, pollen/spikelet fertility	Vales (1985), Brar and Khush (1997, 2003), Khush and Brar (2002) and Chen et al. (2009)
O. meridionalis	Elongation ability, drought avoidance, sheath blight	Brar and Khush (1997) and Prasad and Eizenga (2008)
O. punctata	Resistance to brown planthopper, zigzag leafhopper	Jena and Khush (2000)
O. minuta	Resistance to sheath blight, blast, bacterial blight, brown planthopper and green leafhopper; grain size, awn length, heading date, panicle number, spikelet number	Amante-Bordeos et al. (1992), Jena and Khush (2000), Liu et al. (2002), Jin et al. (2004), Linh et al. (2006) and Rahman et al. (2007)
O. eichingeri	Resistance to yellow mottle virus, brown planthopper, white brown planthopper and green leafhopper	Jena and Khush (2000) and Patra et al. (2016)
O. officinalis	Resistance to thrips, brown planthopper, green leafhopper and white brown planthopper	Jena and Khush (2000), Ammiraju et al. (2010) and Patra et al. (2016)
O. rhizomatis	Drought avoidance, rhizomatous	Jena and Khush (2000) and Patra et al. (2016)
O. alta	Resistance to striped stemborer, high biomass production	Jena and Khush (2000), Ammiraju et al. (2010) and Patra et al. (2016)
O. grandiglumis	High biomass production, resistance to disease and insect pests	Jena and Khush (2000) and Patra et al. (2016)

(continued)

Table 1 (continued)

Oryza species	Agronomically useful traits	References
O. latifolia	Resistance to brown planthopper, white brown planthopper, green leafhopper	Jena and Khush (2000) and Patra et al. (2016)
O. australiensis	Drought avoidance, resistance to blast, brown plant hopper	Jena and Khush (2000) and Ammiraju et al. (2010)
O. brachyantha	Resistance to yellow stemborer, leaf-folder, whorl maggot and bacterial blight; tolerance to laterite soil	Jena and Khush (2000), Ammiraju et al. (2010) and Ram et al. (2010)
O. granulata	Shade tolerance, adaptation to aerobic soil	Jena and Khush (2000), Ammiraju et al. (2010) and Patra et al. (2016)
O. meyeriana	Shade tolerance, adaptation to aerobic soil	Jena and Khush (2000) and Patra et al. (2016)
O. neocaledonica	Not known	Hequet (2017)
O. longiglumis	Resistance to blast and bacterial blight	Jena and Khush (2000)
O. ridleyi	Resistance to stem borer, whorl maggot, blast, bacterial blight and yellow stem borer	Jena and Khush (2000), Ammiraju et al. (2010) and Patra et al. (2016)
O. schlerchteri	Not known	Naredo et al. (1993)

[a] Adapted and modified from Liakat Ali et al. (2010) and Lu et al. (2010)

CCDD, HHKK and HHJJ) with broad geographical distribution and ecological adaptation have been identified as best for crop improvement. It also suggests that variety with AA diploid genome appears to be the progenitor of modern cultivated rice. Drastically changed climatic conditions can cause biotic and abiotic stresses that threat the yield of rice. Wild relatives of cultivated rice can be used to increase the adaptive capacity of cultivated rice varieties around the world through molecular breeding systems. Wild rice species are large pools of genetic diversity that can provide new allelic variations required for breeding programs. These wild relatives are extremely potential in adapting cultivated rice varieties to disease pressures, farming practices, market demands and changing climatic conditions. Several measures have been taken to protect wild rice species, in the form of both in wild and in gene banks (Hajjar and Hodgkin 2007; Mammadov et al. 2018).

2.2 Genetic Diversity of Cultivated Rice

Approximately Asian cultivated rice *O. sativa* includes 120,000 different, named traditional and elite cultivars (Khush 1997). In addition to the two mainly cultivated rice varieties, many other minor rice varieties such as Aus cultivars (drought-tolerant), Ashinas varieties (deep-water) and basmati rice (aromatic) have been identified through molecular marker studies (Second 1982; Bautista et al. 2001; Garris et al. 2005). The origin of these minor rice types is still unknown. The genetic diversity studies reveal that *japonica* and *indica* are genetically distinct, suggesting that these two may have originated from two different gene pools (Londo et al. 2006). All 27 rice species are having nearly 15 million years of evolutionary history, with 11 different genome types. In that, 6 are diploid ($n = 12$: AA, BB, CC, EE, FF and GG) and 5 are polyploid ($n = 24$: BBCC, CCDD, HHJJ, HHKK and KKLL). Cultivated species of rice belong to the AA genome group (Stein et al. 2018). The evolutionary relationship in rice species having AA genome represents the primary gene pool. The pantropical distribution of rice relatives was justified by the genetic clustering analysis that indicated strong geographical differentiation. These were differentiated into two primary South American and African clades having *O. glumaepatula* and *O. longistaminata*. The Australian clade, the African and the Asian clades were included in one largest clade of genetic diversity (Stein et al. 2018).

The AA genome includes eight diploid species, in which one of the cultivated species, *O. sativa* L. has two subspecies, *O. sativa* L. ssp. *japonica* and *O. sativa* L. ssp. *indica*. Another African cultivated species *O. glaberrima* Steud. distributed in West Africa. It is widely accepted that African cultivated rice was domesticated from *O. barthii* (Li et al. 2011; Wang and Wing 2014). As the African clade includes *O. glaberrima* variety clustered with four accessions of *O. barthii*. The rice gene pools of South America/Africa and Australia clades are genetically isolated with important traits from domesticated rice thus remain genetically pure with no gene flow from Asian domesticated rice populations. It is assumed that approximately 10–20% of the wild genetic diversity is present in cultivated rice (Zhu et al. 2007; Palmgren et al. 2015). The genetic diversity among these cultivated and wild species stands valuable for rice crop improvement (Wambugu et al. 2013). The number of germplasm of Aus, Indica, Japonica, Aromatic and Deep Water rice cultivars with their landraces has been conserved in global gene banks (Khush 1997; Brar 2003; Garris et al. 2005; Lu et al. 2005; Tripathy et al. 2018).

2.3 Wild Rice Germplasm Database

The functions of several thousands of genes can easily be discovered through reverse genetics. OryGenesDB (http://orygenesdb.cirad.fr/index.html) is an open-access database mainly developed for reverse genetics in rice. It contains 171,000 flanking sequence tags (FSTs) and annotations among 10 specific categories in 78 annotation layers of various mutagens and functional genomics data from international insertion collections and literature. Oryza Tag Line and GreenPhylDB are the two related databases containing phenotypic descriptions of Genoplante insertion lines (Larmande et al. 2008) and facilitate comparative functional genomics between *O. sativa* with other genomes (Conte et al. 2008; Droc et al. 2008). The genus *Oryza* includes nine genome types and 23 species with rich genetic resource. Genotypic information and genomic variation of imputation and imputation-free deep sequencing derived *O. rufipogon* wild rice accessions (446 and 17 respectively) are available in Oryzabase, whereas information about phenotype and habitat is available in

OryzaGenome repository (Ohyanagi et al. 2016). Oryzabase also includes approximately 1700 accessions from closely to distantly related wild accessions to solve unrevealed tetraploidy issues (Huang et al. 2010; Huang et al. 2012). OryzaGenome mainly provides easy access to information about geographical origins, phenotypic traits, mutations, genome sequence and genetic variation information of wild *Oryza* species with many cultivated species, in collaboration with Oryzabase (http://www.shigen.nig.ac.jp/rice/oryzabase/) (Ohyanagi et al. 2016). International Rice Gene Bank Collection Information System (IRGCIS) displays data about 4645 accessions of wild rice species present in the International Rice Research Institute (IRRI) Gene Bank.

2.4 Indian Wild Rice Database

Indian Wild Rice (IWR) Database provides complete passport data with morphological descriptors and DNA fingerprints for 614 diverse rice accessions collected from India. This information is useful for further yield, quality and adaptive improvement of rice cultivars. IRGCIS provides data about 838 accessions of wild rice species of Indian origin present in the IRRI Gene Bank (http://www.irgcis.irri.org/). Indian gene bank at the Indian Council for Agricultural Research—National Bureau of Plant Genetic Resources (ICAR-NBPGR) consists 307 accessions of wild rice species *O. rufipogon* and 726 accessions of *O. nivara* (http://www.nbpgr.ernet.in:8080/PGRPortal/ as on 14 March 2018) (Singh et al. 2018; Tripathy et al. 2018). Rice Annotation Project Database (RAP-DB) (http://rapdb.dna.affrc.go.jp/) provides a comprehensive set of gene annotations for the genome sequence of *O. sativa* (*japonica*) cv. Nipponbare.

The collaboration of Rice Annotation Project (RAP) and Michigan State University Rice Genome Annotation Project (MSURGAP) (Ouyang et al. 2007) has released the genome assembly, Os-Nipponbare-Reference-IRGSP-1.0 (IRGSP-1.0) (http://rapdb.dna.affrc.go.jp/; http://rice.plantbiology.msu.edu/) (Sakai et al. 2012; Wang et al. 2013). In 40 years, 889 accessions of Indian wild rice germplasm were collected by ICAR-NBPGR from Gangetic Plains and Eastern region of India to facilitate the characterization, evaluation, conservation and utilization. Germplasm of 4 accessions of *O. meyeriana* var. *granulata*, 484 accessions of *O. nivara*, 50 accessions of *O. officinalis*, 367 accessions of *O. rufipogon*, 18 accessions of *O. sativa* var. *spontanea* and 10 accessions of *Porteresia coarctata* were collected from Gangetic Plains and Eastern region of India. The passport information of these varieties is used to study habitat specificity, diversity pattern and distribution of Indian wild rice species (Semwal et al. 2016).

3 Diversity Prospecting of Wild Rice

The wild relatives of rice species possess useful gene sources for high yield, sustainable resistance ability, mainly tolerance to drought and wetlands (Heinrichs et al. 1985). Unfortunately, drastic destruction of respective habitats has led to the extinction of important populations and a decrease in number and sizes of remaining populations (Vaughan and Chang 1992). Conservation of rice genetic resources and evaluation is the primary step towards ensuring germplasm sources for the effective breeding techniques. The preservation of genetic resources mainly accepted in terms of in situ conservation allows sustainable evolution of genetic populations along with effective and more prominent ex situ conservation. Conservation of natural habitats not only helps in in situ conservation of natural species of wild crops but also provides evolutionary relationship among the domesticated crop varieties. Agricultural developments mainly influenced by rapidly emerging technological innovations, scientific and economic forces continuously (Goicoechea et al. 2010). It is impractical and bias to anticipate farmers to preserve their conventional rice crop varieties in a state of the suspended animation. The conservation of wild rice crops should therefore be the focus and necessary conservation strategies have to develop more effectively (Rerkasem and Rerkasem 2005). Sow et al. (2014) conducted documentation come characterization studies on wild species of *Oryza* in Niger and its diversity analysis with microsatellites markers provides comprehensive idea on diversity and population structures within rice geographical areas of Niger.

O. officinalis is one of the agriculturally potential wild rice varieties and it is under the endangered category. Gao (2005) investigated genetic diversity to get more accuracy of population structure for effective conservation studies. Genetic variability was studied using 14 microsatellite DNA loci and was examined in the populations covering most of the rice species across the selected area of China. Acceptable genetic changes were detected at 14 loci in 442 individuals of 18 populations. The evaluation of genetic differentiation [Fixation index (FST) = 0.442] illustrates high genetic variations among Chinese *O. officinalis* populations. Maximum populations exhibited heterozygote deficits in Hardy-Weinberg equilibrium analysis. This difference could be due to the inbreeding in this predominantly outcrossing variety. For sustainable in situ conservation and genetic restoration of significant processes, it is important to include high-throughput breeding, effective gene flow and large population sizes. The experimental results of high FST values among the populations are instructive for adopting a systematic conservation plan that includes representative varieties with the distinct genetic variation for either in situ conservation or expeditions of germplasm collection.

Rice prospecting was performed in twelve African countries; nearly 4000 samples were collected for the analysis, 28% of which belongs to the *glaberrima* complex. Biochemical polymorphism reveals that wild accessions were unique and highly diversified. The very few loci were needed to demonstrate the existence of West-East cline in all African wild types. In West Africa, the Senegal-Mali region can be distinguished from the Chad-Cameroon-Nigeria region. The direct morphological observations, with respect to immersion conditions of plant, exhibit the same type of ecological variations in the two wild and cultivated rice varieties. The vegetative development and reproductive phase of plants were analysed to confirm that cultivated and wild plants do not use same propagation and dissemination strategies. The hybridization in F1 and F2 between samples belonging to two species *O. glaberrima* and *O. breviligulata* was studied. A hierarchical cluster model gives information on the genetic structure of these populations and exhibits the notable natural interspecific hybridization (Brar and Singh 2011; Wambugu et al. 2013).

Therefore, it was found necessary to develop appropriate and efficient conservation strategies to secure survival studies on rice diversity and population structure. In a study by Vaughan (1994), about 21 wild relatives of cultivated rice are severely endangered in the wild. Moreover, sound knowledge regarding their intraspecific population structure is largely limited. Hence, it is necessary to update population genetic analysis in endangered wild rice varieties. Wild rice have generated notable information about population genetics which has proven extremely efficient in providing the perspective of evolutionary relationship for sustainable utilization and conservation actions on these potential genetic resources (Vaughan 1994; Melaku et al. 2013).

4 Pre-Breeding in Rice: Enhancing Utilization of Wild Rice Relatives

Future agriculture is mainly challenged by food needs for the ever-increasing populations and crop-based materials. Food crop improvement, mainly through genomics-based approaches, might be one of the potential and effective methods to mitigate the above critical challenge. The method requires resources of genes that can be brought into breeding materials before it can be transferred to crops to have the desired characteristics. This may be fulfilled through pre-breeding activities (Khush 1997; Hajjar and Hodgkin 2007; Jain and Omprakash 2019). Pre-breeding programs focused to identify and evaluate desirable traits and genes from plant samples that cannot directly be used in breeding (Stander 1993). The proper use of genetic diversity from wild relatives and selection potential parental plants is the necessary first step in achieving expected results from breeding (Nass and Paterniani 2000).

Plant breeding for quality rice varieties includes traditional hybridization technique and selection for successive crossing methods (Jeon et al. 2011). The strategy in the success of breeding technique mainly depends on the selection of parental varieties for crosses in order to develop superior line (Chandraratna 1964). Pre-breeding is one of the key aspects of diversification of germplasm strategies and is the most reliable alternative to connect genetic resources and plant breeding programs. The outcome of pre-breeding being performed is expected to have positive results to be included in conventional breeding programs (Kumar et al. 2014). The key objectives of rice pre-breeding are to minimize genetic uniformity and consequent genome vulnerability. In pre-breeding, genetic improvement becomes essential, persistent and planned aspect of all breeding programs. Genetic enhancement is considered as second important strategy for improvement of rice yield to new heights. This aim is more often anticipated for than achieved, but it is true that most popular rice cultivars have extremely diverse parentage (Chandraratna 1964). Pre-breeding program was applied to adapt various cultivars of rice germplasm to new genetic frameworks and geographical locations. Genetic enhancement in rice cultivars brings new advancement in traits which lack in local cultivars (Dixit et al. 2014; Meena et al. 2017).

Application of molecular maps and genome sequences allows tagging and identifying genes transferred from exotic/semi-exotic sources, including those of adapted, have been screened to any method of selection for possible improvement in order to make the selection more efficient and reliable (Nass and Paterniani 2000). For certain breeding objectives, such as the development of C4-rice, the new traits for improvement cannot be found in landraces and wild rice relatives. Pre-breeding will be the only viable option to reach the targets. Though various disease/drought resistance rice cultivars are available, pre-breeding for broadening the genetic base is very much necessary to fulfil not only genetic transformation but also rice food diet expectation by consumers. Especially wild rice varieties were not being utilized adequately in breeding programs (Jeon et al. 2011). The main limitation is due to the linkage drag and various incompatibility conditions between wild and cultivated species. Under such conditions, pre-breeding methods give a great opportunity to explore primary genetic pool by analysing variability among wild rice plants and cultivated germplasm. Further provides new and useful genetic variability into breeding programs to produce potential cultivars exhibiting diverse genetic information and high resistance (Leung et al. 2015). Rice production needs to rise in the future in order to meet increasing demands. Pre-breeding is a method to improve plants genetically for the benefit of humankind. The objective of the rice pre-breeding work is to grow new genetic resources using genomic tools to help predict the effect of introducing different genes from rice wild relatives into cultivated varieties of *indica* and *japonica* rice. The International Rice Research Institute (IRRI) was one of the pioneer institutes well known for the development of quality rice cultivars with more genetic stability and in 1962, a team of IRRI scientists worked on crossing experiments where Dee-geo-woo-gen, the same Chinese variety which had given TN 1 its semi-dwarf plant stature, crossed with Peta, a vigorous cultivar from Indonesia. Later in 1966, IRRI released variety IR8 from this cross and due to its superiority, IR8 was worldwide accepted as one of the potential rice cultivars (Khush 1997; Jain and Omprakash 2019).

The rice pre-breeding will definitely provide improved germplasm and breeding technique that enhances resistance and genetic diversity. Improvement in the selection of parental lines and advanced methodologies will definitely help in the production of quality rice varieties (Babu et al. 2012). Application of potential genes from a documented gene bank in well-organized and design strategies lead to the development of improved rice germplasm. Rice pre-breeding is a collective responsibility, which strengthened by effective communication between breeders and gene bank curators.

5 Marker-Assisted Rice Breeding Using Potential Traits from Wild Rice

Conventional methods of breeding in rice are time-consuming and varies drastically based on environmental factors. Breeding to obtain an improved variety takes years with no guarantee of success. Therefore, new technologies that help aid in efficient breeding are necessary. Marker-assisted selection (MAS) breeding is one such strategy (Akhtar et al. 2010). Molecular markers have several advantages over morphological markers that are used in traditional plant breeding. Some of them include:

- Time saving: DNA can be isolated from any plant tissue and based on required trait information obtained from the associated marker, informed crosses can be carried out thus saving a lot of time.
- Consistency: DNA markers remain unaffected by environmental factors.
- Biosafety: The presence of disease resistance trait can be analysed using DNA markers linked with the target gene without inoculating pathogen in the field.
- Easier selection of the desired trait: Polygenic traits can be identified using quantitative trait locus (QTL) which is otherwise difficult in conventional breeding programs (Jena and Mackill 2008).

MAS breeding is not a replacement for traditional breeding but can aid in improving its efficiency. There is no transfer of genes which makes it different from genetic engineering. Instead, it helps to screen existing plant materials for targeted selection and breeding (Wijerathna 2015).

5.1 DNA-Based Molecular Markers

DNA-based markers are small DNA sequences that can aid in the identification of individuals based on existing polymorphism. They can arise as a result of point mutations, insertion, deletion and due to errors during DNA replication. These are non-coding regions and significant because of their abundance. DNA markers are unlimited in terms of number and are unaffected by environmental factors, thus making them different from morphological and biochemical markers (Winter and Kahl 1995).

Desirable properties that make DNA markers ideal include polymorphism, codominance, frequent distribution throughout the genome, easy detectability, reproducibility and availability. Detection of polymorphism can be achieved through nucleic acid hybridization technique such as Southern blotting or polymerase chain reaction technique (Collard et al. 2005). These techniques followed by electrophoresis can be used to detect polymorphism based on mobility or band size of the product.

Extensively used techniques based on molecular markers include restriction fragment length polymorphism (RFLP), random amplified Polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), microsatellites, inter simple sequence repeats (ISSR), cleaved amplified polymorphic sequences (CAPS), expressed sequence tag (EST), single nucleotide polymorphism (SNP), diversity arrays technology (DArT) and sequence characterized amplified regions (SCAR) (Wijerathna 2015). These techniques can be broadly divided into two, based on the type of primers used for amplification namely arbitrary techniques based on unknown DNA sequences (RAPD, AFLP, ISSR) and site targeted techniques based on prior sequence information (SSR, EST, CAPS, STS, SCAR, SNP). Markers based on PCR are more preferred for MAS because of the requirement of only small volume of template (Kumar et al. 2009).

5.2 Marker-Assisted Selection

Marker-assisted selection involves the indirect selection of a desirable trait using a marker. The desirable trait could be disease resistance, stress tolerance, better grain quality or higher yield. Plants with regions in genome that are responsible for the expression of the desirable trait are selected (Das et al. 2017). DNA markers linked to trait loci are identified. The markers can be located within the desirable gene or can be linked to the gene of interest, later being the most common (Wijerathna 2015). Hence marker-assisted selection is a method that is based on the traits' genotype rather than its phenotype.

5.3 QTL Identification for MAS

DNA markers and QTLs are identified through QTL mapping. The accuracy of QTL mapping is affected by factors such as environmental aspects, errors in genotype, size of the population and replication of phenotype (Das et al. 2017). QTLs are detected using genetic linkage analysis and statistical methods. Some of the commonly used statistical approaches include interval mapping and single-marker analysis. Apart from preliminary mapping methods, QTL validation and fine mapping is widely being used. The markers tightly linked with the gene of interest are identified through mapping and used for MAS (Akhtar et al. 2010).

5.4 Molecular Markers as Tools for Rice Breeding

Development of molecular tools has added abundant prospects to the development of novel rice varieties. In plants, SNPs or single nucleotide polymorphisms widely distributed over the genome. This abundance makes SNPs a reliable tool for marker-assisted breeding. Presently, in major cereal crops, microsatellites or SSRs are the most widely used molecular markers because they are easily available and procedure involved is comparatively easier (Gao et al. 2016).

5.5 Applications of Marker-Assisted Selection

Marker-assisted selection can be used to develop rice varieties resistant to biotic and abiotic stresses.

5.5.1 MAS for Biotic Stresses

Biotic stresses include rice diseases such as bacterial blight, blast and sheath blight, which are known to significantly affect crop productivity. Molecular tools can aid in genetic improvement of rice and yield disease-resistant varieties. This can help in the reduction of application of pesticide and thus contribute to a sustainable environment. Research over the last few years has lead to the identification of genes and mechanisms contributing to defence responses in rice. MAS has been used for conferring disease resistance to rice cultivars.

5.5.1.1 Blast Resistance

Rice blast, caused by pathogen *Magnaporthe grisea*, is one of the major widespread rice diseases (Jena and Mackill 2008). The symptoms include lesion on leaves, stems, seeds, peduncles, panicles and roots (Zhang and Xie 2014). This disease can lead to huge losses for the farmers. Identification and exploitation of the resistance gene is an effective way of disease control. So far 40 significant blast resistance genes have been identified. Out of these 30 have been mapped and DNA markers have also been developed. In the recent past, a novel gene, *Pi40* responsible for conferring blast resistance has been derived from wild rice species namely *O. australiensis* and linked DNA markers have been identified (Jeung et al. 2007). This gene has the potential to provide durable broad-spectrum blast resistance in rice. On the other hand, several genes capable of conferring blast resistance can be combined using marker-assisted selection approaches to develop rice varieties with broad-spectrum blast resistance (Jena and Mackill 2008).

5.5.1.2 Bacterial Blight Resistance

Bacterial leaf blight was mainly caused by *Xanthomonas oryzae* pv. *oryzae*. The extent of yield losses due to the disease depends upon the rice variety and the environment. Till date, 35 genes responsible for bacterial blight resistance have been identified, out of which nine were reported from wild species (Zhang and Xie 2014). Some of these genes have been cloned and used for developing bacterial blight resistant cultivars. Based on DNA markers, gene pyramiding approaches have been used to develop broad-spectrum resistant varieties (Jena and Mackill 2008). Indian rice cultivar has been pyramided with resistance genes namely *xa5*, *xa13* and *Xa21* using MAS that expressed high resistance to Indian BB races (Singh et al. 2001).

5.5.1.3 Virus Resistance

One of the most destructive viruses known to infect rice is the rice tungro bacilliform virus. There are two types of virus, spherical and bacilliform. DNA markers that are linked with the resistance towards spherical virus have been mapped (Sebastian et al. 1996). Recently, a resistance gene, *rymv*, that can confer resistance to another virus namely rice yellow mottle virus was located. Rice stripe disease is another viral disease common in rice. *Stvb-i*, a resistance gene, has been fine mapped that could be used in MAS for virus resistance breeding (Jena and Mackill 2008).

5.5.1.4 Insect Resistance

Insects are one of the major impediments to rice cultivation. In certain cases, insects cause damage by feeding on plant tissues whereas in some other cases, insects act as vectors of rice viruses. All growth stages of rice are equally vulnerable. Brown planthopper is a destructive insect pest that attacks rice. It also acts as a vector for several viruses. With the aid of advancements in molecular approaches, researchers have identified 19 resistance genes and tightly linked DNA markers for 10 of these (Jena and Mackill 2008). Three of these genes namely *Bph1*, *Bph2* and *Bph18* have been used for marker-assisted selection of brown planthopper resistance genes (Jena et al. 2006). Another destructive rice pest is the green leafhopper. Six corresponding resistance genes have been identified and mapped. These genes were linked with SSR markers (Jena and Mackill 2008).

5.5.2 MAS for Abiotic Stresses

Abiotic stresses include high salinity, high and low temperatures, drought and flood. Hossain (1996) reported that abiotic stresses have more impact on rice production than biotic stresses. Hence, there is an immediate need for rice varieties that are resistant to abiotic stresses.

5.5.2.1 Drought Resistance

At present, scarcity of water is one of the most alarming environmental issues. The climate changes and unavailability of water resources have seriously affected global rice production. In Asia, over 23 million hectares of rice fields are drought-prone (Pandey et al. 2005). Therefore, the development of rice cultivars resistant to drought has become one of the major priorities of rice breeding programs. Genes involved in drought tolerance are those involved in osmotic balance, signal transduction and transcriptional regulation. Fu et al. (2010) proved that the Dongxiang wild rice has high drought resistance. In spite of identification of several QTLs associated with drought tolerance, MAS have been difficult because the associated markers exhibit small effect QTL or are not tightly linked (Steele et al. 2006).

5.5.2.2 Cold Tolerance

Ability to tolerate low temperature is a primary rice breeding objective since cold stress results in reduced growth, poor germination and withering. Genetic study of cold tolerance at the stage of seedling has led to the identification of several associated QTLs. A major QTL for cold resistance was identified in a japonica cultivar named Silewah and using markers, the cold tolerance gene *Ctb1* has been introduced into japonica cultivars. Thus major effect QTLs associated with cold tolerance can be used in MAS (Zhang and Xie 2014).

5.5.2.3 Salinity Tolerance

Soil salinity beyond a certain limit affects rice productivity worldwide. Rice being highly salt-sensitive, exposure to saline soils leads to reduced seed germination, stunted growth and low survival rates of seedlings. *O. coaretata*, found in the Indian coastal areas, is a wild rice species and can tolerate high levels of salinity (Bal and Dutt 1986). Many associated QTLs have been identified and mapped.

5.5.3 MAS for Grain Quality

Since the last few years, there is a high demand for higher quality rice. The amylose content is an important factor that determines grain quality. The gene majorly responsible for amylose content is the *waxy* (*wx*) gene. An SSR marker closely associated with grain quality is linked to *wx* gene and contains a number of CT repeats. Another important trait determining the quality of grain is gelatinizing temperature, controlled by the gene starch II synthase and associated SNPs have been identified (Jena and Mackill 2008). Using MAS, four traits (amylose content, gelatinization temperature, gel consistency and translucency) have been improved in indica hybrid rice (Zhou et al. 2003).

6 Challenges in Gene Transfer from Wild to Cultivated Rice

The genus *Oryza* consists of twenty-two wild varieties and two cultivated varieties (Sanchez et al. 2014). Wild variety of rice has economically important genes that could be transferred to cultivated varieties of rice which provides resistance and tolerance to abiotic and biotic stresses and also provides resistance to pests like yellow stem borer, green leafhopper and brown planthopper (Jena 2010). Wild varieties of rice consist of elite genes than cultivated rice because of natural selection and survival competition which is very long (Fu et al. 2008). Initially beneficial genes were identified from the wild variety *O. rufipogon* and later genes which enhance yield were identified from species like *O. minuta*, *O. glaberrina*, and *O. longistaminata* (Gaikwad et al. 2014). *O. minuta* consists of genes that provide resistance against brown planthopper, bacterial blight and white-backed planthopper (Asaf et al. 2016). Even though wild varieties have useful genes, they possess some negative aspects like weak plant type, grain quality and yield of seeds (Sanchez et al. 2014). Genus *Oryza* consists of nine genome varieties, the chromosomes of the wild variety are closely related and cultivated species which are similar, denoted as AA genomes. The chromosomes of some wild species which differ from those of cultivated varieties are denoted as FF, GG, CC, BB and EE. Certain other species have genomes CCDD, HHJJ and BBCC (Zeliang and Pattanayak 2013). Gene transfer results in the change of genetic makeup of the target population (Jin et al. 2017).

Tissue culture and molecular techniques along with traditional plant breeding are used to transfer useful genes from wild species to cultivated species. The advancement in the fields of genomics, molecular biology, tissue culture and fluorescence in situ hybridization has provided new opportunities to exploit the beneficial genes from wild varieties. The techniques used to transfer genes from wild varieties to cultivated species are dependent on nature of the target species, incompatibility barriers and how the cultivated variety is related to the wild variety and several methods are there to overcome these barriers (Sanchez et al. 2014). Wild gene pools have been classified as primary or sativa complex, secondary or officinalis complex and tertiary or other complex based on the ease of transfer of genes. It is difficult to produce distant hybrids by the crossing of wild varieties and cultivated varieties belonging to the secondary and tertiary complex because of the premature death of the embryo (Sah et al. 2007). By using traditional breeding techniques, the gene of our interest from AA genome could be easily transferred to cultivated rice (Jena 2010). Non-AA genome present in wild species is considered to be important germplasm for the breeding of rice because they have genes which provide resistance to abiotic stresses like extreme cold, drought and acidic soil and biotic stress (Fu et al. 2008). The first step in gene transfer is to search for the beneficial gene which includes the screening of wild varieties that possess the gene of interest. The second step involves the production of interspecific hybrid varieties by crossing rice with the wild species having the desired gene. The alien introgression lines produced in the second step is evaluated through field test and laboratory work to transfer the target traits. Finally, molecular markers are used for tracking the required alien traits which are required for the marker-assisted selection (Sanchez et al. 2014). Certain incompatibility barriers affect the transfer of beneficial genes to cultivated species from wild varieties. Major challenge is to transfer the useful genes without linkage drag, that is the association of the unwanted gene with the beneficial gene (Pratap and Kumar 2013; Sanchez et al. 2014).

Limited recombination of the chromosomes of the cultivated and wild variety, and crossability inhibition are other two challenges in the transfer of genes from wild variety to cultivated variety. The crossability inhibition among the parents before pre-fertilization occurs when pollen grains fail to germinate or pollen tube fail to reach the ovary or fusion between male and female gametes do not occur (Pratap and Kumar 2013). Chromosome pairing between wild and cultivated species is another important aspect of gene transfer but it has been observed that in the metaphase I of F1 hybrids of wild and cultivated species, chromosome pairing is very low which makes it challenging for transferring genes (Pratap and Kumar 2013).

7 Conclusion

All species of wild rice provided as an important gene pool. During breeding programmes, it can be applied to expand the genetic background of domesticated rice. *O. rufipogon* and *O. sativa* have been used to develop resistance against stem rot (Tseng and Oster 1994), rice tungro virus (Angeles et al. 1998), blast (Reimers et al. 1993), bacterial leaf blight (Sun et al. 1992; Kaushal et al. 1998) and submergence tolerance (Mandal and Gupta 1997). Several weed types of wild rice grown occasionally are used as food by collecting seeds (Morishima et al. 1991). Wild rice is also useful in the emergency like the drought that can be used as the famine food (Baksha et al. 1979). Wild rice is an annual grass which is grown naturally. Wild rice species have very special characteristics like drought tolerance, disease resistance, comparatively high in protein and complex carbohydrates; wild rice comes out ahead in the minerals potassium and phosphorus as well as the B vitamins niacin, riboflavin and thiamine. These characteristics of wild rice can be used to hybrid with cultivated rice and improve the qualities of cultivated rice by using different techniques of hybridization.

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Improvement of Rice Quality: The New Revolution

Debabrata Panda, Swati S. Mishra, and Prafulla K. Behera

Abstract

Rice is the staple crop for more than half of the world population. Along with yield, the grain quality of rice is also the principal requirement for producers and consumers. The quality of the grain depends on a combination of several traits including environmental factors. Both conventional and transgenic approaches are used for the quality improvement of rice. Genetic engineering has provided new tools for effectively ensuring food and nutritional security to improve agriculture across the world. With the recent advances in analytical tools, molecular markers, applied genomics, proteomics, and metabolomics, the scope for improving grain and nutritional quality in rice, and combining that with high yield, seems more promising than before. The modification of product quality characteristics using gene technology depends on a well-established understanding of the pathways for biosynthesis of plant products. There is an urgent need to boost more research in this field to help people in understanding the relationship between diet and health, and to ensure that everyone benefits from the genomic revolution. This chapter provides insights into how to link grain quality attributes and sensory perception to support breeding superior rice varieties.

Keywords

Genome editing · Metabolomics · Molecular breeding · Proteomics · Rice quality

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1 Introduction

Over the last 300 years, plant science research has provided vital information and innovations for propelling the sustainability of agriculture. Rice (*Oryza sativa* L.) is a major cereal crop and the predominant staple food feeding more than half of the world's population approximately over 25% of the calorific needs (Kusano et al. 2015; Birla et al. 2017). Like yield, rice grain quality has now become an essential consideration for producers and consumers. As Asian rice production grew faster than the population from 1965 to 1980, the real price of rice declined in world markets and several Asian countries (Mishra et al. 2018). Greater supply and falling prices have increased concern for rice grain quality improvement in national and international research programs in tropical Asia (Mishra et al. 2018). Improvement of rice quality has now become a foremost consideration for rice buyers and breeding programs.

World rice production has witnessed a significant increase during the last half-century due to increase in harvest index by the use of semi-dwarf varieties, that requires high inputs of fertilizers, pesticides which indirectly cause environmental problems, outbreak of diseases and pest resistant insects and also affects human health as a primary concern (Birla et al. 2017). However, since mid-1980, no significant increase in rice yield is observed. The rapidly increasing world's population, ongoing severity of global climate change effects, water scarcity due to decreasing water table, reduction of cultivable land of rice, and an increase in frequency and severity of extreme weather conditions (Stocker et al. 2013) are the major concern, have potentially affected plant growth and rice yield, and also the grains' physical and chemical characteristics (Chen et al. 2012; Zhao and Fitzgerald 2013; Goufo et al. 2014; Halford et al. 2014; Birla et al. 2017). Adding to the problem, malnutrition has been a major concern for developing countries; it has been estimated that micronutrient malnutrition affected about more than three billion people and 3.1 million people die each year for malnutrition, and day by day more increasing of number of deaths (FAO 2009; Johnson et al. 2011; Gearing 2015).

The conventional breeding approach in grain quality and rice yield improvement has met with limited success because it requires sufficient genetic variation for a given trait in a species, time-consuming, and sometimes shows linkage drag due to which adverse genes along with desirable ones are introduced in a generation. Therefore, it is indeed the necessity to find novel methods such as molecular markers, genomics, and transgenic approaches to complement rice breeding approach that could be helpful in higher yield potential and grain quality improvement (Birla et al. 2017). Therefore, new varieties with high yield potential, good quality, and high resistance to biotic and abiotic stresses are needed to meet the demand of rapid human population growth and concurrent decrease in arable and fertile land (Bao 2014).

Recent days the emerging innovative genetic improvement techniques such as molecular breeding, i.e., marker-assisted selection and identification of QTLs for grain quality traits (Brar et al. 2012; Bao 2014; Rao et al. 2014), availability of high-quality genomic sequence of the rice (Goff et al. 2002; Yu et al. 2005), and functional genomics have accelerated identification, isolation, characterization, and cloning of novel genes controlling rice yield and grain quality (Duan and Sun 2005; Jiang et al. 2012). Advancement in genetic engineering approach, genome editing, etc., has accelerated new strategies for quality improvement in rice to overcome the food and nutritional security. This chapter highlights the yield and grain quality improvement strategies through the use of new emerging genetic improvement tools which could help breeders to in-deep understand in the characterization of new high yielding and superior quality rice varieties that are more resilient to stress/climate change and contain a higher nutritive value that provides health benefits to the society (Khush 2005; Birla et al. 2017).

2 Quality Attributes in Rice

Quality is defined as "the totality of features and characteristics of a product or service that bears its ability to satisfy stated or implied needs" (International Standard Organization (ISO) 8402 1986) (Bao 2014). Features are identified properties of a product which can be related to the quality characteristics (Bao 2014). The quality traits vary largely among rice cultivars and are also influenced by environmental factors such as the soil status, fertilizer applications, and climate variations (Mukamuhirwa et al. 2018). The concept of grain quality includes grain shape, size and appearance, milling efficiency, cooking easiness, eating palatability, nutrition, etc., and also covers other physical and biochemical properties. Many countries have set up methods to evaluate some quality parameters viz. international organizations such as ISO, Association of Analytical Communities International (AOAC), American Association of Cereal Chemists International (AACCI), etc. The various quality attributes have been summarized in Fig. 1.

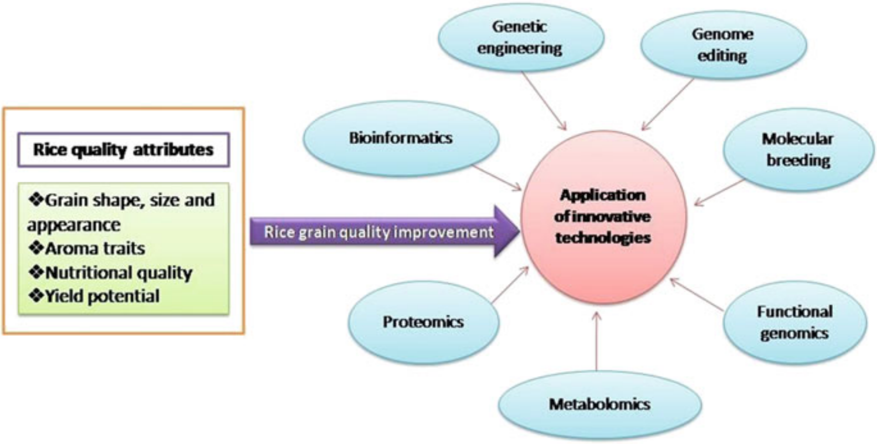

Fig. 1 Quality attributes in rice and different approaches for rice grain quality improvement

2.1 Grain Shape, Size, and Appearance

Appearance is one of the most important properties of rice grain affecting its local and international market acceptability. After milling, the grain appearance is associated with size, shape (long vs. round), chalkiness, and translucency. Mostly rice industries determine the clarity, the virtuousness, and the translucency of the endosperm as the parameters of grain appearance (Bao 2014). Grain yield in rice is determined by three major components: number of panicles per plant, number of grains per panicle, and grain weight. Among these, grain weight is the most reliable trait, which is measured as the 1000-grain weight (Huang et al. 2012). Grain length, width, and thickness are used to describe the physical dimensions of rice kernels, while the grain shape is expressed as the ratio of length to width. Grain shape is characterized by a combination of grain length, grain width, grain length-to-width ratio, and grain thickness. These four parameters are positively correlated with grain weight (Huang et al. 2012). Besides yield, grain shape is also an important quality trait that has a major impact on the market values of rice grain products. In the countries like Southern China, the USA, and South and Southeast Asian countries, people prefer a long, slender rice grain, whereas Japan, Korea, and Northern China consumers prefer short and round grain (Asante 2017).

2.2 Aroma Trait

Aromatic or fragrant rice is an important commodity worldwide and it contributes special features such as pleasing aroma, kernel elongation after cooking, and taste that has been in great demand in the international market (Ghosh and Roychoudhury 2018). Aroma is considered as an important grain quality trait in rice. 2-Acetyl-1-pyrroline (2AP) is the volatile compound responsible for pop-corn-like smell, i.e., aroma of the Indian Basmati and Thai Jasmine rice (Buttery et al. 1983). Fragrance (*fgr*) is a recessive trait that is controlled by a major gene present on chromosome 8 which encodes for betainealdehyde dehydrogenase 2 (badh2) (Lorieux et al. 1996; Jin et al. 2003). Transgene complementation and RNAi-induced suppression have confirmed that a mutation in OsBADH2 is responsible for aromatic phenotype (Chen et al. 2008, 2012). The synthesis of 2-acetyl-1-pyrroline (2AP) is inhibited by the dominant badh2 allele by exhausting 4-aminobutyraldehyde, a presumed precursor of 2AP (Chen et al. 2008). Recently, transcription activator-like effector nuclease (TALEN) has been used as the most acceptable genome editing technique. In another way, homozygous mutant aromatic rice is created from non-aromatic via targeted knockout of the OsBADH2 gene by transcription activator-like effect or nuclease (TALEN) technique with a significant amount of 2-acetyl-1-pyrroline (Shan et al. 2015). Functional markers derived from *fgr* are sufficient to carry out molecular marker-assisted breeding to improve the sensory quality of rice (Shi et al. 2008; Jin et al. 2010; Bao 2014).

2.3 Nutritional Quality

Human health mainly depends on the nutritional quality of the food. Worldwide rice is used as an important staple food. The milled rice constitutes protein as the second most abundant compound after starch. The nutritive value of rice is determined by protein and lysine content as two important constituents (Bao 2014). As per human requirement in diet, lysine is the first limiting essential amino acid in rice. With increasing population further social development, people eating rice require distinct nutritional quality. In the case of the underdeveloped region, genetic study and biofortification of micronutrients by breeding approach are important for nutritional quality improvement in rice as micronutrient deficiency (vitamins and minerals) is apparent in the underdeveloped regions. Whole grain rice (brown rice) provides more minerals, vitamins, dietary fibers, and phenolics to human health than milled rice (Bao 2012). The consumption of brown rice becomes popular to combat chronic disease, e.g., diabetes, for those with improved living standards (Bao 2014). Much effort is needed to facilitate high nutritive rice to each section of the society.

3 Genetic Engineering Approach for Quality Improvement

Gene transfer between unrelated species of plants has been a common practice of crop improvement for many decades, through which many useful traits have been transferred from non-cultivated species. The genetic information such as resistance to insects, stress, and disease has been transferred by the use of recombinant DNA methods (Rashid et al. 2017). Genetic engineering is an exceptional recombinant DNA technique, new innovative breeding because, more effective, efficient, and less time-consuming as compared to conventional breeding (Ali et al. 2011; Ahmad et al. 2015; Rashid et al. 2017). Many successful genetically engineered crops have been commercialized, e.g., insect-resistant cotton and maize, herbicide-resistant soybean and canola, virus-resistant potato, etc. (Dunwell 2000; Akhtar et al. 2014; Amin et al. 2014; Dar et al. 2014; Tariq et al. 2014; Khan et al. 2015; Puspito et al. 2015). Numerous evidences have shown the improvement of nutritional value in rice by the use of various genetic engineering approaches such as through biolistic and *Agrobacterium*-mediated transformation method (Zimmermann and Hurrell 2007; Dias and Ortiz 2012; Roychoudhury et al. 2012). It was first observed that genetic transfer of the *ferritin* gene of soybean resulted in a three-fold enhancement of Fe in the starchy endosperm of rice (Goto et al. 1999). Similarly, in 2003, Vasconcelos et al. introduced *ferritin* gene from common bean into rice and observed a three-fold increase of Fe in milled rice and two-fold in rough rice (Rashid et al. 2017). Different methods of genetic engineering techniques were used to transfer different genes/traits.

3.1 Direct Gene Transfer

In 1940s, methods were developed for direct DNA transfer from one organism into another, based on the chemical nature of inheritance of DNA. Direct DNA transfer methods are the source of diversity and allow controlling of gene expression that provides tissue specificity, timing and level of gene expression and can be modified by gene modification into the new host (Qamar et al. 2015; Rashid et al. 2017).

3.2 *Agrobacterium*-Mediated Gene Transfer

Since 1907, *Agrobacterim tumefaciens*, a soil pathogen, has been extensively studied when it was identified as the causative agent of crown gall disease (Sah et al. 2014). *Agrobacterium tumefaciens* is a plant-pathogenic bacterium that holds the ability to transfer genetic material through the transformation process. This bacterium is modified in the lab and it transfers gene of interest into plants through T-DNA of Ti plasmid without causing symptoms of the disease. By using this method, genes for insect and disease resistance have been transferred. Recombinant DNA technique enabled many genetic engineered plant crops tolerant to broad spectrum and environmentally safer herbicide (Rashid et al. 2017). The use of this unique technique for quality attributes enhancement in rice is the need of the hour.

3.3 Biolistic Transformation

Biolistic transformation is one of the methods of direct gene transfer that enables the delivery of microprojectile. The biolistic transformation was initially invented in 1987 by American Geneticist, John C. Sanford from Cornell University and his colleagues who showed the delivery of small microprojectiles into a cell without killing it (Sah et al. 2014). The microprojectiles are of tungsten or gold coated with DNA which is delivered by acceleration and push into the target cells. The electric charge, CO_2, gun powder, and gases provide acceleration and DNA can be introduced into the tissue. It is otherwise called a gene gun or particle bombardment technique. Rapid gene transfer, efficient, non-specific to tissue, complex cloning strategies with no biological constraints or host limitations and simultaneous multiple gene transfer are the many advantages of a biolistic transfer technique (Sah et al. 2014). Further, the delivery of long fragment DNA is challenging and complex pattern of transgene integration, etc., are the major limitation of biolistic transformation methods (Hansen and Wright 1999; Rashid et al. 2017). Major research on the transfer of quality traits in rice by biolistic transformation is required.

3.4 Genome Editing in Rice Improvement

Now-a-day's rice genetic manipulation is an important more suitable, feasible, and acceptable practice by researchers and plant breeders because of the availability of huge genomic resources together with the small genome size of rice used for genetic manipulation (Mishra et al. 2018). Therefore, rice has been increasingly used to test the efficiency of different types of genome editing technologies viz. CRISPR/Cpf1 system and base editors (Li et al. 2012; Feng et al. 2013), to study the functions of various genes and demonstrate their potential in rice improvement and have superseded the limitations of traditional breeding methods starting a new era of crop improvement (Xu et al. 2014, 2016; Wang et al. 2016; Mishra et al. 2018). The applications of genome editing in plants were reported in various plant species such as *A. thaliana* and *N. benthamiana* (Feng et al. 2013; Li et al. 2013; Nekrasov et al. 2013), as well as crops such as rice (Jiang et al. 2013; Miao et al. 2013; Shan et al. 2013; Wang et al. 2016), wheat (Shan et al. 2013; Wang et al. 2014), maize (Char et al. 2016), etc. Genome editing involves the usage of engineered site-specific nucleases (SSNs) such as zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) to modify specific genes at desired locations in the genome (Miglani 2017; Mishra et al. 2018). In the last several years, CRISPR/Cas9 is the most advanced genome editing tool, has been used to produce new rice varieties with improved traits, including disease resistance, stress tolerance, nutritional enhancement and yield increment (Belhaj et al. 2015; Weeks et al. 2016; Mishra et al. 2018).

3.5 Genome-Wide Sequencing

The DNA sequence of the entire genome provides organism's total genome description as well as genetic makeup and can act as a bridge between the structural and the functional phases of genomics (Tyagi et al. 2004). Rice was the first crop plant to be fully sequenced (International Rice Genome Sequencing Project 2005; Yu et al. 2005). The availability of whole-genome sequence provides an understanding of the genetic basis of complex traits by genome-wide association studies, e.g., grain quality (Huang et al. 2010, 2012; Zhao et al. 2011; Bao 2019). The sequencing of the rice genome alone, however, is not the ultimate goal of genomic research, further needs to understand genome functioning in the organism. Technological advancement made it possible to understand the principle of cellular functions at different levels by "omics" data derived from large-scale experiments, including genomics, proteomics, and metabolomics as well as Bioinformatics (Tyagi et al. 2004; Bao 2019). These "omics" data are an important prerequisite for biotechnological applications, particularly for the rice quality improvement or engineering/transgenic rice production (Tyagi et al. 2004).

3.6 Proteomics Application in Rice Seed Development

Proteomics has provided an efficient large-scale solution complementing traditional and genomic approaches. Over the past 20 years, proteomics has become a major tool for surveying protein profiles which involves global comparative studies, allowing for large-scale identification and expression analysis of proteins active in specific seed compartments or regions at certain developmental stages in rice seed, providing useful information at the proteomic level (Roychoudhury et al. 2011; Deng et al. 2013). The flavor and quality of rice is dependent on various important factors such as the amount, composition, and distribution of seed storage proteins contained in the rice. In a study, Kim et al. (2009) compared and analyzed the seed protein quality through seed proteomes study between a relatively high-quality and a relatively low-quality cultivars and found some specified proteins that may affect rice flavor and quality (Kim et al. 2009; Bao 2019). The mature rice seed contains about 5 to 10% of total protein content, most of which are seed storage proteins (SSPs), viz. glutelins, albumins, globulins, and prolamines (Deng et al. 2013). Glutelins are the predominant SSPs, constitute about more than 80% of the total seed proteins (Deng et al. 2013; Houston et al. 1968). The seed storage proteins (SSPs) are precursor proteins first synthesized, processed, and finally deposited in protein bodies in rice seeds. Therefore, SSPs are highly abundant in both developing and mature seeds (Deng et al. 2013). The appearance and milling quality rice is adversely affected by Grain chalkiness—a complex trait and hence it has been one principal target for rice improvement. In a study, Lin et al. (2014) identified 113 proteins among them 70 proteins are upregulated and 43 proteins downregulated, responsible for chalkiness formation (Deng et al. 2013; Lin et al. 2014). Chalky grains of rice are usually increased by high-temperature stress during grain filling, and proteomic analysis revealed deregulations in the expression of multiple proteins implicated in metabolic and physiological functions (Kaneko et al. 2016).

3.7 Metabolomics

Rice produces many metabolites, and in addition to carbohydrates serve as nutrients for human beings. Metabolomics or rice metabolic studies show versatility has contributed to metabolite biomarkers identification in rice to various stress conditions (Degenkolbe et al. 2013; Maruyama et al. 2014; Bao 2019) and also nutrition starvation (Masumoto et al. 2010; Okazaki et al. 2013). The metabolomics study of developing rice seed and mature grain contributes a better understanding of the genetic and biochemical mechanism underlying seed development and grain quality of rice (Bao 2019). Metabolomics technology can be used to identify taste and flavor compounds in cooked rice (Fitgerald et al. 2009; Calingacion et al. 2012, 2014, 2015; Mumm et al. 2016) and grain quality among different varieties (Kim et al. 2013; Mumm et al. 2016; Song et al. 2016). Hu et al. (2016) found 216 metabolites from japonica and indica rice cultivars using a non-targeted metabolomics approach. The metabolic profile study between waxy and non-waxy rice cultivars found lipid metabolites accumulation viz. fatty acids, phospholipids, glycerophosphocholine, etc. (Song et al. 2016). Further, the physiological and molecular foundation of chalkiness formation is still only partially understood, because of the complex interactions between multiple genes and growing environments (Bao 2019). Lin et al. (2017) conducted the untargeted metabolomics analysis of developing grains, bottom chalky part and upper translucent part, from which 214 metabolites were identified including amino acids, carbohydrates, lipids, cofactors, peptides, nucleotides, phytohormones, and secondary metabolites (Lin et al. 2017; Bao 2019). Furthermore, the metabolomics combined with genome-wide association studies allows identification of metabolite (metabolome) quantitative trait loci (mQTLs) through which the genetically controlled rice metabolism can be dissected (Bao 2019).

3.8 Bioinformatics Tools in the Improvement of Rice Crops

Bioinformatics tools are promising for crop improvement these days. Bioinformatics resources and web databases are essential for the most effective use of genetic, proteomics, and metabolomics information important in increasing agricultural crop productivity and for food security (De Filippis 2013). Genome sequence projects of economically important crops have been completed and regarded as the gateway to genetic manipulation studies and with rich annotations help to study gene families more precisely (Rashid et al. 2017). The World Wide Web is the source for the accession of the many bioinformatics resources. MIPS rice database or the MOsDB is the recently developed informatics resource specifically dedicated to rice (http://mips.gsf.de/proj/rice; Karlowski et al. 2003; Tyagi et al. 2004). This provides rice genome sequence as well as annotation information along with a complex characterization of all annotated rice genes. Major events like gene duplication along with other abnormalities are being manipulated by the help of bioinformatics tools. Furthermore, advances in the technology and data acquisition sites ease the task to access crucial data necessary for the improvement in traits of crops (Rashid et al. 2017). Recently developed technologies like microarrays and SAGE have accumulated a vast amount of analyzed genome expression data which is one of the great achievements in the field of bioinformatics (Gerstein and Jansen 2000; Tyagi et al. 2004). Furthermore, other databases, such as the Rice Expression Database (RED; http://red.dna.affrc.go.jp/ RED/; Yazaki et al. 2002) and the Rice Microarray Opening Site (RMOS; http://microarray.rice.dna.affrc.go.jp/; Yazaki et al. 2002), have been created to allow the processing and mining of expression data (Tyagi et al. 2004).

3.9 Role of Functional Genomics Study in Rice Grain Quality Improvement

Due to the advancement in research and number of special characteristics of rice such as small genome size, high transformation efficiency, rich in rice germplasm resources, and genetic stocks availability, rice has now become a model plant species forfunctional genomics studies (Jiang et al. 2012; Mishra et al. 2018). In 1997, the term "functional genomics" described by Hieter and Boguski is the development and application of global or genome-wide experimental approaches to assess gene function through the use of structural genomics information and reagents (Tyagi et al. 2004). Functional genomics studies assess the functions of various genes and also demonstrate its potentiality for rice quality improvement (Xu et al. 2014, 2016; Wang et al. 2016; Mishra et al. 2018). Rice is the first sequenced crop plant with high precision and genome size is about ~370 Mb (Goff et al. 2002; International Rice Genome Sequencing Project 2005; Yu et al. 2005; Jiang et al. 2012). The overall goal of rice functional genomics studies helps to understand genes and regulatory elements functions in regulating plant development at the whole genomic level (Jiang et al. 2012). The appearance quality, cooking quality, eating quality, milling quality, and nutritional quality are the major attributes that contribute to rice grain quality (Jiang et al. 2012). The amylase content, gelatinization temperature, and gel consistency of the grain starch are contributing toward rice cooking and eating quality. *Wx* gene encodes granule-bound starch synthase I required for the synthesis of amylose in the endosperm (Wang et al. 1995; Zeng et al. 2007), whereas *ALK* gene encodes the soluble starch synthase II, indirectly controlling the gelatinization temperature (Gao et al. 2003; Jiang et al. 2012). Tian et al. (2009) studied 18 starch synthesis-related genes coordination controlling rice eating and cooking quality by using candidate gene association and further constructed a regulatory network underlying eating and cooking quality of rice (Jiang et al. 2012). In 2010, a study was conducted by Fu and Xue to identify genes controlling starch biosynthesis by using coexpression strategy. In 2008, Zhang et al. studied on enhancement of nutritive value in rice and they detected 2, 4, 3, and 4 QTLs for protein fractions, albumin, globulin, prolamin, and glutelin, respectively, and further these QTLs might be useful for identification of genes responsible for the nutritional improvement in rice (Bao 2014). In addition, genes controlling rice fragrance, storage time, and nutritional quality were also characterized by various research workers (Furukawa et al. 2007; Kawakatsu and Takaiwa 2010; Jiang et al. 2012; Yu et al. 2008).

4 Molecular Breeding

Over the past decades, rice cultivar innovation has been depending on the conventional breeding approach. Molecular breeding is one of the alternative efficient methods, involvement of molecular biology tools in plant breeding, basically having two strategies marker-assisted selection (MAS) and genetic engineering (genetic transformation) to develop genetically modified crops (Wijerathna 2015). The rice grain quality improvement has been carried out by both genetic engineering and marker-assisted selection. Nutritional quality improvement has been carried through the use of genetic engineering approach, whereas grain cooking and eating quality have been successfully implication of marker-assisted selection (Wijerathna 2015; Bao 2019). The marker-assisted selection approach has been summarized as follows:

4.1 Marker-Assisted Selection: An Efficient Plant Molecular Breeding Technique

Marker-assisted selection (MAS) is a method that uses molecular markers closely linked to a target gene as a molecular tag for indirect selection of the target gene (Bao 2019). The molecular markers are a string or sequence of nucleic acid which makes up a segment of DNA codes for assayable phenotype, for which alleles at individual loci segregate and transmit according to Mendelian laws of inheritance from one generation to next (Wijerathna 2015). There are various types of molecular markers being used for crop species viz. amplified fragment length polymorphisms (AFLPs), restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), sequence tagged sites (STS), simple sequence repeats (SSRs) or microsatellites, and single nucleotide polymorphism (SNPs). Molecular-assisted selection technique is more efficient and does not replace traditional breeding and also does not transfer isolated gene sequences but offers existing plant material as a target for plant breeding (Wijerathna 2015). There are two strategies to conduct MAS in the breeding program such as foreground selection and background selection. Foreground selection is particularly useful for grain quality traits, which is a time-consuming phenotyping screening procedure whereas background selection is for yield potential and high resistance good quality rice improvement such as basmati rice (Bao 2014). Grain quality markers are used as background selection to avoid good quality traits loss during the introduction of the other traits (Bao 2019). As per MAS strategies, there are three main uses of molecular markers in rice breeding: marker-assisted evaluation of breeding material, marker-assisted introgression, and marker-assisted pyramiding (Wijerathna 2015). One good way to improve the quality of maintainer lines is by marker-assisted selection. Furthermore, MAS with the *Wx* gene marker for quality improvement of the conventional rice has been reported (Yi et al. 2009; Jantaboon et al. 2011; Jairin et al. 2009). Generally, most of the consumers prefer fragrant rice to non-fragrant rice. Functional markers for *fgr* gene have been developed and successively used to transfer this gene from fragrance rice to the target non-fragrance rice (Yi et al. 2009; Jin et al. 2010; Salgotra et al. 2012; Jantaboon et al. 2011; Bao 2019). Breeding does work for not only a single trait but also for the formation of the new variety by taking other traits. In case of good quality variety such as basmati and jasmine are premium quality rice accepted by consumers worldwide. Furthermore, marker-assisted selection has been carried out to successfully introduce some other important traits into the basmati or jasmine rice such as bacterial blight resistance (Pandey et al. 2013; Win et al. 2012), blast resistance (Singh et al. 2012), submergence tolerance (Jantaboon et al. 2011), etc. (BAO 2019).

5 Yield and Quality Improvement Via Genome Editing Tools

Yield and quality traits in rice are typical quantitative traits governed by multiple genomic loci (Table 1). The numbers of panicles per plant, numbers of grains per panicle, and grain weight are the three major components that mainly determined as the yield of rice crop (Mishra et al. 2018). Rice yield has been improved by knocking out genes including *GS3*, *DEP1*, *GS5*, *GW2*, *Gn1a*, and *TGW6* that are known to be negative regulators of grain size and number and grain weight (Mishra et al. 2018). By using the CRISPR/Cas9 system, four genes such as *Gn1a*, *DEP1*, *GS3*, and *IPA1* have been independently edited resulting in enhanced grain number, dense erect panicles, and larger grain size (Li et al. 2016). In another study by Xu et al. (2016), using CRISPR/Cas9 genome editing system resulted in 29.3% increase in 1000 grain weight in the triple null mutant by the simultaneous mutation of three grain weight-related genes—grain weight 2 (GW2), GW5, and *thousand grain weight* 6 (TGW6) (Mishra et al. 2018). This suggests that pyramiding null mutants of major yield-related genes in a single cultivar via multiplex gene editing would be crucial in regulating the yield components of rice (Mishra et al. 2018). TALEN technology has been used to develop a modified ligation-independent cloning method (LIC) for efficient mutation of the LOX3 gene using a pair of TALEN monomer (Ma et al. 2015). The seeds of LOX3 knockout lines exhibited improved storability. Recently, CRISPR/CAS9 has been used to edit the fragrant gene *Badh2* in the indica rice line, Zhonghua 11 (Shao et al. 2017; Mishra et al. 2018).

5.1 "Golden Rice": Nutritionally Improved Transgenic Rice

Lack of protein amount, iron, zinc, and vitamins is a major micronutrient deficiency disorder. "Golden Rice" represents a genetic engineering concept, a solution against malnutrition, for the development of nutritionally enabled staple rice for the developing countries. Rice lacks the ability to produce *β-carotene,* the precursor of Vitamin A. Ye et al. (2000) developed golden rice by introgression of genes viz. *phytoene synthase, phytoene desaturase, β-carotene desaturase*, and *lycopene β-cyclase* into rice through *Agrobacterium*-mediated transformation, that yields 1.6–2.0 μg/g of β-carotene of dry rice, further, ultimately led to decrease of night blindness (Khan et al. 2015). Now-a-days Taipei 309, the transformed rice is no longer cultivated but efforts are now underway at IRRI to transfer the genes for *β-carotene* (Khan et al. 2015) (Table 1).

Table 1 List of genes for yield and quality improvement in rice using genome editing techniques

Application perspective	Target gene	Genome editing strategy	Molecular functions	References
Yield and quality improvement	*LOX3*	TALENs	Enhanced storage tolerance	Ma et al. (2015)
	GW2, GW5, and TGW6	CRISPR/Cas 9	Improvement of grain weight	Xu et al. (2016)
	Hd2, Hd4, and *Hd5*	CRISPR/Cas 9	Early maturity of rice varieties	Li et al. (2017)
	CSA	CRISPR/Cas 9	Photoperiod controlled male sterile lines	Li et al. (2016)
	Gn1a, *DEP1*, *GS3*, and *IPA1*	CRISPR/Cas 9	Improvement of grain number, panicle architecture, grain size, and plant architecture	Li et al. (2016)
	CCD7	CRISPR/Cas 9	Increased tiller number	Li et al. (2016)
	PYLs	CRISPR/Cas 9	Improved growth and productivity	Miao et al. (2018)
	OsBADH2	TALENs	Enhanced fragrance	Shan et al. (2015)
	BADH2	CRISPR/Cas 9	Enhanced fragrance	Shao et al. (2017)
Nutritional improvement	*SBEIIb* and *SBEI*	CRISPR/Cas 9	Generation of high amylose rice	Sun et al. (2017)
	OsPDS, *OsSBEIIb*	Base editing	Nutritional improvement	Li et al. (2017)

Source: Mishra et al. (2018)

5.2 Understanding Pathways for Biosynthesis of Plant Products

Many of the semi-dwarf but high-yielding crop varieties that were developed during the Green Revolution are defective in gibberellins biosynthesis or unresponsive to gibberellin (Peng et al. 2009). Auxin and gibberellin enhancement with quality traits is of utmost importance for quality enhancement. Two major pathways for IAA biosynthesis have been proposed in plants: the tryptophan (*Trp*)-independent and *Trp*-dependent pathways (Woodward and Bartel 2005; Chandler 2009; Normanly 2010). In *Trp*-independent IAA biosynthesis, indole-3-glycerol phosphate or indole is the likely precursor, but little is known about the biochemical pathway to IAA (Ouyang et al. 2000; Zhang et al. 2008). Of the other kinds of plant hormones, brassinosteroids (BRs) seem to be among the most useful for controlling crop productivity—BR mutants can also be dwarfs and overexpression lines can be high yielding (Choe et al. 2001; Mori et al. 2002; Sakamoto et al. 2006). Enhancement of the brassinosteroid biosynthesis pathway improves grain yield in rice. Golden Rice technology is based on the simple principle that rice plants possess the whole machinery to synthesize β-carotene, and while this machinery is fully active in leaves, parts of it are turned off in the grain. By adding only two genes, a plant phytoene synthase (*psy*) and a bacterial phytoene desaturase (*crt I*), the pathway is turned back on and β-carotene consequently accumulates in the grain. In all rice genetic backgrounds tested so far, complementation with these activities is not required to proceed down the pathway.

Starch is the major form in which carbohydrates are stored and is present in almost all plant organs at one or another time during development. Knowledge about starch biosynthesis is required to be able to modify starches for industrial or food uses. This knowledge has been acquired by studies in a broad range of plants using both mutants and reverse genetic approaches. In this way, the function and impact of a number of the essential genes in the starch biosynthetic pathway has been assessed. Modification of starch biosynthesis pathways holds an enormous potential for tailoring granules or polymers with new functionalities. Until recently a lot of effort was put into investigating the individual components in the starch biosynthetic pathway. Also reducing the phytic acid content of seeds is a major breeding target, because it would both increase the availability of mineral nutrients and decrease the environmental phosphorus load. The first step in phytic acid biosynthesis and inositol metabolism is the formation of 1D-myo-inositol 3-phosphate (*Ins*(3)P1) from D-glucose-6-phosphate, catalyzed by *Ins*(3)P1 synthase. The isolation of a cDNA corresponding to the rice Ins(3)P1 synthase gene, RINO1, and suggested that RINO1 plays a key role in phytic acid biosynthesis in developing rice seeds (Yoshida et al. 1999). Hence, thorough investigation and knowledge of these pathways will be of immense help in enhancement quality traits in rice.

5.3 Future Prospects in Health and Genomic Revolution

The dual role places rice at the center of an enormous challenge facing agriculture: how to leverage genomics to produce enough food to feed an expanding global population and provide holistic nutrition for the population. Scientists worldwide are investigating the genetic variation among domesticated rice species and their wild relatives with the aim of identifying loci that can be exploited to breed a new generation of sustainable crops known as Green Super Rice. Ensuring that advances in genomics are applied to the health improvement of people living in developing countries is an important contemporary challenge. In the near term, such advances are likely to alleviate infectious diseases, with longer-term benefits envisaged for chronic disorders. To ensure that benefits are shared by developing countries, attention must be paid to complex ethical, legal, social, and economic issues, as well as to public education and engagement. Creative and equitable international mechanisms and goodwill are needed to turn high hopes into reality and allow the use of genomics to reduce health inequities between rich and poor nations. This genomic breeding is now feasible in almost any species and has promise to help meet the need to feed and nourish over nine billion people by 2050. Genomic techniques can accelerate our response to food security challenges of yield, quality and resilience and also address environmental security challenges. To achieve its potential there will need to be widespread and ongoing investments in the human capital to promote genomic breeding.

For better food security, crops of the future will also need to be more resilient to overcome the threats posed by changing and suboptimal growth conditions, such as resource limitation, and unfavorable weather. Past breeding efforts produced so-called "elite" varieties, to be grown under "optimal" conditions. However, farmers must create these desired environments through irrigation and fertilizer application, leaving a large environmental footprint. Not only will we need to reduce this footprint, but also most of the world's food production, does not, and will not, take place under optimal conditions. Most farmers have only limited agronomic options. Genomic breeding could also help improve food security by accelerating plant breeding for ecosystem services. Potentially, varieties from crop and non-crop species could be selected for the ecosystem services they provide: biodiversity-promotion (animals, plants, and microbes), erosion control, water and soil health, climate change mitigation, etc. (Godfray and Garnett 2014; Sayer and Cassman 2013). Targeted breeding of crop and natural vegetation for ecosystem services may improve environmental security, both on farm and on larger scales (Swinton et al. 2007).

6 Conclusion

Exploring the "omics revolution" for better food quality and security is the pragmatic approach for crop research. Advanced plant science and genomics have revolutionized breeding and crop improvement, and will continue to do so. Innovation in collecting genotypes, phenotypes, and intermediate characteristics is allowing new crop varieties to be selected faster and more accurately than ever before. With genomic techniques researchers can help address food security challenges of yield, quality, resilience, and other environmental and social needs. Investing in the human capital to perform genomic breeding is needed to improve food security, environments, and livelihoods.

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Sequencing the Rice Genome: Gateway to Agricultural Development

Anindita Paul

Abstract

For 10,000 years, rice, the most important staple crop in the world, has played a central role in human nutrition and culture. Ensuring a stable supply of this crop to an ever-increasing global population in the face of climate change has become essential. The focus, therefore, has shifted on maximising rice productivity, yield potential and stability. This requires an in-depth understanding of the functional genomics of rice and its breeding pipeline. Spearheaded by the Rice Genome Research Program (Japan), with multinational contribution from ten countries and integration of draft sequences from private organisations, the International Rice Genome Sequencing Project (IRGSP) published a map-based, high-quality genome sequence for *Oryza sativa* ssp. *japonica* variety 'Nipponbare' in 2006. With the release of the first crop genome sequence data, the global rice community identified novel genes underlying agronomic traits, developed new tool sets for marker-assisted breeding and positional cloning and advanced towards sequencing other cereal genomes. Enhanced comparative and functional genomic studies delivered crucial insights into genome diversity and evolution, speciation and domestication. Integration of the whole-genome data into diverse omics data like transcriptome, protein-protein interaction network and metabolome allowed high-throughput analysis and orthologous gene identification. The rice genome sequence simultaneously laid the foundation for the international 3000 rice genomes resequencing effort and for identification of candidate loci that can be exploited to breed Green Super Rice. More than a decade later, this milestone continues to serve as an information platform for leveraging the genomics of agroecosystems.

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Rice genome sequencing · Nipponbare · Systems biology · Map-based cloning · Green Super Rice

1 Introduction

A comprehensive review of global demographic trends and future prospects published in the *World Population Prospects: The* 2017 *Revision*, released by the United Nations Department of Economic and Social Affairs (UN DESA), predicted the current world population of 7.6 billion to grow each year by roughly 83 million people (https://population.un.org/wpp/). Hence the global population is projected to reach 8.6 billion in 2030, 9.8 billion in 2050 and 11.2 billion in 2100. Within this period from 2017 to 2050, half of the global population growth is expected to be concentrated in a small number of countries, and thus such a status in the poorest countries will present a significant challenge to the respective governments, which strive to implement the 2030 Agenda for Sustainable Development. The nine countries listed in the review that are expected to make a significant contribution to the global population are Indonesia, Uganda, the United States of America, the United Republic of Tanzania, Ethiopia, Pakistan, the Democratic Republic of the Congo, Nigeria and India. The unavoidable question that arises in the face of this global crisis is that how to ensure access of safe, nutritious and sufficient food to all the people?

1.1 Rice: The Right Choice

Rice is a very old crop plant, with a long evolutionary and cultivation history. Since time immemorial, it is regarded as an excellent staple crop, as cooking of rice is very simple in contrast to bread making which requires fermentation by yeast. Not only is rice a rich source of carbohydrates but also contains all the essential amino acids except lysine. Rice is mainly harvested and consumed in Asia and Africa, but at a global level, rice is the staple crop for half of the world's population and constitutes nearly 50% of the daily calorie intake. Similar to religion or tradition, rice is deeply ingrained in our lives.

The genus *Oryza* contains 27 species, 2 of which were domesticated independently ~6000 years apart (Wing et al. 2018). The domestication of the Asian rice (*Oryza sativa*) and African rice (*Oryza glaberrima*) occurred on two different continents, at different time points in the evolutionary history of rice. Therefore, rice has an extensive germplasm collection of wild and domesticated species, with some extinct wild species which were progenitors of the present-day rice. Thus the present-day picture of rice evolution is quite complex, as it emerges that divergent ancestral populations gave rise to extant rice populations (Cíváň et al. 2015). One or more de novo domestication events may have led to the different varietal groups that exist today, e.g. *Oryza sativa* subspecies, *japonica* and *indica*.

Since its domestication and adaptation, rice has been cultivated for thousands of years in the Old World and for hundreds of years in the New World (Wing et al. 2018). Throughout this duration, rice has undergone significant improvement, adapting to range of geographical locations, soil textures, climate or environmental conditions and also cooking preferences. These have also led to positive selection of agronomically desirable traits, which are still applied to current-day breeding programmes. Identifying these desirable traits and crossing plants to develop new varieties with high-yielding phenotype or with resistance towards adverse climatic conditions are already known. For achieving higher yield and accelerated growth, hybrid vigour or heterosis has also been exploited, where genetically distinct parents are crossed to produce a hybrid offspring with an improved or enhanced function of a desirable biological quality. An early example of rice improvement by hybridisation, hailing from the Neolithic age, is the origin of *O. sativa* varietal group *indica* (Wing et al. 2018). Introgression of genomic regions from subspecies *japonica* to *indica* led to integration of a number of agronomically desirable genes, for example:

(a) *SH4*: non-shattering allele, enhances sustainability of mature seeds on the panicle.
(b) *RC*: colour allele, imparts the white grain colour which is desirable.
(c) *PROG1*: important for erect growth of the crop, imparts lodging resistance.

Interestingly the *SH4* orthologue in African rice is associated with mutation, which drives the selection for the non-shattering phenotype in this domesticated crop (Wang et al. 2014). Other genes regulating plant height, grain quality, disease and stress resistance, fertility, nutrient uptake and cooking or eating quality were eventually incorporated as a result of geographical spread and adaptation. Genome-wide association studies have revealed genomic regions that have undergone selection related to geographical adaptation and identified genes of agronomic potential (Meyer et al. 2016) (summarised in Table 1).

With the ever-increasing trend of the global population and the current status of food resources, it is clear that rice will play a crucial role to fulfil the global demands. But increasing quality and quantity only by implementation of traditional crop improvement programmes will undermine the crucial contribution that this cereal can deliver in this lingering crisis. Also there are additional factors associated with breeding of sustainable crops that cannot be ignored (summarised in Fig. 1). With population growth, constant urbanisation and increasing misuse of land, there is an evident dearth of cultivable land. Excessive use of resources such as fertilisers, pesticides and water has had adverse effects on the environment. Increasing rice production by developing high-yielding varieties therefore necessitates incorporating other attributes such as reducing dependency on fertilisers and pesticides, increasing efficiency of water and nutrient use, enhancing tolerance to biotic and abiotic stresses (e.g. disease, drought, salinity, temperature, etc.), being able to grow on limiting lands and reducing emissions of greenhouse gases (Zhang

Table 1 List of genes that impart agronomically desirable traits and were positively selected during domestication and diversification of cultivated rice

Genes	Agronomically desirable traits
SH1/SH4 (*non-shattering allele*)	*SH1* locus for seed shattering on chromosome 1; *SH4* locus for grain shattering on chromosome 4; role in retention of mature rice grains on the panicle (Li et al. 2006)
PROG1 (*prostate growth 1*)	Encodes a single Cys2-His2 zinc-finger protein. Naturally occurring mutants in this gene affect plant architecture (erect growth) and grain yield (Tan et al. 2008)
hd1	A homolog of CONSTANS from *Arabidopsis*, encodes a protein with a zinc-finger domain, regulates flowering time and heading time (Zhang et al. 2012)
sd1 (*semidwarf*)	The semidwarfing varieties contain a defective gibberellin 20-oxidase gene, whereby the deficiency in gibberellin biosynthesis affects plant height (Spielmeyer et al. 2002)
Gn1a	Encodes cytokinin oxidase/dehydrogenase, regulates rice grain yield (Wang et al. 2015)
Waxy	Mutation in this gene leads to change in endosperm starch content and imparts the glutinous or sticky texture in rice (Olsen et al. 2006)
Rc	A predominant frameshift deletion in this gene is found in the white rice varieties (Sweeney et al. 2007)
HAK5	Encodes a high-affinity potassium transporter, role in K+ acquisition by roots and upward transport (Yang et al. 2014)
Cyp2 (*cyclophilin2*)	Upregulated during salinity stress and other types of stresses, serving as a general integrator of environmental stresses (Ruan et al. 2011)
AMT1	Encodes an ammonium transporter, regulates ammonium uptake and assimilation in rice (Hoque et al. 2006)
Xa26	Encodes a leucine-rich repeat (LRR) receptor kinase-like protein, which confers resistance against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), at both seedling and adult stages (Sun et al. 2004)
Rf-1 (*fertility restorer gene*)	A nuclear-encoded gene which recovers pollen fertility, thereby cytoplasmic male sterility (CMS), inherited by the mitochondrial genome (Kazama and Toriyama 2003)

2007; Roychoudhury et al. 2013). Other qualities that will contribute to the better acceptability of rice as a staple diet include improving plant architecture (e.g. number of panicles, number of grains per panicle, grain weight, more productive tillers, spikelet number, uniformly mature kernels, free of empty or half-filled grains); manipulating flowering time, spikelet fertility or heading date; and improving the appearance (uniform size and shape, colour, translucency), milling (high milling recovery, high head rice recovery, no discoloration), cooking (amylose content, gel length and gelling temperature) and nutritional (both micro- and macronutrients) quality of the rice grain (Zhang 2007). To achieve this, scientists and researchers, over the years, had to leverage genomics to obtain a thorough understanding of the genetic components that regulate the abovementioned attributes. Hence this was preceded by deciphering the entire rice genome sequence.

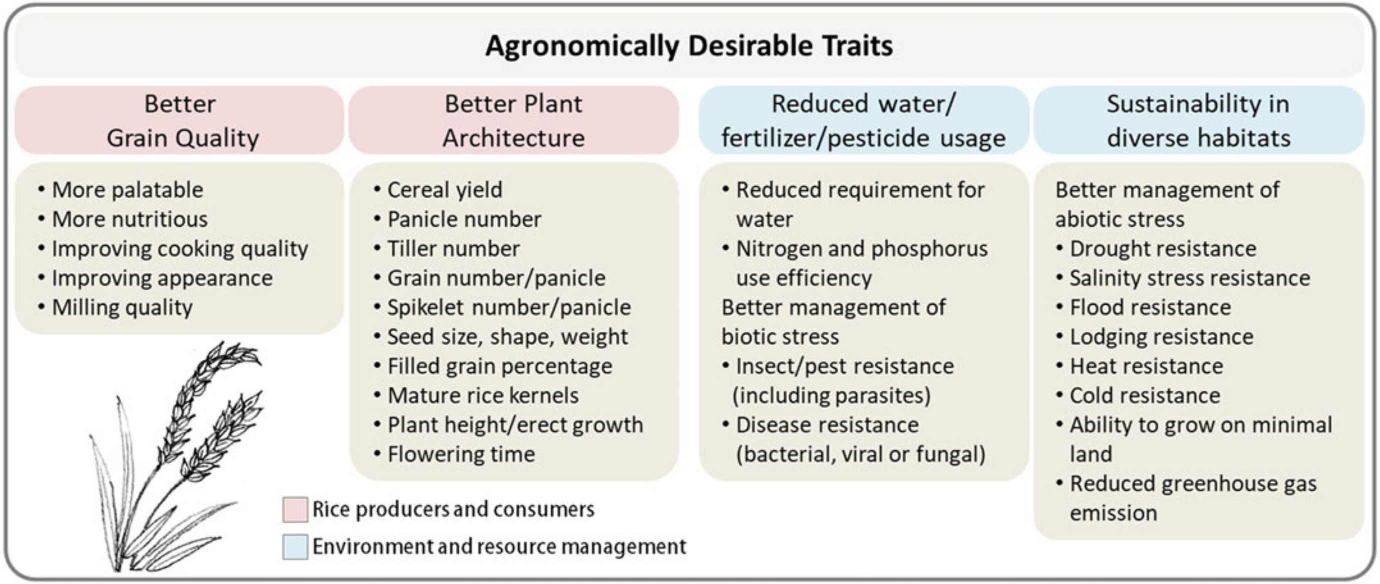

Fig. 1 Expected deliverables from a typical present-day crop improvement programme that aims for breeding of sustainable rice crop

Rice is the first cereal crop, the first grass species and the first monocot genome to be sequenced. Rice was an attractive target for genome sequence analysis because it has a relatively small genome (430 Mb, $2n = 24$) compared to that of other common cereals like sorghum (750 Mb), maize (3000 Mb), barley (5000 Mb) and wheat (16,000 Mb). Cereal genomes are highly conserved and exhibit considerable synteny among the genes (Freeling 2001). Rice, with a small genome size, was predicted to have higher gene density compared to the other candidates. Moreover, rice has an elaborate germplasm of wild and domesticated species. Also, once genetic markers were identified, they had to be tinkered with to yield the desirable phenotypes, and rice was an easy candidate for genetic manipulation. Therefore, rice emerged to be an excellent model for plant genomics, following *Arabidopsis*.

The following sections aim to highlight how the International Rice Genome Sequencing Project (IRGSP) was conceived as well as its contribution and impact on the present-day understanding of rice genomics. Recently, resequencing efforts through high-throughput platforms and mapping this data into high-quality reference sequence genomes is helping to identify novel targets for genomic breeding and making development of Green Super Rice (GSR) varieties scientifically and technically feasible.

2 Mapping the Rice Genome

The profound importance of rice as a food crop and as a model plant by virtue of its syntenic relationships with other cereal crops has significantly stimulated rice genome analysis. Genome analysis in rice comprised of the following goals: (1) mapping of rice genome, (2) sequencing the rice genome and (3) functional association of the mapped genes. Large volumes of structural and functional data of the rice genome were generated in the twentieth century which facilitated mapping of the rice genome. This created a strong foundation for subsequent sequencing of the rice genome, which was possible with major advancements in sequencing strategies. Initial efforts included cytogenetic mapping, linkage mapping, genetic mapping and physical mapping, which have been utilised extensively to build sequence-ready physical map of the rice genome (Tyagi et al. 2004).

1. Cytogenetic maps conventionally were based on microscopic examination of chromosomes. With the advent of novel effective techniques like fluorescence in situ hybridisation (FISH) and genomic in situ hybridisation (GISH), an enhanced-quality rice chromosome map could be generated (Heng et al. 1997). GISH was used extensively to dissect the genomic constitution of rice somatic hybrids and tetraploid species, as this technique could efficiently identify a chromosome complement belonging to a particular genome (Fukui et al. 1997). FISH, on the contrary, allowed mapping of restriction fragment length polymorphism (RFLP) markers, rDNA loci, bacterial artificial chromosome (BAC) and yeast artificial chromosome (YAC) clones on the respective rice chromosomes (Jiang et al. 1995).

2. Khush and Brar proposed 12 linkage groups which corresponded to the haploid number of chromosomes in rice (Khush and Brar 2001). These linkage groups were associated with the rice chromosomes by primary trisomics and reciprocal translocations, while centromere positions and orientation of the linkage groups were determined using secondary trisomics and telotrisomics. All these efforts culminated into a comprehensive molecular linkage map of rice.
3. The advent of molecular genetic markers provided significant impetus to the construction of genetic maps, which were being eventually supplemented with phenotypic markers. Several genetic maps of rice were being constructed from RFLP, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), cleaved amplified polymorphic sequence (CAPS) and microsatellite or simple sequence length polymorphism (SSLP) markers (Mohan et al. 1997). Most of these markers were developed from expressed sequence tag (EST) clones derived from cDNA libraries of rice.
4. The immense progress that was feasible while constructing the physical maps was accomplished mainly due to the availability of libraries of large insert clones in vectors like BAC, YAC and P1-derived artificial chromosomes (PACs) and the information about DNA markers on genetic maps. The YAC clones were originally mapped onto the rice chromosomes by chromosome landing, integrating information from the genetic maps. Such integrated YAC physical map was subsequently used for positional cloning of several genes (e.g. rice blast resistance gene, *Pib*; bacterial blight resistance gene, *Xa-1*; and gibberellin-insensitive dwarf mutant gene, *d1*), for the assignment of chromosomal locations of more than 6000 EST markers to generate a rice transcript map and also has been utilised as a backbone for construction of PAC- and BAC-derived physical maps (Yoshimura et al. 1996; Ashikari et al. 1999; Wang et al. 1999; Wu et al. 2002). Tao et al. (2002) developed one large insert plant-transformation-competent BIBAC library and three BAC libraries for the temperate *japonica* rice cv. Nipponbare, which significantly aided functional analysis of the genome, comparative genomics of grass species and subspecies and molecular breeding in rice and other cereals (Tao et al. 2002).
5. With constructions of these abovementioned maps, the next approach was to integrate all the information obtained from the cytogenetic, genetic and physical maps to constitute a comprehensive physical-genetic map, which will cover a large fraction of the genome and facilitate the study of organisation and functional aspects of the genome. For instance, a standardised rice karyotype was constructed from meiotic pachytene chromosomes of *O. sativa* spp. *japonica* rice cv. Nipponbare, using centromere-specific DNA probes and chromosomal arm-specific BACs, following which it was fully integrated with the most saturated rice genetic linkage maps in which Nipponbare was used as one of the mapping parents (Cheng et al. 2001a). A physical-genetic map of rice chromosome 10 was developed by integrating the pachytene chromosome-based FISH mapping of BAC clones with a genetic linkage map, which revealed the precise genetic position of the centromere on chromosome 10 and reflected on

the genetic recombination frequencies among the chromosomal arms (Cheng et al. 2001b).

2.1 Rice Genome Research Program (RGP), Japan, Established 1991

In 1991, the Japanese government initiated its Rice Genome Research Program (RGP). The aim of this programme was to clarify the genome sequence of rice in entirety (Sasaki 1998). The RGP was implemented in two phases: the first phase of 7 years dealt with the mapping of the rice genome which included large-scale cDNA analysis, genetic mapping by construction of fine-scale RFLP map and YAC-based physical mapping; the second phase was initiated in 1998, when RGP stepped into the new era of genome sequencing and matured into the International Rice Genome Sequencing Project (IRGSP). This section highlights the first phase when the main motive was to construct a genome-wide physical map. This phase was coupled with advances in the field of computation in terms of next-generation web technologies and bioinformatics analysis platforms, which became extremely important for scientists and bench biologists to visualise, annotate and analyse the high-throughput experimental data.

2.1.1 Linking Genomics and Genetics

RGP adopted cDNA analysis, a quick and easy strategy to clone several genes expressed in rice. This involved random cloning, partial sequencing and developing cDNA libraries from various tissues at different developmental stages, for example, green and etiolated seedlings, young roots, panicles at the flowering stage and calluses cultured with 2,4-dichlorophenoxyacetic acid (Yamamoto and Sasaki 1997). By 1997, 36,000 cDNA clones from 15 main cDNA libraries were sequenced for 400–500 5′-terminal bases (Sasaki 1998). Additionally, more than 40,000 cDNA clones were partially sequenced to generate ESTs. Therefore, sequencing the cDNA from these samples helped: (1) to designate ESTs for genomic regions of expressed genes, (2) to understand the expression profile of genes from various tissues at separate growing stages and (3) to functionally annotate the genes depending on similarity search in public databases. Generating ESTs was of profound importance as it was exploited for linkage analysis by utilising RFLP markers and also for determining hinge markers for YAC contigs. So to link the information in the DNA sequence to phenotypic traits, the basic tool required for rice genome analysis is a genome-wide physical-genetic map.

At the time, a high-density genetic linkage map based on polymorphisms within DNA sequences, such as RFLPs, CAPSs and simple sequence repeats (SSRs), was derived from F2 plants from a single cross between the *japonica* variety Nipponbare and the *indica* variety Kasalath (Harushima et al. 1998). It was composed of 2275 genetic markers with a cumulative genetic distance of 1550 cM for 12 linkage groups. Nearly 70% of the DNA markers were rice ESTs (clones from Nipponbare callus, root and shoot libraries), while the remaining were clones from genomic

DNA and cDNA of rice and other cereal crops. The position of the centromere, assigned using the secondary trisomics and telotrisomics developed at the International Rice Research Institute (Philippines), revealed the chromosomal orientation of individual linkage groups and suggested that the meiotic recombination frequency is very low at each centromere but reasonably high in the flanking regions (Singh et al. 1996). Additionally the clarity on the knowledge of DNA markers on the genetic map was used for accurate genotyping of candidate progenies obtained by backcrossing for a particular trait (Yano et al. 1997).

A physical map assembled using YACs was also available. The YAC library was derived from Nipponbare variety and comprised of ~7000 clones with an average insert size of 350 kb (Umehara et al. 1995). Thus the library size was 5.5 times of the rice genome (430 Mb), indicating overlapping YAC clones. Also chimerism (non-contiguous DNA fragments present within the same YAC clone) and difficulty of separating YACs from other yeast chromosomes posed challenges in using YACs as templates for DNA sequencing. This redundancy was removed by the following strategies: (1) colony hybridisation of YACs using RFLP markers, (2) using sequence-tagged site markers for identifying positive YACs and (3) mapping many ESTs onto YAC physical map, by assembling them using primers designed from the 3′ untranslated region (UTR), specific for each gene (Antonio et al. 1996, Saji et al. 1996, Shimokawa et al. 1996, Umehara et al. 1996, 1997, Wang et al. 1996, Koike et al. 1997, Tanoue et al. 1997). BAC/PAC vectors were also used extensively to construct new rice genomic libraries (Budiman et al. 1999; Baba et al. 2000). Clones were generated using several combinations of restriction enzymes (e.g. Sau3AI for PACs and HindIII and EcoRI for BACs), partially sequenced from 5′ and 3′ termini and subsequently fingerprinted to develop a BAC physical map. Similarly as mentioned before, PAC contigs were identified by EST and confirmed by fingerprinting. Sequencing of the selected PAC contigs resulted in a significant coverage of the rice genome and indicated a higher gene density than expected.

The combination of ESTs obtained from extensive cDNA analysis, DNA markers identified on RFLP linkage maps and the information derived from the physical maps generated from YAC clones and BAC/PAC libraries indicated to a large extent the exact location of the expressed genomic region. A properly annotated genome-wide physical-genetic map was the need of the hour, such that each locus, supplemented with positional information on the chromosomes, could be associated with a phenotypic trait, thereby facilitating the cloning of biologically and agronomically important genes. RGP was a prerequisite for generating a reliable sequence-ready physical map. These efforts not only prepared the foundation for rice genome sequencing, but triggered and inspired the entire sequencing project that was to be undertaken in the subsequent years worldwide (Sasaki 1998; Sasaki and Burr 2000).

2.1.2 Genomics Meets Informatics

The sequences that were generated by the concerted efforts were subsequently annotated by searching with various software and prediction tools against databases. The following approaches were adopted to generate a final annotation of genes and associated elements, along with assigning their proper coordinates in the genome sequence: (a) BLASTX to search the nonredundant protein database, (b) BLASTN to search the rice EST database, (c) GENSCAN to predict open reading frames (ORFs) within the sequence, (d) SplicePredictor to identify potential exon-intron splice sites, (e) Miropeats to predict inverted or tandem repeats and assemble the shotgun sequence and (f) using *gag* and *pol* genes as references to recognise transposable elements, followed by identification of long terminal repeats (LTRs) (Parsons 1995; Kleffe et al. 1996; Altschul et al. 1997; Burge and Karlin 1997). Similarly other tools such as Gene Finder, GeneMark and NetPlantGene were also employed (Hebsgaard et al. 1996; Solovyev and Salamov 1997; Lukashin and Borodovsky 1998). Even after integration and correlation of all this genetic information with the genome sequence, powerful information retrieval system was necessary to easily access and manipulate the data and link this information to other genetic resources.

A new rice genome database, called INtegrated rice genome Explorer (INE, pronounced as ‘i-ne’, also referring to rice plant in Japanese language), was developed to accelerate the release of all this genetic information to the public (Sakata et al. 2000). This web interface based on a Java applet allowed rapid viewing capability of the integrated maps, accompanied by smooth navigation options. INE incorporated and integrated large data volumes from various experimental outputs to enhance the overall density of the markers within the genome. The high-density linkage map with 2275 DNA markers also included the image of each probe used for RFLP and the sequence of the corresponding markers (Harushima et al. 1998). The physical map generated using the YAC clones covered a significant portion of the genome and also showed the actual physical distance between genetic markers. Incorporation of ESTs in the INE allowed review of syntenic relationships and also enhanced the significance of high-density markers. Also a physical map constructed from sequenced PAC clones was incorporated to enhance the reliability of the data. Additional quality control was also applied to ensure good-quality data. INE provided a page for accessing ‘Low Quality Information’, which harboured sequences that failed to meet the standards or any thresholds set up by RGP. INE not only increased its extent of data incorporation but also allowed integrated view of the data for efficient data mining. Each chromosome was associated with an integrated map which showed the linkage map, the physical map constructed from ordered YAC clones, the EST map and the PAC contigs. Therefore the value of genetic information was greatly enhanced with this integrated display. The following examples corroborate the usefulness of the genetic map: by integrating the PAC contig map or an EST map into the linkage map, (1) the existence of a PAC or EST adjacent to the genetic marker of interest was verified, (2) the exact positions of genetic markers and other genetic elements were evaluated, and (3) functional annotation of a PAC near a genetic marker revealed an EST within a PAC contig responsible for a particular trait. These would facilitate subsequent map-based cloning of agronomically desirable genes. INE was later modified to meet the demands of the genome sequencing project (Sasaki and Burr 2000). Upon further elucidation of signal transduction pathways in rice, more valuable information on the physiological and biochemical aspects of rice genes was incorporated into INE. For exploration of syntenic relationships of rice with other cereal crops, INE was subsequently linked to other genomic resources of important cereal crop species.

3 Sequencing the Rice Genome

The genome-wide physical-genetic maps could leverage genomics if the nucleotide sequence of the rice genome was available. In fact, the DNA sequence can bridge the gap between the structural organisation of the genome and its functional dimensions. Towards the end of the twentieth century, with the progress in sequencing methodologies, adoption of automation and advances in computation, several genome sequencing projects of higher organisms were undertaken, including *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, *Drosophila melanogaster*, *Arabidopsis thaliana* and *Homo sapiens* (Mewes et al. 1997; The C. elegans Sequencing Consortium 1998; Adams et al. 2000; The Arabidopsis Genome Initiative 2000; International Human Genome Sequencing Consortium 2001; Venter et al. 2001). In that new era of sequence-based genomic research, the obvious choice for a genome sequencing project on a cereal crop was rice. By then the rice genome was well mapped because of the decade-long extensive research on rice genomics, thereby providing a solid foundation for the upcoming sequencing project. Among the major cereal crop genomes, rice has the smallest genome of 430 Mb. Moreover, rice serves as an excellent model as it shares a large extent of synteny with other grass genomes, like rye, wheat, maize, barley and sorghum, and can be easily transformed and genetically manipulated (Tyagi and Mohanty 2000). With the establishment of the International Rice Genome Sequencing Project, in the second phase of RGP, rice became the first crop and the first monocot genome to be sequenced. The following section discusses the inception of IRGSP, inclusion of the participating nations, adoption of optimal methodologies for sequencing, choice of rice cultivar, nucleotide sequence accuracy, finishing standards and sequence release policies.

3.1 International Rice Genome Sequencing Project (IRGSP), Established 1997

The International Rice Genome Sequencing Project (IRGSP) was initiated at a workshop in September 1997, at the International Symposium on Plant Molecular Biology held at Singapore. Scientists and representatives from several nations attended the workshop and agreed to collaborate internationally to sequence the rice genome (Eckardt 2000). To avoid any instance of allelic polymorphism, a single variety of rice cultivar was used as the common source of DNA: *Oryza sativa* ssp. *japonica* cultivar Nipponbare. Participants from Japan, Korea, China, the United Kingdom and the United States decided to share and contribute materials and agreed to release physical maps and DNA sequences to the public databases in a timely manner. The IRGSP eventually evolved to include 11 nations (listed in Table 2) and also revised their policies and finishing standards to ensure a high-quality sequence-ready genome map of rice. Like other large-scale genome sequencing projects accomplished at the time, the modus operandi for this project was similar (Sasaki and Burr 2000; International Rice Genome Sequencing Project and Sasaki 2005). Large insert genomic libraries constructed in BACs and PACs were used as the primary sequence templates. Using polymerase chain reaction (PCR) screening, fingerprinting, end-sequencing and physical mapping, minimal tiling paths were constructed such that a contiguous set of minimally overlapping BAC/PAC clones can be anchored to physical positions along the length of the chromosome, thereby generating a sequence-ready BAC/PAC contig. These clones belonging to a contig were subjected to a map-based hierarchical clone-by-clone shotgun approach, to produce shotgun libraries, which were then sequenced and assembled using in silico tools to reconstitute the entire intact insert within the corresponding BAC/PAC clone (see Fig. 2). The second focus of this project was annotating the raw nucleotide sequence data and associating useful biological information with it, for example, positioning of genes, prediction of splice sites, transcriptional start sites and regulatory sites, repeat elements, prediction of tRNAs, analyses of the predicted proteins to dissect any functional domains or motifs, etc. Therefore this mammoth task, inclusive of so many dimensions, finally culminated into a high-quality genome sequence of rice. The following sections discuss the methodologies adopted by IRGSP and some significant outcomes of the rice genome analysis.

3.1.1 Physical Map and Sequencing

The hierarchical clone-by-clone methodology adopted by IRGSP used a high-density genetic linkage map generated using a single F2 population (2275 markers), ESTs, YAC- and BAC-based physical maps and 2 draft sequences from 2 separate private sources, Monsanto (now Pharmacia, New Jersey) and Syngenta (San Diego) (Yamamoto and Sasaki 1997; Harushima et al. 1998; Mao et al. 2000; Barry 2001; Saji et al. 2001; Chen et al. 2002; Goff et al. 2002; Wu et al. 2002). In total, nine genomic libraries from *Oryza sativa* ssp. *japonica* cultivar Nipponbare were utilised to establish the physical map (International Rice Genome Sequencing Project and Sasaki 2005). Marker-aided PCR screening, fingerprinting with restriction enzymes and end-sequencing of approximately 3400 BAC/PAC clones were used to construct the minimal tiling paths. Majority of the physical gaps in the BAC/PAC tiling path were filled with the help of PCR fragments, 10-kb plasmids and 40-kb fosmid clones. A typical workflow with BAC clones included the following steps: (1) fingerprinting of HindIII and EcoRI BAC libraries, (2) assembly of the fingerprinted BACs into contiguous contigs, (3) anchorage of these BACs onto the physical map with RFLP and end-sequencing analysis and, finally, (4) connection and extension of contigs by chromosome walking. Another technique that was adopted for extending contigs was the use of ‘overgo probes’ (Eckardt 2000). 24-bp sequences were designed from BAC end-sequences with an 8-bp overlap. The 24-bp sequences were then joined to create a 40-bp ‘overgo’, which was then used to probe a high-density BAC library in order to search for additional BAC clones that may potentially extend a contig. Using similar workflows, plant ESTs were also mapped onto the physical map of rice chromosomes. The PAC, BAC and fosmid clones on the

Table 2 Chromosome assignments for sequencing for IRGSP participants

Rice chromosomes	Participating nations	Initiatives and/or institutions (acronym)
1,6,7,8	Japan	Rice Genome Research Program (RGP)
3,10,11	United States of America	• Arizona Genomics Institute (AGI) and Arizona Genomics Computational Laboratory (AGCol) • Cold Spring Harbor Laboratory (CSHL) • Washington University School of Medicine Genome Sequencing Center • University of Wisconsin–Madison • The Institute for Genomic Research (TIGR) • Clemson University Genomics Institute (CUGI) • Plant Genome Initiative at Rutgers (PGIR), Waksman Institute, Rutgers University
1	Korea	Korea Rice Genome Research Program (KRGRP) • National Institute of agricultural science and technology
2	United Kingdom	• John Innes Centre (JIC)
4	China	National Center for Gene Research (NCGR) • Shanghai Institutes for Biological Sciences Chinese Academy of Sciences (CAS)
5	Taiwan	Academia Sinica Plant Genome Center (ASPGC) • National Cheng Kung University • National Yang-Ming University
9	Thailand	National Center for Genetic Engineering and Biotechnology (BIOTEC) • Rice Gene Discovery Unit, Kasetsart University

(continued)

Table 2 (continued)

Rice chromosomes	Participating nations	Initiatives and/or institutions (acronym)
9	Canada	- McGill University - York University
11	India	Indian Initiative for Rice Genome Sequencing (IIRGS) - University of Delhi South Campus (UDSC) - Indian Agricultural Research Institute (IARI)
12	Brazil	Brazilian Rice Genome Initiative (BRIGI) - Centro de Genomica e Fitomelhoramento, UFPel
12	France	Genoscope - Centre National de Séquencage, INRA-URGV and CNRS

physical map were next subjected to a shotgun sequencing approach, originally employed by RGP. In this procedure, the extracted DNA from individual PAC/BAC clones (ranging between 100 and 200 kb) belonging to a sequence-ready contig was subjected to random shearing by sonication or nebulisation, following which the fragments were subcloned to produce shotgun libraries with an average insert size of 1 to 3 kb (see Fig. 2). Random clones from the shotgun libraries were then sequenced, using both universal primers and the dye-terminator or dye-primer methods, to acquire the desired degree of 'coverage' of the total sequence. The shotgun sequencing approach finally led to an assembled, ordered and finished quality rice genome, with approximately tenfold sequence coverage and less than 1 error per 10,000 bases. As mentioned above, small physical gaps were bridged by long-range PCRs. FISH and optical mapping were the other two techniques that were adopted for the remaining gaps. The profound application of FISH to rice mapping was already well established (Jiang et al. 1995; Cheng et al. 2001b). FISH

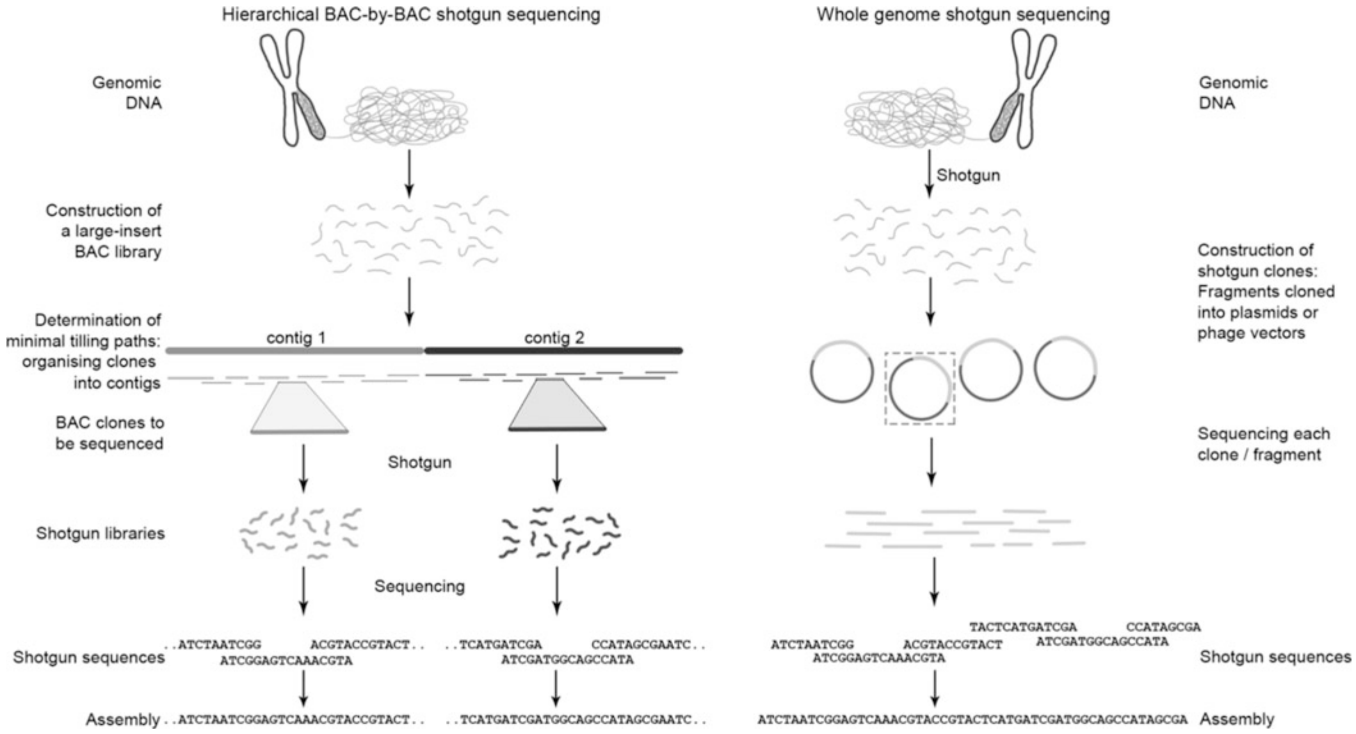

Fig. 2 Schematic workflow of two distinct shotgun sequencing approaches used in rice genome sequencing: hierarchical clone-by-clone approach was adopted by IRGSP and Monsanto (*left*), while whole-genome shotgun approach was employed by Syngenta (*right*)

was established as a useful technique for easy identification of rice chromosomes, determination of physical positions of uncertain clones and examination of the physical nature of large linkage gaps. The last feature facilitated sequencing at chromosomal ends, centromere and telomere regions and turned out to be effective for characterising BAC clones that contain complex repetitive DNA sequences, very common in rice (Moore et al. 1995). The optical mapping technique was previously used to create whole-genome restriction maps of the microorganisms *Deinococcus radiodurans* and *Plasmodium falciparum* (Lin et al. 1999; Lai et al. 1999). In this technique, fluid flow capillary action was employed to extend and align DNA molecules onto a specially prepared glass surface. DNA was then digested with restriction enzymes, and fluorescence microscopy imaging was finally used to map the fragments into an ordered array. Typically, charting a minimal tiling path (i.e. a subset of clones with minimally overlapping sequences) which encompasses a chromosome or a region of interest along a chromosome is dependent on the physical map and the assembly of sequences from a selected subset of clones in an unambiguous fashion with help from their overlapping regions. Unfortunately, the centromeric regions of majority multicellular eukaryotic organisms emerge to be resistant to this method, largely because they contain highly repetitive satellite DNA sequences and transposable elements (Eckardt 2004). The recalcitrance of these regions impeded the efforts to understand their functionality. Findings from previous cytological work indicated that rice centromeres contain multiple repeats of a 155-bp satellite DNA sequence called CentO and many rice chromosomes limited amounts of the satellite DNA repeat sequences compared to other species (Dong et al. 1998; Cheng et al. 2002). The centromere of rice chromosome 8 (Cen8) had the least amount of satellite DNA sequence (∼64 kb) among the 12 rice chromosomes and was an obvious choice for obtaining the sequence for this region. Two groups put significant efforts towards this goal: Wu et al. (2004) employed repeated subcloning, transposon-based sequencing and fingerprinting of BAC/PAC clones, while Nagaki et al. (2004) used similar methods, mapped the Cen8 region using CentO repeat sequences and centromere-specific retrotransposon sequence probes and constructed a minimal tiling path of 12 BAC clones encompassing this region (Nagaki et al. 2004; Wu et al. 2004). Breakthrough findings reported from two groups indicated a large fraction of the Cen8 sequence to contain known repetitive elements, like inverted repeats, transposons, *gypsy*-type retrotransposons, CentO satellite repeats, etc. Therefore to fill in the large physical gaps arising from the centromeric regions in the sequences, they were estimated on the basis of the CentO satellite DNA content of the respective chromosomes (International Rice Genome Sequencing Project and Sasaki 2005). The length of CentO arrays therefore provided approximate measures of centromere gaps, telomere gaps and chromosomal arms.

3.1.2 Integration of Draft Sequences from Monsanto and Syngenta with IRGSP

In early 2000, Monsanto announced that the company was set to release a draft sequence of the rice genome. The sequences would be made available to academic scientists under an access agreement with Monsanto. A separate agreement between the company and IRGSP facilitated incorporation of the Monsanto BAC clones and sequences into the IRGSP sequences. The Monsanto rice genome sequencing project also undertook the BAC-to-BAC sequencing approach on ~3400 BAC clones of the cultivar Nipponbare (Barry 2001). The draft sequence represented 393 Mb of the rice genome, with ~5X coverage. This development significantly accelerated public efforts to sequence the genome. Drafts of the rice genome sequence from ssp. *japonica* cultivar Nipponbare were completed and published separately by Syngenta (San Diego) in 2002 (Goff et al. 2002). This draft, generated using whole-genome shotgun sequencing approach, represented 93% of the rice genome (~390 Mbp), with ~6X coverage, and a 44% GC content. The Syngenta draft sequence, also referred to as Syd, provided useful insights into the rice genome and proteome. Gene predictions on the assembled sequences revealed 32,000 to 50,000 genes contained by the genome, with homologs in maize, wheat, barley and other cereal crops. Extensive synteny and gene homology was also indicated between rice and other cereal genomes. Another draft sequence of the *O. sativa* subsp. *indica* cultivar 93–11 was released by the Beijing Genomics Institute (BGI), which was also generated through a whole-genome shotgun sequencing approach (Yu et al. 2002). This represented ~360 Mb of the genome and emerged to be an important resource for gene discovery, comparative genomics, syntenic associations and SNP discovery. BGI sequence analysis suggested that the rice genome encodes for 46,000–55,000 proteins, which was consistent with the estimate made from the Syd data. The BGI data were made publicly available through GenBank/DDBJ/EMBL, unlike Monsanto or Syngenta. Contigs from BGI and Syngenta whole-genome assemblies were aligned against the IRGSP Nipponbare pseudomolecules using MUMmer (Delcher et al. 1999). The number of IRGSP full-length cDNA-supported gene models that aligned with the contigs was tabulated. To estimate the location of centromeres, the 155-bp CentO consensus sequence was used as BLAST query against 93–11 and Nipponbare whole-genome shotgun contigs, and their coordinates were recorded (International Rice Genome Sequencing Project and Sasaki 2005). The CentO sequence positions on each IRGSP pseudomolecule for a particular chromosome served as the benchmark to estimate the location of centromeres for each *indica* chromosome.

3.1.3 Assembly and Annotation

Fingerprinted BACs were assembled into contigs using the software called FingerPrinted Contigs (FPC) (Soderlund et al. 2000). The shotgun sequences were assembled into intact inserts within the BAC/PAC clone using PHRED, PHRAP (http://www.phrap.org/phredphrapconsed.html) and CONSED software packages or using the TIGR Assembler (Sutton et al. 1995; Ewing et al. 1998; Ewing and Green 1998; Gordon et al. 1998). The primary goal of IRGSP was to obtain a complete and accurate sequence of the rice genome. Consistent with the Bermuda sequencing standards (the international human genome sequencing community organised meetings in Bermuda in 1996 and 1997 to set finishing standards for DNA sequences), ‘complete’ was initially defined as less than 1 error in 10,000 bases (Eckardt 2000; International Rice Genome Sequencing Project and Sasaki 2005). An empirically determined PHRAP score of 30 or above was found to be consistent with the level of accuracy. Sequence ambiguities, therefore, were indicated by PHRAP scores less than 30 and were usually regions containing repetitive DNA sequences. These problem regions were resolved by changing sequencing chemistries or using alternate polymerases. Assemblies of BAC and PAC clones were checked for accuracy by comparing the experimentally determined fingerprint patterns with computationally derived patterns of restriction enzyme digests. Sequence quality was also tested by comparisons of overlapping sequences obtained independently.

Another important step in the rice genome sequencing project was the construction of pseudomolecules, which were generated by using an in-house, database-based, semiautomated process (http://rice.plantbiology.msu.edu/). These are virtual contigs that are constructed by selecting an optimal tiling path of BAC/PAC clones representing all the 12 chromosomes and by resolving discrepancies between overlapping BAC/PAC clones. Overlap regions were trimmed in accordance with information from similarity searches and tiling path information, while physical gaps were represented by a stretch of 1000 nucleotides. The overlapping clones were also validated by comparing the pseudomolecule sequence with the optical map for rice. The unique sequences were then linked to form a contiguous sequence for each rice chromosome. Location of centromeres was ascertained on the basis of gaps and presence of the CentO repeats. Information on gene models and features associated with the BAC/PAC clones were transferred to the pseudomolecules, which underwent quality control using the whole-genome shotgun sequences of Nipponbare. All BAC/PAC clones were not incorporated into the pseudomolecules due to different reasons: some belonged to subspecies *indica*, some clones were unfinished or redundant or overlapping, some were released later than the release of the version of pseudomolecules, while some were difficult to map to their correct positions. Sequences from BACs and the Syngenta whole-genome shotgun assembly of Nipponbare that could not be aligned on the existing pseudomolecules were represented on separate pseudomolecules, the Unanchored and the Syngenta chromosomes. Release of this assembly of pseudomolecules, termed Os-Nipponbare-Reference-IRGSP-1.0, was made in collaboration with the Agrogenomics Research Center at the National Institute of Agrobiological Sciences, Tsukuba, Japan. The annotation files for the pseudomolecules exclude partial genes, pseudogenes and small gene models (<50 amino acids).

Many bioinformatics resources were used to leverage sequence annotation of the rice genome. Several gene prediction software like GENSCAN, FGENESH, RiceHMM, GlimmerM and MZEF were employed to predict gene models (Burge and Karlin 1997; Zang 1997; Sakata et al. 1999; Salamov and Solovyev 2000; Pertea and Salzberg 2002). Monocot trained versions of the tools were used for accuracy. Gene models that corresponded to organellar DNA, those with incomplete open reading frames and those encoding proteins less than 50 amino acids were omitted. The SplicePredictor programme was employed to correctly ascertain the exon-intron boundaries (Kleffe et al. 1996). Another automated annotation system, RiceGAAS (the Rice Genome Automated Annotation System), was also used extensively to predict genes and long terminal repeat sequences on the basis of homology search

(Sakata et al. 2002). The predicted proteome was analysed, using various tools from RiceGAAS, for the presence of any functional domains (ProfileScan and MOTIF), intracellular localisation (PSORT) and solubility (SOSui). Conserved domains and motif searches and deciphering the gene ontology (GO) associations were completed using InterproScan in combination with Interpro2Go programme (Zdobnov and Apweiler 2001; Camon et al. 2005). The predicted proteome of rice was also searched using BLASTP against the proteome of several model species, including *Arabidopsis*, to identify homologs that could reflect on the potential functions of the genes. MUMmer and RepeatMasker software were used to delineate repetitive sequences in the rice genome (Delcher et al. 1999; Smit et al. 1996–2000). The Simple Sequence Repeat Identification Tool was used to identify SSR motifs and estimate the copy number of SSR markers (Temnykh et al. 2001). The TIGR Oryza Repeat Database, together with other sequence data on rice transposable elements, was used to create a rice transposable element database (RTEdb) (Juretic et al. 2004). The coordinates of these transposable elements were determined on the rice pseudomolecules. Organellar DNA sequences were also used to mask pseudomolecules, using BLASTN and MUMmer. Prediction of noncoding RNAs was also addressed. Prediction of transfer RNA genes was executed by the programme tRNA-scanSE (Lowe and Eddy 1997). miRNAs, spliceosomal and snoRNA sequences were retrieved from the Rfam database (http://rfam.xfam.org/) and used as BLASTN queries. In a different approach, experimentally validated miRNAs from other species, excluding *Arabidopsis*, were used for BLASTN queries against the pseudomolecules. To identify single nucleotide polymorphisms in the Nipponbare cultivar, end-sequencing of BAC clones from an *O. sativa* ssp. *indica* var. Kasalath BAC library was carried out, and the sequences were subjected to BLASTN analysis against the pseudomolecules (International Rice Genome Sequencing Project and Sasaki 2005).

3.1.4 Salient Features of the Rice Genome Reported by IRGSP

In 2005, the IRGSP presented the map-based, finished quality sequence of the rice genome that at the time included virtually all the euchromatin and two complete centromeres (International Rice Genome Sequencing Project and Sasaki 2005). The estimated genome size of the rice nuclear genome was 388.8 Mb (~389 Mb). The draft sequence was used to construct pseudomolecules representing the 12 rice chromosomes (haploid number), which were expected to cover 95.3% of the entire genome and 98.9% of the euchromatin. In a separate representation, 8391 of 8440 unique EST markers (99.4%) could be identified in these pseudomolecules. The centromere allocations were done for two chromosomes, 4 and 8. As discussed previously, all rice centromeres contain the repetitive CentO satellite DNA sequences, along with flanking centromere-specific retrotransposons (Dong et al. 1998; Cheng et al. 2002). Complete sequencing of the centromeric regions for chromosomes 4 and 8 indicated these chromosomes to contain ~59 kb and ~ 69 kb of clustered CentO repeats, respectively, arranged in tandem head-to-tail arrays within the clusters (Nagaki et al. 2004; Wu et al. 2004; Zhang et al. 2004). These CentO clusters varied in length and orientation between the two chromosomes and were frequently associated with centromere-specific transposable elements, like the retrotransposon RIRE7.

To figure out the gene content, distribution and density in the genome, the pseudomolecules were masked for repetitive sequences, and the gene prediction tools were employed to identify potential non-transposable element-related genes. At the time, a total of 37,544 non-transposable element-related protein-coding sequences were predicted, of which 22,840 genes could be correlated with ESTs or full-length cDNA models (International Rice Genome Sequencing Project and Sasaki 2005). A lower gene density of one gene per 9.9 kb was predicted in rice. Homolog search in cereals and dicots like *Arabidopsis* indicated a total of 2859 genes unique to rice and other cereals, which provided useful cues for differentiating between monocots and dicots. While majority of the genes encode for proteins with unknown function or hypothetical proteins, families of seed storage proteins called prolamins, hormone response proteins and defence proteins, such as proteinase inhibitors, chitinases, pathogenesis-related proteins and seed allergens, are absent in dicots. But with improvements in gene annotation methods, the number of true protein-coding genes in rice has been revised in the present day. Functional classification of 2296 rice genes according to agronomically desirable traits indicated the following percentage of genes in rice to have distinct functional roles (Wing et al. 2018):

Stress response 12.2% (367), disease resistance 7.4% (221), insect resistance 1.0% (31), growth and development 17.1% (513), phytohormone 15.8% (472), flower organ and heading date 9.2% (276), fertility 5.8% (174), yield 6.3% (189), grain quality 2.1% (63), domestication 1.0% (28), nutrient-use efficiency 6.9% (207) and others 15.2% (455).

Functional analysis of genes and relating them to phenotypes is the basic essence of rice research, and gene disruption with transposable elements is still an ongoing trend. *Tos17* is an endogenous *copia*-like retrotransposon in rice, which prefers to insert into gene-rich regions, making it a suitable tool for functional analysis of the rice genes by gene knockout (Hirochika et al. 1996). A total of 11,487 *Tos17* retrotransposon insertion sites were identified within the rice genome at the time, of which 3243 were in genes, suggesting the density of *Tos17* insertions to be higher in euchromatic regions, compared to retrotransposons, which were frequently found in the paracentromeric regions (International Rice Genome Sequencing Project and Sasaki 2005). Another interesting outcome of the annotation was a large percentage of genes were arranged in tandem repeats, for example, the genes that encoded for protein kinase domains. Predictions of various types of noncoding RNAs distributed across the rice pseudomolecules indicated a total of 763 transfer RNA genes, 158 miRNAs, 215 small nucleolar RNAs (snoRNA) and 93 spliceosomal RNA genes. rDNA loci could also be ascertained on a number of chromosomes, including the nucleolar organiser at the telomeric end of the short arm of chromosome 9.

To accommodate the original endosymbionts inside the cells as organelles, a continuous transfer of organellar DNA to the nucleus has took place in the course of evolution, diminishing the sizes of the organellar genomes to their present-day sizes. Based on the parameters used during homology searches, IRGSP detected 421–453 chloroplast insertions and 909–1191 mitochondrial insertions in the rice genome, and on the basis of their distribution across the 12 chromosomes, it was suggested that mitochondrial and chloroplast transfers occurred independently (International Rice Genome Sequencing Project and Sasaki 2005). The rice genome was also found to be populated with transposable elements, belonging to Class I, Class II or neither of the two families. The transposon content of rice was predicted to be at least 35%, with elements belonging to all known transposon superfamilies. The Class I elements (e.g. long terminal repeats (LTRs) and non-LTR retrotransposons) were less in number compared to Class II elements (e.g. *h*AT, CACTA, IS*256/Mutator*, IS*5/Tourist* and IS*630/*Tc1*/mariner* superfamilies), and majority of Class I elements were found to be concentrated in heterochromatic regions such as the centromeric and pericentromeric regions. Class 1 SSRs which behave as hypervariable loci were also found in the rice genome. These repeats of >20 nucleotides in length have immense application in genetics and breeding and have been shown to be polymorphic in diverse cultivars of rice (McCouch et al. 2002). A total of 18,828 Class 1 SSRs (di-, tri- and tetra-nucleotide) were identified and annotated on the rice genome that represented 47 distinct motif families. Finally, intraspecific sequence polymorphisms were detected between the two cultivated rice subspecies, *japonica* and *indica*. Comparison of orthologous sequences between *O. sativa* ssp. *indica* cv. Kasalath and *O. sativa* ssp. *japonica* cv. Nipponbare identified a total contingent of 80,127 polymorphic sites, resulting in a high-resolution genetic map for rice. Insertions and deletions were also detected (International Rice Genome Sequencing Project and Sasaki 2005; Yu et al. 2002).

Genome-wide comparisons of the two published whole-genome shotgun assemblies of draft-quality rice sequences with the IRGSP pseudomolecules were also done. These assemblies predicted genome sizes of 433 Mb for *O. sativa* ssp. *japonica* cv. Nipponbare (6X coverage) and 466 Mb for *O. sativa* ssp. *indica* cv. 93–11 (6.28X coverage), which differed from the 389 Mb genome size reported by IRGSP. A detailed comparison of the sequences indicated that a substantial percentage of the contigs in both assemblies were misaligned or nonhomologous or provided duplicate coverage, suggesting the draft sequences to be inadequate for gene annotation or functional genomics.

3.2 Updates on the Current Rice Genome Data

The Michigan State University Rice Genome Annotation Project Database and Resource (MSU RAP-DB) is a project funded by the National Science Foundation (NSF). This provides sequence and annotation data for the rice genome, using the sequence of IRGSP, that of *Oryza sativa* spp. *Japonica* cv. Nipponbare (International Rice Genome Sequencing Project and Sasaki 2005). The parallel and complementary annotation efforts of the MSU RGAP and IRGSP/RAP are unified on the set of pseudomolecules that represent the 12 chromosomes, and therefore the gene loci, gene models and associated annotations are comparable. The findings from the latest unified Os-Nipponbare-Reference-IRGSP-1.0 pseudomolecules and MSU Rice Genome Annotation Project Release 7 are summarised in Table 3 (Kawahara et al.

Table 3 Summary of MSU RGAP Release 7 (2011)

Total loci	55,986
Non-TE loci number	39,045
Gene models	49,066
Gene size	2853 bp
Exons/gene	4.9
Introns/gene	3.9
TE loci number	16,941
Gene models	17,272
Gene size	3223 bp
Exons/gene	4.2
Introns/gene	3.2

TE transposable element-related genes and gene models, *Non-TE* non-transposable element-related gene models. In Release 7, there were 373,245,519 bp of nonoverlapping rice genome sequence pertaining to the 12 rice chromosomes, derived from 3184 BAC/PAC clones. 55,986 genes (loci) had been identified, of which 6457 had 10,352 additional alternative splicing isoforms resulting in a total of 66,338 transcripts (or gene models) in the rice genome. Small gene models (<50 amino acids) were excluded. Two approaches were employed for identification of TE-related gene models: BLASTN searches against the MSU Oryza Repeat Database and by identifying gene models containing TE-related Pfam domains

2013). Continuous revision and revalidation of the minimal tiling paths were undertaken to update the sequence assembly. Advances in sequencing strategies, platforms and whole-genome sequencing allowed identification of sequencing errors in the revised assembly. Resequencing the genome of two different Nipponbare individuals using the Illumina Genome Analyzer II/IIx platform identified a total of 4886 sequencing errors in 321 Mb of the assembled genome. This indicated an error rate in the original IRGSP assembly to be only 0.15 per 10,000 nucleotides. Five insertions/deletions were also identified using the Roche 454 pyrosequencing platform. This resequencing data, generated from two different individuals, gave information regarding allelic differences between the original Nipponbare individual used in the IRGSP and the two individuals used in this resequencing effort.

Sequence annotation efforts have designated ~400 distinct gene families in rice. In the Pfam database (http://pfam.xfam.org/), which harbours a large collection of protein families, 42,365 domains are reported for 48,930 protein sequences from the rice proteome, while 5891 unique domain organisations or architectures are also listed. The kinase domain (Pkinase) is maximally represented in the proteome, with currently 517 sequences containing this unique domain. This is in agreement with several instances of tandem repeats, already known for the rice genome.

4 Systems Biology Resources for Rice Genomics

The major challenge after achieving completion of ‘gold standard’ rice genome sequence was to organise all the available primary and secondary data from diverse experimental sources. INE, discussed previously, served as a unified platform for integrated map displays for each chromosome (Sakata et al. 2000). But advances in bioinformatics have led to development of species-specific or general databases, which allowed efficient genome browsing, extension of the genetic information to multiple plant species and other organisms as well as cross-referencing data with functional capabilities. Apart from various genome browsers with novel features that have been developed over the last decade, a plethora of new generation analysis tools were also enabled for functional studies, transcriptome analysis, gene coexpression analysis, protein-protein interaction networks, metabolic pathway analysis, orthology identification analysis and assessment of indexed rice mutant genes (Chandran and Jong 2014; Hong et al. 2019). All these tools have their own significance and greatly enhance the functional implication of rice genes.

4.1 Genome Browsers

The concurrent release of genome sequences for *Oryza sativa* enabled generation of huge volumes of functional genomics data, made easy by high-throughput analysis tools. Therefore an integrated genome browser to visualise, navigate, analyse and annotate the rice genome was becoming indispensable for researchers and biologists. To serve this purpose of data organisation and visualisation, browsers such as Rice-Map (http://www.ricemap.org/), Rice Genome Annotation Project (RGAP, http://rice.plantbiology.msu.edu/), Rice Annotation Project Database (RAP-DB, https://rapdb.dna.affrc.go.jp/), Rice Functional Genomic Express Database (RiceGE, http://198.202.69.30/cgi-bin/RiceGE) and Gramene (http://gramene.org/) were eventually developed, each built on datasets differing from each other (Wang et al. 2011, Kawahara et al. 2013, Sakai et al. 2013, Tello-Ruiz et al. 2018). Rice-Map delivers several precomputed *japonica* and *indica* subspecies annotations and also provides an interactive interface for users to browse the different genomic features at multiple levels (Wang et al. 2011). RGAP provides the fundamental rice genome data, supplemented with various analysis tools such as BLAST search, GO retrieval, domain and motif search, etc. (Kawahara et al. 2013). In contrast, Gramene allows for extension of data to other candidates within the plant kingdom, or subspecies within *Oryza* (Tello-Ruiz et al. 2018). These genome browsers allow bulk data retrieval, batch query searches and efficient identifier mapping between loci listed in discrete databases.

4.2 Orthology Identification Databases

These databases have significantly augmented genome-wide analysis of a group of genes and comparative genomics research. For transferring the knowledge of economically important QTLs from one crop species to another, it is crucial that the orthologues are correctly identified. This knowledge can be exploited for development of markers in agronomically important crops. Such analysis can also boost the expanse of the predicted protein-protein interaction networks: experimentally proven interactions between a pair or group of orthologous protein interactors can be extrapolated to other species, thus facilitating functional studies. Phytozome (https://phytozome.jgi.doe.gov/pz/portal.html) provides a centralised comparative hub for plant genome including land plants and algae (Goodstein et al. 2012). Each plant gene's evolutionary history can be browsed starting right from its sequence, gene structure, gene family and organisation within the genome, thus providing a platform to compare genes across plant species. GreenPhylDB (http://www.greenphyl.org/cgi-bin/index.cgi) is another web resource containing a comprehensive catalogue of gene families, automatically clustered and manually annotated into orthologous groups (Conte et al. 2008). It is supplemented with a powerful tool, GreenPhyl Ortholog Search Tool (GOST), which can predict orthologous relationships between protein sequences across a broad taxonomy of green plants.

4.3 Transcriptome Databases

The ultimate goal in rice molecular biology is to correctly assign a functional role to all the predicted genes in the rice genome. Despite major developments in high-throughput analysis tools, the number of fully characterised genes is far more less than the number of predicted genes, still classified as 'hypothetical', 'unknown' or 'unannotated'. Large-scale gene expression profiling from different organ, tissue or cell types at various developmental stages greatly enhances the efforts put into functional characterisation of rice genes (Roychoudhury et al. 2011). The rapid accumulation of microarray data for rice in public repositories allowed hand-in-hand development of diverse informatics platforms and data analysis tools (Roychoudhury and Banerjee 2015). Rice Expression Profile Database (RiceXPro, http://ricexpro.dna.affrc.go.jp/) was built on the Agilent 44 K microarray platform, with probes derived from manually curated gene models in RAP-DB and full-length cDNA sequence information extracted from Knowledge-based Oryza Molecular biological Encyclopedia (KOME, cdna01.dna.affrc.go.jp/cDNA/) database (Rice Full-Length cDNA Consortium 2003; Sato et al. 2011). The microarray analysis was done from organs/tissues of the rice plant at various growth stages under natural field conditions, rice seedlings treated with different phytohormones and specific cell types or tissues isolated by laser microdissection (LMD) techniques. The Rice Oligonucleotide Array Database (ROAD, http://www.ricearray.org/) integrates information from six rice microarray platforms, including the Affymetrix, Agilent 22 K and 44 K, BGI/Yale and the NSF 20 K and 45 K, thus making it very comprehensive, and provides a user-friendly web interface with various functional analysis tools (Cao et al. 2012). ROAD allowed meta-profile analysis for different anatomic tissues at various developmental stages, gene coexpression analysis and creation of coexpression networks and gene ontology (GO) and KEGG orthology (KO) analyses of query genes. With the emergence of next-generation sequencing technologies (NGS), RNA sequence-based profiling is rapidly gaining ground and is already replacing the databases built on microarray data, thus overcoming the limitations of microarray technique. Rice Expression Database (RED, http://

expression.ic4r.org/) is a repository of gene expression profiles derived from curated and high-quality RNA-Seq data from tissues at various developmental stages or encompassing a wide variety of biotic and abiotic treatments (Xia et al. 2017). Similarly, AgriSeqDB (https://expression.latrobe.edu.au/agriseqdb) is an online RNA-Seq database with features for visualisation, analysis and interpretation of transcriptome data from various stages of development and tissue/cell types from several species, for major agricultural crops such as rice, wheat, maize, barley and tomato (Robinson et al. 2018). Transcriptome ENcyclopedia Of Rice (TENOR, http://tenor.dna.affrc.go.jp) provides comprehensive large-scale mRNA-Seq data obtained from rice sampled from a variety of conditions: ten abiotic stress conditions such as high salt stress, osmotic stress, high and low phosphate or cadmium levels, drought, cold and flood and two plant hormone treatments (abscisic acid and jasmonic acid) (Kawahara et al. 2016).

4.4 Promoter Databases

Promoter databases provide information regarding the core promoter structures and regulatory elements, which have been experimentally verified or predicted from consensus. Various informative resources for promoter detection and analysis are available for different plant species like rice, *Arabidopsis*, poplar, etc. Plant Promoter Database (PPDB, http://ppdb.agr.gifu-u.ac.jp/ppdb/cgi-bin/index.cgi) is a web-based promoter database which comprises of sequence lists of bioinformatically identified promoter elements, extracted by local distribution of short sequence analysis (LDSS) (Kusunoki and Yamamoto 2017). Promoter recognition is accomplished by annotating genome sequence to these lists of TATA boxes, initiators, GA and CA elements, Y patches and regulatory element groups (REGs), supplemented with information on experimentally demonstrated transcription start sites (TSSs). Additionally, REGs are linked to the information in the Plant cis-acting regulatory DNA elements (PLACE, http://www.dna.affrc.go.jp/htdocs/PLACE/) database (Higo et al. 1998). PLACE is a database of nucleotide sequence motifs of regulatory elements extracted from published reports on genes in vascular plants and other plant species. It is appended with a Signal Scan programme that allows users to search for cis-regulatory elements in their query sequence. The outputs are assigned PLACE accession numbers and are hyperlinked to PubMed or GenBank identifiers. The Plant Promoter Analysis Navigator (PlantPAN, http://plantpan2.itps.ncku.edu.tw/) tool allows users to search for transcription factor binding sites (TFBSs), respective transcription factors (TFs) and several important cis- and trans-regulatory elements in their query promoter sequences or set of promoter sequences in plants (Chang et al. 2008). It also allows determining co-occurrence of TFs and their binding sites for the promoters of the input gene groups and construction of gene-regulatory networks using coexpression analysis. PlantPAN also provides cross species promoter search utilising paralogs and orthologues.

4.5 Databases for Rice Coexpression Analysis

Gene coexpression analysis is performed to associate genes of known or unknown function with biological pathways, by discerning the correlation patterns among them across microarray and other transcriptomic datasets. But with recent advances in next-generation sequencing, gene coexpression networks built on RNA-Seq data enable improving the definitions of gene function and associating noncoding genes and splice variants with some biological roles. RiceFREND (http://ricefrend.dna.affrc.go.jp/) is a gene coexpression database built on large volumes of rice microarray data, derived from various tissues at different developmental stages under natural field conditions or under the influence of some phytohormone treatments – the same Agilent 44 K array dataset available in the RiceXPro database (Sato et al. 2011, 2013). RiceFREND provides a platform with two modes of search: single guide gene and multiple guide genes mediated search for coexpressed genes or functionally related genes in various signalling or metabolic pathways. As already mentioned previously, PlantPAN also has options for gene coexpression analysis and for construction of regulatory networks based on co-occurrence TFs and protein-protein interactions (Chang et al. 2008). OryzaExpress is another integrated database, providing interactive user interface to compare gene expression networks in rice with that of other plants (Arabidopsis Gene Expression Network data from ATTED-II) (Hamada et al. 2011).

4.6 Phylogenomics Databases

Experimental determination of biological functions of genes within large families becomes problematic because redundancy limits exactly assigning the contribution of individual genes. As of 2019, 1072 genes have been reported in the collection of the Overview of functionally characterized Genes in Rice Online database (OGRO, http://qtaro.abr.affrc.go.jp/ogro/) (Yamamoto et al. 2012). Therefore, a combinatorial approach of incorporating phylogenetics into comparative genomics had been undertaken to predict the functions of candidates within large-gene families considering the similarity among gene products: a phylogenomics analysis. In rice, phylogenomics databases have been constructed for six gene families (http://ricephylogenomics.ucdavis.edu/index.shtml): kinases, glycosyltransferases (GTs), glycoside hydrolases (GHs), transcription factors (TFs), transporters and cytochrome p450 monooxygenases (P450s). The Rice Kinase Database (RKD) derives information from the NCBI Gene Expression Omnibus (GEO) and provides a platform to integrate functional genomics data into phylogenetic trees built for individual families (Dardick et al. 2007; Jung et al. 2010). The RKD includes an interactive chromosomal map depicting the position of kinase genes, protein-protein interaction maps and meta-expression data developed on microarray data analysis. Thus RKD facilitates effective estimation of functional redundancy or dominance among closely related kinases within subfamilies. The Rice GT database integrates functional genomics information of all putative rice glycosyltransferases on a phylogenetic tree (Cao et al. 2008). All these putative GTs have been identified through similarity searches against annotated GTs in the Carbohydrate-Active enZymes (CAZy) database (http://www.cazy.org/) and subsequently classified into subclasses, based on domain composition and sequence similarities (Lombard et al. 2014). Similarly, the rice GH database lists all the GHs identified through sequence similarity searches in the CAZy database and shares a lot of features with the GT database (Sharma et al. 2013). It incorporates several features such as gene expression patterns, orthologous relationships, structural information and mutant availability for individual GH families in a phylogenomics context. These efforts have provided some significant insight into understanding of cell wall structure and biotic and abiotic stress tolerance. The Rice TF database (http://ricephylogenomics.ucdavis.edu/tf/) integrates information on putative and predicted rice TFs and transcriptional regulators, retrieved from the Plant Transcription Factor Database (PlnTFDB, http://plntfdb.bio.uni-potsdam.de/v3.0/) (Jin et al. 2017). Another database that provides comprehensive information regarding rice TF expression during drought and salinity stress and at various developmental stages is the Rice Stress-Responsive Transcription Factor Database (RiceSRTFDB, http://www.nipgr.res.in/RiceSRTFDB.html) (Priya and Jain 2013). This enables a holistic view of the TF of interest involved in stress response at a particular developmental stage, the cis-regulatory elements in the promoters, mutant availability and phenotype information. The Rice Transporter database (http://ricephylogenomics.ucdavis.edu/transporter/) contains all the potential rice membrane transporter genes, retrieved from the Transporter Protein Analysis Database (TransportDB; http://www.membranetransport.org/), while the Rice CytP450 Database (http://ricephylogenomics.ucdavis.edu/p450/) was developed to integrate functional genomics information for all putative rice P450s in a phylogenomics context (Elbourne et al. 2017). Both these databases are supplemented with similar features like orthologous relationships, gene expression patterns, phenotype information, etc., as discussed above for other databases.

4.7 Proteome Databases

Proteome databases in rice have been built on diverse platforms or data acquisition types, thereby each having their own significance. Rice Proteome Database (RPD, http://gene64.dna.affrc.go.jp/RPD/main_en.html) contains a catalogue of rice proteins, extracted from various tissues and subcellular compartments and separated and analysed on two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) (Komatsu 2005). The database contains an ensemble of reference maps based on 2D-PAGE, with sequences of individual proteins, as well as functional characterisation of major proteins. Plant Proteomics Database (PPDB, http://ppdb.tc.cornell.edu/) contains an assembly of all protein-encoding gene models in *Arabidopsis*, maize and rice, which are linked to each other via BLAST options (Sun et al. 2009). Each gene model is associated with some experimental information which has been derived from in-house proteome and mass spectrometry analysis and is also supplemented with curated information regarding protein function, properties and subcellular localisation. Nowadays, with the advances in mass spectrometry, large-scale and quality-controlled peptide sequencing data are available. OryzaProteogenomics Database (OryzaPG-DB, http://oryzapg.iab.keio.ac.jp/) is a data repository of shotgun proteomics data, built from the outputs of 27 nanoLC-MS/MS runs, on a hybrid ion trap-orbitrap mass spectrometer, of tryptic digests from undifferentiated cultured rice cells (Helmy et al. 2011). Peptides were identified, when the product ion spectra were searched against protein, cDNA, transcript and genome databases and were subsequently mapped onto the rice genome. OryzaPG is the first proteogenomics-based database of the rice proteome, which associated peptide-based expression profiles with corresponding genomic origin.

4.8 Protein-Protein Interaction Databases

These databases have flourished due to the recent developments in techniques employed to identify the potential interactors of a protein candidate. Understanding the protein interactomes from yeast two-hybrid (Y2H), split-ubiquitin (sUbq), bimolecular fluorescence complementation (BiFC), fluorescence resonance energy transfer (FRET), affinity purification (AP), co-immunoprecipitation (Co-IP) and mass spectrometry (MS) has reignited the possibilities of comparative analysis of protein-protein interactions (PPIs). Thus based on the assumption that protein complexes across different species share significant structural and functional similarity, evolutionary conserved proteins will not only retain their structure and function but also the interactions with other protein partners. Predicted Rice Interactome Network (PRIN, http://bis.zju.edu.cn/prin/), a well-annotated PPI database for rice, extended the current expanse of the PPI data by integrating information from experimentally verified interologs of six model organisms (*Saccharomyces cerevisiae* (yeast), *Caenorhabditis elegans* (worm), *Drosophila melanogaster* (fruit fly), *Homo sapiens* (human), *Escherichia coli* K12 and *Arabidopsis thaliana*), using computational approaches (Gu et al. 2011). Supplemented with GO assignments, subcellular localisation data and gene expression data, PRIN provides a user-friendly web interface for easy database search and effective network visualisation. STRING (Search Tool for the Retrieval of Interacting Genes/Proteins, https://string-db.org/) is another database of established and predicted protein-protein interactions, which are of direct (physical) or indirect (functional) nature (Szklarczyk et al. 2015). Information regarding interactions in STRING database stems from genomic context predictions, high-throughput lab experiments, coexpression data, automated text-mining, identifying interologs in other organisms and interactions reported in other primary databases.

4.9 Databases for Metabolome Analysis

Metabolomics addresses the global metabolic changes in biological systems and has immense application in disease diagnosis and functional genomics. Metabolic profiling of cells, tissues or organisms, biomarker identification and pathway analysis are an integral part of it and demand easy-to-use analysis tools. MetaboAnalyst (https://www.metaboanalyst.ca/) provides a web-based user interface with metabolomics data processing tools, options for data normalisation, statistical analysis, graphing, metabolite identification and characterisation as well as pathway mapping (Chong et al. 2018). MetPA (Metabolomics Pathway Analysis, https://www.metaboanalyst.ca/) is a web-based tool, which combines advanced pathway enrichment analysis and pathway topology analysis, to visualise and analyse metabolic pathways involved in a particular metabolomics study (Xia and Wishart 2010). MetPA currently allows visualisation and analysis of a total of 1600 pathways for 21 model organisms, including rice. EXPath (http://expath.itps.ncku.edu.tw) uses public large-scale microarray datasets, derived from samples under abiotic or biotic stress or under different hormone treatments, for gene coexpression analysis and identification of differentially expressed genes (DEGs) (Chien et al. 2015). Down the pipeline, this information is finally utilised for inferring enriched KEGG pathways and GO terms for three plant species, namely, *Arabidopsis*, rice and maize.

4.10 Rice Gene Indexed Mutant Databases

The rice research community has greatly benefited from the high quality of the rice genome and also the appreciably fair annotation in other cereal crop species. To elucidate the function of all the predicted coding and noncoding regions within the rice genome, the International Rice Research Institute (IRRI) in close association with the International Rice Functional Genomics Consortium (IRFGC) developed an enormous collection of indexed rice mutant genes. The members within a population of gene indexed mutants are distinguished on the following criteria: mutagenesis methods adopted (T-DNA insertion, transposons, chemical or physical mutagens), rice variety, mutant phenotypes, seed availability, reporter-gene expression patterns and mutated loci per genome. All this information facilitated further functional characterisation of the genome. Rice Mutant Database (RMD, http://rmd.ncpgr.cn/) encompasses the information regarding ~129,000 rice T-DNA insertion (enhancer trap) lines generated by an enhancer trap system and delivers three distinct functional classes: (1) novel gene identification, (2) identification of regulatory elements and (3) identification of patterns of ectopic expression of target gene at particular growth stage or tissue type (Zhang et al. 2006). Oryza Tag Line (http://oryzatagline.cirad.fr/) is a phenotypic mutant database for the French genomics initiative 'Genoplante' rice insertion line library (Larmande et al. 2008). Based on the molecular characterisation of the mutagen insertion sites, the sequence information of flanking sequence tags (FSTs) was retrieved. This database also allows forward genetic search through queries based on mutant phenotype or reporter-gene expression, coupled with other categories such as organ, developmental stage or trait. OryGenesDB (http://orygenesdb.cirad.fr/data.html) displays sequence information on T-DNA and *Ds* FSTs in insertional rice mutants, produced in the framework of Genoplante and the EU consortium Cereal Gene Tags (Droc et al. 2006). This database is supplemented with tools for reverse genetics, allowing a molecular geneticist to readily find T-DNA, *Tos17* and/or *Ds* insertion lines in genes of interest and to retrieve all the annotations associated with these sequences, derived from external rice molecular resources (e.g. cDNA full length, gene, EST, markers, expression data, etc.).

4.11 Other Databases

Apart from the above listed categories of databases, there are other web resources available, each built on diverse data sources and possessing unique features and tools compatible for functional genomic annotations. RiceVarMap (http://ricevarmap.ncpgr.cn/v2/) provides curated information of genomic variations—single nucleotide polymorphisms (SNPs) and small insertions/deletions (INDELS)—from sequencing data of ~4700 rice accessions (Zhao et al. 2015). The variations are associated with comprehensive resources of functional annotations, chromatin accessibility data, risks associated with gene expression, phenotype data and images, agronomic and metabolic traits, geographical details, etc. DroughtDB (http://pgsb.helmholtz-muenchen.de/droughtdb) is a valuable tool for researchers working on drought stress (Alter et al. 2015). This database is a manually and expert-curated compilation of drought stress-responsive genes in plants, which have been molecularly characterised. Each drought stress-responsive gene, already characterised either in *Arabidopsis* or *Oryza sativa*, is supplemented with information on its sequence, physiological or molecular function, mutant phenotypes and their homologs in nine model crop species, like maize, barley, etc. With new insights into the role of microRNAs in plant growth and development, repositories of microRNA data are also being developed. The plant microRNA database (PMRD, http://bioinformatics.cau.edu.cn/PMRD/) congregates available information on plant microRNA data, for example, sequence of microRNAs and their target genes, secondary dimension structure, expression profiling, genomic origins, etc. (Zhang et al. 2010). Gene duplications are widespread in plant species. For example, receptor-like kinase families in *Arabidopsis* and rice have nearly 600 and 1000 members, respectively. This implies gene duplication and subsequent mutations have generated new genes with diverse functions. In similar lines, recent developments in gene coexpression networks have implied that not only genes but pathways can also undergo multiplication and diversification to perform related functions in various parts of an organism. Such pathways are called modules, which may give us a lead to understand the biological processes and functions well. FamNet (http://aranet.mpimp-golm.mpg.de/famnet.html) is an interactive platform for exploration and visualisation of these multiplied modules in gene coexpression networks of eight plant species (Ruprecht et al. 2016). With this tool, multiplied modules involved in tip growth in pollen tubes and root hairs or in secondary metabolite synthesis were identified and functionally characterised in separate plant species.

Recent advances in development and refinement of new and existing high-throughput analysis tools have significantly impacted the functional annotation of the rice genome. From browsing the genome to deciphering phylogenomic relationships, from mapping orthologues to identifying SNPs and INDELs, from identification of gene coexpression networks to metabolic pathways, these tools have leveraged the quality of meaningful predictions. Table 4 lists the diverse omics tools—browsers, databases, mapping tools, etc.—that have not been touched upon in the above section, but are used at large for functional genomics.

5 Rice Genomics Propelled Research on Important Agronomic Traits

The availability of the rice genome sequence data and development of new tool sets to analyse the data has greatly revolutionised the research in rice genetics and breeding. A search in the NCBI PubMed database with the keywords 'map-based cloning', 'rice' and 'Oryza' has retrieved nearly over 4700 publications corresponding to rice research, from the past three decades (see Fig. 3). Following the establishment of the Rice Genome Research Program in Japan, there was a major boost in rice research, with 40–60 publications per year. This was nearly 2–3 times the publication volume before RGP was established. But following the public release of the rice genome sequence by IRGSP in 2005, which incorporated the draft sequences from Monsanto and Syngenta, there were, on an average, more than 200 publications per year pertaining to rice research. It is evident from the volume of publications in the last 10 years that the numbers are on the rise, which has been propelled with the genomes of several wild and domesticated rice varieties and other cereals being sequenced. This rough analysis, which does not encompass any manual curation of the retrieved citations, simply indicates the enormous advancement in the rice research area with the completed rice genome sequence, with a multitude of genes being associated with metabolic, developmental or stress-related signalling pathways (Wing et al. 2018; International Rice Genome Sequencing Project and Sasaki 2005). Moreover, the reference genome of rice allowed exploration into the genomes across the entire *Oryza* genus as well as studying of genetic variations among domesticated rice species and their wild relatives (Wing et al. 2018; Civáň et al. 2015; Wang et al. 2014; Meyer et al. 2016). Sequence-based analysis of the variations in different species allowed breeders to exploit such variations for rice improvement. But none of this was possible without the significant increase in the number of molecular markers, and access to the knowledge about their physical order in chromosomes and proximity to annotated genes, all of which was utilised to predict gene-trait associations. Another noticeable impact of the rice genome sequence was an opportunity to gain an understanding of the molecular and genetic basis of the traits such as efficient mineral utilisation, resistance towards biotic and abiotic stress, physical features and so on. All this insight helped rice

Table 4 List of genome browsers, databases and diverse omics tools which facilitate high-throughput analysis of the rice genome

Databases	Resource links	References
Genome browsers		
TIGR Rice Genome Annotation project	http://blast.jcvi.org/euk-blast/index.cgi?project=osa1	Yuan et al. (2003)
OsGDB	http://www.plantgdb.org/OsGDB/	Dong et al. (2005)
Rice FPC Genome Browser	https://www.genome.arizona.edu/fpc/rice/gbrowse/	Pampanwar et al. (2005)
Oryzabase	https://shigen.nig.ac.jp/rice/oryzabase/	Kurata and Yamazaki (2006)
Rice-Map	http://www.ricemap.org/	Wang et al. (2011)
MSU Rice Genome Annotation Project (RGAP)	http://rice.plantbiology.msu.edu/	Kawahara et al. (2013)
Rice Annotation Project Database (RAP-DB)	https://rapdb.dna.affrc.go.jp/	Sakai et al. (2013)
Gramene	http://www.gramene.org/	Tello-Ruiz et al. (2018)
Resources for rice orthology analysis		
GreenPhylDB	http://www.greenphyl.org/cgi-bin/index.cgi	Conte et al. (2008)
Phytozome	https://phytozome.jgi.doe.gov/pz/portal.html	Goodstein et al. (2012)
Putative Orthologous Groups (POGs) DB	http://cas-pogs.uoregon.edu/#/	Tomcal et al. (2013)
InParanoid	http://inparanoid.sbc.su.se/cgi-bin/index.cgi	Sonnhammer and Östlund (2015)
Plaza	https://bioinformatics.psb.ugent.be/plaza/	Van Bel et al. (2018)
Resources for rice transcriptome analysis		
RiceXPro	http://ricexpro.dna.affrc.go.jp/	Sato et al. (2011)
Rice Oligonucleotide Array Database (ROAD)	http://www.ricearray.org/	Cao et al. (2012)
PLEXdb	http://www.plexdb.org/plex.php?database=Rice	Dash et al. (2012)
Transcriptome Encyclopaedia Of Rice (TENOR)	https://tenor.dna.affrc.go.jp/	Kawahara et al. (2016)
Rice Expression Database (RED)	http://expression.ic4r.org/	Xia et al. (2017)
AgriSeqDB	https://expression.latrobe.edu.au/agriseqdb	Robinson et al. (2018)
Resources for rice promoter analysis		
Plant Promoter Analysis Navigator (PlantPAN)	http://plantpan2.itps.ncku.edu.tw/	Chang et al. (2008)
Osiris	http://www.bioinformatics2.wsu.edu/Osiris	Morris et al. (2008)
GrassPROMDB (Grassius)	https://grassius.org/grasspromdb.php	Yilmaz et al. (2009)

(continued)

Table 4 (continued)

Databases	Resource links	References
Plant Promoter Database (PPDB)	http://ppdb.agr.gifu-u.ac.jp/ppdb/cgi-bin/index.cgi	Kusunoki and Yamamoto (2017)
Resources for rice coexpression analysis		
OryzaExpress	http://plantomics.mind.meiji.ac.jp/OryzaExpress/	Hamada et al. (2011)
RiceFREND	http://ricefrend.dna.affrc.go.jp/	Sato et al. (2013)
PLANt co-EXpression database (PLANEX)	http://planex.plantbioinformatics.org/	Yim et al. (2013)
RECoN: Rice Environment Coexpression Network	https://plantstress-pereira.uark.edu/RECoN/	Krishnan et al. (2017)
Resources for rice phylogenomics		
Rice Kinase Database (RKD)	http://ricephylogenomics.ucdavis.edu/kinase/	Dardick et al. (2007) and Jung et al. (2010)
Rice GlycosylTransferase Database (RGTD)	http://ricephylogenomics.ucdavis.edu/index.shtml	Cao et al. (2008)
Stress-responsive TranscrIption Factor Database (STIFDB)	http://caps.ncbs.res.in/stifdb2/	Shameer et al. (2009)
Rice glycoside hydrolase database	http://ricephylogenomics.ucdavis.edu/cellwalls/gh/genInfo.shtml	Sharma et al. (2013)
Rice Stress-Responsive Transcription Factor Database (RiceSRTFDB)	http://www.nipgr.res.in/RiceSRTFDB.html	Priya and Jain (2013)
Rice Transporter Database	http://ricephylogenomics.ucdavis.edu/transporter/	Jung et al. (2015)
Rice CytP450 Database	http://ricephylogenomics.ucdavis.edu/p450/	Jung et al. (2015)
Rice TF Database	http://ricephylogenomics.ucdavis.edu/tf/	Jung et al. (2015)
Resources for rice proteogenomics		
Rice Proteome Database (RPD)	http://gene64.dna.affrc.go.jp/RPD/main_en.html	Komatsu (2005)
Plant Proteomics Database (PPDB)	http://ppdb.tc.cornell.edu/	Sun et al. (2009)
OryzaPG-DB	http://oryzapg.iab.keio.ac.jp/	Helmy et al. (2011)
Resources for rice protein-protein interaction analysis		
Predicted Rice Interactome Network (PRIN)	http://bis.zju.edu.cn/prin/	Gu et al. (2011)
Database of interacting proteins in *Oryza sativa* (DIPOS)	http://comp-sysbio.org/dipos/?id=5	Sapkota et al. (2011)
STRING	https://string-db.org/	Szklarczyk et al. (2015)
Resources for rice metabolic pathway analysis		
KEGG	https://www.genome.jp/kegg/pathway.html	Kanehisa and Goto (2000)
MapMan	http://mapman.gabipd.org/home	Thimm et al. (2004)

(continued)

Table 4 (continued)

Databases	Resource links	References
MetaboAnalyst/MetPA	https://www.metaboanalyst.ca/	Xia and Wishart (2010) and Chong et al. (2018)
RiceNetDB	http://bis.zju.edu.cn/ricenetdb/	Liu et al. (2013)
RiceCyc	http://archive.gramene.org/pathway/ricecyc.html	Dharmawardhana et al. (2013)
EXPath	http://expath.itps.ncku.edu.tw	Chien et al. (2015)
PANTHER	http://www.pantherdb.org/pathway/	Mi et al. (2017)
Plant Reactome	http://plantreactome.gramene.org/index.php?lang=en	Naithani et al. (2017)
Resources for rice gene indexed mutants		
Rice Mutant Database (RMD)	http://rmd.ncpgr.cn	Zhang et al. (2006)
OryGenesDB	http://orygenesdb.cirad.fr/data.html	Droc et al. (2006)
Taiwan Rice Insertional Mutants (TRIM)	http://trim.sinica.edu.tw/	Chern et al. (2007)
Oryza Tag Line	http://oryzatagline.cirad.fr/	Larmande et al. (2008)
Other database resources		
Plant MicroRNA Database (PMRD)	http://bioinformatics.cau.edu.cn/PMRD/	Zhang et al. (2010)
NIASGBdb (National Institute of Agrobiological Sciences planttfdb database)	http://www.gene.affrc.go.jp/databases_en.php	Takeya et al. (2011)
RiceVarMap	http://ricevarmap.ncpgr.cn/v2/	Zhao et al. (2015)
DroughtDB	http://pgsb.helmholtz-muenchen.de/droughtdb	Alter et al. (2015)
FamNet	http://www.gene2function.de/famnet.html	Ruprecht et al. (2016)

Resource links and references of corresponding publications are also provided

researchers to engineer ‘Green Super Rice’, a new generation of sustainable crops that will tackle the food demands of the growing population.

Limitations in arable land and water resources in rice-producing countries and the issues related to the increasing trend of global population growth can be addressed to a great extent by increasing per unit area yield of rice. This has been largely possible in the last decade because of correctly identifying the molecular markers determining the agronomically desirable traits and using them effectively in rice production.

5.1 Improving Plant Architecture and Yield

The semidwarf trait has been introduced into a number of rice cultivars growing worldwide, as it improves light-interception properties, harvest index, nitrogen

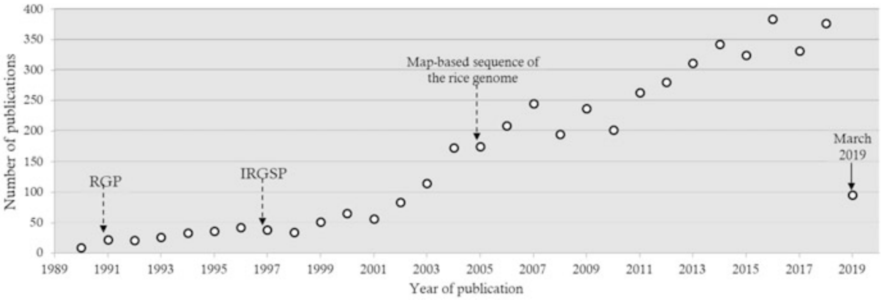

Fig. 3 A timeline for the period 1990–2019 depicts nearly 4783 publications related to research on gene cloning in rice, retrieved from the NCBI PubMed database. For the year 2019, the records listed are till the month of March. The dotted arrows indicate the years when RGP and IRGSP were established and the map-based genome sequence of rice was released

responsiveness and lodging resistance, without having non-detrimental effects on the grain yield. The International Rice Research Institute developed a semidwarf variety of rice IR8, from a cross between Peta, a tall *indica* variety, and Dee-geo-woo-gen (DGWG), a Taiwanese indigenous semidwarf rice variety. This new variety led to a dramatic improvement in rice yields, thereby bringing about the Green Revolution to tropical Asia (Hargrove and Cabanilla 1979). IR8 had a culm length of 90–100 cm, compared to its parent line, Peta, characterised by longer culm (150–180 cm) and long hanging leaves. Sequence data from the rice genome and supporting mapping studies facilitated identification of genes responsible for the semidwarfism trait in rice (Monna et al. 2002; Spielmeyer et al. 2002). The incomplete recessive gene, *sd1*, located on the long arm of chromosome 1, encodes for GA20-oxidase with loss-of-function mutations (OsGA20ox2). This gene product regulates synthesis of gibberellins, which regulates the GA biosynthesis pathway. Semidwarfism is one of the traits that got positively selected during domestication of *japonica* rice. In addition to affecting plant height, it has positive effect on the tiller number and enhances erection of the blade.

Apart from plant architecture, other component traits that determine yield of a rice plant are tiller number, number of grains per panicle, grain weight and grain filling rate. The *MONOCULM 1* (*MOC1*) gene is the first gene identified related to controlling rice tiller number (Li et al. 2003). *MOC1* is a member of the plant-specific GRAS transcription factor family. *MOC1* is required for growth of axillary meristems at both vegetative and reproductive stages. Consequently, loss of function of *MOC1* affects formation of both tiller and panicle. *Ghd7* encodes a CCT domain containing transcriptional activator that simultaneously controls number of grains per panicle, plant height and heading date (Xue et al. 2008). Another QTL with similar effects was *Ghd8*, which encodes the OsHAP3 subunit of a heterotrimeric heme activator protein (HAP) (Yan et al. 2011). This gene manipulated flowering time in rice and upregulated *MOC1*, thereby increasing the number of tillers and grain yield. Along with *Ghd7.1*, which encodes a PSEUDO-RESPONSE REGULATOR (PRR), *Ghd7* and *Ghd8* render pleiotropic effects (delays heading, increases plant height and grain yield) under long-day conditions. Map-based cloning facilitated identification of *Hd1* gene, which largely affected the interaction between *Ghd7* and *Ghd8* (Zhang et al. 2015). Recently it has been demonstrated that combinatorial loss-of-function alleles of *Ghd7*, *Ghd8* and *Hd1* allow expansion of rice cultivars to higher altitudes, therefore defining the ecogeographical adaptation and yield potential in rice cultivars. High-yielding varieties can also be associated with high grain numbers per ear. Several genes, such as the *DENSE AND ERECT PANICLE* genes, *DEP1*, *DEP2* and *DEP3*, have been identified that affect this trait (Xu et al. 2016). *Gn1* gene encoding a cytokinin oxidase OsCKX2 has been identified by map-based cloning. Downregulation of *Gn1* expression leads to an increment in the quantity of glumous flowers, thereby grain number per ear (Yeh et al. 2015). *IPA1* (*Ideal Plant Architecture 1*) encodes the transcription factor OsSPL14, which regulates the plant architecture through DEP1 (Zhang et al. 2017). Fewer but more productive tillers are produced upon achieving optimal expression levels of *IPA1*, thereby regulating plant height and ear length.

5.2 Improving Grain Quality

This demand of enhanced grain quality is primarily determined by consumers and is thereby important to farmers and millers. The quality of rice grain depends on a number of features, which vary according to the preferences of consumers across the world. The grain size, shape and its translucent appearance are important features determining grain quality and are directly related to yield. A complement of genes has been indicated to control these traits. *GS3*, *GW2* and *GW5* are negatively correlated with grain length and width, grain weight and grain fullness (Zheng et al. 2015). Upregulation of *GS5* expression is involved in seed yield, size, thousand seed weight and seed setting rate as well as grain width and weight. *GW8* expression promotes cell division and regulates grain size, thousand seed weight and filling speed, thereby increasing yield and affecting milling quality. Milling quality is largely determined by the chalkiness and intact nature of the milled rice. Grain chalkiness is highly undesirable and a major QTL, *Chalk5*, influences this trait. Elevated expression of *Chalk5* increases chalkiness of the endosperm, thereby posing a major problem in milling and post processing (Li et al. 2014). A perfect combination of amylose content and gelling temperature influences the cooking quality and palatability of rice. The *WAXY* gene codes for the starch synthase enzyme, which is responsible for synthesis of amylose in the endosperm. Based on the amylose content (AC), rice is commercially classified into five categories: high (25–33%), intermediate (20–25%), low (12–20%), very low (5–12%) and waxy (0–5%) (Juliano 1992; Yamanaka et al. 2004). Grains with high AC result in dry and well-separated rice after cooking but eventually turn hard upon cooling. Cooking of rice varieties with intermediate AC leads to a soft, nonsticky texture in rice, while low and very low AC results in a soft and sticky texture. Waxy rice (also called, sweet rice or glutinous rice) becomes sticky when cooked. Using map-based cloning strategy, *ALK* was identified to be the key gene controlling gelatinisation temperature and therefore the gel consistency in rice (Gao et al. 2003). Although rice is a major carbohydrate and even a potential protein source for rice-eating populations, it is a poor source of essential micronutrients. For countries where rice is the staple food, the primary cause of micronutrient malnutrition, including iron, zinc and vitamin A deficiencies, is a rice-based diet. All these deficiencies can be related with reduced working capacity, decreased mental capacity, blindness, stunting and elevated morbidity and mortality. To address this issue, food-based approaches have been adopted that involve biofortification of genotypes resulting in increased levels of vitamins and minerals. Golden Rice, expressing two genes encoding phytoene synthase and carotene desaturase, produced the provitamin A carotenoid β-carotene in rice endosperm and is one of the earliest examples of biofortification (Schaub et al. 2005). Increasing the seed iron content by overexpression of the iron storage protein ferritin in rice grains and controlling the mineral status by overexpressing ion transporters are other strategies adopted. Ongoing efforts aim at improvising the rice grain with other micronutrients, specifically high-quality protein and vitamin E. Other approaches include screening germplasm collections for detecting variation in mineral content (Gregorio et al. 2000) and initiating rice breeding programmes to develop mineral-rich genotypes, using high iron/zinc germplasms and crossing it with plants containing other micronutrient traits (e.g. vitamin E, high protein, β-carotene). A different approach of rice biofortification can be adopted to regulate/reduce the levels of compounds such as phytate and tannins, both of which complex with minerals and prevent their absorption by the body during digestion. This approach simply aims to enhance mineral bioavailability (Welch and Graham 2004).

5.3 Enhanced Efficiency of Nutrient Use

Reducing dependency on fertiliser application can be achieved by gaining a deep understanding of the molecular mechanisms underlying nitrogen and phosphorus usage. Overexpression of *OsNRT1.1A* (*OsNPF6.3*), a member of the *Oryza sativa* nitrate transporter 1/peptide transporter family, in rice significantly improved nitrogen utilisation and grain yield; additionally, the maturation time was also shortened (Wang et al. 2018b). Overexpression of *OsNPF7.2*, a low-affinity nitrate transporter, significantly enhanced nitrate influx and promoted tillering, thereby improving grain yield (Wang et al. 2018a). Likewise, overexpression of *OsNRT2.1*, a high-affinity nitrate transporter, increases yield and manganese accumulation, while high expression of *OsNRT2.3b*, a pH-sensitive nitrate transporter, enhances the pH-buffering capacity of the plant, promoting plant adaptation, and also facilitates increased uptake of nitrogen, iron and phosphorus (Fan et al. 2016; Luo et al. 2018). The phosphate transporter gene *OsPht1;8* (*OsPT8*) increases Pi uptake and is involved in phosphate homeostasis in rice (Jia et al. 2011). Members of the sulphate transporter family in rice (OsSULTR) play an important role in regulating sulphur demand by the plant (Sasaki et al. 2016). OsHAK5, a high-affinity K transporter, controls potassium acquisition and distribution in the rice plant (Chen et al. 2017). Therefore, identification and manipulation of potentially useful genes have to be done with an aim to maximise uptake, allocation and utilisation.

5.4 Increased Resistance to Abiotic and Biotic Stress

Developing rice varieties that can withstand frequent and harsh changes in the climatic conditions is an important goal of rice research worldwide. These abiotic stresses include drought, flood, temperature (heat and cold), salinity and diverse soil problems like iron toxicity. Tremendous efforts are being put to identify genes or genomic regions responsible for conferring resistance or susceptibility to these conditions (Roychoudhury and Paul 2012; Golldack et al. 2014). Breeders have adopted a breeding method called marker-assisted breeding, to accurately incorporate specific desirable traits into novel varieties at a faster rate. Through the use of advanced technology and contribution from national institutions, the International Rice Research Institute has developed ‘climate change-ready rice’ that are able to tolerate harsh climatic conditions (https://www.irri.org/climate-change-ready-rice). Several QTLs have been identified, which are being extensively studied to gain a better insight into their molecular and physiological processes. OsNAC10 and OsAHL1 confer drought tolerance and drought avoidance by regulating root development under stress conditions (Jeong et al. 2010; Zhou et al. 2016). The SUB1A gene that was derived from an Indian rice variety was found to conserve energy till floodwater reduces, thereby conferring resistance to submergence. Two ethylene response factors SNORKEL1 and SNORKEL2 enabled adaptation of rice to deepwater (Hattori et al. 2009). QTLs, e.g. *Ctb1* and *COLD1*, were associated with chilling tolerance, while *TT1* and *OsRab7* are linked to thermotolerance (Gardener and Kumar 2015). A major region of the rice genome, named as Saltol, has been identified by scientists that confers tolerance to salinity. Saltol is being exploited at large to develop crop varieties that can tolerate exposure to salinity stress at both seedling and reproductive stages (Thomson et al. 2010). This approach, combined with submergence tolerance trait, is targeted to rice varieties cultivated in coastal areas, where during rainy season, salinity and submergence are major problems. Additionally several QTLs have been identified from genetic maps that could be associated with iron or aluminium toxicity tolerance and mineral homeostasis. Several of these genes have also been integral parts of breeding programmes of climate change-ready rice.

The increase in the number of cloned disease resistance genes has been greatly accelerated by the map-based sequence of the rice genome. A molecular and functional understanding of these genes gave insights into devising strategies to prevent yield loss during bacterial blight and fungal blast infections, whose causal pathogens are *Xanthomonas oryzae* pv. *oryzae* and *Magnaporthe oryzae*, respectively. Additionally many genes have been fine-mapped, paving the path to identify functional markers. Marker-assisted selection was employed to introgress such genes into parent varieties to develop elite hybrids. The R gene in rice *Xa3* (also known as *Xa26*) displayed enhanced resistance level and enlarged resistance spectrum (i.e. resistance to multiple *Xoo* races) when expressed under native promoter in *japonica* rice (Cao et al. 2007). The atypical protein with armadillo repeats, encoded by the *Ptr* gene, confers broad-spectrum blast resistance (Zhao et al. 2018). Involvement in conferring resistance to bacterial and fungal pathogens was also demonstrated for *OsGAP1* encoding a GTPase-activating protein (GAP), receptor-like kinases like OsBRR1 and XA21 and transcription factors like WRKY45 (Singh et al. 2018). Although marker-assisted selection provides an effective strategy for pyramiding of disease resistance genes, it can be combined with the transgenic approach using cloned genes for developing disease-resistant rice cultivars.

6 Conclusion and Future Perspectives

6.1 Rice Genome Sequence: A Milestone in an Expanse of Untrodden Information

Access to the sea of genomic sequence information of a cereal crop plant was a revolutionary achievement in the twenty-first century. This is solely because rice is a quintessential component of the diet and livelihoods of several million people globally. There is always a befitting demand to increase the yield, productivity, health and acceptability of the plant, which has been largely addressed across generations through traditional crop-breeding programmes. The nature of demands has undergone a noticeable change in the present global scenario, considering uncontrollable population growth, climate change, loss of cultivable land, greenhouse gas emissions and excessive use of pesticides and fertilisers. The rice genome sequence at hand has enabled map-based cloning of QTLs, marker-based characterisation of beneficial genes, functional analysis by developing functional knockouts with mutation or gene tagging and exploration of possibilities of introgression of these genes or genomic regions to develop new agronomically competent hybrids. Moreover, it also facilitated a better understanding of the successful ecogeographical adaptation of rice, genetic variation between wild and domesticated species and also the molecular components underlying agronomically beneficial phenotypes.

The opportunity to map genes to genome provides useful information regarding features of micro- and macro-level synteny of the rice genome to other cereal crops. Such comprehensive molecular linkage maps are the primary components of the toolkit required to breed improvised crops. Following functional assignments of genes, a large amount of information can be extended to other plant species, simply by the virtue of extensive colinearity in the conservation of gene order and content within members of the dicot and monocot families. Comparative mapping of grass genomes, using rice as the reference genome, revealed considerable macro-colinearity, represented by a graphical consensus map, ‘Circle Diagram’ (Moore et al. 1995). Further refinements of such maps gave the general impression that despite significant variations in DNA content or chromosome number, all the cereal crops examined tend to maintain similar gene order, thus maintaining significant micro-colinearity at the mega-base level. Deletions, insertions, small-scale rearrangements or even gene amplification, duplication and translocations can account for the deviations observed. This extensive genome colinearity is exploited for fine mapping and map-based cloning, as several crop plants have large genomes and the extra information derived from the closely related model crop, rice, greatly facilitates cloning of genes in other crops.

Recently, an international resequencing effort of 3000 rice germplasm accessions in the Illumina-based next-generation sequencing (NGS) platform had been undertaken to understand the total genetic diversity within the *Oryza sativa* gene pool (3000 rice genomes project 2014). The International Rice Research Institute (IRRI) contributed 2466 accessions from its International Rice Genebank Collection (IRGC), while the remaining 534 accessions were obtained from the China National Crop Gene Bank (CNCGB) in the Institute of Crop Sciences, Chinese Academy of Agricultural Sciences (CAAS). The temperate *O. sativa* spp. *japonica* Nipponbare (Os-Nipponbare-Reference-IRGSP-1.0) was used as the reference genome for mapping of the selected reads, simply indicating that the significance, versatility, expanse and quality of this genome sequence. When aligned with the reference genome, innumerable single nucleotide polymorphisms could be mapped, depending on which the *O. sativa* gene pool could be differentiated into five varietal types of diverse origins: *indica*, *aus/boro*, *basmati/sadri*, tropical *japonica* and temperate *japonica*. This effort not only gave access to enormous volumes of SNP data but also a knowledge-based tool to discover allelic variants and associate important rice traits with diverse alleles. Additionally, a number of high-quality reference genomes also became accessible for rice genome research.

Another revolutionary development that happened alongside was the emergence of diverse omics web-based tools. Genome browsers and databases were developed and were gradually getting equipped with high-throughput analysis tools for comparative and functional genomics, transcriptomics, metabolomics, phylogenomics, interactomics, etc. Integration of information from different experiments—microarray, RNA-Seq, promoter analysis, yeast two-hybrid, mass spectrometry, co-immunoprecipitation, bimolecular fluorescence complementation and fluorescent protein localisation, to name a few—has now enabled researchers to understand the temporal and spatial expression patterns of a target gene, identify other targets that coexpress with the gene of interest, predict the functionality based on sequence and structural similarities, predict potential interactors and, thereby, possibly identify the signalling or metabolic pathway the candidate gene is involved in. In fact, several computational systems biology studies have been undertaken to clearly understand the mechanisms of salt tolerance or the regulatory crosstalks in the hormone biosynthesis pathways for modulating responses to biotic and abiotic stress (Wang et al. 2013; Deb et al. 2016). This advancement in the diversity, user accessibility and versatility of the omics-driven tools, software and programmes is gradually clearing up the ambiguity related with the rice genome.

However, this information explosion is meaningless and stagnated until a rice researcher is able to associate functional, structural or phenotypic implications with the corresponding genes. Despite the total number of genes worked out from the rice whole-genome sequence, a total of ~3100 gene loci have been analysed till date for biological functions using various approaches (http://funricegenes.ncpgr.cn/). This disparity between analysed genes and total number of genes is due to the restrictions imposed by functional redundancy between the genes and the limited data available on gene function. Additionally several QTLs have been associated with different traits, but very few have been fine-mapped, identified or even explored for proper functions. At this stage of rice research, where there is a need for delivering a sustainable crop plant, more initiatives have to be undertaken to understand the functionality of the genome in entirety, to delineate the effect of allelic differences on gene functions, to dissect the molecular basis of ecogeographical adaptation of rice varieties and to explore the feasibility of manoeuvring these traits to our benefits.

Development of new improved varieties of cereal crops was a significant achievement of Green Revolution, which was possible through the efforts of Norman Borlaug, the father of the Green Revolution. Nearly half a century ago, the International Rice Research Institute (IRRI) developed IR8 variety of rice, also known as 'miracle rice' (Hargrove and Cabanilla 1979). This variety yielded more grains than the traditional ones, when grown with proper irrigation and nitrogen-rich fertilisers. But this miracle also cost a high price: excessive and indiscriminate use of chemical fertilisers and pesticides affected the environment, commercial fertilisers posed an additional cost to the rice farmers, and irrigation demands adversely affected the global water supply, which also faces additional pressure due to climate change, pollution, population growth, industrial requirements, etc. So the demand of an environment-friendly solution was addressed in a collaborative project between IRRI and the Chinese Academy of Agricultural Sciences (CAAS). Funded by the Bill & Melinda Gates Foundation (BMGF), the project aims to develop Green Super Rice (GSR) varieties that can ensure a stable and sustainable yield potential even when supplemented with fewer inputs or even if grown in unfavourable environmental conditions. In contrast to the breeding approaches undertaken during Green Revolution, where all other traits took a backseat compared to high yield, the GSR researchers adopted a different methodology. In this, a large number of backcrossed second-generation lines (BC2F2) and their succeeding generations were subjected to a range of biotic and abiotic stresses, following which weak lines could be eliminated and promising transgressive high-performing segregants could be identified. Several GSR varieties that are drought-tolerant; salinity-tolerant; submergence-tolerant; weed-tolerant; suitable for rainfed lowlands; multiply resistant to rice blast, rice planthoppers and gall midge; zero-input (no fertilisers and no pesticides); and high-yielding are now ready for seed exchange and germplasm distribution. GSR varieties that combine several of these traits are also in the pipeline, such that they can perform well in the toughest conditions, providing a stable and sustainable solution to the poorest farmers who cultivate rice. In recent times, the focus of GSR research has considerably changed as the additional challenge of balancing food security with preservation of natural resources and protection of the environment has come to light. The achievable solution is development of improved GSR varieties and development and diffusion of conventional agricultural practices that are environment-friendly as well.

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Genomic Approaches to Understand Varietal Differences in Rice Species and Genotypes with Respect to Stress Response and Quality Traits

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Abstract

Rice is one of the major food crops feeding more than 3.5 million people worldwide, especially in developing countries. The emerging population demands increased production of rice with improved quality and traits which is a challenge for sustainable rice cultivation. Although the rice production over the past decade is increasing annually, yet it is not sufficient to meet the need. Elevated demands for rice need increased production for which abundant arable land is unavailable, especially in Asian countries where more than 90% of rice is produced and consumed. Under a variable range of agroclimatic conditions, rice is produced across the world, so it is highly prone to a wide range of abiotic stress conditions including extreme temperature changes, heavy metal toxicity, salinity, and drought stress. Conjunction of various abiotic stresses is more common in field conditions, and abiotic stresses seldom occur singly. Different rice genotypes respond differentially to abiotic stress, and variance of intraspecific responses can be noted within *indica* and *japonica* varieties. Responses to abiotic stress tolerance is a complex trait, although many genes related to abiotic stress response have been identified, yet there remain gaps which are needed to be bridged for complete understanding of the differential varietal traits. Functional genomics has revealed more than 2000 genes which have been cloned and partially characterized; genome-wide expression profiling of rice varieties, mutants, and transgenics have also aided the development as well. Molecular regulatory networks involved in several agronomic traits have been investigated. Newer fields of proteomics, metabolomics, phenomics, and epigenomics have also aided the functional genomics research in the recent times. We briefly review here the recent developments in rice functional genomics research that are of

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paramount significance in understanding the responses and quality traits of rice varieties to abiotic stress.

Keywords

Rice · Salt stress · Drought stress · Cold stress · Antioxidants · Genomic approach · MAPK

1 Introduction

The present magnitude at which the population is increasing is of serious concern. The present population of 7.6 billion is expected to reach 9.8 billion by 2050 (United Nations 2017). The variability in climatic conditions and exposure to drastic and intense climatic changes are alarming. The severe climate extremities can also reverse the previous efforts made to meet the hunger demands. This constant change is one of the leading contributors to food crisis of recent times. The profound effect of drought and temperature changes on food security and agricultural production is also distressing. The gradual rise in the sea temperature is leading to higher mobilization of harmful heavy metals, thereby facilitating the metals to enter the food chain causing major health concerns. In some countries high salinity caused deficits in agricultural production contributing to the ever-increasing food security (FAO 2018).

Rice is the staple food for most part of Asian population which is about 4.5 billion. In Asia, the production and consumption of rice is more than 90%. It is consumed by more than four billion people worldwide. Rice contributes to 20% of the global energy supply which is higher than maize (5%) and wheat (19%). One of the essential factors for global food security is balanced rice production and consumption (Food and Agricultural Organization (FAO) 2017). Rice consumption is statistically predicted to increase to 465 million metric tons by 2035 (Maclean et al. 2013, http://ricepedia.org). The rice cultivars which are cultivated presently account to 4.5 tons per ha production which are needed to be increased by at least 0.6 times to meet the demands. Rice cultivation is challenged with various environmental factors. These include various abiotic conditions like lack of optimum temperature, saline soil conditions, heavy metal contamination of soil, and lack of enough water for irrigation leading to drought conditions. The changing climatic condition is a major threat to the world food security and slows down the crop production required to meet the demands. This has led to the development of stress-adaptive crop varieties by scientists in order to increase the yield under unfavorable climatic conditions, although various factors have obstructed the progress like (1) complex nature of tolerance mechanism, (2) unclear understanding of tolerance inheritability, and (3) inefficient screening techniques (Lafitte et al. 2006).

In comparison to other crops, a large amount of water is required by rice plants for proper growth and development (Pandey and Shukla 2015). When the required amount of water is unavailable to the plants due to drought conditions, it results in reduction of the crop yields among the rice cultivars (Wassmann et al. 2009; Dixit et al. 2014). Physiological and biochemical processes are compromised in addition to various morphological changes under stressful environment. Changes in root and shoot length have been seen (Nahar et al. 2016b); as a consequence of water loss, stomatal closure takes place inhibiting the gas exchange disturbing the water potential and stomatal function. Shrinkage in leaf area followed by leaf rolling, necrosis, and inhibition of root elongation also occurs (Singh et al. 2012). Reduced photosynthesis and ion uptake and hampered nutrient delivery and metabolism lead to plant death (Jaleel et al. 2008; Farooq et al. 2008; Razmjoo et al. 2008).

Soil salinity is one of the leading threats to crop production worldwide (Flowers and Flowers 2005). Plant growth in the saline soil is problematic due to salt toxicity, altered nutrient metabolism, and reduced water absorption (Munns et al. 2006). Rice can be said to be salt sensitive, and a reduction in grain yield is seen on exposure of the rice plants to more than 1.9 to 3 dS/m (Grattan et al. 2002). Rice also has an advantage of being able to grow on standing water conditions which allows salt leaching from the topsoils (Abrol and Bhumbla 1978). Saline soil conditions adversely affect the growth and development of rice plant in the germinating, vegetative, and reproductive phases (Fujino et al. 2004; Roychoudhury et al. 2008; Paul and Roychoudhury 2019). Due to cost-effectiveness, direct seeding method is of high demand in Asian countries for crop improvement at the germination stage (Wang et al. 2011).

Rice generally grows in tropical or subtropical region, in temperatures ranging from 20° C to 40 °C (Sridevi Chellamuthu 2015). So, rice is a relatively cold-sensitive plant. The optimum temperature for cultivation of rice plants generally ranges from 25 °C to 35 °C, so even the rice that grows in winters or in high-altitude areas are prone to stress conditions in germination, vegetative, and reproductive stages. Various threshold temperatures are set for different growth stages, such as 10 °C for seedling stage, whereas 17 °C for reproductive stage, below which a variable degree of damage is seen depending on the growth stage of the plant (Yoshida 1981; Andaya and Mackill 2003; Xu et al. 2008; Ye et al. 2009).

Another factor which poses a serious threat to rice cultivation is contamination of agricultural field with varying concentrations of metals. These metals get gradually accumulated in the soil due to constant usage of fertilizers, insecticides, and pesticides (Ali et al. 2017; Yousaf et al. 2017). Understanding water conditions, the rice plants accumulate the metals present in the soil from different sources; these metals not only alter the soil chemical equilibria but also affects the growth and grain yield of the plants (Fan et al. 2017). The metals are transported from the roots to the upper parts of the plant, ultimately getting accumulated in the food grains, gradually making its move into the food chain, posing a threat to human rice consumption.

Different rice varieties have different responses to the abiotic stress. Some of the varieties are able to tolerate the stressful conditions, while some are not able to tolerate the stress; as a result the yield and development of these varieties are compromised. Rice varieties tolerant to the various abiotic stresses (mainly temperature, drought, salinity, heavy metals) will be able to bypass the adverse effects of the constant climate change without compromising the total rice production

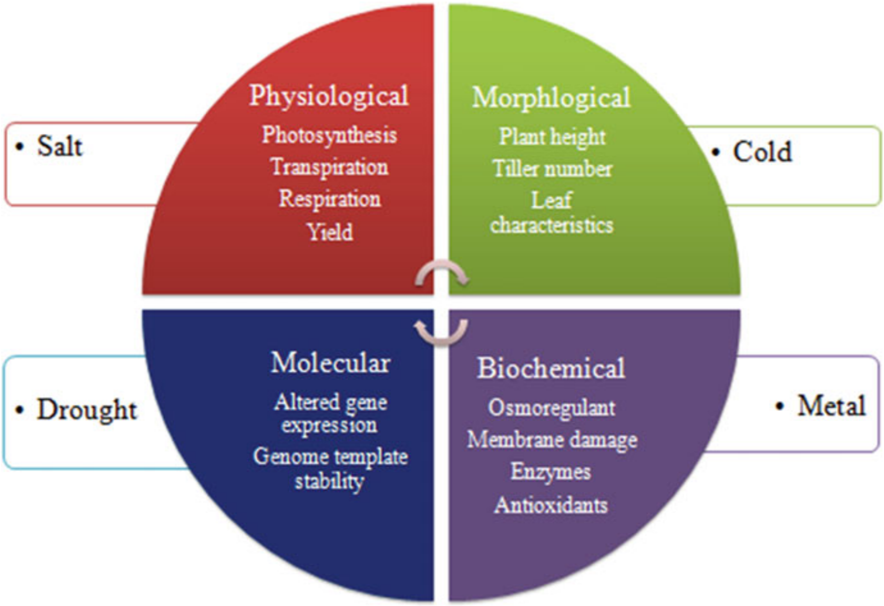

Fig. 1 Responses due to the different abiotic stress conditions

(Roychoudhury and Chakraborty 2013). Stress-tolerant high-yielding varieties (HYV) are of more importance for commercialization. There lies a basic difference between the stress-tolerant and stress-sensitive varieties. The understanding of this differentiation might be aided by genomic tools. Responses of several stress factors are represented in Fig. 1.

In the past decade, enormous progress has been made in rice genomics, which primarily aims at high-throughput gene identification, genomic analysis for pathways and gene networks that can be correlated with important agronomic and biological traits, and identification of novel genes by development of new genomic tools. More than 2000 genes have already been identified and cloned that have been reported to control agronomic trait of profound importance; the molecular biological mechanism behind this is also partially characterized (Li et al. 2018a, b).

Understanding and pinpointing the basis for differentiation of stress-tolerant and stress-sensitive varieties is a complex process, and it requires combination of various strategies together. Genetic repertoire enables identification of the alleles responsible for the extreme difference in responses toward stress; the genomic tools facilitate successful introgression of these alleles into the genome for further development (Sevanthi et al. 2019).

2 Drought Stress in Rice

2.1 Responses to Drought Stress

2.1.1 Major Morphological Responses

A variety of responses are monitored under drought stress; different morphological responses have already been identified which are reliable indicators of drought stress, which include leaf shrinkage, decrease in stomatal number, closure of stomata, and cell wall thickening in leaves (Anjum et al. 2011; Zokaee-Khosroshahi et al. 2014; Zaher-Ara et al. 2016). Reduced water potential in the soil reduces the leaf area, number, and size and ultimately decreases the leaf biomass, adversely affecting the plant growth and development. Severe and prolonged drought conditions also affect the shoot length of the plants (Abdullah et al. 2015; Farooq et al. 2009a, b). Exposure of plants to drought conditions at the preanthesis stage is reported to have profound effect on the grain number and grain yield of the plants (Dickin and Wright 2008). The ability of the plants to produce a distinct phenotype in response to the stress conditions is known as phenotypic plasticity. Rice plants are reported to exhibit phenotypic plasticity in the root and shoot for adaptation to the drought stress (Kadam et al. 2017). During drought stress, panicle length, tiller number, and grain weight per panicle are highly affected in rice (Muthurajan et al. 2011; Wei et al. 2017).

2.1.2 Physiological Responses

A variety of physiological responses are exhibited in response to drought stress in rice. It affects the leaf water potential, transpiration rate, and plant-water relations. The relative water content of stressed plants is greatly reduced (Farooq et al. 2009a, b; Fahad et al. 2017). To achieve the osmotic homeostasis, rice plants produce osmoregulants. These osmoregulants play a key role to maintain the stomatal conductance, leaf water volume, photosynthesis, and growth of the plants; stomatal closure and loss of water potential lead to downregulation of photosynthesis-related genes. Nitrogen metabolism is also hampered during drought stress (Naik et al. 2015). Also, decrease in nitrate reductase activity is also observed (Xu et al. 2015). Drought stress increases the tendency of photodamage in crops due to limited carbon dioxide availability. Loss of photosynthetic pigments is triggered by the deficits in water potential; it also hampers the activity of enzymes; thereby activation of photosystem II is compromised. Chlorophyll a:b ratio is decreased in drought-stressed plants (Jain et al. 2010). Drought stress affects the proper uptake and transport of important mineral nutrients like nitrogen, magnesium, calcium, and silicon, thereby decelerating the plant growth and development.

2.1.3 Biochemical Responses

A cascade of biochemical reactions occur as drought-induced stress responses in plants. Transduction of energy in the inner mitochondrial membrane or thylakoid membrane by electron transfer or redox reaction is a natural cellular metabolism process. Carbon metabolism through NAD(P)H and ATP is involved in the ETC which takes place in cellular organelles like mitochondria and chloroplasts. During drought stress conditions, there are alterations in the metabolic processes in these organelles. To combat the oxidative stress, a well-regulated system is required to coordinate the processes in the organelles, by maintaining the reversible redox cycles of proteins through thiol-disulfide exchange, activating the ROS-responsive gene-induced cell signaling cascade and ROS-antioxidant reactions, and regulating the phosphoproteins (Foyer and Noctor 2009; Suzuki et al. 2011). Under drought stress, harmful ROS is generated that damages the biomolecules like carbohydrates, lipid, nuclei acid, and protein eventually leading to oxidative stress (Gill and Tuteja 2010; Basu et al. 2010a, b). The elevated level of ROS is counterattacked by increased production of antioxidant enzymes like SOD, CAT, APX, GR, MDHAR, DHAR, GPX, and GST or nonenzymatic antioxidants like ASA, GSH, phenolic compounds, alkaloids, alpha tocopherols, and nonprotein amino acids. Several osmolytes are accumulated in the plant body in order to combat the oxidative stress. The accumulation of ROS is enhanced due to decreased proline production; this increases the plants sensitivity to stress conditions and hikes the oxidative damage (Miller et al. 2010). It is reported that drought stress condition results in oxidative stress which induces the activation of the antioxidant defense system; tolerant cultivars are reported to have enhanced antioxidant production in comparison to susceptible ones (Nahar et al. 2016a).

2.1.4 Molecular Responses

A vast range of molecular responses occurs in rice on the onset of drought stress conditions. Studies have shown numerous changes in gene expression pattern due to stress conditions; the expression patterns revealed that most of the genes induced by drought stress respond to ABA treatment (Osakabe et al. 2014: Fu et al. 2017; Du et al. 2018). There are four signal transduction pathways at the least, of which two are ABA-dependent and the rest are ABA-independent pathways. In the ABA-dependent pathways, ABA response element is the key regulator, whereas in ABA-independent ones, drought/dehydration response elements are the key players. Various transcription factors like MY, MYC, DRE/CF, ABF/AREB, NAC, and WRKY TFs are induced to transcribe the drought-responsive genes (Wang et al. 2011; Roychoudhury et al. 2013; Dey et al. 2017; Nahar et al. 2016b: Zhang et al. 2016).

2.2 Genomics in Drought Stress

2.2.1 QTL-Aided Genomics

Tolerance to drought stress is a highly complicated mechanism which is controlled by particular genes and loci on chromosomes called quantitative trait loci (QTLs). Variations in traits are associated with the QTLs. These genetic variations may be utilized for the development of drought-tolerant cultivars by screening under drought conditions or by mapping of the QTL using molecular marker-assisted selection (MAS). Various drought-tolerant QTLs have already been mapped in various crops,

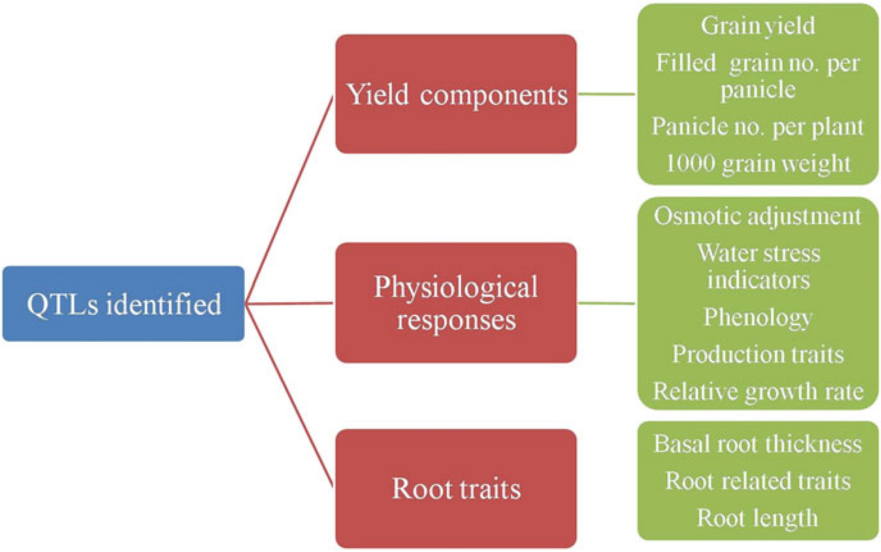

Fig. 2 QTLs of major traits related to drought stress in rice

and many of them have been done in rice (Shamsudin et al. 2016; Dixit et al. 2017). One of the important QTLs is Deeper Rooting 1 (DRO1); it has been shown to increase the root length and is proven to be beneficial for drought tolerance studies. It has been shown to result in significant improvement in grain yield specifically in rice cultivars with shallow roots (Uga et al. 2013). In contrast to conventional breeding, in marker-assisted breeding, a trait is selected on the basis of the marker associated with it. Introgression of regions involved with tolerance at various stages of growth; early reproductive stages are possible with identification of QTL and fine mapping studies. The MAS is highly advantageous as it provides opportunities to screen for plant with the desired genetic makeup; this can bypass the hideous process of field trials making it time- and cost-efficient. This helps in the selection of traits with high agronomic value. There are several reports of identification of QTLs associated with drought tolerance in rice and other cereal crops; three QTLs were identified by MAS, viz., qDTY2.2, qDTY3.1, and qDTY12.1, which were associated with reproductive phase drought stress, and the grain yield of the elite Malaysian rice cultivar MR219 was improved (Khan 2012; Shamsudin et al. 2016). A synergistic relationship among the QTLs is exhibited by different combinations of the QTLs which correspond to variations in the crop yields. A set of three QTLs, viz., qDTY3.1, qDTY6.1, and qDTY6.2, corresponding to grain yield under drought stress have shown to have high effect in the rice variety TDK1; such combination of QTLs has resulted in variations in yield and yield-related traits (Dixit et al. 2017). So far, qDTY3.1 is reported to be the most exploited QTL affecting the crop yield during drought stress followed by qDTY6.1 and qDTY6.2, respectively. QTLs for the main traits are schematically represented in Fig. 2.

2.2.2 Chromosome Regions Affecting a Trait (CRATs)

Analysis of various physiological and morphological parameters like grain number per panicle, yield, flag leaf characteristics, estimated sink size, and SPAD value at the heading, with chromosome segment substitution lines (CSSLs), can confer the function of chromosome regions affecting a trait (Madoka et al. 2008; Ujiie et al. 2012; Ujiie and Ishimaru 2013, 2014). Similar to genetic maps, the two varieties should exhibit significant phenotypic differences. This is statistically able to identify the chromosome regions affecting the traits on the genetic maps of the varieties (Ujiie et al. 2015).

2.2.3 Transgenics-Aided Genomics

The use of transgenics to produce improved crop varieties is a reliable approach to combat drought stress. It involves the modification of traits through the transference of desired genes that encode TFs, growth regulators, antioxidants, and enzymes involved in drought stress tolerance (Su and Wu 2004; Fahad et al. 2017; Ghneim-Herrera et al. 2017; Li et al. 2017; Liu et al. 2017). Isolation of stress-associated protein (SAP) from *Aeluropus littoralis* and overexpressing it in rice resulted in alleviation of leaf drying and rolling induced by drought stress; it also enhanced the biomass. Functional genomic tools have played a great role in identification of drought stress-responsive genes since quite some time. Drought-tolerant transgenic rice are being developed by gene transformation techniques. Isolation, characterization, and overexpression of stress-related genes in rice have shown tolerance to drought stress (Todaka et al. 2017; Selvaraj et al. 2017). Several important genes have been overexpressed in rice to provide drought tolerance in them (Table 1).

2.2.4 MicroRNA Technology

Regulation of gene expression by cleaving the target mRNA is done by microRNAs (miRNAs) which are small noncoding regulatory RNA. Modern advancements of technologies like proteomics and genome-wide gene expression have led to the identification of several genes involved in the drought stress response (Bartels and Sunkar 2005; Yamaguchi-Shinozaki and Shinozaki 2006). MiR393 with target gene Auxin transporter gene (OsAUX1) and a rice tiller inhibitor gene (OsTIR1) has shown to increase the tiller number and induce early flowering, thereby providing tolerance drought and salt stress (Xia et al. 2012). MiR396 with target gene growth-regulating factor 6(OsGRF6) gene has shown to improve the crop yield by regulation of the shape and structure of inflorescence (Zhou et al. 2010; Gao et al. 2015). MicroRNA amiRNA with target for DST gene, the loss of function of which causes closure of stomata and induces genes for ROS homeostasis providing tolerance to drought stress in DST amiRNA-overexpressing rice plants (Banerjee et al. 2016; Faisal et al. 2017).

Table 1 Some of the transgenes used for conferring drought stress tolerance in rice plants

Gene	Gene origin	Phenotypic feature	References
OsAHL1	*O. sativa* (upland rice)	Drought avoidance and drought tolerance	Zhou et al. (2015)
Osdrap1	*O. sativa*	Activation of stress-responsive genes that regulate water balance, redox homeostasis, and vascular development	Huang et al. (2018)
OsNAC14	*O. sativa*	Mediated drought tolerance by recruiting factors involved in DNA damage repair and defense response	Shim et al. (2018)
AtGolS2	*A. thaliana*	Increases relative water content, higher photosynthetic ability, greater recovery against stress	Selvaraj et al. (2017)
CaMSRB2	*C. annuum*	It plays an important functional role in chloroplast for conferring drought resistance in rice	Kim et al. (2014)
OsRab7	*O. sativa*	Production of osmolytes, antioxidants, and genes involved in abiotic stress response	El-Esawi and Alayafi (2019)
OsASR5	*O. sativa*	Regulation of ABA biosynthesis, acts like chaperone-like proteins and helps in prevention of stress-related protein inactivation	Li et al. (2017)
C4PEPC	*Zea mays*	Regulation of Ca signals and repression of ROS	Liu et al. (2017)
TIOsm	*Tripogon loliiformis*	Retention of higher water content, better membrane integrity, and improved survival rate	Le et al. (2018)
AtDREB1A	*A. thaliana*	Osmolyte production, increased spikelet fertility and grain yield	Ravikumar et al. (2014)
GSMT and DMT	*Aphanothece halophytica*	Dehydration and cold stress tolerance	Niu et al. (2014)
GDH	*Magnaporthe grisea*	Prevention of toxic accumulation of ammonia	Zhou et al. (2015)

3 Salt Stress in Rice Plants

3.1 Responses to Salt Stress

3.1.1 Morphological Responses to Salt Stress

One of the major abiotic stresses hampering rice health and production is salinity stress; due to the varying range of soil salinity conditions, rice is more vulnerable to salt stress (Flowers and Yeo 1989; Gao et al. 2007). Germination of seed is highly important to factors like total biomass and yield production along with other biological and physiological processes leading to the activation of embryo (Parihar et al. 2014). Salinity negatively affects the seed germination percentage and time required for seed germination (Kaveh et al. 2011).

Salinity stress has been reported to negatively affect the plant height. One of the most harmful consequences of salinity stress is stomatal closure which causes elevation of the leaf temperature and inhibition of shoot elongation (Rajendran et al. 2009; Siraul et al. 2009). The increasing concentration of soil salinity tends to interfere with the water potential and proper absorption of nutrients (Gain et al. 2004). Reports suggest that, although salinity stress negatively affects the shoot length, the root length is positively affected (Amirjani 2011); the root length was found to increase under salinity stress which also suggests the reallocation of photosynthetic materials to the root as an adaptation to limit biomass assimilation in the shoots (Riaz et al. 2019).

As a response to salinity stress, plant decreases the water and nutrient uptake, and the leaf area also decreases as an adaptation to decrease the salt stress. The salt stress conditions also lead to ion toxicity due to accumulation of sodium in the leaf blade and transpiration stream, especially in older leaves which cannot expand in adaptation; the younger leaves are able to expand in area and are able to dilute the ion toxicity conditions; consequently the older leaves die decreasing the photosynthetic efficiency resulting in highly reduced overall growth and development of the plant (Munns and Tester 2008).

Salinity stress hampers the plant height, biomass, and number of total tillers produced in the plants. Although the tiller numbers tend to decrease on increasing the salt concentration, yet it is reported to be a less sensitive factor compared to grain and panicle production. Saline conditions also reduce the fresh and dry biomass of the rice plant (Gain et al. 2004).

3.1.2 Physiological Responses to Salt Stress

A number of intermediate reaction cascade occur in rice plants in response to salt stress, ultimately leading to reduction in plant growth; the intermediate responses include changed Na^+/K^+ ratio, reduced photosynthetic activity, decreased stomatal conductance, elevated ROS generation, and increased production of antioxidants to fight the oxidative stress. Higher accumulation of Na leads to premature senescence in leaves by hampering the process of translation and disruption of enzyme activity (Munns 2002; Munns and Tester 2008; Carillo et al. 2011). Under prolonged exposure to stress (salinity, ionic, Na^+ and Cl^-), an alteration in enzyme production is coupled with swelling of cellular compartments that reduces energy production and transfer along with additional physiological changes (Fig. 3) (Larcher 1980; Cramer and Nowak 1992; Carillo et al. 2011). Diagrammatic representation is shown in Fig. 3.

Upon exposure to salinity stress, plants immediately reduce the stomatal aperture leading to decreased transpiration and carbon dioxide assimilation (Ashraf 2001; Romero-Aranda et al. 2001; Munns and Tester 2008). Important factors governing reduced photosynthesis include restricted photosynthetic electron transport system due to shrunken intercellular spaces. Uptake of nitrate by root is hampered leading to Cl^- toxicity and reduced photosynthetic rate (Iyengar and Reddy 1996). Under salt

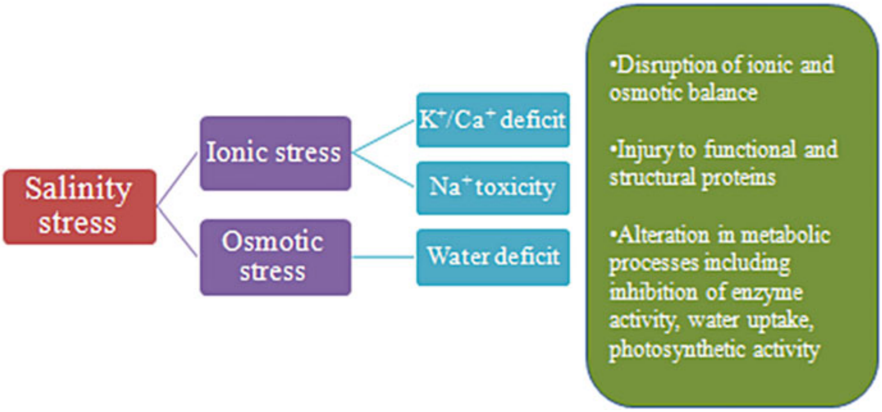

Fig. 3 Physiological changes in rice under salt stress

stress significant change in plant chlorophyll content indicated by chlorophyll fluorescence and membrane permeability are important markers for photosynthetic inhibition (Baker 2008; Ghosh et al. 2016).

3.1.3 Biochemical Responses to Salt Stress

The first phase of biochemical response to salt stress is generation of ROS. Plants combat the stressful effects of photoinhibitory condition by altering the metabolic pathways, xanthophyll pigment-aided heat dissolution, and transfer of electrons to ROS producing O_2 acceptor molecules (Apel and Hirt 2004). Electrolytic leakage during transfer reacts with O_2 under aerobic conditions to produce toxic ROS like superoxide, hydroxyl radical, and hydrogen peroxide (Lee et al. 2003). Salinity hampers the antioxidant-mediated removal of ROS (Allan and Fluhr 1997; Foyer and Noctor 2003; Carillo et al. 2011). The enzymatic antioxidant SOD scavenges and detoxifies the ROS; they are metalloenzymes that catalyze the conversion of H_2O_2 and O_2 and protect cells against oxidative stress (Lee et al. 2011a, b; Banerjee and Roychoudhury 2018). Reports suggest that reduction in SOD activity is observed under salinity stress in rice seedling; variation in responses is observed among different cultivars (Dionisiosese and Tobita 1998). However, a hike in SOD, APX, and GPX activity was reported in response to salinity stress, but contrasting response was seen in CAT activity (Mishra et al. 2013). Salinity stress resulted in increased CAT and APX activity in salt-tolerant rice genotypes and decreased in salt-sensitive rice genotypes; on the contrary POX enzyme followed the exact opposite trend in all the rice genotypes (Kibria et al. 2017).

3.1.4 Molecular Responses to Salt Stress

The salt sensors or receptors present at the plasma membrane perceive the salt stress signal. This signal activates the SOS pathway which regulates the expression of several transporter genes. Some of these transporters include K^+ inward rectifying channel (OsAKT1), K^+/NA^+ symporter (OsHKT1), K^+ outward rectifying channel

(OsKCO1), vacuolar Na^+/H^+ exchangers (OsNHX1–4), and vacuolar chloride channel (OsCLC1).

H^+-ATPases generate electrochemical gradient across the membrane which results in extrusion of Na^+ ions from the plant cells; this allows the Na^+/H^+ antiporters to simultaneously aid the H^+ ion movement along the gradient and movement of Na^+ out of the cytosol. OSA3, a H^+ATPase gene, is highly expressed in salt-tolerant mutant M-20, but it was not expressed in the salt-sensitive rice variety 77–170, which confers an important role of OSA3 in providing salt stress tolerance. A comparative study revealed a differential expression of the OsCLC1 gene among the genotypes under salt stress. In the salt-sensitive cultivar IR29, the expression of OsCLC1 gene was downregulated, whereas in the salt-tolerant cultivar Pokkali, higher expression was reported in roots, suggesting the relation of the CLC1 gene to salt tolerance (Diedhiou and Golldack 2006). Under salt stress the expression of OsAKT1 was highly repressed, and the inward influx of K^+ was significantly hampered in the root protoplasts due to salt stress, conferring the role of OsAKT1 as a dominant salt-sensitive K^+ uptake channel (Fuchs et al. 2005).

3.2 Genomics in Salt Stress

3.2.1 QTL-Aided Genomics

Significant progress has been made in the area of functional genomics as the whole genome sequence of rice is available; it has contributed immensely in the identification of new genes and pathways associated with salt tolerance. Transgenic- and genomic-aided breeding approaches are reliably and socially acceptable approaches. The more the number of germplasms studied, the greater is the genetic diversity associated with the studied traits. For successful breeding choice of germplasm is highly critical. The tolerance to salt stress is a complicated phenomenon as it requires combination of various pathways which are interdependent or independent. A salt stress-tolerant rice cultivar is expected to possess the desired trait of maintaining lower NA+/K+ and higher biomass. Conventional breeding aided with the use of molecular markers associated with the tolerance trait being introgressed is known as marker-assisted selection (MAS). Variation in the allele associated with the gene of interest can serve as novel genomic source for MAS and provides high possibility for designing of reliable molecular marker for salt tolerance which can be easy and cheap as well (Reddy et al. 2017). One of the major QTLs for salt tolerance is *saltol* which has already been transferred to seven popularly commercialized rice varieties like Pusa 44, ADT45, Sarjoo 52, CR1009, PR114, Gayatri, and MTU1010 (Singh et al. 2016). Tiwari et al. (2016) reported a newer method for identification of QTLs related to salt tolerance by analysis of bulk segregants of biparental recombinant inbred lines; this was associated with the reproductive phase in the rice plants. Diversity analysis was performed utilizing 376 single nucleotide polymorphism (SNP) markers designed genome wide, across 107 germplasm accessions; allelic variation was also characterized against the QTL saltol (Rahman et al. 2016). The seven landraces (Kutipatnai, Akundi, Kalarata, Ashfal, Jatai Balam, Chikirampatnai, capsule) maintain lower Na^+/K^+ ratio in leaves due to lower Na^+ and higher K^+ accumulation. Reports suggest that the N^+ concentration is lower in plants that are tolerant to salt stress; they also restrict the transport of Na^+ to shoot. Mutation breeding program can also produce salt-tolerant high-yielding varieties of rice (Das et al. 2014). Isolation and characterization of the mutant rst1 showed its higher tolerance to salt stress, with higher shoot mass and chlorophyll content, lower membrane damage, and leakage of electrolytes under salinity stress conditions. Gene expression analysis of the mutant rice d1(RGA1) showed its key role in providing tolerance to several abiotic stresses including heat, cold, drought, and salinity (Jangam et al. 2016). The major QTL saltol is identified on rice chromosome 1 and is derived from Pokkali, it maintains Na^+/K^+ ion homeostasis, whereas SKC1 (OsHKT1) is derived from Nona Bokra (Thomson et al. 2010; Platten et al. 2013). Novel salt tolerance-associated SSR markers were identified from rice (Molla et al. 2015). SNPs targeting yield and stress-specific genes in rice were reported (Kurokawa et al. 2016). A SNP array consisting of 51,478 markers was developed (Chen et al. 2014).

3.2.2 Transgenics in Salt Tolerance

This approach involves the improvement of present traits by the introduction of transgenes conferring the desired trait. OsPP1a overexpression lines were shown to exhibit enhanced tolerance to salt stress; OsNAC5 TFs and SnRK1a protein kinase expression were also upregulated (Liao et al. 2016). The vacuolar Na+/H+ antiporter gene was constitutively overexpressed, the gene was derived from Pokkali, and it conferred considerable salt tolerance to the rice plants (Amin et al. 2016). SIDP361 can be efficiently used as a tool for genetic crop improvement of the salt tolerance trait in rice (Li et al. 2016). The photosynthesis and antioxidant defense system can be maintained by the rice SUV3 gene which functions as DNA-RNA helicase, conferring salt tolerance in rice plants (Sahoo et al. 2014). The PDH45 gene is able to confer salt tolerance in rice plant by limiting Na + concentration in rice shoots (Nath et al. 2016). The summary of some of the genes used for transgenic study is represented in Table 2.

3.2.3 Whole Genome Expression Profiling

Genome-wide expression analysis in cultivars or varieties of contrasting response to salt stress might be helpful in identification of pathways, genes, and gene families related to salt stress (Kawasaki et al. 2001; Walia et al. 2005, 2007). Two rice genotypes, one tolerant (FL478) and one sensitive (IR29), were taken into account; it was reported that induction of two distinct groups of genes occurred during salt stress conditions; during the vegetative phase, the flavonoid biosynthesis pathway-related genes were highly upregulated in the sensitive cultivar, whereas the cell wall-related genes were induced in both the cultivars (Walia et al. 2007). In the sensitive rice genotypes, the expression of many cation transporters was upregulated except SKC1 (Ren et al. 2005), the expression of which was found to increase in the root of salt-tolerant lines on increasing salt stress (Walia et al. 2007).

Table 2 Some of the transgenes studied for conferring salt stress tolerance in rice plants

Salt stress-responsive components	Genes	References
Aquaporin	*OsPIP1;1*, *OsTIP2;1*, *OsTIP2;2*	Katsuhara et al. (2003) and Sakurai et al. (2008)
Hormones: Abscisic acid, brassinosteroid	*OsAP2–39*, *OsPSY1*, *OsDMI3* *OsGSK1(BIN2)*, *OsBRI1*	Yaish et al. (2010), Shi et al. (2012), Koh et al. (2007) and Zhao et al. (2013)
Calmodulin (CaM)	*OsCam1–1*, *OsCML4*, *OsCML8*, *OsCaM*	Saeng-ngam et al. (2012)
Intermediate filament	*OsIF*	Soda et al. (2018)
Zinc finger protein	*OSISAP1*	Mukhopadhyay et al. (2004)
Protein kinases: Ca21-dependent protein kinase, mitogen-activated protein, kinases	*OsCDPK7*, *OsCPK4/12*, *OsMAPK5*, *OsMAPK44*, *OsMKK6*, *OsMPK4*	Asano et al. (2012), Campo et al. (2014), Chen et al. (2013), Diedhiou et al. (2008), Jeong et al. (2006), Kim et al. (2003), Kumar and Sinha (2013) and Lee et al. (2011a, b)
Ion channels/transporters/ ion exchangers	*OsCLC1*, *OsNRT1;2*, *OsHAK5*, *OsHAK1*, *OsHAK21*, *OsAKT1*, *OsNHX1*, *OsCAX1*, *OsHKT1;4*, *OsHKT1;1*, *OsHKT2:1*, *OsHKT1;5*, *HKT2;3*, *OsSOS1*	Diedhiou and Golldack (2006), Yang et al. (2014), Chen et al. (2015), Shen et al. (2015), Ahmad et al. (2016), Wang et al. (2012), Kumar et al. (2013), Bassil et al. (2012), Amin et al. (2016), Cotsaftis et al. (2012), Takagi et al. (2015), Mishra et al. (2016), Suzuki et al. (2016) and Hoa and Thanh (2017)
Late embryogenesis abundant (LEA)	*Rab16A*	Ganguly et al. (2012)
Transcription factors	*OsDREB1A*, *OsDREB1F*, *OsDREB2A*, *OsNAC/SNAC*, *OsNAC6*, *OsMYB3R-2*, *OsWRKY13*, *OsbZIP23*, *OsABI5*, *DST*, *ONAC*, *OsABF2*, *OsNAC10*, *OsNAC5*, *OsbZIP71*, *OsMYB2*, *OsMYB91*, *OSISAP1*, *OsTZF*, *OsTZF1*, *OsWRKY42*, *OsERF922*, *OsNAP*, *ONAC022*, *OsRAB1*, *SERF1*, *OsbHLH001*, *OsbHLH*	Dubouzet et al. (2003), Wang et al. (2008), Hu et al. (2006), Dai et al. (2007), Nakashima et al. (2007), Qiu et al. (2008), Xiang et al. (2008), Zou et al. (2008), Huang et al. (2009), Zheng et al. (2009), Hossain et al. (2010), Jeong et al. (2010, 2013), Takasaki et al. (2010), Mallikarjuna et al. (2011), Song et al. (2011), Liu et al. (2012), Redillas et al. (2012), Yang et al. (2012), Jan et al. (2013), Schmidt et al. (2013), Chen et al. (2014), Liang et al. (2014), Yoshida et al. (2014) and Hong et al. (2016)
Antioxidant enzymes	*OsIM1*, *PDH45*, *SERF1*, *OsCatC*, *OsAPx2*, *catalase*	Kong et al. (2003), Wutipraditkul et al. (2011), Guan et al. (2012) and Schmidt et al. (2013)

4 Cold Stress in Rice Plants

4.1 Responses to Cold Stress

4.1.1 Morphophysiological Responses

Exposure of rice plants to low-temperature stress not only affects the growth and development and crop yield but also hinders the natural process of geographical diversification (Xin and Browse 2001). Loss of crop yield due to cold stress is a serious concern worldwide (Peyman and Hashem 2010). Temperature plays a crucial role in the seed germination of rice. Retardation in germination rate is seen in temperature lower than favorable temperature range required for seed germination which lies between 18 °C and 33 °C. Cold temperature causes disruption in the natural imbibition process causing the solutes to come out of the seeds. Temperature less than 10 °C can cause unsuccessful seed germination.

Low temperature affects the rice plants during all the stages of plant growth and development; the responses vary differently at different phases of the plant growth. Exposure of the plants to low temperature leads to growth retardation and loss of seedling vigor (Ali et al. 2006); cold stress also causes reduction in tiller number (Shimono et al. 2002) and also causes tissues necrosis leading to death of the plant (Farrell et al. 2006; Baruah et al. 2009; Fujino et al. 2004). It also delays the maturation of the crops (Alvarado and Hernaiz 2007). The chlorophyll a and b contents reduce drastically under cold stress (Aghaee et al. 2011). *Japonica* varieties, reported to be more tolerant to cold stress, were found to maintain a higher level of chlorophyll content than *indica* rice cultivars in response to cold stress. The photosynthetic activity of plants is severely affected by low temperatures (Mohabbati et al. 2013; Banerjee and Roychoudhury 2019). Among the other organelles, chloroplast is severely and rapidly affected by cold stress, and inhibition of chlorophyll synthesis is also associated with the imbalance in PSII, due to alterations in chlorophyll antenna complexes (Ensminger et al. 2006; Habibi et al. 2011).

Low temperature causes a number of anomalies in the reproductive phase of the rice plants; it inhibits the ripening of the pollen grains, also stops the emergence of the anthers from the spikelets, inhibits the pollen shedding, and causes unsuccessful germination of the pollens after they reach stigmas (da Cruz et al. 2013). The microspore development is impaired that leads to the production of sterile pollens and sterile spikelets (Satake and Hayase 1976). Male sterility is most commonly caused during exposure to cold stress during the young microspore stage (Yoshida 1981). The final crop yield is greatly hampered due to cold stress and also results in spikelet degeneration and incomplete panicle exertion (Shimono et al. 2007; Ye et al. 2009).

4.1.2 Biochemical Responses to Cold Stress

Changes in a number of responses are accompanied as a response to cold stress including alterations in the electrolytic leaking, proline contents, malondialdehyde contents, and total glutathione content (Tian et al. 2011; Zhang et al. 2011; Yang et al. 2012). There are differences reported among the *japonica* and *indica* varieties with respect to changes in biochemical responses, *japonica* varieties tend to exhibit lesser electrolytic leakage in comparison to the *indica* varieties, whereas the *indica* varieties show higher accumulation of stress parameters like MDA, proline, and GSH (Kim and Tai 2011). The differential biochemical response shown by the two varieties can be utilized to determine the varieties with higher degree of cold tolerance; as ROS generation is linked with the membrane damage-induced MDA production, analysis of MDA content can also help in the identification of transgenic lines with higher degree of ROS signaling-mediated cold tolerance.

Changes in antioxidant enzymes like POD, SOD, CAT, and APX can also be analyzed to determine cold tolerance potential of different cultivars in rice. Higher generation of antioxidant enzymes is seen in transgenic lines in comparison to the wild-type plants which show their higher tolerance to cold stress conditions (Bonnecarrère et al. 2011; Huang et al. 2009). The activities of important antioxidant enzymes like OsPOX1, APXa, and OSTrx23 increase in order to maintain the functionality of other genes to provide cold stress tolerance (Kim et al. 2011; Sato et al. 2011; Xie et al. 2012). Cold stress also triggers the accumulation of various soluble sugars like sucrose, trehalose, glucose, fructose, and raffinose; these sugars play the role of compatible solutes and protect the rice plants from freezing dehydration damage by acting as osmoprotectants (Nagao et al. 2005; Shao et al. 2007; Yuanyuan et al. 2009). Some of the metabolites like trehalose, raffinose, and stachyose can be used as indicators for the evaluation of cold tolerance potential of rice plants (Morsy et al. 2007). As discussed before, cold stress also triggers the accumulation of proline which not only protects the cellular enzymes from denaturation but also serves as a source for carbon and nitrogen (Shah and Dubey 1997).

4.1.3 Molecular Responses to Cold Stress

OsSAP8 gene of SAP gene family encodes for a cytosolic zinc finger protein that mediates various cold stress-induced signal transduction (Kanneganti and Gupta 2008). The expression of OsAsr1 (ABA-responsive gene) is also induced that provides increased tolerance to cold stress (Kim et al. 2009). ABA is a phytohormone which is not only associated with the growth and development of the plant but also provides adaptive measures against various environmental stress factors (Hossain et al. 2010). Under cold stress ABA production leads to the initiation of ABA signaling cascade which induces the expression of ABA-responsive genes via the cis-acting ABA response elements (ABRE) and the ABRE- transcription factor bZIP (Hossain et al. 2010).

To provide cold tolerance, NACRS-containing genes are expressed, by the OsNAC-transduced ABA signaling through ABRE in its promoter (Fig. 4) (Nakashima et al. 2007, 2012; Song et al. 2011). The cold stress activates MAPK signaling cascade, which is triggered by the increase in membrane rigidity and alteration in ion conductance within cells (Sangwan et al. 2002).

The entry of calcium ions into the cytosol during cold stress induces the Ca^{2+}-mediated channels which are activated by membrane rigidification, ligands, or membrane stimuli (Chinnusamy et al. 2006). This in turn activates the DREB-CRT/DRE (dehydration-responsive element-binding proteins-C-repeat/

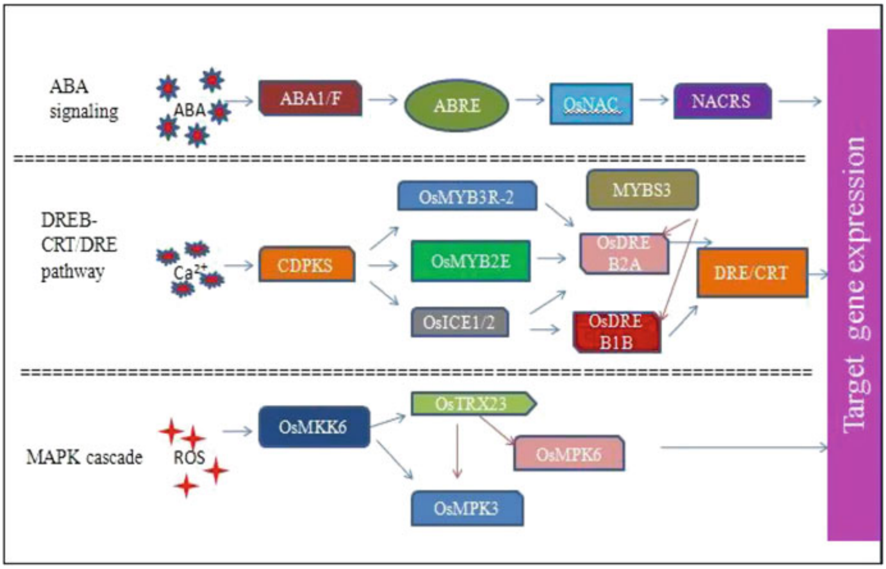

Fig. 4 Mechanisms of signaling in rice in response to cold stress; ABA signaling is activated by the accumulation of ABA and DREB-CRT/DRE pathway initiated by calcium; ROS generation leads to the induction of MAPK cascade

dehydration-responsive elements) pathway in rice plants, which is highly important as a cold stress tolerance mechanism. ROS accumulation also induces the expression of various cold-responsive genes and cold-responsive signaling cascade via OsMKK6-OsMPK3 (MAPKK-MAPK) pathway (Xie et al. 2009). Schematic representation of the different signaling pathways is given in Fig. 4.

4.2 Genomics in Cold Tolerance

4.2.1 QTL-Assisted Genomics

With the new achievements being made in the field of molecular biology, efforts are being made for the identification of new QTLs and genes involved in the cold tolerance of rice plants. Till date, more than 270 QTLs have been identified in rice, conferring tolerance to cold stage at different stages of rice growth and development. The stability for the majority of these identified QTLs is a factor, like five and six QTLs were identified at seedling stage under a constant 9 °C and 25/9 °C (day/night) at laboratory conditions, respectively, but in both the cold environments, only one QTL was found to common and constant (Andaya and Mackill 2003). Four QTLs were identified on chromosome numbers 1, 6, 9, and 12 at seedling stage, for providing cold stress tolerance under 9 °C cold water irrigation conditions made in tank; five QTLs were identified on chromosome numbers 7, 8, 9, 11, and 12 when exposed to cold stress in low-temperature growth chamber; only two QTLs, qCTS-9 and qCTS-12, were common to both the conditions. Most of the QTLs are unstable at different environmental conditions, listing behind only few stable QTLs. Instability of these QTLs might be related to the difference in physiological conditions at different developmental stages at variable environmental conditions. More than 30 main effect QTLs were reported for cold tolerance.

4.2.1.1 QTLs at Germination Stage

QTLs specific for germination at low temperature showed that it is a polygenic trait; more than 60 QTLs were identified for this (Sheng et al. 2001; Miura et al. 2001; Fujino et al. 2004; Zhang et al. 2005; Chen et al. 2006; Ji et al. 2008; Jiang et al. 2006; Wang et al. 2011; Li et al. 2013; Satoh et al. 2016; Yang et al. 2018). Most of the identified QTLs were positioned on the chromosomes 2, 3, 7, and 8. Among 30, the main effect QTLs account to only eight, of which only three were utilized for fine mapping, namely, LTG3–1 (Fujino et al. 2008), qSV1, and qSV-5c (Xie et al. 2014).

4.2.1.2 QTLs at Vegetative Stage

Higher than 170 QTLs have been identified conferring to cold stress tolerance at the seedling stage. These QTLs are positioned throughout all the chromosomes, majorly on chromosome numbers 1, 2, 6, 8, and 11. Among them, only a few QTLs conferring to main effect were identified, which were common with differential marker intervals. Of the 13 QTLs for seedling, stress tolerance positioned over the chromosomes 1, 2, 4, 6, 8, 10, 11, and 12; only a single main effect QTL, qCTS-12, located on chromosome 12 at marker intervals of 35.3–38.4 cM was identified; later on, this QTL was mapped (Andaya and Tai 2006). Another major QTL of seedling stage, qCTS-2, was derived from the cross between a *japonica* variety and an *indica* variety (AAV002862, Zhenshan97B) (Lou et al. 2007). Another major QTL qCsst1 1 located on chromosome number 11 was identified (Koseki et al. 2010). In a RIL population study involving a cross of cold-tolerant *japonica* cultivar Jinbubyeo and cold-sensitive *indica* cultivar BR29 (Kim et al. 2014), six QTLs including two QTL combinations (qSCT1 and qSCT11) were identified. These QTLs were fine mapped and analyzed; qSCT1 was found to be related with calmodulin-binding transcription activator on chromosome 1. Four molecular markers significantly related to electrolyte leakage, and one marker associated with photoinhibition (PSII) was also identified (Bonnecarrere et al. 2015).

4.2.1.3 QTL at Reproductive Stage

In comparison to the germination and seedling stage, QTLs conferring cold stress tolerance in the reproductive phase are positioned mainly on chromosomes 3, 4, 6, 10, and 12. The traits controlled by these QTLs are highly associated with the seed setting rate. Approximately 40 QTLs conferring to cold tolerance during the reproductive stage have been reported. A first few QTLs associated with this stage to be mapped were chromosomes 3 and 4 (Saito et al. 1995). Later the two QTLs, Ctb-1 and Ctb-2, were identified on chromosome 4; it was reported to govern the spikelet fertility under cold stress conditions (Saito et al. 2001). Ctb-1 was fine mapped, and it was found to be associated with the putative gene encoding F-Box protein of E3 ubiquitin ligase complex (Skp1) (Saito et al. 2010). Eight QTLs including QTLs with higher contribution to cold stress tolerance, qCT-7 located on chromosome 7, the ones associated with culm length (qCL-1) on chromosome 1, the ones related to heading, qHD-3-2 on chromosome 3, and qHD-6 on chromosome 6 were mapped across the DH lines of Akihikari/Koshihikari. The QTLs associated with the booting stage of cold tolerance, namely, qCTB2a and qCTB3, were also mapped using RIL population between M202 and IR50 (Andaya and Mackill 2003). The three QTLs qPSST-3, qPSST-7, and qPSST-9 were identified as main effect QTL on chromosomes 3, 7, and 9, and they were reported to be associated with spikelet fertility under cold stress. Reports suggest that a strong correlation exists between cold tolerance QTLs and anther length QTLs; the amount of pollen produced is a significant part of cold tolerance mechanism at the reproductive stage (Saito et al. 2001).

4.2.2 Transgenics in Cold Stress

Several genes have been overexpressed to know the related functionality of the gene. Identification of cold stress-responsive genes is also done by screening of T-DNA-tagged rice plants exposed to cold stress, and identification of several genes was done in this manner like OsRLK1 (a putative leucine-rich repeat-type receptor-like protein kinase), OsDMKT1 (a putative demethylmenaquinone methyltransferase), and OsGSK1 (glycogen synthase kinase3-like 1) (Lee et al. 2004a, b; Koh et al. 2007).

Transgenic rice plants overexpressing OsDREB1 or AtDREB1 showed higher tolerance to several abiotic stresses; however growth retardation was seen in these transgenic lines (Ito et al. 2006). Later, OsDREB1F gene conferred increased cold tolerance to the transgenic rice plants without any negative impact on plant growth. Maize CBF gene (ZmCBF3) was overexpressed in rice plants, the resultant transgenic lines were cold tolerant, and no yield penalty was observed on full maturation (Xu et al. 2011). Overexpression of OsCOIN transcription factor resulted in cold-tolerant transgenic lines that produced higher levels of proline post cold treatment as an adaptive measure (Liu et al. 2007). Overexpression of OsZFP245, a zinc finger protein, also showed higher tolerance to cold stress (Huang et al. 2009). Transgenic lines also exhibit higher enzymatic activities of superoxide dismutase and peroxidase than wild-type plants on exposure to cold stress. Many genes that were overexpressed showed higher tolerance in the transgenic lines including the SNAC2 gene, OsNAC5, and OsMYB3R-2 (Hu et al. 2008; Song et al. 2011; Ma et al. 2009). A summary of transgenes studied for cold stress tolerance is represented in Table 3.

Table 3 Some of the transgenes studied for conferring cold stress tolerance to rice plants

Gene	Gene origin	Phenotypic feature	References
OsDREB1	*O. sativa*	Tolerance to abiotic stress and elevated levels of osmoprotectants	Ito et al. (2006)
AtDREB1	*A. thaliana*	Elevated osmoprotectants and soluble sugars	Ito et al. (2006)
OsDREB1F	*O. sativa*	Enhanced tolerance to cold, drought, and salinity	Wang et al. (2008)
ZmCBF3	*Zea mays*	Increased survival rates and reduced membrane damage and relative conductivity	Xu et al. (2011)
OsCOIN/maize ubiquitin	*O. sativa*	Enhanced cold tolerance and cellular proline	Liu et al. (2007)
OsZFP245/ CaMV 355	*O. sativa*	Elevated expression of rice pyrroline-5-carboxylatesynthase, proline transporter genes, enhanced activity of antioxidative enzymes	Huang et al. (2009)
OsZFP182/ CaMV355	*O. sativa*	Increased accumulation of proline and free sugars	Huang et al. (2012)
AISAP/CaMV 355	*Aeluropus littoralis*	Enhanced photosynthetic rates, restored leaf gas exchange rates	Ben-Saad et al. (2012)
SNAC2/maize ubiquitin	*O. sativa*	Tolerance to abiotic stress factors, lower cell membrane permeability	Hu et al. (2008)
OsNAC5/ maize ubiquitin	*O. sativa*	Enhanced production of osmoregulants, lower membrane damage and H_2O_2 production	Song et al. (2011)
OsMYB3R-2/ maize ubiquitin	*O. sativa*	Increased proline accumulation and higher mitotic index	Ma et al. (2009)
OsMYBS3/ maize ubiquitin	*O. sativa*	Increased tolerance to cold stress	Su et al. (2010)
OsMYB2/ maize ubiquitin	*O. sativa*	Increased tolerance to cold, drought, and salinity stress	Yang et al. (2012)
LeTERF2/ CaMV355	*O. sativa*	Increased accumulation of osmoregulant, decreased MDA, ROS production	Tian et al. (2011)
OsMAPK5/ CaMV 355	*O. sativa*	Increased tolerance to several biotic and abiotic stresses	Xiong and Yang (2003)
TaWFT1 and TaWF2/CaMV 355	*Triticum aestivum*	Enhanced cold tolerance and accumulation of fructans	Kawakami et al. (2008)
OsAsr1/maize ubiquitin	*O. sativa*	Enhanced cold tolerance and twofold higher photosynthetic efficiency	Kim et al. (2009)
OsPR3/maize ubiquitin	*O. sativa*	Higher cold tolerance, protected cell wall integrity and cellular structure of mesophyll cells	Gothandam et al. (2010)
OsRAN2/ maize ubiquitin	*O. sativa*	Enhanced cold tolerance by maintenance of cell division, decreased no. of cells with intranuclear tubulin	Chen et al. (2011)
OsTPS!/CaMV 355	*O. sativa*	Enhanced cold stress tolerance with increased proline levels	Li et al. (2011)

(continued)

Table 3 (continued)

Gene	Gene origin	Phenotypic feature	References
OsOVP1/maize ubiquitin	*O. sativa*	Enhanced tolerance with decreased MDA and increased proline levels	Zhang et al. (2011)
OsAPXa/ E0082	*O. sativa*	Higher spikelet fertility, lower ROS and MDA generation	Sato et al. (2011)
OsGST/maize ubiquitin	*O. sativa*	Reduced days required for germination	Takesawa et al. (2002)
TaABA8’OH/ OsG6B	*Triticum aestivum*	Reduced sterility percentage	Ji et al. (2011)
COLD1	*O. sativa*	Tolerance to chilling stress	Ma et al. (2015)
CTB4a	*O. sativa*	Enhanced cold tolerance, seed setting, crop yield	Zhang et al. (2017)
OsSAP16	*O. sativa*	Enhanced germination at low temperature	Wang et al. (2018)
ICE1	*A. thaliana*	Lower mortality rate under cold stress and increase in proline accumulation	Xiang et al. (2008)
OsTMP14	*O. sativa*	Increased tolerance to cold stress	Liao et al. (2017)

5 Metal Stress in Rice Plants

5.1 Responses to Metal Stress

5.1.1 Physiological Responses to Metal Stress

The major events associated with the accumulation of metals within the plant body comprise of metal adsorption on the roots, uptake of metals from the soil, compartmentalization and sequestration of the metals, loading of the xylem, and transportation to the aerial parts of the plant body and storage in the tissue. Multiple factors influence the metal uptake process like nature of the rhizosphere, soil characteristics, and microbial pool within the rhizosphere (Tak et al. 2013). The *indica* and *japonica* rice varieties show differences in metal accumulation patterns; the *indica* cultivars tend to accumulate more metals in comparison to the *japonica* cultivars (Uraguchi et al. 2009). Manifestations of heavy metal toxicity first appear on younger leaves as dark green rib formation. Higher toxicity causes the leaves to turn yellowish and gradually white. High Zn concentration in soil causes plant death 10 days post germination (Silva et al. 2014). Exposure to heavy metals (Cu, Pb, Zn, Cd) causes retarded germination and plant growth. The heavy metals are reported to interact among themselves; Pb stress results in increased accumulation of Cd and Cu in the shoots and roots (Fahad et al. 2019). High Ni^{2+} accumulation leads to chlorotic and necrotic tissue development in rice plants (Samantaray et al. 1997). Pb toxicity impairs normal growth and development in rice and also reduces the crop yield

(Ashraf et al. 2015). High Cd accumulation leads to structural changes in the leaf and root and disrupts the photosynthetic machinery (Aina et al. 2007; Anjum et al. 2015). Lead retards the seed germination and decreases root/shoot ratio and fresh and dry weight in rice (Sethy and Ghosh 2013).

5.1.2 Biochemical Responses to Metal Stress

High Ni toxicity not only disturbs the nutrient homeostasis but also disrupts the H-ATPase of the plasma membrane by interfering with the lipid composition (Ros et al. 1992). The first line of defense for heavy metal toxicity is production of ROS through Haber-Weiss reaction; ROS generation leads to oxidative stress (Wojtaszek 1997; Mithöfer et al. 2004; Shahzad et al. 2018; Fahad et al. 2016a, b, c, d). The second line of stress caused by the metal toxicity includes disturbance in nutrient homeostasis (Dong et al. 2006) and disturbs the electron transport chain (Quadir et al. 2004), and it also interferes with antioxidant-mediated defense response.

Lipid peroxidation and biomembrane disintegration is another manifestation (Türkan et al. 2005). The oxidative stress is elevated in rice seedlings due to cadmium stress, which is also accompanied by increase in ROS production, MDA accumulation, and electrolyte leakage (Srivastava et al. 2014; Yuet Ping et al. 2013). Metal toxicity is accompanied with structural changes in roots and leaves, and photosynthetic machinery is also disintegrated (Aina et al. 2007). High cadmium levels induce regulation of many genes (Lee et al. 2013). Cadmium stress results in high accumulation of thiobarbituric acid reactive substance (TBARS), accompanied by hampered seed germination and decreased shoot growth, water content, and biomass. Several proteins are involved in processes like antioxidant activation, protein biosynthesis, germination, and detoxification (Ahsan et al. 2007a, b; Roychoudhury et al. 2012). Lead toxicity is found to decrease the level of chlorophyll, carotenes, nitrogen, and protein contents (Chatterjee et al. 2004), but enzymatic activities of acid phosphatases, ribonucleases, and catalases were elevated. The toxicity also caused alteration in the thylakoid membrane lipid composition (Stefanov et al. 1995); restricted chlorophyll biosynthesis resulted in highly decreased uptake of Fe and Mg (Burzynski 1987).

5.1.3 Molecular Responses to Metal Stress

The molecular responses are mediated through a two-component signaling system that comprises of a membrane-bound receptor with an extracellular domain that can act as a site for ligand binding, an intracellular kinase domain, and a transmembrane domain. On perception of a signal by the extracellular sensor domain, autophosphorylation of the cytoplasmic histidine domain takes place; an aspartate receiver receives the phosphoryl group which can be a part of a sensor protein. The sensors can now interact with a downstream mitogen-activated protein kinase (MAPK) cascade or can directly phosphorylate targets (Xiong and Zhu 2002). The roots are the first plant parts that come in contact with heavy metals, and root cell walls have been reported to contain the maximum amount of heavy metals when seen under electron microscope (Arduini et al. 1997). In response to cadmium stress, several genes are activated, leading to signal transduction cascade activation

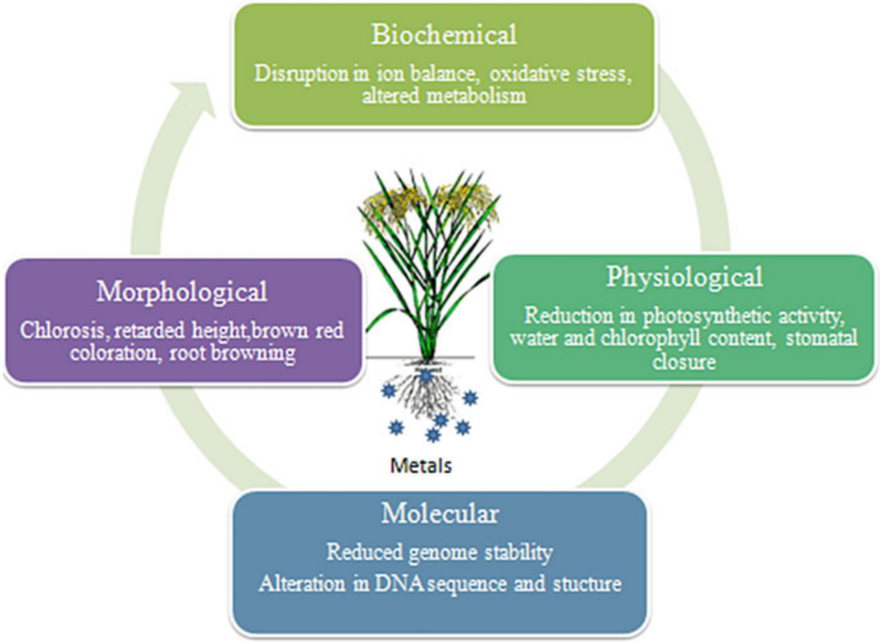

Fig. 5 Metal stress-induced responses in rice plant

responsible for the regulation of differential gene expression. Mitogen-activated protein kinase (MAPK) pathways that involve the activation of three protein kinases in a sequential manner represent a mechanism of signaling, and these include MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK (Schaeffer and Weber 1999; Widmann et al. 1999). MAPKKKs lead to phosphorylation of MAPKKs on the Ser/Thr residues. After the phosphorylation and activation of MAPKKs, MAPKs are phosphorylated on Thr and Tyr residues. Phosphorylation activates these enzymes. In addition to the transcription factors, the MAPKs are able to phosphorylate and activate a variety of substrates in different cellular compartments. Exposure of plants to cadmium stress enzymatically activates several enzymes of MAPK family (Jonak et al. 2004). The cascade of reactions involving phosphorylation and activation is reported to be involved in heavy metal stress-induced signaling to the nucleus (Fig. 4). In response to heavy metal stress, the second messenger calcium ions and calmodulin participate in the signaling (Suzuki et al. 2001). In response to cadmium stress, the calcium content of cells undergoes an increase (DalCorso et al. 2008) which in turn causes the calmodulin-like proteins to interact with calcium ions; this binding to calcium ions changes the conformation of calmodulin proteins that in turn regulate gene, transportation of ions, and tolerance to stress including response to cadmium (Yang and Poovaiah 2003). The various responses of metal stress are diagrammatically represented in Fig. 5.

5.2 Genomics Heavy Metal Stress Tolerance

5.2.1 QTL-Assisted Genomics

Researchers are trying to investigate the mechanisms associated with metal tolerance so that improved rice lines can be produced with increased heavy metal tolerance. The aim is to strategize for increase in crop yields on the basis of gene function, regulatory networks, and growth and development associated with metal stress (Takeda and Matsuoka 2008). High-throughput analysis-aided comparative genomics like expressed sequence tags, targeted or random mutagenesis, gene expression analysis, loss/gain of function or mutant complementation, and novel gene discovery have made our understanding of metal tolerance mechanism better (Cushman and Bohnert 2000). The two primary approaches for development of stress tolerance in plants using genomic approaches include (1) natural selection of plants under heavy metal stress or through mapping of the quantitative trait loci (QTLs) followed by marker-assisted selection and (2) development of transgenic rice lines carrying newly incorporated genes or modified exiting genes to enhance the stress tolerance (Blumwald et al. 2004). Domestication of rice and its breeding and genetic have provided a platform for modern-day rice research (Ma et al. 2007). Several QTLs have successfully been identified conferring to metal-induced toxicity in rice, although the physiological basis of this tolerance and their association with QTLs is not yet well understood (Xue et al. 2007; Zhang et al. 2013; Dufey et al. 2015). Marking-assisted selection process is very promising as it allows the selection of crosses between low-yielding, stress-tolerant and high-yielding, stress-sensitive lines; it allows a greater pool of genetic diversity into the breeding process and eliminates the chances of unreliable phenotypic screening (Lafitte et al. 2004).

5.2.2 Transgenic Approaches

To develop rice transgenic lines with improved traits, transgenes are introduced, overexpressed for providing tolerance to metal stress. Various techniques have been widely applied including electroporation, microprojectile bombardment, PEG-mediated gene transfer, and *Agrobacterium*-mediated transformation for the transference of the gene into rice plants (Kathuria et al. 2007; Fraiture et al. 2016). The overexpression of the rice gene OsHMA3 showed increase in root to shoot transport of cadmium (Ueno et al. 2010). Low-cadmium-accumulating transgenic rice were also developed by alteration of genes involved in cd transportation like OsIRT1 and OsLCT1 genes (Lee and An 2009; Takahashi et al. 2011). Overexpression of the OsNAS3 gene exhibited increased tolerance of the rice plants toward Zn, Cu, and Ni stress (Lee et al. 2009). The two genes STAR1 and STAR2 when co-expressed were reported to form a complex with bacterial-type ABC transporter for its function to provide tolerance against Al toxicity (Huang et al. 2009). Some of the genes studied for metal stress tolerance is listed in Table 4.

5.2.3 MicroRNA Technology

This approach has also been exploited lately for producing engineered crop plants tolerant to a wide range of abiotic stresses, as abiotic stresses are known to cause

Table 4 Genes studied for metal tolerance in rice plants

Gene	Phenotypic feature	References
OsPDR5/ABCG43	Cd tolerance	Oda et al. (2011)
OsCDT1	Cd tolerance	Kuramata et al. (2009)
OsHMA3	Compartmentalization of cadmium	Miyadate et al. (2011)
LCD	Accumulation of Cd and tolerance	Shimo et al. (2011)
Os08g01480	Tolerance to As, Cd, Cr	Rai et al. (2015)
OsMTP11	Tolerance to Cd, Ni, Zn, Mn	Zhang and Liu (2017)
OsMTP1	Tolerance to Cd, Zn	Yuan et al. (2012)
OsNAS3	Tolerance to Zn, Cu, Ni	Lee et al. (2009)
STAR1 and STAR2	Tolerance to Al	Huang et al. (2009)
OsSUV3	Tolerance to Zn and Cd	Sahoo and Tuteja (2014)
ARS5	Detoxification of Al	Arenhart et al. (2013)
AtPCS	Tolerance to Cd	Venkataramaiah et al. (2011)

anomalous expression of many microRNAs (Banerjee et al. 2016). The microRNA390 (miR390) was found to be linked with cd tolerance, and the overexpression of miR390 in transgenic rice lines showed decreased cd tolerance and increased cd accumulation in comparison to wild-type plants (Ding et al. 2016). Overexpression of the 1.74 kb rice SUV3 (OsSUV3) gene in sense and antisense orientations in transgenic IR64 lines showed increased tolerance to cd and zn stress (Sahoo and Tuteja 2014). Transgenic rice carrying the cadmium tolerance gene YCF1 showed higher cd accumulation; as the accumulated cd was sequestered in the vacuoles, the grains were protected from cd contamination (Islam and Khalekuzzaman 2015). Overexpression of the putative abscisic acid, stress, and ripening gene in *japonica* rice cultivar exhibited its role in detoxification of aluminum and providing tolerance (Arenhart et al. 2013).

6 Conclusion

To achieve sufficient productivity, plants depend on various abiotic factors like water availability, optimum soil conditions, feasible temperature, and mineral bioavailability. The tremendous increase in population brings increasing demands for crop production, but the productivity is threatened by several abiotic stresses like drought, salinity, cold, and metal stress. These abiotic stress factors lead to loss in crop yield, eventually resulting in global food crisis. An efficient alternative to deal with this difficulty is natural selection of genotypes that hold high tolerance potential against these stresses.

Considerable assessments have been undertaken to understand the severity and the effects of the various stress factors such as drought, salinity, cold, and heavy metal on the morphological, physiological, biochemical, and molecular responses exhibited by the rice plants at the various stages of its growth and development. Several research inputs toward better understanding of the stress-mediated response and tolerance mechanisms have been directed. Several techniques have been developed to screen the rice genotypes with higher tolerance against stress conditions, and these stress-tolerant genotypes will be able to sustain in the unfavorable environments without compromising its yield.

Natural selection of tolerant genotypes is quick and easy by nature but certainly not highly reliable. QTL-assisted breeding will make the selection process highly reliable, due to the large number of molecular markers generally used for QTL detection. The platform of genomics-assisted agriculture is highly evolving, with the recent advent of many new technologies. Use of transgenics to improve the traits developed many tolerant rice lines. Understanding the differential stress responses of the tolerant and sensitive rice genotypes and the different genomic approaches to develop improved, tolerant lines will be highly beneficial to the abiotic stress researcher community.

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Selection and Hybridization Techniques for Stress Management and Quality Improvement in Rice

Muhammad Yousuf and Danish Alim

Abstract

Rice (*Oryza sativa* L.) is the staple food of population in the greater part of the total world. The Green Revolution through development of semidwarf varieties and hybrid rice techniques exploiting heterosis increased rice production worldwide. Advances in cellular and molecular biology are important for further increasing rice productivity. Several biotic and abiotic strains like submergence, diseases, insects, salinity, drought, heat, and cold are major challenges for increasing rice production. Grain quality of rice is complex and is mainly determined by grain length, aroma, head rice recovery, chalkiness, and amylose content. The present chapter describes the selection and hybridization techniques for stress management and quality improvement in rice. Conventional interbreeding and selection procedures, ideotype crossing, heterosis breeding, wide hybridization, marker-assisted breeding, and genetic engineering (transgenic) are techniques being used by rice breeders. Many genes for biotic and abiotic strains tolerance and various quality traits have been identified, and functional markers have been established for the selection of these traits. Marker-assisted assortment is an appropriate strategy for stress management and grain quality improvement in rice. Breeders should also exploit current marker technologies, for example, genomic selection (GS) to take precaution of genes with small impacts for the improvement of biotic and abiotic stress tolerance, grain quality and high yield simultaneously.

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Keywords

Rice · Selection · Hybridization · Biotic · Abiotic · Grain quality · Ideotype · Heterosis · Molecular markers · Wide hybridization and transgenic

1 Introduction

World population is currently over six billion and expected to reach eight billion by 2030. Cropland area is shrinking worldwide due to population growth and urbanization. Due to this severe population pressure and reduction in cropland area, the food security is a real challenge. The only viable solution is to improve crop yields per unit area by use of innovative techniques of plant breeding. Rice (*Oryza sativa* L.) is a cereal plant with high economic and social morals. It is estimated that this is the main food of 3.5 billion people over the world, as it has a lot of carbohydrates, low fat, and a lot of proteins, vitamins, and minerals. It is evaluated that 50% of total world's populace exists completely or incompletely on rice. The interest in rice is increasing consistently because of changes in customer needs and populace growth. Therefore, rice production must be increased. Sadly, the capability of extending the growing area is restricted, and, therefore, rice production must be expanded by improving yields per area (Khush 2013). Rice along with wheat and maize gives 50% calories utilized by the world population. Although rice production of all world shows rise every year, all-world populace grows quickly (Hadiarto and Tran 2011). An estimated 870 million people worldwide suffer from chronic malnutrition, the vast majority of whom live in growing countries, in which rice is nearly linked to food security and political strength. Food security is the point at which all individuals have physical and financial access to a sufficient measure of sheltered and nutritious food whenever to fulfill their nutritional needs and preferences for an active and healthy life (FAO 1996). In Asia, food security has generally been characterized as maintaining stable rice costs in the country's major urban markets.

Rice crop is self-pollinated, and growing rice (*Oryza sativa* L.) belong to genus *Oryza*, strain Oryzeae, in subfamily Pooideae of Poaceae (Gramineae) family of grass. There are 24 recognized species in genus *Oryza* which show a remarkable range of adaptation to different habitats. Among these only two are growing species; the African *O. glaberrima* and Asian *O. sativa*. *Oryza sativa* is more distributed into three subspecies, namely, Javanica, Japonica (Sinica), and Indica.

Since the spread of the Green Revolution, South Asia has made a significant rise in rice production. With 41% of cultivatable land, it is the second biggest rice-growing region in the world. Just India and Pakistan are independent in rice growing; still Bangladesh, Sri Lanka, and Nepal are not independent and rely upon exports from different nations. Climate, H_2O, environment, rainfall and conditions of soil, agricultural production resources, and the hereditary potential of germplasm are main factors in plant productivity. Growth of population, increased demand of water, water crisis and drought, insufficient adaptation to change in climate, agricultural land decline, soil moisture, soil properties and deterioration of nutrient content,

Table 1 In Pakistan % change in area, production, and yield of rice

Year	Area (000 hectare)	% change in area over the last year	Production (000 tons)	% change in produce over the last year	Yield (kg/ha)	% change in yield over the last year
2017–2018	2889	6.4	7442	8.7	2567	2.1
2016–2017	2724	−0.6	6849	0.7	2514	1.2
2015–2016	2739	−5.3	6801	−2.9	2483	2.5
2014–2015	2891	3.7	7003	3.0	2422	−0.6
2013–2014	2789	–	6798	–	2437	–

Source: Pakistan Economic Survey 2017–2018

weed effectiveness, increased strength, and rate of biotic/abiotic pollution will be problems in satisfying future food needs strengthening. This will influence the financial development and social soundness of areas with food deficiencies. Farmers will gain a benefit in particular in the event that they effectively tackle the arithmetical riddle of cultivating. Staple food of human populace and causes of feed for livestock such as wheat, rice, maize, and other grains represent over 60% of total crop evapotranspiration necessity.

The contribution of plant rearing to improving economically main crops, like maize, rice, cotton, wheat, and pearl millet, at worldwide level is noteworthy. Prior to the Green Revolution, conventional wheat and rice varieties were tall, photoperiod sensitive, low yielding, and dry season tolerant, having a wide-ranging development term and good grain quality.

In Pakistan, rice is the main food and cash crop, the second largest main crop after wheat and the second largest export product after cotton. It considers for 3.1% of value addition in agriculture and 0.6% in GDP. Rice is growing on an area of almost 2.8 million ha yearly with the yearly produce of 7.4 million tons approximately. Pakistan is a fourth largest rice-exporting country with annual export of over four million tons (2017–2018). Pakistan is one of the best producers of basmati rice in the globe, but failed to conquer large markets. Over the past decade, Pakistan has regressed in every progressive rice market as the overall rice market has grown, but Pakistan has fluctuated with uncertain ups and downs. Situation of coarse rice is also not different as per acre production is not more than 50 maunds/acre against the progressive farmers yield of 105 maunds/acre. Pakistan has to position its rice products according to international scenario to increase its share of rice exports to China and other rice-eating countries. The area, production, and rice yield since the past 5 years are presented in Table 1.

In Pakistan, per acre rice yield is very low (2.5 t/ha) as compared to the rest of rice-producing countries of the world due to multifarious factors. There is lot of potential to increase per ha yield through cultivation of improved rice varieties/

Table 2 Province-wise area and production of rice

	Area (000 ha)			Production (000 tons)		
Province	2016–2017	2017–2018	Change (%)	2016–2017	2017–2018	Change (%)
Punjab	1701.7	1823.5	7.15	3409.1	3853.6	13.03
Sindh	750.5	828.3	10.36	2678.6	3007.3	12.27
KPK	64.9	67.4	3.85	154.1	158.9	3.11
Balochistan	1170.0	169.8	−0.12	554.5	553.8	−0.12
Pakistan	2724.0	2889.0	6.4	6849	7442	8.7

Source: Economic Wing M/O NFS&R, Govt of Pakistan

hybrids, using better seed of recommended varieties, better nutrient and water management, integrated pest management, and minimizing harvest and postharvest losses (Table 2).

The consumption rate of rice in Pakistan is about 0.99 kg/person/month. The yield gap between progressive and average farmer exists due to less adoption of certified and quality seed, delayed transplanting of nursery, imbalanced use of fertilizer, lesser use of micronutrients, lack of awareness about weed and pest management, and nonadoption of innovative technologies like mechanical methods of rice cultivation. Fulfillment of food needs of the ever-increasing population of the country at yearly growth rate of 2.4% is also a matter of concern for sitting government. It is also of worth mentioning that while population density is growing, land-to-man ratio is reducing day by day putting huge pressure on agriculture sector.

Improved or high-yielding varieties of rice available in Pakistan have yield potential of 71 maunds/acre for basmati and 105 maunds/acre for coarse, whereas our national average yields for basmati and coarse are very low. There is need to increase per acre yield to increase productivity and profitability of rice farmers and overall rice production to meet growing requirements of our increasing populace and have surplus for export as well. There is almost 50% yield gap in rice productivity which needs to be bridged/narrowed down. The major reasons for low productivity of rice include:

1. Manual transplanting leading to less plant population
2. Less adoption of certified and quality seed
3. Delayed transplanting of rice nursery
4. Imbalanced use of fertilizers
5. Lesser use of micronutrients
6. Lack of understanding about weed and pest management
7. No awareness or nonadoption of innovative technologies

The critical factors in rice productivity are:

1. Timely transplanting of nursery
2. Use of certified and treated seed

3. Balanced use of fertilizers and micronutrients
4. Timely control of weeds and pests
5. Optimum plant population

A definitive objective of crop reproducing is to create varieties with high produce potential and attractive agronomic attributes. Yields are affected by plant species, environmental conditions, and agronomic factors. In rice farming, the important traits that breeders pursued were high yield potential, struggle to serious diseases and insects, and improving the quality of grain and food. Although, there appears to be conflict between these goals, importance on high grain quality unstable yield. On the other hand, an excessive amount of emphasis on insect and disease effects and stable production prompts poor grain quality. Subsequently, rearing endeavors may focus on varieties with possibility to limit production misfortunes under critical conditions and to expand produce when situations are promising. The biggest challenge for rice research and production scientists around the globe is finding appropriate solutions to important problems, for example, the effects of climate change. Moderate water efficacy and pollution will play a key role in established food security in large part of the globe. The ecological crisis, plant diseases, and pests have also led to a reduction in rice yield in many countries of the globe. A variety of breeding strategies to increase yield potential, stress tolerance, and quality improvement include (1) ideotype breeding, (2) conventional hybridization, (3) heterosis breeding, (4) male sterility, (5) wide hybridization, (6) genetic engineering, and (7) molecular marker-assisted breeding.

There are two methodologies in the biotechnological use in molecular rice breeding: the first one is by marker-assisted selection (MAS), also termed marker-assisted breeding (MAB), and the other one is by building up hereditarily modified crops. A hereditary marker is any observable character or, in any case, assayable phenotype, for what alleles at singular loci isolate in a Mendelian way. MAS is a strategy that doesn't replace conventional breeding but can assist with making it progressively effective. It doesn't include the exchange of isolated gene successions, for example, hereditary engineering, but offers devices for the focus on determination of the current plant material for further breeding.

2 Conventional Hybridization

From the beginning of agriculture 8–10,000 years ago, farmers changed the hereditary composition of their plants. Early farmers chose the most attractive plants and seeds and stored them to plant for next year. At that point, once the study of hereditary qualities turned out to be improved and understood, plant breeders utilized what they thought about the genes of plant to choose for explicit wanted characteristics to create developed varieties. Conventionally plant breeding has been continuing for a long time and is still usually utilized today. Early ranchers found that certain crop plants could be misleadingly bred or cross-pollinated to enhance yields. Descendants may also be given desirable traits from different parent plants.

As the plant breeding science evolved in the twentieth century, growers began to enhance understanding how to choose the best plants and grow them to produce new and improved varieties of various crops. This has significantly expanded the profitability and nature of plants we develop for food, feed, and fiber. The art of perceiving the desired traits and fusing them in the future generation is significant in plant rearing.

It is important to recognize desired traits and transfer them into future generations. Sometimes the breeders travel long distances to discover plants with wanted traits. Occasionally, the breeders find plants with desirable traits spontaneously. It is very rare and is due to mutation. Conventional breeding and hybridization is important in stress management and quality improvement in rice. However, conventional hybridization has some limitations as well. Some unwanted and undesirable traits are also transferred along with desirable traits through conventional hybridization.

The specialty of perceiving desired traits and adding them into the generation in the future is significant in plant breeding. Breeders examine their fields and travel large distances looking for singular plants that show desirable characteristics. Some of these traits sometimes appear instinctively in a practice called a mutation, but the natural speed of the mutation is very moderate and unreliable to produce all of plant traits that breeders want to see.

3 Ideotype Breeding

The word "ideotype," exactly "a form denoting an idea," was designed for biological models. In the widest perception, an ideotype is a biological model that has to function or behave predictably in specific environment. In particular, the ideotype of culture is plant model that is estimated to produce a large amount or excellence of grain, oil, or other beneficial products when established as variety.

Ideotype rearing planned for changing the plant design is additionally time-tested technique to expand the yield potential. The ideotype reproducing approach can be viewed as a technique to improve the limit of the pedigree strategy to advance additions for quantitative characteristics, particularly yield. It depends on the hypothesis that one can improve complex traits by changing similar traits that are positively related to them. It should be progressively proficient to characterize a plant type which was hypothetically productive and afterward strain for this (Hamblin 1993).

The International Rice Research Institute (IRRI) has been working on different rice ideotype or a new plant type (NPT) with crop index of 0.6 (40% straw, 60% grain) and enhanced photosynthesis capability to achieve an absolute organic crop. Peng and Kimmel (2005) examined the accompanying parts of this NPT: low planting capacity, several unfertile plantings and 200–250 seeds/panicle, plant height 90–100 cm, thick and strong stems, strong root system, and 100–130 days of cycle development. These properties permit the rice plant to change over new energy into grain production, expanding the potential of yield up to about 20%, however with higher sources of input and expenses.

Various breeding lines with the wanted ideotype have been developed and used in national rice enhancement programs (Khush 1995). To further increase rice yield, a new type of plant was developed (IRRI 1989; Khush 2005). Semi-predominates rice crop varieties yield countless unfertile tillers and extreme leaf area that causes shared shading and a decrease in shelter photosynthesis. IRRI researchers have suggested more changes to plant design with reduced tillering (9–10 tillers for transferred environments): no unfertile tillers; 200–250 grains per panicle; dark green, thick, and vertical leaves; and vigorous and profound root framework. The projected ideotype has developed viewed as “new plant type” (NPT) of rice. The goal has been to create enhanced germplasm with 15–20% better production compared with current good varieties. Recently, new genetic factor and QTL have been recognized to manage the projected characteristics for NPT varieties.

Peng et al. (2008) informed that the first-age lines of new plant type (NPT), which were produced in IRRI from tropical Japonica, did not produce good results due to the restricted biomass produce and the poor grain content. Improvement has been made on second-generation NPT lines that have emerged from the overlap of first-class India with better tropical Japonica. Some second-generation NPT lines resulted in first-generation NPT lines and controlled various indicators. The Chinese “super” rice cultivation project has created numerous F1 hybrids that use mixture of an ideotypical method and a subspecific heterosis. In the demonstration fields of the farm, these crossed varieties gave a grain yield of 12 t/ha, 8–15% higher as compared with hybrid control varieties. The achievement of Chinese “super” crossed rice was partly the outcome of combining the best constituents of the IRRI’s NPT construct in addition to using interspecific heterosis. The achievement of “super” hybrid rice growing in China and the improvement in crossing breed the NPT in the IRRI proposed that ideological method is effectual to exceed the yield limit of irrigated rice.

4 Heterosis Breeding

Heterosis is characterized as unrivaled presentation in development, vigor, stress resistance, vitality, reproductive capacity, grain quality, grain yield, adaptability, and other physiological attributes of F1 populace of two hereditarily different parents (P) contrasted with any of the midparent (MP) or better parent (BP) of crossbreed or to the check (CK) (Nanda and Virmani 2000). Both positive and negative heterosis is helpful for crop enhancement, relaid upon the rearing goals and environment of the trait. The exploitation of heterosis has been the best functional accomplishment of study of hereditary qualities and plant breeding (Alam et al. 2004). Between 1962 and 1967, various proposals originated from well area of the globe for marketable utilization of heterosis to turn into significant segment of rice enhancement programs at the national and global levels. For instance, rice reproducers from China, India, the United States, Japan, former Soviet Union, and the Philippines began its activities to use rice heterosis. However, advancement hadn't been sound as a result of trouble for the rice crop to be carefully self-pollinated crop not at all like corn that made out crossing significant for crossed seed yield very hard.

Great quantitative hereditary clarifications for heterosis focus on two ideas, predominance and overstrength (Crown 1952). By progress on the hereditary investigation of computable traits and high-density molecular association maps, various investigation groups incline toward epistasis as significant hereditary premise of heterosis (Hallauer and Miranda 1988). "Predominance" initially implies that heterosis has come about because of activity and communication of good prevailing genes united in hybrid F1 from two parents. This theory expects that genes which are good for force and development are prevailing, and the genetic factor contributed by another parent brings about an increasingly great combination of predominant genes in F1 as compared with either parent. A second ancient description for heterosis is "overdominance" that states allelic contacts in crossbreed, so the class of heterozygotes works well than any class of homozygotes.

The epistasis hypothesis suggests that heterosis is genetically organized by numerous genes due to complex nature, for example, yield contains various components. Heterozygosis in the collaboration of genes is the primary hereditary source for explaining heterosis, since the crossed breed is heterozygous for all genetic loci which varies b/w parents. Therefore, level of heterosis relies upon loci that are heterozygous and how inside locus alleles and between locus alleles associate through one another. Association of inside locus alleles brings about strength, incomplete domination, or overpredomination, with theoretic scope of domination point from 0 (no strength) to <1 (overdominance). Cooperation between locus alleles brings about epistasis. Hereditary charting outcomes have demonstrated that greatest QTLs include in heterosis and further computable attributes have predominance consequence. Epistasis has been seen even new routinely and exhibited as regular wonder in the hereditary control of measureable characteristics comprising heterosis (Yu et al. 1997; Luo et al. 2001; Hua et al. 2003). Investigation of Yu et al. (1997) gave solid proof to two-loci and multi-loci cooperation (epistasis) particularly for characters, for example, yield of grain, that are difficult in nature. They come to know that heterosis isn't constrained via single locus, although the locus carried on the prevailing or overdominant pattern, linkage, and epistasis has a significant role. In this way, the influences of predominance, overstrength, and epistasis of different structures are not commonly exclusive in hereditary premise of heterosis, instead of what was recently discussed for various theories. These parts have a role to carry out relaid upon the hereditary design of the populace (Hua et al. 2003).

Enhancing the efficiency of rice by novel hereditary methodologies like crossbreed rice is essential. The manipulation of heterosis is viewed as one of the exceptional accomplishments of plant rearing. The occurrence of adequate crossed energy is a significant preessential for the effective yield of crossed varieties. Firstly heterosis was exposed by Jones in 1926 in rice that originate the best production of grain return and culm in population F1 and then to their maternal lines. The estimations of heterosis may be seen through crossed breed performances above their midparents (MPH), better parents (BPH), and standard check assortment (STH) (Rahimi et al. 2010; Yildirim et al. 2014). The estimation of heterosis moreover positive or negative relies upon the level of chosen parental lines (Rahimi et al. 2010; Bhatti et al. 2015), and both demonstrate critical data of preferred characteristics for quality and gene activity that is valuable for the rice improvement program (Raju et al. 2014). According to Malthus (1989), the grain of food increases in arithmetic progress, while the population increases in geometric progress. Therefore, improved technology is needed to fill this gap and feed the growing population. To overcome the yield limit and make rice growing more attractive, alternative approaches are now needed. Among all possible alternatives, heterosis is the main methodology to increase rice production. This not only subsidized to food security but also profited the environment (Duvick 1999). In the different crop species where crossed varieties were utilized commercially, rice positions remarkably high. Heterosis was used economically in rice with produce of 20–25% compared with the best pure lines (Rather et al. 2001). Crossbreeds offer prospect to overcome the produce roofs of semidwarf rice varieties. In rice, revelation of cytoplasmic male sterility (CMS) (Athwal and Virmani 1972; Erickson 1969; Shinjyo 1969) proposed that breeder could build up an economically feasible F1 half-breed; however minimal serious attention was compensated until Chinese researchers announced the successful F1 rice crossbreeds production in China (IRRI 1977). Such crossbreeds produced 20–30% higher as compared with conventionally reproduced varieties (Lin and Yuan 1980; Shen 1980).

5 Male Sterility System in Rice

Rice is a self-pollinated crop. Hence, male infertility method is prerequisite for crossed seed production. Cytoplasmic genetic male sterility (CMS) has been observed in rice that may be utilized for crossed seed produce in bulk. The three-line framework contains CMS or A line, sustainer or B line, and the restorer or R line.

CMS or A line produces no pollen or abortive pollen and no seed is set on it by selfing. However, CMS line has the normal pistil and seed is set when pollinated by normal pollen. A good CMS line must have the subsequent characteristics.

Stable Sterility: Sterility will be stable generation after generation without affecting the environment, especially temperature.

Easy Restoration: The restoration of the CMS line should be easy, and the seeds of restored hybrids would be less exposed to unfavorable ecological conditions.

Good Flowering Structure and Flowering Behaviors: The line of CMS should normally bloom and synchronize with male parent. His stigma must be well implemented.

The sustainer line is a specific pollen variety that produces progenies that are still sterile. The CMS line and the maintainer line are similar in appearance. If there is no maintainer line, then it is not possible to multiply line of CMS.

The restorer line is a pollinator variety which is utilized to produce F1 hybrids. The hybrids F1 are productive and can produce seeds through selfing. The best restorer line must have good restoring capability, good agronomic characteristics, and good combining ability. The height of the restorer line should be more than the CMS line. The growth duration of the restorer line is generally equal to or longer than that of CMS line.

The environmental genic male sterility (EGMS) is of prime importance in the seed production of crossed rice. The photoperiod profound genic male sterile (PGMS) and thermosensitive genic male sterile (TGMS) lines are effectively utilized to deliver crossover rice seeds.

6 Wide Hybridization

Wide hybridization refers to the process of development of hybrids between different species of similar genus or even of different genera. In rice for all practical purposes, it refers to crosses between the cultivated rice and different *Oryza* wild species. However, in rice inter-subspecific crosses like japonica × indica hybrids are also mentioned under wide hybrids. Wide hybridization is challenging to achieve because numerous wild rice species of *Oryza* genus fail to crossbreed with cultivated rice as of variances in genome organization and chromosome number. In some cases pollination may happen but the embryo is aborted. Employing embryo rescue technique, the hybrid embryos are rescued.

Rice belongs to the Oryzeae tribe and Poaceae family (Gramineae). *Oryza* is just one genus with cultivated species out of 11 genera of Oryzeae tribe. The *Oryza* genus has 2 cultivated and 22 wild species reported by Khush and Brar (2002). The two cultivated species are *O. sativa* and *O. glaberrima*. *Oryza sativa* is a rice produced all over the globe, and *Oryza glaberrima*, a small-scale African rice grown up in several countries in West Africa. The wild yearly *O. nivara*, which is obtained from *O. rufipogon*, is observed the forerunner of *O. sativa*. *O. longistaminata* has been derived from *O. breviligulata*, which is the ancestor of *O. glaberrima* (Chang 1976). The genus *Oryza* has 10 genomes, and the wild kinds of this family have also $2n = 24$ or 48 chromosomes that are disseminated into fourcomplexes. The cultivated rice and associated wild species having a place with the *O. sativa* composite are effectively bredable and share typical AA genome. The wild species belonging to *O. officinalis* complex are *O. punctata* (BB and BBCC), *O. rizomaties* (CC), *O. officinalis* (CC), *O. eichingeri* (CC), *O. latifolia* (CCDD), *O. minuta* (BBCC), *O. alta* (CCDD), *O. grandiglumis* (CCDD), and *O. australiensis* (EE). The species of *O. meyeriana* complex are *O. granulata* (GG) and *O. meyeriana* (GG). The species of *O. ridleyi* complex are *O. longiglumis* (HHJJ) and *O. ridleyi* (HHJJ). The wild species *O. brachyantha* (FF) and *O. schlechteri* (HHKK) can’t be assembled in these sets (Vaughan 1989). Species from secondary rice genetic supply are partly homologous or nonhomologous to species of the AA genome (Khush 1977), which leads to a limited crossing. All indirectly related wild species which belongs to *O. meyeriana* complex and the *O. ridleyi* complex from the tertiary genetic supply.

Rice is cultivated under diverse agroclimatic conditions. However, its production is influenced by numerous biotic (insect pests, diseases, and weeds) and abiotic (drought, flood, adverse soil conditions and temperature) stresses, limiting the rice production. Several main diseases and insect pests influencing rice production contain bacterial leaf blight (BLB), brown planthopper (BPH), blast (BL), yellow stem borer (YSB), sheath blight (ShB), white-backed planthopper (WBPH), etc. The heritable variability for some useful traits is either limited in cultivated rice germplasm or the cultivars become susceptible to the pests because of changes in insect biotypes or disease competitions. Wild species continue to grow in their natural habitat and are subjected to diverse environmental biotic and abiotic stresses. They are potential reservoirs of many useful traits, which can be utilized for the genetic enhancement of rice. In this way, it is necessary to widen the genetic supply by introgressing different qualities from wild rice classes to cultivated rice to address difficulties for rice yield. Alien gene transfer from wild rice species through wide hybridization is nonhazardous and environment friendly, which is most important for safety of human beings (Tables 3 and 4).

7 Genetic Engineering

Rice crop is influenced by different biotic and abiotic stresses. Abiotic stress negatively affects plant development and profitability. It prompts a progression of morphological, physiological, biochemical, and subatomic changes that unfavorably influence plant development and profitability (Holmstrom et al. 2000). In general, plants can cope with moderate stress. However, if stress exceeds a threshold, the physiological mechanism that gives tolerance breaks down and causes plant death (Roychoudhury et al. 2008). The most common abiotic strains are drought, cold, and salinity, which limits crop yields, including rice. Understanding the stress tolerance mechanism, which includes many genes that are involved in a network of stress signals, is critical to improving crop yields. Since a significant portion of rice-growing areas suffers from abiotic stress, it would be difficult to meet the future food needs of an ever-growing world population (Hayashi et al. 1997). Efforts to use traditional selection methods to improve traits that confer resistance to abiotic strains have had limited success (Sakamoto and Murata 2000). Therefore, to meet the food needs of a growing world population, traditional breeding methods must be combined with tools such as molecular markers and genomics. Various biotechnological approaches are used to improve the quality and quantity of rice, as well as its resistance to pests, diseases, and environmental pollution (Prasad et al. 2000; Paul and Roychoudhury 2018). Transgenic gold rice with a high source of provitamin A (carotene) was obtained (Capell et al. 1998) by manipulating the biosynthetic pathway using tissue-specific promoters. They presented three genes, lycopene

Table 3 Agronomically main genes interbreeding from wild *Oryza* species into cultivated rice (Khush and Brar 2002)

Trait	Donor species	Genome
Transferred to *O. sativa* grassy stunt virus resistance	*O. nivara*	AA
Bacterial blight resistance	*O. longistaminata* *O. officinalis* *O. minuta* *O. latifolia* *O. australiensis* *O. brachyantha*	AA CC BBCC CCDD EE FF
Blast resistance	*O. minuta*	BBCC
BPH resistance	*O. officinalis* *O. minuta* *O. latifolia* *O. australiensis*	CC BBCC CCDD EE
WBPH resistance	*O. officinalis*	CC
Cytoplasmic male sterility	*O. sativa* f. *spontanea* *O. perennis* *O. glumaepatula*	AA AA AA
Tungro virus	*O. rufipogon*	AA
Progenies under assessment		
Yellow stem borer Sheath blight resistance Increased elongation ability Tolerance to acidity, iron, and aluminum toxicity	*O. longistaminata* *O. minuta* *O. rufipogon* *O. glaberrima* *O. rufipogon*	AA BBCC AA AA AA
Tolerance to nematode	*O. glaberrima*	AA

cyclase, phytoene synthase, and phytoene desaturase (two genes from daffodil and one gene *Erwinia uredovora*, respectively) and stated them in the endosperm. The improvement of recombinant DNA innovation permitted the investigators a more profound understanding of transcriptional guideline of genes and encouraged overproduction of endogenous or outside proteins in plants, other than unraveling the biochemical and subatomic procedures. A large body of writing on hereditary engineering of rice is presently accessible.

Plant genetics strategies for resistance to abiotic stress are based on the expression of genes that participate in signaling and regulatory pathways, or genes that encode proteins that provide stress tolerance, or enzymes that are present in pathways responsible for the synthesis of functional and structural metabolites (Roychoudhury et al. 2013). Numerous gene and gene items have been recognized which get endless supply of plants to different abiotic stresses. Along these lines, stress-inducible genes have been used to improve stress resilience through gene exchange. In spite of the fact that gene change in japonica rice is performed routinely in a few research centers, change in indica rice is nearly troublesome. Therefore, in order to select the desired transformants and study the expression of introduced genes, it is necessary to develop a relatively large number of transgenic plants in species of the indica. Over the past two decades, a large number of genes have been isolated and cloned

Table 4 List of genes for monogenic characters recognized using the *O. glumaepatula* (GLU-ILs) and *O. meridionalis* (MER-ILs) introgression lines

Gene or allele sign	Chromosomes	Gene name	Right marker	Left marker	ILs used for gene identification	Reference
Reproductive barriers						
S22	2S	STERILITY22	C1357	–	GLU-ILs	Sobrizal et al. (2000a)
S23	7L	STERILITY23	R1789	C213	GLU-ILs	Sobrizal et al. (2000b)
S27	8L	STERILITY27	mtRPL27	(Cloned)	GLU-ILs	Sobrizal et al. (2001a), Yamagata et al. (2010)
S28	4L	STERILITY28	mtRPL27	(Cloned)	GLU-ILs	Sobrizal et al. (2002)
hwf1	4S	HYBRID WEAKNESS F1	G3006	C933	GLU-ILs	Sobrizal et al. (2001b)
Rhw	8	RESTORATION HYBRID WEAKNESS	C1115	–	GLU-ILs	Ikeda et al. (1999)
Domestication-related traits						
Sh3-glum	4L	SHATTERING3	R1427	C107	GLU-ILs	Sobrizal et al. (1999)
Sh3-mer	4L	SHATTERING3	R1427	C107	MER-ILs	Nagai et al. (2002)
Sh5-glum	5L	SHATTERING5	R118	R1553	GLU-ILs	Sanchez et al. (2002)
Sh6-mer	5S	SHATTERING6	C119	Y1060L	MER-ILs	Sanchez et al. (2002)
An6-mer	8L	AWN 6	–	RM3496	MER-ILs	Matsushita et al. (2003)
An7-mer	5S	AWN 7	Y1060L	C249	MER-ILs	Kurakazu et al. (2001)
An7-glum	5S	AWN 7	RM3419	RM289	GLU-ILs	Matsushita et al. (2003)
An8-mer	4L	AWN 8	XNbp237	C891	MER-ILs	Kurakazu et al. (2001)
An8-glum	4L	AWN 8	RM261	RM1359	GLU-ILs	Matsushita et al. (2003)
An9-mer	1S	AWN 9	RM811	RM8051	MER-ILs	Matsushita et al. (2003)
An10-mer	1L	AWN 10	RM237	RM265	MER-ILs	Matsushita et al. (2003)

S short arm of chromosome, *L* long arm involved in signal transduction, transcriptional regulation, ion transporters, and metabolic pathways. It has recently been reported that some genes from signaling pathways and nucleic acid pathways are activated in response to stress from cold and salinity, which indicates the presence of crosstalk between these pathways. The role of calcium as an important signaling molecule in response to various stress signals has also been described.

Flash flooding and the resulting submergence of plants in various parts of the world adversely affect at least 16% of the rice fields (Khush 1984), particularly in the flood-inclined rainfed lowlands in South and Southeast Asia. Over 22 million ha of rice in Asia are considered submergence ranging from flash flood to deepwater conditions (Mackill et al. 2012). Among various cereal crops, rice is the only crop that can withstand waterlogging. However, under submergence stress, based on the duration and height of the water stagnation, the productivity reduces to 50% to complete loss of the rice crop. Submergence stress is generally caused by flash flood throughout the monsoon season with fluctuating intensity and periods. The flash flood immerses the rice fields totally at the beginning period previously or following the crop establishment for not many days to weeks. At the point when rice is submerged for over 5 days, the crop dies and can't improve from the stress after water is subsided (Roxas et al. 1997).

8 Molecular Marker-Assisted Breeding

Around 50 years back, breeders found a strange and old variety of rice that could withstand 2 weeks of complete submergence. Utilizing conventional techniques, breeders attempted to bring this submergence resilience quality into rice varieties supported by farmers in India and Bangladesh. However, since the breeding was inaccurate, the varieties obtained were rejected by farmers. During the 1990s, rice breeder David Mackill (University of California, Davis) and graduate student KenongXu (University of California, Davis) showed that tolerance to finish submergence mapped to the Submergence resistance 1 (Sub1) quantitative trait locus (QTL). Utilizing detailed hereditary data about the Sub1 locus, the teams of McKill and Abdel Ismail from the International Rice Research Institute (IRRI) have created and released several strains of Sub1A (developed by marker selection) in seven countries. In 2015, with help from the Bill and Melinda Gates Foundation, Sub1 rice was developed on 2.2 million ha and came to over 4.9 million farmers.

In a study of rice breeder's interests in South and Southeast Asia (Datta et al. 2012), 51% of respondents appraised flash flooding as one of the three most significant abiotic constraints on rice yields (the others being drought and salinity). Traditional varieties adjusted to these submergence-inclined conditions are, be that as it may, low yielding because of their poor tillering capacity, long sagging leaves and vulnerability to lodging, and poor grain quality. Improved varieties that can consolidate high-yield properties with submergence resistance are required. Most rice cultivars can't endure if the plants are totally submerged for over 7 days (Ye et al. 2000). The current new varieties are not very much adjusted to submergence.

Submergence resilience is constrained by a solitary gene Sub1 that represents 70% of phenotypic variation which is mapped (Chen et al. 2008) on an FR13A cultivar and can be partitioned into three significant sub-genes, Sub1A, Sub1B, and Sub1C. Submergence resistance was presented by the overarticulation of Sub1A and downregulation of Sub1C in a Japonica cultivar to monitor the impact and was seen as tolerant (Shima et al. 2007).

Marker-helped backcrossing (MABC) is the most suitable technique for the consolidation of desired quantitative trait loci (QTL) into the hereditary foundation of a mega variety (Holmstrom et al. 2000; Hayashi et al. 1997). The MABC approach is a clear advantage over conventional breeding, since this strategy leads to the progress of an ideal genotype in a short time, which is not possible with conventional breeding.

9 Rice Grain Quality

The quality of the rice grains includes milling, appearance, cooking, and nutritional qualities. Among these, individuals give more consideration to the appearance and cooking quality (Huang et al. 1998). The appearance quality in China is often estimated based on the grain content with a white core and a square white core. The quality of cooking is determined by the amylose content, alkali-spreading score, and gel consistency. The inheritance of rice cooking quality has been broadly studied (Mckenzie and Rutger 1983; Sano et al. 1986; Pooni et al. 1992; Zhu and Weir 1994; Mo 1995). Pooni et al. (1992) proposed that amylose content may be identified with the impacts of the maternal plant or cytoplasm, though Xu et al. (1995) revealed that rice amylose content was basically constrained by the triploid endosperm genotype with no cytoplasmic impact. Shi et al. (1997) recommended that immediate seed impacts, maternal impacts, and cytoplasmic impacts were the fundamental factors in controlling amylose content, alkali-spreading score, and gel consistency, respectively, and furthermore recognized strong genotype/environment collaboration impacts for these qualities. Up until this point, a few hereditary models have been created for analyzing the inheritance of endosperm characteristics. Pooni et al. (1992) proposed a model for investigating the immediate seed impacts and maternal/cytoplasm impacts of endosperm attributes. Zhu and Weir (1994) set forward an endosperm model for the investigation of the cytoplasmic and maternal impacts. Mo (1995) introduced a mating plan, and a relating measurable technique, with which the hereditary impacts of the endosperm and maternal genotype, as well as impacts because of a cytoplasm contrast, can be autonomously tested. Despite the fact that the previously mentioned models were utilized to recognize different hereditary parameters, aside from the wx gene (Sano et al. 1986), the quality loci for rice grain quality have not been situated on the chromosomes. Besides, the heterozygotic individuals in F2 or F3 descendants, which were generally utilized in the previously mentioned research, can’t give countless hereditarily indistinguishable seeds for the exact examination of rice grain quality.

Fig. 1 Rice grain quality improvement in Pakistan. (Source: Rice Research Institute, Kala Shah Kaku, Pakistan)

The inheritance of grain quality is more complex than the inheritance of other agronomic characteristics in cereals due to epistasis, maternal and cytoplasmic impacts, and the triploid nature of the endosperm. He et al. (1999) examined an established rice DH populace got from another culture of an indica/japonica hybrid that was utilized for hereditary investigation of rice grain quality. Five parameters were evaluated for DH lines and parent varieties: alkali-spreading score (ASS), amylose content (AC), percentage of grain with a white core (PGWC), gel consistency (GC), and the square of the white core (SWC). They found that for each parent, the estimation of every parameter was generally constant in three areas, Beijing, Hangzhou, and Chengdu, while the contrasts between the parents were significant for every one of the five parameters.

Some researchers examined the inheritance of qualitative traits of rice appearance (Ebata and Tashiro 1973; Takeda and Saito 1983) and found that PGWC and other qualitative traits depend not only on the genotype but also on environmental impacts and are controlled by some minor genes with additive effects.

There are different consumers' preferences for rice grain quality in various parts of the world. The world markets have divisions according to the following rice types:

1. High-quality long-grain rice
2. Medium-quality long-grain rice

3. Short-grain rice
4. Parboiled rice
5. Aromatic rice
6. Glutinous rice

The European Union countries demand high-quality long-grain rice. Medium-long-grain rice is sold at a loss in Asia. Short-grain and parboiled rice is mostly marketed to African and Middle East countries, respectively. Aromatic rice is mostly demanded in the subcontinent and the Gulf States (Fig. 1).

In rice varieties grain quality is an economically important character. Any information on the hereditary system of rice grain quality will profit rice breeders. Therefore, the main and minor genes that affect the quality of rice grain should accelerate the process of growing new rice varieties with higher yields and better quality. Grain quality improvement will involve different adjustments as it is influenced by environmental and genetic factors.

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Transformation Techniques and Molecular Analysis of Transgenic Rice

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Abstract

The genetic modification of plants paved the way for new breeding strategies, including for recalcitrant crops. Rice is a staple food for more than 60% of the population worldwide. However, the accelerated growth rate of the world population calls for an enhancement in rice production and modified rice varieties that can produce improved yields and are resistant to environmental stresses. This can be achieved through production of transgenic rice that is attained by the introduction of a transgene into the plant cells using methods such as electroporation, polyethylene glycol-mediated transformation, biolistics, transformation via *Agrobacterium* and *in planta* floral-dip transformation. The presence of transgenes in the regenerated plants can be confirmed using several molecular tools. However, a real transgenic cannot be confirmed based on initial

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transformants under the confinements of a laboratory and a long term approach is necessary. Several factors affect the efficiency of transformation and regeneration of rice, including type of explant used, components of culture medium, gelling agents and type of *Agrobacterium* strains. Currently, transgenic rice production is hindered by drawbacks such as low transformation efficiency, tedious and lengthy protocols, genotype specificity, failure of transgenes to express the required protein due to silencing and generation of sterile plants. Thus, effective breeding of enhanced varieties of rice can be possible through development of improved protocols to ensure stable expression of transgenes and proper regulatory framework.

Keywords

Genetic transformation · Marker genes · Rice genotypes · Transgene confirmation · *Agrobacterium tumefaciens* · Tissue culture · NGS technologies

1 Introduction

A decrease in crop yields alongside high food consumption has raised the question of food security for the global population. Several factors have influenced the crop yields, including rapid population growth and changes in the climate resulting in the shortage of suitable growing land and availability of raw materials for crops (Ray et al. 2013; Zhao et al. 2017). Grass crop plants are the major source of food, feed, fibre and fuel for human use. Among the various grass crops, the most important grasses are rice, wheat, maize, sorghum and sugarcane. Globally, more than three billion people from Asia and other countries depend on rice as their staple food, and it is estimated that by 2025 approximately 60% more rice will be required to fulfil the needs of the growing population (Khush 1997). Because rice has become the major food source for half the world's population, mainly living across Asia, more than 90% of this rice has been produced and consumed in Asia (Papademetriou 2000). However, there is an approximate 52% loss in the production of rice due to damage caused by biotic factors, among which almost 21% is due to the attack of insect pests (Brookes and Barfoot 2003). India has become the second country to commercialize hybrid rice (Pandey and Tiwari 2012). In the present scenario, in India, hybrid rice cultivation has expanded to approximately 2 million hectares out of 44 million hectares under rice cultivation (Anonymous 1985). Abiotic stresses such as drought, salinity, waterlogging and temperature aberrations also cause serious economic losses in rice production and productivity.

Rice is also considered a model system for studying gene expression, regulation and crop improvement in monocots similar to *Arabidopsis* and tobacco in dicots (Izawa and Shimamoto 1996; Bennetzen 1999). Obtaining plants through classical plant breeding by specific targeted genotypes requires the crossing of the proper selection of multiple plant generations which, in fact, causes a disturbance in the traits that do not currently exist in the species. Horizontal specific gene transfer is another novel technique which benefits the agricultural industry by producing desirable plants with increased yield quality, tolerance capacity against abiotic stress and disease and pest resistance (Abdallah et al. 2015).

In general, the recent advanced genetic transformation in plants consists of two important steps: delivery of targeted genes and studying its regeneration. Efficient genetic transformation mainly depends on the type of DNA delivery method used. The regeneration procedure consists of three parts: inducing totipotent tissue to develop into callus, then selection of calli and segregation of progeny. Compared to animal cells, targeted gene delivery in plants faces challenges due to the presence of the multilayered and rigid plant cell wall. Thus, delivery of intracellular biomolecules has become an obstacle to the use of genetic engineering techniques in plants due to slower implementation (Azencott et al. 2007).

2 Rice Transformation: Methods and Protocols

An improvement of crop plants through genetic transformation depends on the expression of transgenes across multiple generations. Integration of a single or intact copy of transgene expression causes complex integration events such as unintended insertional inactivation and silencing of transgene with reduction in the potential (Schubert et al. 2004; Chawla et al. 2006; Meng et al. 2006).

In the field of plant biology, such as in cell biology and gene regulation and expression studies, transgenesis and genetic transformation has several applications with respect to the production of improved agricultural traits at a significantly faster rate compared to any conventional breeding practice. Genetic transformation is based on the delivery of targeted desired genes of interest into plant genomes to produce improved traits in crops (Roy Choudhury et al. 2012). Rice regeneration using the tissue culture technique was first reported in 1968 (Kawata and Ishihara 1968; Maeda 1968; Niizeki and Oono 1968; Tamura 1968). DNA can be delivered through two main methods, i.e. *Agrobacterium*-mediated gene transfer and biolistic DNA transfer (Birch and Bower 1994; Slater et al. 2008; Chawla 2009). *Agrobacterium*-mediated transformation depends on the ability of *Agrobacterium tumefaciens* (*A. tumefaciens*) to transfer a portion of its DNA, called transferred DNA (T-DNA), into plant cells (Tzfira and Citovsky 2006). The transfer of DNA to rice plants has been achieved through various methods such as electroporation, protoplast transformation using polyethylene glycol (PEG) and *Agrobacterium*-mediated transformation (Datta et al. 1990; Tada et al. 1990; Hiei et al. 1994).

2.1 Electroporation

Electroporation is a widely adopted new method in plant genetic transformation (Neumann et al. 1982; Potter et al. 1984). The efficient transfer of DNA into cells is facilitated by transient pore formation on the cell membrane because of the current pulse. Presently, electroporation can be used for both dicots and monocots (Fromm et al. 1985). It provides an efficient expression system for the transient gene in crop plants, and this transient expression system plays a significant role in the characterization of novel genes. Electroporation allows the stable integration of a transgene with plant chromosomes. Stable genetic transformation via electroporation has been achieved in corn and tobacco (Shillito et al. 1985; Fromm et al. 1986; Schocher et al. 1986). Direct gene transfer to plant protoplasts has been successful using electroporation, which has also become the alternative to the *Agrobacterium*-mediated gene transfer in several cases (Paszkowski et al. 1984). The method is based on an efficient uptake of a transgene into protoplasts of DNA from the surrounding medium, which can be enhanced by chemical treatment, e.g. with PEG (Negrutiu et al. 1987), by the application of electric pulses (electroporation) (Fromm et al. 1986) or a combination of both (Shillito et al. 1985).

Electroporation is a successful methods for DNA delivery to regenerable tissues. Generally, tissue electroporation involves the transfer of DNA to wounded zygotic or somatic maize embryos. However, transgenic plants obtained through electroporation were reproducible, and the transferred neo-gene segregated according to Mendelian rules (D'Halluin et al. 1992). Several reports suggest that electroporation is a promising method for gene transfer in the scutellar cells of wheat (Kloti et al. 1993). In addition, despite early successes, rice genetic engineering has encountered a challenge because traditional and widely used gene transfer methods, such as electroporation and PEG-mediated gene transfer into protoplasts, are limited by constraints imposed by the culture systems. As a result, only a few japonicas and an even smaller number of indica varieties could be engineered routinely.

2.2 Polyethylene Glycol Mediated Transfer

In 1986, for the first time, the PEG method was used to obtain the rice protoplast for genetic transformation by transferring chimaeric plasmid and produced colonies of rice callus resistant to kanamycin. However, these transformed colonies were unable to regenerate into plants (Uchimiya et al. 1986). In comparison, plants can be regenerated from mesophyll protoplasts of monocotyledons species, but in the case of cereals, there are only a few reports on plant regeneration using leaf protoplasts with efficient sustained divisions (Hahne et al. 1990). Plant regeneration from cereal protoplasts is regarded as a difficult task because several parameters are uncontrollable during experimental conditions (Potrykus 1989). Currently, protoplast regeneration is an efficient and routinely used method for rice and maize.

By employing PEG or electric pulses, protoplasts can be made to intake DNA. Several reports have proved that both methods have achieved stable transformation for cereals and transformed cell lines (Fromm et al. 1986; Rhodes et al. 1988; Lazzeri et al. 1991). The direct DNA uptake method may achieve successful transformation, but regeneration of the transgenic plants remains difficult. Usually, in the case of highly stressed cells, PEG is preferable (Genschik et al. 1992), but they are suitable for detailed protein localization (Reyes et al. 2010).

2.3 Particle Bombardment Transfer

Christou et al. (1991, 1992) developed a variety-independent method based on the bombardment of immature embryos that produced the recovery of fertile transgenic plants from many varieties, both indicas and japonicas, at high frequencies. In the particle bombardment method, biologically active DNA can be transferred to plant cells using high-velocity metal particles for the transfer (Sanford 1988). Generally, for intact cells and tissues, the particle bombardment technique uses DNA coated microprojectiles to transfer the transgenes, and after *Agrobacterium*-mediated gene transfer, this technique is the most widely used method for genetic transformation (Gray and Finer 1993).

Compared to other methods, the particle bombardment technique has the ability to transfer transgenes into regenerable cells, tissue and organs by achieving truly genotype-independent transformation by avoiding *Agrobacterium* host specificity and tissue culture-related regeneration difficulties. The particle bombardment method has become the choice for producing largely genotype-independent efficient transformation in many crops (Altpeter et al. 2005; Christou 1995). Salt-tolerant transgenic rice has been generated using particle bombardment via overexpression of a *late embryogenesis abundant* (*lea*) gene (Ganguly et al. 2012). Compared to *Agrobacterium*-mediated transfer, this method tends to produce higher average transgene copy numbers with complex integration events (Kohli et al. 2003). Transfer of a foreign gene to a wide range of plant tissues and species through the microprojectile method has already been achieved and proven by several laboratories. However, there is no difference achieved in the case of transformation of monocotyledonous and dicotyledonous species (Sanford 1990).

An advantage of this method is that it can be used for the transfer of multiple plasmids as co-transform multi genes (Hadi et al. 1996; Chen et al. 1998). In addition, even larger fragments of DNA such as yeast artificial chromosome can be introduced using this method (Van Eck et al. 1995). Particle bombardment conditions can be changed or modified to give highly reproducible results in transient assays by delivering a large number of copies of potentially transcribable DNA into the nuclei of target plant cells.

Compared to the *Agrobacterium*-mediated transfer particle bombardment method, the particle bombardment method has lower transformation efficiency and is more cost effective. In this method, due to the random targets, DNA damage cannot be prevented and there may be a chance of gene silencing and variation in transgene expression owing to the complex integration patterns and multiple copy insertions (Dai et al. 2001; Darbani et al. 2008). In the future, this method can be improvised by changing the parameters and culture conditions of explants.

2.4 *Agrobacterium*-Mediated Transformation

Transfer of DNA into the dicotyledonous genome can be achieved using soil bacteria *A. tumefaciens,* and its associated plasmid can be used as a vector for introducing

DNA into the genomes of dicotyledonous plants. In the early 1980s, the first transgenic plant was generated using *A. tumefaciens* as a gene vector, and the healthy transgenic plants had the ability to transmit the replaced T-DNA to their progeny (Zambryski et al. 1983). Genetic transformation of rice through *Agrobacterium*-mediated gene transfer is a common method to study gene function and improve agricultural traits, for example, disease, insect and pest resistance; drought and salt tolerance. Generally, the *Agrobacterium*-mediated gene transformation requires an aseptic condition and a long process for the conversion of the transformed tissue into regenerated shoots and roots. In addition, there may be a chance of an undesirable mutation such as somaclonal variation in transformed plants (Clough and Bent 1998; Bent 2000). It is proven that *Agrobacterium*-mediated transformation presents a higher frequency of multi-locus insertion of transgenes. Experimentally, using a 'super-binary' vector and *Agrobacterium*-mediated transformation, Komari et al. (1996) reported a 47% frequency of transferring two T-DNAs of the vector, and the T-DNAs segregated independently in more than half of the co-transformants.

A disadvantage of *Agrobacterium*-mediated transfer is that it typically produces transgenic lines with a low average copy number of the T-DNA (Kohli et al. 2003; Olhoft et al. 2004; Oltmanns et al. 2010). Owing to the limited integration rates, *Agrobacterium*-mediated gene transfer has difficulty in the case of monocotyledonous compared to dicotyledonous species (Frame et al. 2002). However, significant modification in the procedure of *Agrobacterium*-mediated transfer can achieve stable transformation in monocotyledonous species (e.g. maize; Ishida et al. 2007). In the case of both cereals and monocots, *Agrobacterium*-mediated gene transfer has proved unsuitable for many reasons, including because it is not a natural pathogen for the monocotyledonous plants and because all types of cells cannot effectively receive the transgene (Cleene 1985; Binns and Thomashow 1988). Therefore, because of all these disadvantages and failures, many researchers have become interested in other genetic transformation methods such as direct DNA transfer and biolistic transformation (Travella et al. 2005) (Fig. 1).

2.5 Floral Dip Method of Transformation

Floral dip is another method being used in the genetic engineering strategies because it directly produces genetically modified seeds bypassing the laborious tissue culturing procedures. In addition, tissue culture could generate transformed plants harbouring undesirable mutations from somaclonal variation (Bent 2000). Without the requirement of any equipment and tissue culture methods, the floral dip transformation method has become the simplest and most convenient method. *Arabidopsis* plant *Agrobacterium* floral dip transformation was demonstrated successfully for the first time in a flowering plant (Clough and Bent 1998). By using this method, the risk of unwanted microbial contamination can be avoided. It also has some disadvantages such as lower transformation efficiency with no specific target and requires a sufficient number of flowers and seeds. However, through the transformed anthers,

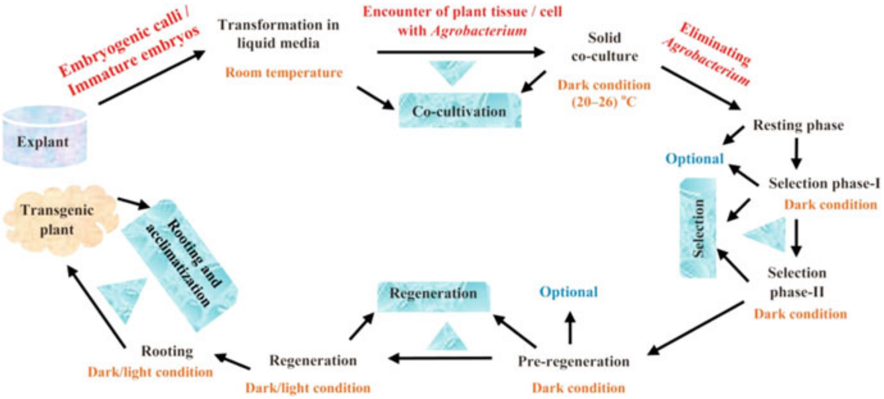

Fig. 1 A generalized scheme of *Agrobacterium*-mediated transformation and recovery of transgenic rice. [Reproduced with permission (Sulaiman et al. 2019), Copyrights reserved to Elsevier Inc., 2019]

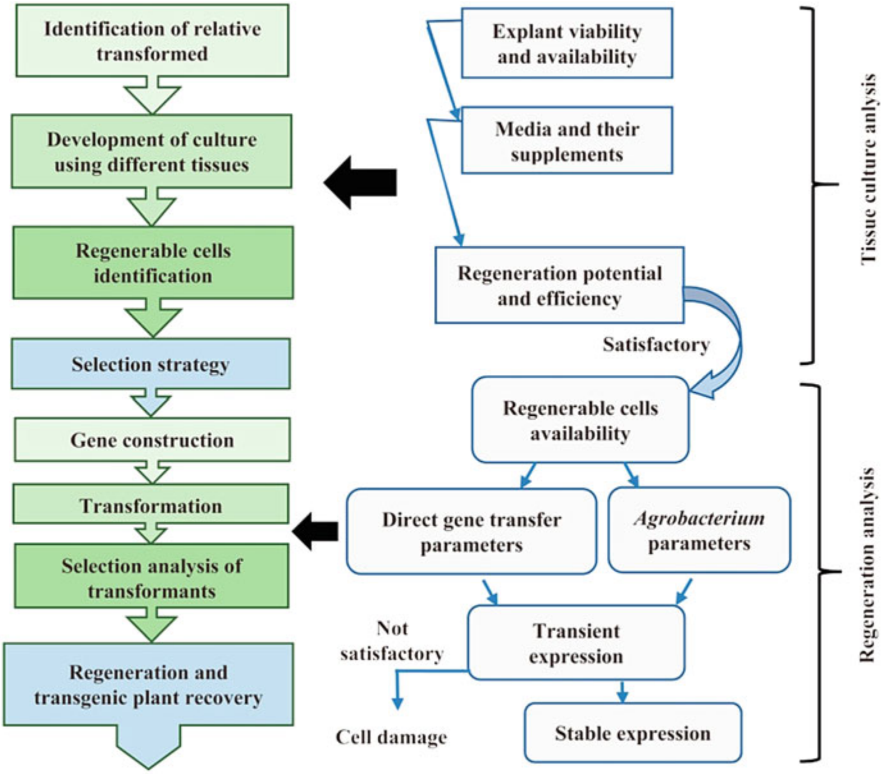

Fig. 2 The process of producing transgenic rice using tissue culture, transformation and regeneration. [Reproduced with permission (Sulaiman et al. 2019), Copyrights reserved to Elsevier Inc., 2019]

the floral dip method can be considered an alternative technique for the rice spikelet (Fig. 2).

3 Confirmation of Transgenes in Regenerated Plants

The analysis of transgenes integration in regenerated plants needs to be performed through proper systems based on a selectable marker, transgene constructs and reporter genes. Transformed plant cells integrated with antibiotic resistance markers are generally screened by the addition of antibiotics in the growth media to identify the transgenic plant cells. However, this process requires high-quantity antibiotics that are quite expensive and is further undesirable due to the risk of horizontal transfer of antibiotic resistant genes to the other bacteria. Therefore, other screening approaches such as PCR as well as reporter genes expression are utilized to enhance the accuracy of confirmation methods for transformed plant cells. Few reporter genes, including GFP, Luc and GUS expression, are colorimetric, where expression of these reporting markers could be monitored visually (Naylor 1999). Quantification of reporter expression is also possible through spectrophotometry. The expression of GUS can be noticed with the use of histochemical assays where localization of transgene may be examined. Furthermore, some other reporter expressions, including LacZ and CAT activities, are screened with several enzyme assays.

The southern blotting molecular technique is commonly used to detect specific DNA fragments, for the identification of transgene copy numbers incorporated into the host genome and to find the transgene rearrangement and transgene integrity (Gheysen et al. 1987; Dai et al. 2001). It is performed by cleaving the DNA molecule into short fragments using restriction endonucleases, separation by molecular weight of fragments through electrophoresis, transfer onto nitrocellulose membrane, incubation of membrane bound DNA fragments with labelled DNA probes and then the hybridization pattern is observed through chromogenic detection. The number of transgenes is directly proportional to the number of DNA bands noticed. Western blotting provides qualitative findings for target proteins present in the transgenic plant samples and is very effective for analysing insoluble proteins (Brett et al. 1999; Lipton et al. 2000). However, in comparison to other target protein based tests, it is a tricky technique and able to analyse only a few regenerated plant samples during one round. Thus, this approach is not regularly applied in transgenic screening events, but it can be used for research activities to verify the preliminary results produced by other screening methods.

The most regular antibody-based protein identification assay for transgene testing is the strip test method also called the dipstick or lateral flow test. This method provides qualitative results and is used to detect proteins of interest in plant samples. In this assay, the strip is composed of a nitrocellulose thin membrane which is supported by the sample pad on one side and wicking pad on the other side. Samples are homogenized in the suitable buffers and the nitrocellulose membrane on the strip wicks up buffer solution and then it steps upwards through capillary transfer and the transgenic protein becomes bound to a specific antibody. The results appear in the form of two visible lines on the strip; the first is for the tested transgenic protein and the other for the control that shows the accuracy of the strip used and all the assay procedure. The presence of only the standard line reveals the absence of a transgenic protein in a sample but that the assay was performed correctly (Conceição et al. 2004). Moreover, it is simple to operate, low-priced, can be accomplished in the open field and does not require particular equipment and trained manpower. These strips now offer detection of several specific proteins in a single assay (Lipton et al. 2000). Another extremely sensitive antibody-based assay is ELISA in which a protein targeted antibody is coated on a multiwell plate that provides the quantitative results for transgenic proteins present in regenerated plant samples (Kuiper 1999). In this method, an enzyme linked antibody binds with a target protein and then unbound antibodies are eliminated by washing. This technique is comparatively more sensitive than the strip assay and can quantify specific proteins even at low concentrations. However, it demands more time, advanced laboratory facilities and trained manpower.

The PCR approach is the most sensitive method compared with all other molecular methods used for the confirmation of transgenes. The conventional PCR is performed using oligonucleotides specific to the site of the transgene and plasmid constructs used for the development of transgenic plants. Amplification of DNA fragments with expected size indicates the presence of the gene of interest, and it could be further validated by DNA sequencing. A real-time amplification provides a sensitive, fast, high-throughput PCR-based analysis compared with traditional southern blotting, especially for zygosity identification in transgenic crops and copy numbers of transgene (Bubner and Baldwin 2004). This method offers quantitative monitoring of reporter genes in real time. Gene expression is analysed in RT-PCR by isolating mRNA from transgenic plant cells. This method relies on mRNA reverse transcription and formation of cDNA which is further employed as a template for amplification of the gene of interest. Similar to RT-PCR, northern blot analysis also requires the mRNA isolation as a screening material for transgene testing, and it provides a relative amount of transgene expression at the RNA level. This method is comparatively cheap, simple to execute and not inundated by artefacts (Ferreira et al. 2012). However, this method can analyse only a few genes for the expression in a single test. Moreover, it can be helpful to monitor up or down transgene regulation for specific analysis, but it is not useful for regulated genes that are unknown. A microarray method is a very promising laboratory practice used to quantify the expression of several transgenes in a single analysis. This method has been encompassed in transgene validation as a powerful technique that can simultaneously detect more than 250,000 targets using a single chip (Leimanis et al. 2006; Elenis et al. 2008; Kim et al. 2010). This analysis comprised an amplification of desired targets followed by a hybridization step with unique probes attached on the chip (Querci et al. 2010; Von Götz 2010). The use of the microarray technique for transgene confirmation is restrained because of cross contamination risks, cost and advanced equipment required for the scanning, and it is comparatively more laborious compared with other techniques.

At present, emerging NGS technologies provide massive parallel targeted sequencing protocols from the whole genome at low cost and in a relatively short time. The PCR-based methods in transgenic confirmation are often limited owing to production of non-specific amplicons, shortened transgene sequences, amplification failure of extensive exogenous DNA insertions in highly complexed repetitive genomes, multiple insertions and hindrances in the particular transgene identification (Park et al. 2017). The accessibility of NGS approaches and bioinformatic tools have facilitated the genome analysis and genetic characterization of highly complex traits. Moreover, NGS data analysis facilitates identifying the exact location of transgenes in the whole genome, especially in transposable elements and repetitive genomic regions which is impossible to accomplish using PCR amplification methods (Elbaidouri et al. 2013). Consequently, NGS technologies provide an alternative high-throughput analysis for the confirmation of transgene insertions in regenerated crops (Lambirth et al. 2015).

4 Factors Affecting Transformation and Regeneration of Rice

4.1 *Agrobacterium* Strains and Vectors

Agrobacterium-mediated gene transformation is a more popular tool than other techniques (Kumria et al. 2001; Sridevi et al. 2005), and it provides high efficiency of gene transformation, integration of small numbers of copies of T-DNA into the host genome programmed by *Agrobacterium* and transfer of a relatively large segment of DNA with defined ends or little rearrangement of T-DNA (Hiei and Komari 2008). Virulence (*vir*) genes on the plasmid of *Agrobacterium* code for processing and transfer of T-DNA (Roy et al. 2000). Many researchers have tested different *A. tumefaciens* strains and vectors in the transformation of rice (Table 1). *Agrobacterium* strain LBA4404 is more frequently used in rice transformation than the EHA105 and EHA101 strains. An efficient gene transformation requires a strain harbouring a vector which is a small piece of circular DNA, taken from a plasmid (*Escherichia coli*) containing a feature of insertion (foreign) or removal of DNA fragment. The choice of vector is based on the origin of replication (*ori*), selectable marker genes (both bacterial and plant resistant antibiotic) and the reporter gene. A binary vector composed of T-DNA borders, sites for multiple cloning and specific restriction and replication functions is a standard tool for plant transformation by *A. tumefaciens* (Komari et al. 2006). The important aspect to consider about a vector is size, whether cloning and transferring large or small DNA fragments. Karthikeyan et al. (2012) reported a binary vector pCAMBIA 1301 with improved efficiency.

Table 1 List of *A. tumefaciens* strains, vectors, promoters and selectable marker genes/reporter genes used for genetic transformation of different rice genotypes (adapted, modified and updated from Sulaiman et al. 2019)

Strain	Vector	Promoter	Selectable marker gene	Reporter gene	Rice genotype	Reference
A281	pTiBo542	–	npt II	*Gus*	Japonica rice cv.	Raineri et al. (1990)
LBA4404	pTOK233	CaMV35S	hpt	*Gus*	Indica rice IR72 and TCS10; Japonica rice Nortai and Radon	Aldemita and Hodges (1996)
EHA101	pTiBo542	CaMV35S	PPT	*Bar*	Tropical Japonica cv. Maybelle	Park et al. (1996)
LBA4404	pTOK233, pBin935SΩGusint, pIG121Hm	CaMV35S	hptB	*Gus*, *aphIV*	Japonica rice cv. Taipei 309	Uzé et al. (1997)
LBA4404	pTOK233	–	hpt, npt II	*Gus A*	Indica rice cv. Pusa Basmati 1(PB1)	Mohanty et al. (1999)
LBA4404	pCGN1589	CaMV35S	hpt	*Chi*	Indica rice cv. Basmati 122, Tulsi (aromatic) and Vaidehi	Datta et al. (2000)
LBA4404	pJD4	CaMV35S	hpt	*Gus*	Japonica rice cv. Taipei 309	Dong et al. (2001)
LBA4404	pWNHG	CaMV35S	nptII, hpt	*Gus*	Japonica rice cv. Taipei 309	Dai et al. (2001)
LBA4404	pMR43	4ABRC1-Act1-Hva22, CaMV35S	Bar	*ADC*	Japonica rice TNG67	Roy and Wu (2001)
LBA4404	pTOK233	–	hpt	*Gus*	Japonica rice cv. Taipei 309	Kant et al. (2001)
LBA4404 pGV2260	pTOK233 pJB90GI	–	hpt	*Gus*	Indica rice cv. Pusa Basmati (PB1)	Kumria et al. (2001)
LBA4404	pMR10	4ABRC1-Act1-Hva22, CaMV35S	bar	SAMDC	Japonica rice TNG67	Roy and Wu (2002)
LBA4404	pMVR1ODC	CaMV35S	hpt	*Gus*, *odc*	Indica rice Pusa Basmati 1 (PB1)	Kumria and Rajam (2002)

(continued)

Table 1 (continued)

Strain	Vector	Promoter	Selectable marker gene	Reporter gene	Rice genotype	Reference
LBA4404	pKUB, pKUC	CaMV35S	hpt	*Gus*, *cry1Ab*, *cry1Ac*	Indica rice Basmati 370	Ahmad et al. (2002)
EHA 105	pC30063	CaMV35S	hpt	*gfp*, *gus A*	Japonica rice cv. Taipei 309, Nipponbare, Zhongzuo 321 and Azucea	Sallaud et al. (2003)
EHA 101	pRIT1, pINA134, pRIT 1DT	CaMV35S	Hyg^R	*Gus*	Japonica rice cv. Nipponbare	Terada et al. (2004)
EHA 105	pX1-H, pX2-H, pX2-HB	CaMV35S	hpt	*Gfp*	Japonica rice cv. ZhongZuo321 (China), Ariete (Italy) and Indica rice cv. KDML105 (aromatic)	Breitler et al. (2004)
EHA 105	pCAMBIA 1301	–	hpt	*Gus*	Indica rice cv. Minghui 63, Zhenshan 97, W9864S and Zhong 419	Lin and Zhang (2005)
LBA4404	pTOK233 pCAMBIA1301-Xa21Mss	–	hpt	*Gus*	Indica rice (Bangladeshi) BR22, BRRI dhan 29, BR5842-15-4-8 and Moulata; Japonica rice cv. Taipei 309	Hoque Md et al. (2005)
LBA4404	pSB1, Pmku-RF2	CaMV35S	hpt	*chi*11, *gus*	Indica rice cv. White Ponni	Sridevi et al. (2005)
EHA 101	pCAMBIA1301	CaMV35S	hpt B	*Gus*, NiR	Indica rice cv. Koshihikari	Ozawa and Kawahigashi (2006)
LBA4404	pSAMαA1	CaMV35S	Hyg^R	*Gus*, α-A1	Indica rice cv. Pusa Basmati (PB1)	Ignacimuthu and Arockiasamy (2006)
EHA 105	pCAMBIA 1301	CaMV35S	hpt	*Gus*	Thai jasmine rice KDML105	Yookongkaew et al. (2007)

EHA 101	pRIT1	CaMV35S	hpt	*Gus*	Indica rice cv. White ponni (WP) and Pusa Basmati (PB1)	Arockiasamy and Ignacimuthu (2007)
LBA4404	pNSP3	CaMV35S, Ubi	Hyg^R, Hyg^S	*chi11*, *Gus*	Indica rice Pusa Basmati 1 (PB1)	Sridevi et al. (2008)
LBA4404, EHA105	pSB134 pNB134 pNB134	–	hpt	*Gus*	Indica rice IR64 and IR72; Japonica rice Nipponbare and Koshihikari	Hiei and Komari (2008)
EHA 101	pCAMBIA 1301	–	Hyg	*Gus*	Japonica rice cv. Nipponbare, Hokkai Siryo 308, Dular and Koshihikari	Ozawa (2009)
EHA 105	pEGAD-At2g47750	CaMV35S	bar	*Gus*, *gfp*	Indica rice cv. R207 and Teqing	Lin et al. (2009)
EHA 101	pCAMBIA1301	CaMV35S	hpt B	*Gus*, NiR	Japonica rice cv. Nipponbare	Ozawa and Takaiwa (2010)
LBA4404	pCAMBIA 1301.1 (+ AMV enhancer)	CaMV35S	Hyg	*Gus*	Indica rice cv. IR64	Ignacimuthu and Raveendar (2011)
LBA4404	pCAMBIA1305.2	CaMV35S	hpt	*Gus*, Am-SOD	Indica rice cv. Pusa Basmati 1 (PB1), Taraori Basmati and IR79485-15-3-2	Sarangi et al. (2011)
EHA 105	pCAMBIA 1301	CaMV35S	hpt	*Gus*, *hpt*	Indica rice cv. ADT 43	Karthikeyen et al. (2011)
LBA4404, EHA105	pCAMBIA1304	–	nptII, hpt II	*Gus*, *gfp*	Indica rice cv. IR64, CSR10, Pusa Basmati (PB1) and Swarna	Sahoo et al. (2011)
EHA 105	pCAMBIA 1301	CaMV35S	Hpt	*Gus*	Indica rice cv. ADT 43	Karthikeyan et al. (2012)
EHA 101	pIG 121-Hm	–	hptII	*Gus*	Indica rice cv. IR-64, Lalat and IET-4786	Shri et al. (2013)
EHA 105	Rice transformation vector	CaMV35, Ubi, Act 1	npt II, hpt	*pmi*/*pat*, *gfp*	Japonica rice Wanjing97 and Nipponbare	Duan et al. (2012)
LBA4404	pCAMBIA 1300	CaMV35S	Hyg	*Hyg*	Indica rice cv. IR64	Tran and Sanan-Mishra (2015)

(continued)

Table 1 (continued)

Strain	Vector	Promoter	Selectable marker gene	Reporter gene	Rice genotype	Reference
EHA 105	pCAMBIA 1301	CaMV35S	hpt	*Hpt*, *Cry2A*	Indica rice restorer line MH86	Ling et al. (2016)
AGL1, EHA105	pCAMBIA1304	CaMV35S	–	*Gus*	RD41	Ratanasut et al. (2017)
LBA 4404	pCAMBIA 1305.1	CaMV35S	hpt	*Gus*	Indica rice ASD16, ADT43, IR64 and Pusa Basmati	Sundararajan et al. (2017)
LBA4404	pCAMBIA 1305.2	CaMV35S	Am-SOD, hpt	*Gus*	Indica rice Sambha mahsuri and Cotton dora sannalu	Reddy et al. (2019)

Hyg, hygromycin; *pmi,* phosphomannose-isomerase; *pat*, herbicide resistance gene; Hyg^R/Hyg^S, ^{R}-resistant, ^{S}-sensitive; *gfp*, green fluorescence protein; *gus*, β-glucuronidase; *Ubi*, maize ubiquitin promoter; *Act 1*, rice actin promoter; *bar*, bacterial phosphinothricine acetyltransferase (herbicide resistance gene-Basta); *4ABRC1*, ABA-inducible promoter complex; *Hva22*, barley Hva22 intron; *SAMDC*, *S*-adenosylmethionine decarboxylase; *odc*, ornithine decarboxylase; At2g47750, *Arabidopsis* putative indole-3-acetic acid-amido synthetase; *PPT*, phosphinothricin; *AtDREB*, *Arabidopsis*-dehydration-responsive elements binding factors; *NiR*, ferredoxin-nitrite reductase; *Am-SOD*, *Avicennia marina*-superoxide dismutase; *α-A1*, α-amylase inhibitor gene; *chi*, chitinase; *cry*, *Bacillus thuringiensis*

4.2 Acetosyringone Induced Gene Transformation

Acetosyringone, a phenolic compound, is an essential component of the transformation of rice (Hiei et al. 1994). It is an inducer that activates the *vir* genes for the transfer of T-DNA from *A. tumefaciens* (Datta et al. 2000). The presence of adequate acetosyringone in inoculation and co-cultivation media results in increased T-DNA delivery by providing the specific temperature and acidic environment for *Agrobacterium vir* genes expression (Tyagi et al. 2007). The efficiency of transformation was low when acetosyringone was omitted (Hiei and Komari 2006) and a higher level might be harmful. The amount also varies with respect to genotype and explants (Ozawa 2009; Karthikeyen et al. 2011).

4.3 Role of Polyamines

Polyamines are a group of ubiquitous polycationic-nitrogen containing osmolytes present in all organisms (Roy and Wu 2002). They have essential roles in various plant cellular and molecular processes such as regulation of growth and development, membrane integrity, synthesis and function of nucleic acids, protein phosphorylation and host-pathogen interactions (Rajam et al. 1998; Mulangi et al. 2012). Low molecular weight metabolites increase their endogenous polyamine levels in response to tolerate biotic and abiotic stresses (oxygen and mineral deficiency, chilling, drought, salinity, low and high temperature, low pH) (Rajam 1997; Pillai and Akiyama 2004). The presence of polyamines in regeneration medium also improved the regeneration ability of long-term callus cultures of rice (Bajaj and Rajam 1995, 1996). It has also been well documented in rice transformation that the host polyamine metabolism responds to *Agrobacterium* infection (Kumar and Rajam 2005). Polyamines increased the *vir* gene induction and T-DNA transfer (Kumar and Rajam 2005). Tyagi et al. (2007) reported that polyamines in the co-cultivation and regeneration medium greatly improved the final transformation efficiency of rice compared with acetosyringone alone. The influence of exogenous polyamines on regeneration and *Agrobacterium*-mediated genetic transformation was investigated in other plant species, such as wheat (Khanna and Daggard 2003), tobacco (Kumar and Rajam 2005), apricot (Petri et al. 2005) and soybean (Arun et al. 2016).

4.4 Competent Rice Tissue

Bacterial infection to host cell depends on certain physiological parameters such as age of plant and tissue and stage of cell type and cell cycle. *Agrobacterium*-mediated gene transformation in rice tissue required wound-induced divisions and competence of cells (Roy et al. 2000). Monocots are not good hosts for *A. tumefaciens*, as cells at infected sites become lignified/sclerified without any further cell division. Acetosyringone in co-cultivation medium greatly influenced *vir* gene induction and gene transformation. The ability of *vir* gene induction was reported in immature endosperm and scutellum tissue of rice (Vijayachandra et al. 1995). The leaf segments neither induced *vir* genes nor inhibited gene induction. Prior to transformation, embryogenic callus increased transformation efficiency (Lin and Zhang 2005; Karthikeyan et al. 2012; Sundararajan et al. 2017).

4.5 Genotype

Agrobacterium-mediated rice transformation has been investigated in a variety of genotypes. Efficiency of regeneration and transformation have been found to be variable due to various manipulations during the transformation procedure and physiological changes occurring in the cell. *Agrobacterium*-mediated gene transformation of different genotypes is listed in Table 1.

4.6 Medium Composition

Agrobacterium-mediated gene transformation protocols include various steps such as *Agrobacterium* infection, co-cultivation, selection of transformed cells and regeneration (Duan et al. 2012; Karthikeyan et al. 2012). The composition of tissue culture media is an important factor that influences the efficiency of transformation. The medium can support the active cell division (Hiei et al. 1997). Optimization of the medium may be necessary, depending on the genotype of rice or the type of material. Successful gene transformation needs a better co-cultivation and regeneration medium encompassing salts, sugars and hormones. Researchers have employed different media compositions in rice transformation (Table 2). Transformed cells with the marker gene grow less vigorously on the selective media than on the non-selective media (Hiei et al. 2014). Type and concentration of plant growth hormones and other synthetic chemicals such as 2,4-D, Dicamba, Picloram and organic salts are effective for regeneration of transformed rice. Osmotic pressure in medium containing sucrose, maltose and mannose (Duan et al. 2012) and addition of antibiotics such as cefotaxime, carbenicillin and timentin after co-cultivation of plant cells and *A. tumefaciens* have substantial influences (Ignacimuthu and Raveendar 2011).

4.7 Different Gelling Agents

Media solidified with gelling agents are better for co-cultivation than liquid media (without solidifying agent). Different gelling agents are available in markets, and they are nontoxic to the plant tissue. A suitable concentration should be found that helps to support utilization of minerals by the tissues for survival and regeneration. The most popular gelling agents are Gelrite (Toki 1997; He et al. 2004; Ozawa 2009; Karthikeyan et al. 2012), Phytogel (Sallaud et al. 2003; Arockiasamy and Ignacimuthu 2007; Shri et al. 2013; Tran and Sanan-Mishra 2015), Agarose (Sallaud

Table 2 Media compositions for rice transformation[a]

Components (mg/l)	Medium				
	MS	N6	B5	AA	LS
Major salts					
KNO_3	1900	2830	2500	–	1900
KCl	–	–	–	2950	–
NH_4NO_3	1650	–	–	–	1650
$(NH_4)_2SO_4$	–	463	134	–	–
KH_2PO_4	170	400	150	–	170
Na_2HPO_4	–	–	–	170	–
$MgSO_4{\cdot}7H_2O$	370	185	250	500	370
$CaCl_2{\cdot}2H_2O$	440	166	150	150	440
Minor salts					
$FeSO_4{\cdot}7H_2O$	27.85	27.85	27.8	27.8	27.8
Na_2EDTA	37.25	37.25	37.3	37.3	37.3
$MnSO_4{\cdot}4H_2O$	22.3	4.4	10	10	22.3
$ZnSO_4{\cdot}7H_2O$	8.6	1.5	2	2	8.6
H_3BO_3	6.2	1.6	3	3	6.2
KI	0.83	0.8	0.75	0.75	0.83
$CuSO_4{\cdot}5H_2O$	0.025	–	0.025	0.025	0.025
$Na_2MoO_4{\cdot}2H_2O$	0.25	–	0.25	0.25	0.25
$CoCl_2{\cdot}6H_2O$	0.025	–	0.025	0.025	0.025
Organic salts					
Glycine	2.0	2.0	–	2.0	–
Thiamine	0.4	1.0	10.0	10.0	0.4
Pyridoxine	0.5	0.5	1.0	1.0	–
Nicotinic acid	0.5	0.5	1.0	1.0	–
i-Inositol	100	100	100	100	100
L-Glutamine	–	–	–	876	500
L-Asparagine	–	–	–	266	500
L-Arginine	–	–	–	174	–
Guanylic acid	–	–	–	–	200
Cytidylic acid	–	–	–	–	200
Tyrosine	–	–	–	–	100
Carbon source					
Sucrose	3%	3%	3%	3%	3%
pH	5.8	5.8	5.5	5.8	5.6

[a] MS, Murashige and Skoog (1962); N6, Chu et al. (1975); B_5, Gamborg et al. (1968); AA, Toriyama and Hinata (1985); LS, Linsmaier and Skoog (1965)

et al. 2003; Terada et al. 2004) and Agar (Ignacimuthu and Raveendar 2011). Gelrite and Phytogel have higher purity and potency in tissue culture media than Agar.

4.8 Co-cultivation Conditions

An *A. tumefaciens* strain harbouring a selectable marker gene was grown on a suitable culture media with appropriate antibiotics (Tables 1 and 2). A single colony of this bacterium was inoculated with an antibiotic containing liquid media, and the culture was incubated on an orbital shaker at 28 °C and 150 rpm. After reaching 0.2–0.4 OD (optical density) at 600 nm, the bacterial culture was centrifuged at 3000 rpm and 28 °C for 10 min and the pellet was resuspended in preincubation medium with acetosyringone. The selected explants were transferred in the bacterial suspension for 10–15 min with occasional shaking. The excess of bacteria were removed from the explants using filter paper and transferred to the co-cultivation medium. After 3 days of co-cultivation at 25 °C, the explants were rinsed 4–5 times with washing medium containing cefotaxime. The explants were blotted on sterile paper to remove excess bacteria and moisture and transferred to the selection medium (Arockiasamy and Ignacimuthu 2007).

4.9 Promoters and Selectable Markers

Promoters are regions of DNA located near the transcription start site that initiates transcription. The CaMV35S promoter is a minimal promoter that promotes enhanced strength of gene expression (Odell et al. 1985; Sridevi et al. 2008). Other promoters, the maize ubiquitin gene and rice actin gene, are also studied in rice transformation. Selectable marker genes are employed to screen for our gene of interest during co-cultivation. In rice transformation, hygromycin phosphotransferase (hpt), phosphomannose-isomerase (pmi), neomycin phosphotransferase (nptII) and phosphinothricin acetyl transferase (pat or bar) are frequently used for selection of the transformed clone. The antibiotic hygromycin resistant or sensitive hpt gene is a potential selection marker for transgenic rice (Hiei and Komari 2008). After co-cultivation, visual reporter gene expression detection is needed in *Agrobacterium*-mediated transformation. Visual marker gene β-glucuronidase (gus) are virtually used (Karthikeyan et al. 2012; Shri et al. 2013).

4.10 Fertility, Stability and Expression of Transgene

Stable genetic inheritance and transgene expression are essential for the production of transgenic plants (Roy et al. 2000). Extensive reports indicate that the gene transformed plants obtained using the *Agrobacterium*-mediated transformation protocol showed normal phenotype. The maximum number of copies of integrated gene was observed in transgenic rice, and several authors proved that the transgenic rice has a fertile condition (Chan et al. 1993; Reddy et al. 2019).

5 Conclusion

Combining the method of DNA introduction into plant cells with an efficient regeneration protocol, avoiding protoplasts or extensive periods of callus culture, we can produce the most convenient system for genetic transformation. Rice transformation has been successfully demonstrated using several techniques such as electroporation, PEG treatment, particle bombardment and the *Agrobacterium*-mediated method. Owing to several advantages such as stable and low copy number integration of T-DNA for the plant chromosome with the capacity of transferring larger DNA segments with defined ends, the *Agrobacterium*-mediated gene transfer method is the preferred genetic transformation method for rice among various methods. However, in the studies of transient gene expression assays, electroporation or PEG treatments provide an alternative system to analyse genetically modified plant genes. Although in the case of the establishment of protoplast regeneration and transformation, systems would improve significantly with the routine combination of both systems which is not yet possible. On the other hand, the development of biolistic transformation systems has allowed rapid progress towards the recovery of transgenic cereals. The recently developed method of tissue electroporation promises to be another very attractive approach for the transformation of primary explants.

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New Approaches for Improving Salt Stress Tolerance in Rice

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Abstract

Rice is the main food crop for more than half the world's population. Environmental stresses lead to significant economic losses with decreased productivity. Alleviation of salt stress is vital to maintain productivity and ensure food safety for a global population worldwide. Rice is tolerant to salinity in the stages of germination, tillering, and maturity. However, at the stages of early seedling and reproduction, it exhibits vulnerability against this stressor. Inhibition of growth and decrease in biomass production are an inevitable effect of salt stress. Efforts to enhance the ability of rice plants to survive under various abiotic stresses are essential toward changing climate. Several strategies were taken for managing salinity stress. In this prospect, this chapter summarizes the role and mechanisms of action of priming, microorganisms, nutrients application (calcium, manganese, silicon), osmolytes organic solutes (trehalose), and small biological molecules such as phytohormones (abscisic acid) that could be taken as stimulators for rice adaptation under salt stress. Application of priming, microorganisms, nutrients, organic solutes, and phytohormones on rice plants is almost certainly due to its practical significance and the economical benefits under salt stress prevailing in most regions of this crop production.

Keywords

Microorganisms · Nutrients · Organic solutes · Phytohormones · Priming · *Oryza sativa* · Salinity

1 Introduction

Rice (*Oryza sativa* L.) belongs to the Poaceae family and is a staple food crop worldwide. Thus, any collapse of this crop production caused by changing environmental conditions may lead to major economic losses. Rice production has increased dramatically over the past few years, but this trend can be sustained through the introduction of new technologies to advance its stress tolerance.

During the last years, climate changes hastened influences of environmental stresses on plants. Among the emerging ecological effects of climate change, abiotic stresses evidently disturb plant growth and lower productivity (Bellard et al. 2012). Environmental stresses, resulting from severe climatic changes, like salinity, UV radiation, drought, heat, and chilling stress, cause a disturbance in rice plant development. The results are difficult to predict, because plants are often at one time subjected to many stresses, such as water stress followed by heat stress (Suzuki et al. 2014). Plants can adapt to stress factors (Yoshida et al. 2014), even multiple, through stress-induced gene expression leading to increased protective metabolite output (Basu and Roychoudhury 2014; Paul and Roychoudhury 2016). In the key rice-growing areas, immense abiotic stress tolerance is needed to meet the agronomic and economic needs. Salt stress can lead to the worst losses of rice yield in the world, among the abiotic stresses mentioned above.

This chapter reviews the response of rice to salt stress and considers new action plans to diminish the salt stress hostile effects on rice plants.

2 Salinity Stress

According to Richard (1954), saline soils are defined as having a particular EC of saturated soil-paste extracts 4 dS m^{-1} ($t = 25$ °C). Bresler et al. (1982) considered soils to be salt-affected starting at 2 dS m^{-1} EC, where the level of salt toxicity for many fruit, vegetable, and ornamental crops is 2–4 dS m^{-1}. Soils are supposed to be salinized if salt concentrations exceed toxicity threshold defined as (1) a salt concentration of 3–5 g L^{-1} in soil solution (Vargas et al. 2018); (2) an amount of 0.05–0.15% toxic salts in water extracts (Vargas et al. 2018), or (3) a particular electrical conductivity of 2–4 dS m^{-1} saturated soil-paste extracts (Bresler et al. 1982).

Analysis of soil solution at soil/water extract of 1:5 is a conventional technique of estimating soil salinity in the Eurasian countries (Vargas et al. 2018). However, the preparation of pastes and their extracts is a labor-intensive process, commonly supplemented by soil/water suspension assessments of 1:2.5, 1:5, or 1:10 with subsequent recalculation by empirical coefficients (Jahn et al. 2006).

$$\mathrm{EC}_{\mathrm{SE}} = (250 \times \mathrm{EC2.5})/\mathrm{WC}_{\mathrm{SE}}$$

where $\mathrm{EC}_{\mathrm{SE}}$ is the electrical conductivity of saturated soil-paste extract, EC2.5 is the electrical conductivity of soil/water (1:2.5) suspension, and $\mathrm{WC}_{\mathrm{SE}}$ is the moisture content of soil paste in g 100 g^{-1}.

Soil salinity leads to significant restrictions in the production of agricultural crops and has a negative impact on food safety. Salinity has induced yield losses in dry territories ranging from 18% to 26% to 43% (Vargas et al. 2018), which can threaten the quality of life of local communities and exacerbate harm caused by land degradation and climate change.

3 Response of Rice to Salt Stress

Rice is the cereal model to understand the environmental stress effects on the yield of crops. Impacts of salt stress on rice growth and the development and type of mechanisms, which regulate stress effects, were reviewed by Hussain et al. (2017). Cereal crops typically are characterized by a high degree of salt stress tolerance. Munns and Tester (2008) reported differences in salt response in dicotyledonous plants are more common than in monocots. Salt-susceptible and salt-resistant species evolved various adaptation mechanisms to this stressor (Roychoudhury and

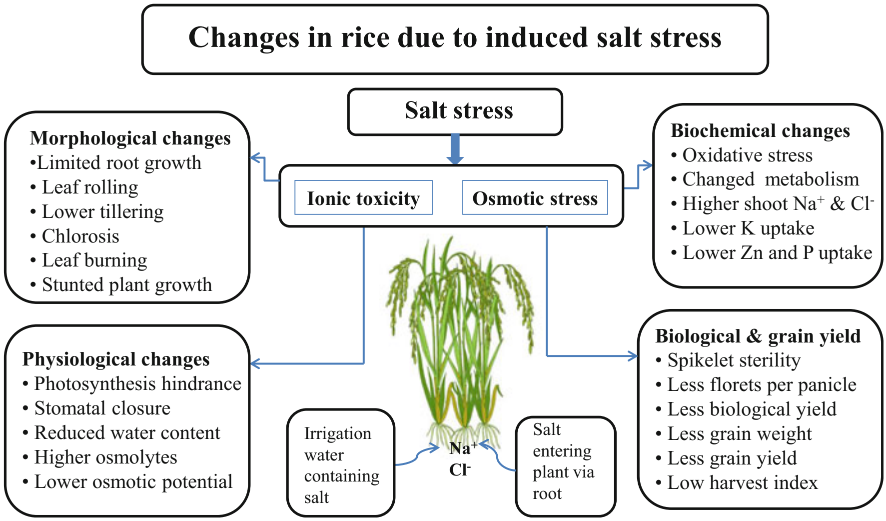

Fig. 1 Schematic diagram shows response of rice plant growth, development, and yield under salt stress

Chakraborty 2013). The reaction of plant to salt stress is caused by reducing osmotic potential accompanied by high toxicity to the ion (Rahman et al. 2000).

Understanding the mechanisms by which plants perceive and transmit signals of salt stress to cellular machinery to generate adaptive responses is important for classical breeding and biotechnology programs designed to improve salt tolerance (Roychoudhury et al. 2008, 2009b). Recent studies on model plants and crops have shown that these adaptive responses focus on complex signaling mechanisms which lead to an integrated physiological response to environmental stress (Hasegawa et al. 2000; Zhu et al. 2004). It is therefore vital to increase crop productivity to recognize morphological, physiological, and biochemical compounds acting as coordinators controlling multiple stress signaling pathways and to show how these enhance the physiology of whole plant stress.

Rice displays tolerance to salt in the developmental stages of germination, tillering, and maturity, but demonstrates susceptibility in the early stages of seedling and reproduction (Moradi and Ismail 2007; Zeng et al. 2001). The unfavorable effects of low salt levels on rice plants are because of osmotic stress, nutritional disorder, and ionic toxicity (Grattan and Grieve 1998; Wahome et al. 2001), while the effects are due to nutritional imbalances and gathering of ions, especially Na^+ and Cl^- ions under moderate to high salt stress (De Pascale et al. 2003a, b). Usually, salt-stressed plants suffer cyclical physiological, morphological, and biochemical alterations from appearance to ripeness (Läuchli and Grattan 2007; Munns 2002). Figure 1 shows salt's multiple effects on plant growth, development, and yield. Nevertheless, salt-stressed plants' response is referred to as interacting causes, which

Fig. 2 Cultivation of rice at salt-affected soil in Kafr El Sheikh Governorate, Egypt

including genotype, growth phase and shape, solution composition, and salt-induced stress concentration (Jenks et al. 2007).

Generally, salt stress affects different metabolic procedures comprising activity of cellular enzymes and its concentration and function (Amirjani 2011; Khan and Panda 2008). Shoot growth and elongation are decreased because of dehydration and shrinking of plant cells. Such modifications result in signs of visual injury for the salt-sensitive rice genotypes (Läuchli and Grattan 2007). Figure 2 shows growing rice in salt-affected soil in Kafr El Sheikh Governorate, Egypt.

3.1 Rice Seed Germination

A complex physiological and biochemical modification phenomenon, which leads to the activation of the embryo. Seed germination is a critical stage, which affects total dry matter and grain yield output (Parihar et al. 2015). Salt stress induced a delay in rice seed germination showing an adverse association between seed germination and salt stress (Shereen et al. 2011).

3.2 Rice Morphology

Plant height is a major feature of plant morphology, which under biotic and/or abiotic stress frequently results in alterations in growth and development. The hostile influence of salt stress in plant height has been reported in several studies. Salinity-induced stress induces stomatal closure resulting in increased temperature of the leaves and reduced elongation (Rajendran et al. 2009; Sirault et al. 2009). Gain et al. (2004) reported a clear negative impact of salinity on plant height and shoot length with a gradual increase in solutions till 31.3 dS m^{-1} in rice cultivar BR11. Such adverse effects are leading to diminishing rice plant ability to consume water and nutrients due to changes in osmotic potential.

The length of the root varies significantly even under salt stress. A rise in salt-stressed root length is attributed to the plant's capacity to enter deeper soil field layers at low water potential over root elongation (Perez-Alfocea et al. 1996).

Salt stress induces changes in the osmotic capacity of leaf parenchyma cells, thereby reducing the capability of plants to absorb water and nutrients. Ali et al. (2004), when they studied leaf and yield parameters of 18 rice cultivars, reported that the size of the leaf depended on reduced cell division and cell elongation processes in stress conditions.

3.3 Rice Physiology

The physiological alterations in salt-stressed plants comprise a range of responses, for example, higher Na^+:K^+ ratio, lower stomatal conductance (gs), lower photosynthesis, and increased accumulation of reactive oxygen species (ROS), which inhibits photosynthesis, and all these changes contribute to plant growth reduction. Salt stress toxic effects trigger the occurrence of mature and older leaves by diminished protein production and function of the enzymes (Carillo et al. 2011; Munns 2002; Munns and Tester 2008). Under salt stress, the accumulation of Na^+ ions disturbs photosynthetic constituents (Davenport et al. 2005). For many plant species, decreased stomatal conductance followed by decreased transpiration and CO_2 assimilation is the immediate response of plants to a large range of salt stress (Ashraf 2001; Munns and Tester 2008; Romero-Aranda et al. 2001). Generally, chlorophyll and stomatal conductance regulate photosynthesis, which are decreased in salinity condition (Dingkuhn et al. 1992; Parida et al. 2004).

ROS production is a basic reaction to plant under salt stress. ROS overload is toxic in the cell but can mediate the induction of stress tolerance mechanisms (Das and Roychoudhury 2014; Lee et al. 2003). If no protective mechanism exists, ROS are highly reactive and can harm all metabolic pathways through lipid, nucleic acid, and protein damage. Degradation of ROS by antioxidant compounds can be substantially affected by salinity (Banerjee and Roychoudhury 2018; Carillo et al. 2011; Foyer and Noctor 2003).

3.4 Rice Growth and Yield

Salinity stress results in reduced rice growth and rice yield quantity and quality (Asch and Wopereis 2001; Hasanuzzaman et al. 2009; Zeng and Shannon 2000).

According to Grattan et al. (2002), field salinity exceeding 1.9 dS m^{-1} could decrease rice grain yield. It was suggested that 3.0 dS m^{-1} (Grattan et al. 2002), while $\geq$3.4 dS m^{-1} are considered as salt toxicity threshold in rice (Zeng and Shannon 2000). Further, $\leq$4.0 dS m^{-1} decreased rice yield by 25% in tolerant cultivars (Beecher 1991). In addition, a reduction of 50% in seed germination and 80% loss in grain yield based on cultivar tolerance were reported when rice was grown at 8 dS m^{-1} (Asch and Wopereis 2001). Yields decreased to reach zero in sensitive salt varieties when rice was grown at 8 dS m^{-1} in hot and dry seasons, and dry matter accumulation decreased by 90% (Asch and Wopereis 2001).

4 Approaches to Mitigate Salt Stress in Rice

Plant species with the ability to withstand high levels of salt are called halophytes, whereas glycophytes are susceptible to salinity in the soil. Plants are categorized into halophytes and glycophytes, taking into account the susceptibility to salt stress (Levitt 1980; Shannon et al. 1994). There are two main strategies to utilize the salt-affected soil: i.e., (1) exploit of reclamation procedures to utilize salt-affected soils appropriate for cultivation and (2) usage of salt-affected soils through halophyte cultivation or salt-tolerant crops/cultivars. The second proposal is called a "biological approach" (Ashraf and Wu 1994), which has a major prospective to deal with salinity all over the world. Conventional breeding programs have made several endeavors to increase crop salinity tolerance (Duan et al. 2008), but progress toward cultivation of these salt-tolerant species is unhurried and the profitable returns are restricted. Consequently, the exploitation of a range of biological strategies that can increase the salinity tolerance of rice such as priming, microorganisms, nutrient management, and the application of phytohormones or compatible solution might be a smart approach to conquer the growth disturbance of immature plants in salt-affected soils. Table 1 presents the role of certain techniques used in salt-stressed rice. Some of them are utilized for mitigating unfavorable influences of salt stress on rice and comprise physical, chemical, and biological management of plants, seedlings, or seeds, before, during, or after exposure to salinity stress. These suggested strategies will be outlined in the following sections.

4.1 Salt Stress Mitigation in Rice by Priming

Priming is a procedure in which seeds are exhibited to limited water availability in well-ordered conditions, enabling metabolic processes such as chemical and physiological pregermination activities to begin with short-term storage redrying until germination is complete (Bradford 1986; Farooq et al. 2009). In practice, large-scale seed priming of profitable cereal crop seeds is tricky to accomplish while benefits are recognized (Murungu et al. 2004). The widely used methods of priming are in general proprietary and are done by seed companies. Seed priming allows most crops to combat salt stress hostile effects (Dawood et al. 2014a; Taie et al. 2013).

Table 1 The role of some strategies in improving rice plant tolerance under salt stress

Strategy/salt	Main findings	Reference
Sodium selenate (Na_2SeO_4) at 2, 4, 6, 8, 10, and 12 mg L^{-1}	Na_2SeO_4 increased activity of antioxidant enzyme (GSH-Px, APX, SOD, CAT), K^+: Na^+, and proline, while reduced H_2O_2 and MDA	Subramanyam et al. (2019)
Calcium chloride ($CaCl_2$) at 10 and 15 mM	$CaCl_2$ at 10 mM increased germination rate index, final germination %, shoot height, root height, and dry weight	Roy et al. (2019)
Sodium silicate (Na_2SiO_3, $9H_2O$) at 2 mM	Na_2SiO_3, $9H_2O$ enhanced ascorbate-glutathione cycle enzymes activity and ascorbate and GSH levels while reduced accumulation of ROS	Das et al. (2018)
Brevibacterium linens RS16	Bacterial inoculation improved photosynthesis and decreased ACC oxidase activity, and organic compounds and 1-aminocyclopropane-1-carboxylate	Chatterjee et al. (2018)
Gibberellic acid (GA3) at 10 μM	GA3 up-regulated chloroplast lipid biosynthesis	Liu et al. (2018)
Sodium nitroprusside (SNP) at 0.25 mmol L^{-1}	Nitric oxide increased biomass production	Adamu et al. (2018)
Trehalose at 0, 10, and 20 mM	Trehalose improved superoxide dismutase, net photosynthetic rate, total soluble proteins, growth, and yield	Shahbaz et al. (2017)
Silicon (Si) ion and silica nanoparticles	Si activated antioxidant defense systems and induced osmolyte accumulation	Abdel-Haliem et al. (2017)
Manganese sulfate ($MnSO_4$) at 0.5 mM	$MnSO_4$ increased water status, flavonoids, phenolic compounds, ascorbate, dehydroascorbate reductase, monodehydroascorbate reductase activities, superoxide dismutase, and catalase, while decreasing Na	Rahman et al. (2016)
Trehalose at 25 mM	Trehalose 25 mM increased antioxidant enzymes activity and solute concentration participating to osmotic adjustment	Abdallah et al. (2016)
Arbuscular mycorrhizal (AM) fungi *Claroideoglomus etunicatum*	AM increased plant biomass production as increased net photosynthetic rate, photosynthetic and rubisco activities, transpiration rate, and stomatal conductance	Porcel et al. (2015)
Trehalose at 10 mM	Trehalose reduced proline, ROS, MDA, and LOX activities while improving RWC, AsA, GSH, chlorophyll, and redox status	Mostofa et al. (2015)

Previous researches on priming presented and offered a plan for seed priming engineering, which explains the current possibility existing on biological, chemical, and physical treatments (Abdelhamid et al. 2019b; Ibrahim 2016). Conversely, efficiency of different priming mediators differs with different environmental stresses and plant species. Growth regulators have received considerable attention from seed priming chemicals due to their strong influence on germination and increased seedling development of numerous species of plants (Afzal et al. 2012; Farooq et al. 2007; Iqbal and Ashraf 2013). Seed germination and initial growth of seedlings of hot pepper were encouraged by polyamine seed priming, i.e., diamine putrescine (Put^{2+}), tetramine spermine (Spm^{4+}), and triamine spermidine (Spd^{3+}) (Khan et al. 2012). Priming of seeds with Spd and Spm also increases salinity tolerance in rice (Paul and Roychoudhury 2016, 2017a, b; Paul et al. 2017).

Abdelhamid et al. (2019b) suggested a schematic diagram demonstrating a series of response after seed priming with a view to relieving salt stress effects on plants. The diagram shows the prospective influence of seed priming on physiological processes leading to increasing plant salt tolerance and the final yield. Chunthaburee et al. (2014) reported that Spd or gibberellic acid (GA3) seed priming enhanced to some extent the salt-induced decrease in the growth and anthocyanin and chlorophyll content of rice seedlings. In addition, salt-stressed seedlings priming with Spd or GA3 had greater phenolic and antioxidant capacity than control seedlings and concluded that Spd was more successful than GA3 in increasing the tolerance of rice to salt stress. Jamil et al. (2014) investigated the effect of 1:100, 1:500, and 1:1000 derived smoke solutions from *Bauhinia* and *Cymbopogon* with priming on the biochemical and physiological traits of the rice cultivar NIAB-IR-9. Comparing with non-primed seeds at high salt concentrations, smoke primed seeds increased significantly in germination rate and seedling vigor (Jamil et al. 2014). Furthermore, smoke primed seeds displayed a significant increase in chlorophyll a, chlorophyll b, carotenoids, protein, N, K^+, Ca^{2+}, fresh weight, and dry weight, whereas decreased Na^+ concentration in comparison with seed hydropriming in salt stress. They concluded that *Bauhinia* was the most successful dilution with 1:500, which improved biochemical and physiological traits. Hathout et al. (2014) reported that soaking rice seeds with 30 μM validamycin A (trehalase inhibitor) increased the weight of 1000 grains, filled grains %, and rice grain yield in both salt-tolerant cultivar Agami M5 and susceptible one Sakha 103.

4.2 Salt Stress Mitigation in Rice by Microorganisms

The beneficial microorganisms, which diminish toxic effects of salt stress on plant production were reported (Talaat et al. 2015). Recently, Bhambure et al. (2018) reported that joined usage of salt-tolerant microorganisms (*Pseudomonas multiresinivorans*, *Microbacterium esteraromaticum*, and *Bacillus subtilis*) and organic fertilizers (farmyard manure) helped rice plants relieving salt stress and increasing growth.

Several studies have reported the importance of arbuscular mycorrhizal fungi (AM fungi) in extenuating salt stress by improving plant growth and crop development, as AM fungi can reform root morphology and increase the rummaged area of nutrient by extensive hyphal netting (Abdelhamid et al. 2019a). AM fungi improve plant salt tolerance over additional effective nutrient uptake (Beltrano et al. 2013), security of enzyme activity (Rabie and Almadini 2005), improved efficiency in photosynthesis (Estrada et al. 2013a), facilitated water uptake by the plant (Sheng et al. 2008), and the reduction of ionic imbalances. (Estrada et al. 2013b). These fungi also reshape the physiology of plant, increase plant growth and nutrient concentration, and diminish the use of mineral fertilizers (Estrada et al. 2013a; Gianinazzi et al. 2010). AM colonization of the fungi enhances K^+ absorption, while reducing Na^+ translocation into the tissue in salt-stressed plants (Giri et al. 2007; Talaat and Shawky 2011). Thus, plants with AM fungi under salt stress also had a higher K^+:Na^+ ratio and a lower Na^+ concentration than plants without AM fungi (Estrada et al. 2013a; Rabie and Almadini 2005), therefore preventing disorder of different enzymatic processes and protein synthesis inhibition.

Dry matter output is an integrated sign of plant quantity in various sorts of environmental stresses, and the symbiotic productivity of AM fungi has been assessed in terms of increasing plant growth (Ruiz-Lozano et al. 2012). Rice inoculation with AM fungi created greater stomatal conductivity, transpiration, and a net photosynthetic level (Porcel et al. 2015). Furthermore, Porcel et al. (2015) proposed that symbiosis with AM fungi under salt stress has a protective outcome on photosynthetic tissues compared to root tissues. The ability of a plant to except Na^+ is considered a key feature of tolerance to salt stress in glycophytes (Abdelhamid et al. 2010; Cuin et al. 2011; Munns and Tester 2008). It was also proposed that Na^+ transportation regulation over time to the shoot is essential for tolerance to salinity (Maathuis et al. 2014). Moreover, rice plants inoculated with AM fungi maintain their growing processes by reducing more Na^+ salt-stressed plants (Porcel et al. 2015). Rice plant inoculation with AM fungi increased solar energy use and CO_2 fixation, which could enhance the tolerance of salt in plants through escaping injury to photosystem centers and improving light energy use in photochemical developments, thus increasing productivity (Porcel et al. 2015).

4.3 Salt Stress Mitigation in Rice by Plant Nutrients Use

With tolerance mechanisms, plants may use multiple mineral ions, i.e., N, P, K, Zn, Fe, and Si, to overcome different environmental challenges (Abdelhamid et al. 2011; Bargaz et al. 2016; Dawood et al. 2014b; El-Lethy et al. 2013; Hellal et al. 2012; Kamel et al. 2010; Mohamed et al. 2016; Rady et al. 2016a; Shedeed et al. 2015). Disturbances to nutrient crops under salt stress restrict the growth via disturbing nutrient transportation and partitioning. Owing to the rivalry of Na^+ and Cl^- with ions, for example, K^+ and Ca^{2+}, salt stress may also cause unbalance in plant nutrient status (Abdelhamid et al. 2010). Salt stress reduced plant growth because of ion toxicity, e.g., $Cl^-$and Na^+, and ion disparities (Forni et al. 2017; Orabi and Abdelhamid 2016). Increased Na^+ and Cl^- concentrations were accompanied by reduced N, P, K^+, Mg^{2+}, and Ca^{2+} in several plant species, e.g., wheat and common bean, in sustainable production systems (Abdelhamid et al. 2013a, b; Awad et al. 2012; Dawood et al. 2016; Rady et al. 2015, 2016a, b, 2019). Utmost fertilizers contain high soluble salt concentrations. It is typically important to avoid application of fertilizers with high salt indices near seedlings. With the addition of gypsum, the protection of soil structure and the improvement of water absorption could prevent sodium accumulation in the soil exchange complex (Sharma and Singh 2017). The role of certain mineral ions in mitigation of salt stress and increase of plant salt stress tolerance, e.g., calcium, manganese, and silicon, will be explained in the following subsections.

4.3.1 Calcium

Calcium (Ca) is upper than the majority of other plant nutrients as the mean Earth's concentration is about 36.4 g kg^{-1} (Mengel and Kirkby 2001). Ca^{2+} is an essential nutrient for plant and plays an indication for particles to regulating the response to environmental stresses (Mahmood-ur-Rahman et al. 2019). Several reports showed that Ca^{2+} application improved plant salt tolerance (Jaleel et al. 2008; Rahman et al. 2016; Sohan et al. 1999). Several protective mechanisms were associated with Ca^{2+}'s beneficial role in security against stress. Ca^{2+} supports membrane structural and functional survival, increases the stability of cell walls, and regulates ion homeostasis, thereby playing a key part in plant growth and development (Arshi et al. 2010; Morgan et al. 2014). In addition, by promoting high K^+:Na^+ ratio selectivity, an exogenous introduction of Ca^{2+} decreases the NaCl toxicity (Rahman et al. 2016). Ca^{2+} also improves the enzymatic antioxidant mechanisms and ROS detoxification processes of Rahman et al. (2016). Cha-um et al. (2012) recorded that 7.92 mM $CaCl_2$ significantly promoted photosynthetic capabilities, including peak PSII quantity (Fv/Fm), PSII photon yield, photochemical quenching (qP), and net photosynthetic frequency (Pn), in two genotypes of salt-stressed rice seedlings, especially in salt-susceptible IR29 genotypes. Cha-um et al. (2012) concluded that exogenous calcium was absorbed by root tissues to the entire plant, performing as a salt defense indicator in the regulatory system for abscisic acid (ABA) and in the process of stomatal closure in salt-stressed plants. $CaCl_2$ application with 10 mM reduced the unfavorable effect of salt in rice, so calcium application-enriched fertilizers in saline soils could be an efficient way to enhance rice adaptation (Roy et al. 2019). Roy et al. (2019) clarified that, for the following reasons, Ca^{2+} is involved in regulatory systems that allow the plants to adapt to the adverse salt stress environment. First, Ca^{2+} exogenous application increased germination rate index, final percentage of germination, shoot mass, root height, shoot mass, root mass, relative water content, and typical evaluation record in rice cultivars that are either salt-tolerant or salt-susceptible. Second, by retrieving the photosynthetic pigments, Ca^{2+} increased the photosynthetic capability. Third, Ca^{2+} adjusted the proline biosynthesis, which balances growth and existence energy and thus helped to increase plant stress tolerance. Fourth, by regulating the antioxidant protection and ROS detoxification system, Ca^{2+} reduced oxidative injury. Calcium signaling functions an important part in enabling rice plants to generate the protection response counter to salt stress (Roychoudhury and Banerjee 2017).

4.3.2 Manganese

Manganese (Mn) is an essential plant nutrient, which acts an energetic part in several metabolic processes such as amino acids, fatty acids, proteins, lipids, flavonoids, respiration, photosynthesis, ATP synthesis, and hormone stimulation (Lidon et al.

2004; Millaleo et al. 2010). Mn deficiency injuries plant through influence on photosystem II (PS II) that offers electrons for photosynthesis. Moreover, Mn deficiency erodes the structural tolerance of pathogens, thus decreasing plant tolerance to abiotic stresses (Millaleo et al. 2010; Rahman et al. 2016). Mn acts as $O_2^{\bullet-}$ and H_2O_2 scavenger though the scavenging process mechanism is not well-known (Ducic and Polle 2005). Supply of Mn is important in plant acclimatization reactions under different abiotic stresses. Mn use improved tolerance of rice seedlings to salt stress by coinciding with increasing of the ionic balance, antioxidant protection system, and glyoxalase system (Rahman et al. 2016). Mn exogenous application reduced metal toxicity via decreasing metal concentration in plant tissues and reducing lipid peroxidation, whereas increased chlorophyll, carotenoids, and antioxidant protection system activity was observed, thus improving the productivity of plants (Paľove-Balang et al. 2006; Sebastian and Prasad 2015).

4.3.3 Silicon

Silicon (Si) is the second element in the Earth's crust next to oxygen (28%). It is a metalloid element as well, which exists in a composite form like SiO_2 (Swain and Rout 2017). The helpful part of Si in several plant species in terms of improving plant growth, development, and yield has been recorded, confirming Si's ability to improve tolerance of plant under different abiotic stresses (Swain and Rout 2017). Furthermore, under drought and heat stress, Si application enhanced photosynthetic pigments, straw yield, grain yield, and grain quality (Qados 2015; Swain and Rout 2017). Under salt and drought stresses, Si also acts a critical part in motivating antioxidant enzymes, expression of plant genes, exchange of gases, and regulation of osmoprotectant accumulation and osmotic adjustment (Qados 2015; Swain and Rout 2017). In addition, under salt stress, Si application also reduced uptake of Na^+ and its transport, while improving uptake of K^+ and its translocation. Nevertheless, these mechanisms vary with the length of the stress, growth conditions, plant types, genotype, etc. (Qados 2015; Swain and Rout 2017).

Numerous studies are published to elucidate Si's constructive part in enhancing plant salt stress tolerance (Farooq et al. 2019; Garg and Bhandari 2016; Swain and Rout 2017). The beneficial function of Si in relieving salt stress may be attributable to impeding the absorption and transporting of Na^+ and Cl^- to shoots and preserving the osmotic capacity of tissues, leading to salt dilution and subsequently increased yield (Garg and Bhandari 2016). Si application under salt-stressed rice plants increased stomatal activity (Romero-Aranda et al. 2006). Si decreased H_2O_2 and increased photosynthesis rate (Al-aghabary et al. 2005). Tuna et al. (2008) reported that under salinity stress, the permeability of the cell membranes was partly preserved at the same level as under no salinity stress as compared to the Si application. It was suggested that increased activities of ATPase and PPase as well as decreased Na^+ and increased K^+ are the possible mechanisms of Si-enhanced salt tolerance (Liang et al. 2007). Some studies (Al-aghabary et al. 2005; Zhu et al. 2004) approved the assumption that Si's function in raising plant salt tolerance was through decreased lipid peroxidation, while increasing enzymatic and nonenzymatic antioxidants (Liang et al. 2003).

A reduction in Na^+ uptake is considered one of the key functions of Si in enhancing salt stress tolerance (Matoh et al. 1986). In addition, Si influences transport of K^+ and Na^+ by inducing root plasma membrane activity, for example, HC-Ppase, HC-ATPase, and HC-ATPase tonoplast (Liang et al. 2005). Additionally, silicon's up-regulated expression of jasmonic acid in plants under salt stress provided with Si ions has activated their antioxidant protection systems and induced the production of osmolytes; consequently Si alleviated salt stress unfavorable effects (Abdel-Haliem et al. 2017).

4.4 Salt Stress Mitigation in Rice by Applications of Osmolytes Organic Solutes (Trehalose)

An important mechanism for sustaining hyperosmotic stress caused by excess salinity is osmotic adjustment (Chen and Jiang 2010). This technique in plants works by gathering different organic compounds called osmoprotectants, osmolytes, or compatible solutes that enable cell turgor to create an energetic grade for water absorption. Osmoprotectants are categorized into three main classes, i.e., quaternary amines (e.g., betaine glycine and dimethylsulfoniopropionate), polyol/sugars (e.g., mannitol and trehalose), and amino acids (e.g., proline). Trehalose (Tre), a non-diminishing glucose disaccharide, protects against stress (Ali and Ashraf 2011; Garcia et al. 1997). Utmost studies on osmolyte involved glycine betaine and proline and their function as osmoprotectors in improving antioxidant systems (Roychoudhury and Banerjee 2016; Roychoudhury et al. 2015); however, less is realized about the role of Tre in improving stress tolerance in higher plants. Tre can act efficiently to settle proteins, dehydrated enzymes, and lipid membranes, and to prevent damage to biological structures (Fernandez et al. 2010). Tre occurs in very minor quantities in plants near to the recognition point, but contributes to metabolic processes correlated with plant stress tolerance (Aghdasi et al. 2008; Duman et al. 2011). Mostofa et al. (2015) explained the function of trehalose in defending rice from salt-induced oxidative injury through reduced ROS accumulation, increased nonenzymatic antioxidants, and co-activated antioxidant and glyoxalase systems. Abdallah et al. (2016) found enhanced osmotic adjustment and elevated activity of antioxidant enzymes in shoots of salt-stressed rice variety Giza 178 with 25 mM Tre application. Theerakulpisut and Phongngarm (2013) reported that Tre (5 mM) was the more effective osmoprotectant compared to proline (10 mM) and sorbitol (without and with 170 mM NaCl) in providing protection against salt stress in rice seedlings and offering an applied implication for growing rice in saline agriculture.

4.5 Salt Stress Mitigation in Rice by Phytohormones Application

It is identified that phytohormones such as ethylene, gibberellins, abscisic acid, cytokinins, auxins, and brassinosteroids all regulate plant growth and development traits (Iqbal et al. 2014). Abscisic acid (ABA) typically guards plants against different environmental stress conditions (Dar et al. 2017; Sah et al. 2016; Tuteja 2007; Vishwakarma et al. 2017). Cabot et al. (2009) reviewed ABA's positive role in decreasing Cl^{-} and Na^{+} concentrations and reducing transpiration in order to maintain water via switching plant stomata. In addition, in rice plants, Na^{+}, Cl^{-}, and Na^{+}:K^{+} were decreased due to exogenous application of ABA (Gurmani et al. 2013). Some studies reported the role of exogenous use of ABA in increasing rice salt stress tolerance (Roychoudhury et al. 2009a, b). Moreover, exogenous application of 100 μM of ABA in rice increased grain yield through more efficient accumulation of soluble sugar and proline as well as maintaining K^{+} and Ca^{2+} equilibrium (Sripinyowanich et al. 2013).

5 Concluding Remarks and Future Perspectives

Rice has been the most ancient and the most important food source for humans. Changing environment generates stress conditions which can negatively affect rice crop leading to major economic losses. Most of the fruit, vegetable, and ornamental crops were exposed to salt stress within a range of EC 2–4 dS m^{-1}. Salt stress contributes to major restrictions in the production of agricultural crops and thus has a negative effect on food safety. Rice is the cereal crop susceptible to salinity stress. Rice displays some tolerance to salinity in the developmental stages of germination, tillering, and maturity. However, it exhibits vulnerability at the early seedling and reproductive stages. Inhibition of rice growth owing to physiological and biochemical modifications leads to a decrease in dry matter production and grain yield due to manifold influences of salt stress. Endeavors to improve the ability of plants to thrive under various environmental stresses are ongoing. In this prospect, the role of priming, beneficial microorganisms, nutrients application (calcium, manganese, silicon), osmolytes organic solutes (trehalose), and small biological molecules such as phytohormones (abscisic acid) might be deemed powerful in improving rice plant adaptability against salt stress. The up-to-date awareness of the biological approaches used in saline rice is reviewed in Table 1. It is highly promising to use biological methods to improve rice plants under salt stress. Identifying morphological, physiological, and biochemical targets that serve to control multiple stress signal pathways and determining how to boost whole plant physiology under salt stress are crucial in order to maintain/increase crop productivity. Upcoming research must focus on genetic, metabolic, and physiological alterations raised in salt-stressed plants and modified by agro-technical approach involving priming, microorganisms, nutrients, organic solutes, and phytohormone mediators. In addition, more research into the directions suggested in this chapter will help in the near future for the effective management of rice production under salt pressure.

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Genomics and Biotechnological Approaches in Generating Salinity and Drought Tolerance in Rice

Poonam Tiwari, Yuvraj Indoliya, and Debasis Chakrabarty

Abstract

Agronomically important crops like rice are highly affected by environmental stresses. Drought and salinity stress are the two dominant forms of abiotic stress which deteriorate crop yield and productivity. Despite the superior varieties and tremendous research in improving technologies, rice productivity has become static in the last few decades. Therefore, biotechnology techniques particularly genomic modification through genetic engineering could be utilized for enhancing rice yield in drought and saline conditions. Plant genetic engineering and molecular-marker approaches enable us to generate transgenic rice with improved genotype which can efficiently survive under extreme drought and salinity conditions. The use of improved tools like gene pyramiding and genome-editing techniques like TALENs and CRISPR/Cas9 will open up new avenues to engineer crops with desired traits.

Keywords

Abiotic stress · Crisper/Cas9 · Epigenomics · Proteomics · QTL · Transcriptomics

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1 Introduction

Rice is undoubtedly a staple food and source of energy and vitamins especially in case of developing countries of South East Asia. The significant challenges that the farmers are facing are the extremely sodic soil and unavailability of sufficient ground or rainwater to grow rice, so that there is a significant yield penalty. Traditional approaches like breeding have numerous demerits which include the low level of variation in gene pools and reproductive barrier, are tedious, and took almost a decade to develop a crop variety resistant to drought and salinity stress. Biotechnological approaches and genomics provide a platform to study myriads of genes induced during abiotic stresses (Roychoudhury et al. 2011; Paul and Roychoudhury 2019). The latest progress in genomic studies leads to the discovery of the considerable number of expressed sequence tags (ESTs) and novel genes which helped us in deciphering their exact role in stress tolerance.

Further improved multi-parallel approaches like microarray and transcriptome sequencing for gene expression profiling, complementation, and promoter-trapping strategies along with random and targeted mutagenesis open the new avenues for identification of specific drought and salinity responsive genes (Roychoudhury and Banerjee 2015). Overlapping of osmotic stress (drought and salinity) responsive gene was witnessed due to the recent advancement of genomics and biotechnological approaches (Chen et al. 2002; Kreps et al. 2002; Buchanan et al. 2005). Genetic engineering and molecular analysis paved the way to introduce the gene of interest to produce cisgenic or transgenic crops with altered high-performance genotype which can withstand severe drought and saline conditions. Osmotic stresses either by drought or excessive salt distort the cellular homeostasis leading to metabolite toxicity and generation of ROS which ultimately negatively affects the plant growth and productivity (Hasegawa et al. 2000). Higher accumulation of osmoprotectants like proline, glycine betaine, and antioxidants helps in ROS scavenging, and efficient compartmentation of ions occurs by transporter/symporters to cope with osmotic stress induced by water scarcity and excess salt (Ingram and Bartels 1996; Hasegawa et al. 2000; Zhu 2002; Sangam et al. 2005; Valliyodan and Nguyen 2006). On the other hand, QTL analysis, epigenetic regulation coupled with high-throughput sequencing, even allows to better understand the genetic basis of stress tolerance and stress memory in subsequent plant generations. This chapter focuses on the detailed study of how biotechnology and genomic advancement helped us to explore the rice genome intensely availing new avenues to developed efficient drought and salinity-resistant variety.

2 Functional Characterization of Potential Targets

2.1 Overexpression Analysis

Genome-wide sequencing of abiotic stressed rice seedling results in the identification of myriads of differential expressed genes. These unique genes were characterized by the researcher to decipher their role in drought and salt tolerance through genetic engineering. Genetic engineering is an innovative technique to alter the plant genome by overexpressing or knockdown of the targets. More recently, genome editing using Crisper/Cas9 comes in the scene which is very precise and distorted the specific portion of the gene to completely silence the activity of targets. Alteration in the transcript of osmoprotectants, transporters and ion channels, antioxidant enzymes, TFs, helicases, molecular chaperones, and signaling molecules would result in the efficient abiotic stress-tolerant transgenic crops (Table 1).

2.1.1 Osmoprotectants

Both salinity and drought stress create osmotic imbalance due to ion disproportion and water deficiency, respectively. In such cases, rice produces compatible organic solutes like proline, glycine betaine, trehalose, mannitol, and myoinositol also commonly known as osmoprotectants. Proline, common yet effectual osmolytes, accumulates in the plant in response to various abiotic stresses. Pyrroline-5-carboxylate synthase (5PCS) and pyrroline-5-carboxylate reductase (P5CR) are the two key enzymes involve in the biosynthesis of proline from glutamic acid (Ashraf and Foolad 2007). Thus researcher tried to enhance salinity and drought tolerance in transgenic rice by upregulating the transcript level of these enzymes through genetic engineering which in turn accumulate the increased amount of proline (Roychoudhury et al. 2015). Overexpression of *P5CS* in rice confers salt tolerance in transgenic rice (Karthikeyan et al. 2011). Similarly, expression of Δ1-pyrroline-5-carboxylate synthetase (P5CS) from *Vigna aconitifolia* in rice under stress-inducible promoter promotes excess production of proline under drought and confers drought tolerance to transgenic rice (Zhu et al. 1998). However, *P5CSF129A* which is a mutagenized homolog of P5CS also found to impede the adverse effect of salinity stress by lowering the lipid peroxidation (Hong et al. 2000).

Glycine betaine is also one of the compatible solutes that accumulate in response to stress to protect from drought and salinity (Chen and Murata 2008; Roychoudhury and Banerjee 2016). Reports were available of transgenic rice overexpressing *codA* (choline oxidase) gene from *Arthrobacter gobiformis* and the *cox* gene coding for choline oxidase from *Arthrobacter pascens* capable of withstanding in extreme saline condition (Sakamoto and Murata 1998; Su et al. 2006). Similarly, homologous expression of *codA* gene in rice from Pusa basmati confers the salinity tolerance (Mohanty et al. 2002).

Another essential molecule categorized as osmoprotectant is trehalose which is a nonreducing disaccharide potentially involved in maintaining homeostasis during abiotic stresses (Redillas et al. 2012). The study by Li et al. (2011) proved that *OsTPS1* overexpressing transgenic rice augments the salt tolerance by accumulating

Table 1 Tabular representation of selected studies depicting overexpressing and gene silencing approaches to generate salinity- and drought-tolerant rice

Technique	Class	Genes	Source	Stress tolerance	References
Overexpression	Osmoprotectants: proline	*P5CS*	*Vigna aconitifolia*	Drought	Zhu et al. (1998)
		P5CS	Rice	Salinity	Karthikeyan et al. (2011)
	Glycine betaine	*codA*	*Arthrobacter gobiformis*	Salinity	Sakamoto and Murata (1998)
		codA	Pusa basmati	Salinity	Mohanty et al. (2002)
		cox	*Arthrobacter pascens*	Salinity	Su et al. (2006)
	Trehalose	*otsA* and *otsB*	*E. coli*	Salinity	Garg et al. (2002)
		OsTPS1	Rice	Salinity	Li et al. (2011)
	Chaperones	*OsDhn1*	Rice	Drought and salinity	Kumar et al. (2014)
		OsDhn-Rab16D	Rice	Drought	Tiwari et al. (2019)
		OsHsfA7	Rice	Drought and salinity	Liu et al. (2013)
		OsHSP50.2	Rice	Drought	Xiang et al. (2018)
	Transporters and ion channels	*AgNHX1*	*Atriplex gmelini*	Salinity	Ohta et al. (2002)
		OsNHX1	Rice	Salinity	Fukuda et al. (2004)
		ApKUPs	*Alternanthera philoxeroides*	Drought	Song et al. (2014)
		OsHAK1	Rice	Drought	Chen et al. (2017)
		AKT1	Rice	Drought	Ahmad et al. (2016)
	TFs	*DREB2A*	Rice	Drought	Cui et al. (2011)
		DREB2B	Rice	Drought	Mizoi et al. (2013)

		OsABF1	Rice	Drought and salinity	Hossain et al. (2010)
		OsbZIP23	Rice	Drought and salinity	Xiang et al. (2008)
		OsbZIP46	Rice	Drought	Tang et al. (2012)
		OsbZIP16	Rice	Drought	Chen et al. (2012)
		ZFP182	Rice	Drought and salinity	Huang et al. (2013)
		OsNAC2/6 OsNAC10, OsNAC6 and ONAC045	Rice	Drought and salinity	Nakashima et al. (2009), Jeong et al. (2010), Nakashima et al. (2007), Zheng et al. (2009)
		OsNAC14	Rice	Drought	Shim et al. (2018)
	Signaling molecules	*OsCDPK21*	Rice	Salinity	Asano et al. (2011)
		OsCDPK7	Rice	Drought and salinity	Saijo et al. (2000)
Gene silencing	RNAi	*RACK1*	–	Drought	Li et al. (2009)
		SQS	–	Drought	Manavalan et al. (2011)
		OsDSG1	–	Drought	Park et al. (2010)
		C3HC4	–	Drought	Kamthan et al. (2015)
		Zat10	–	Drought and salinity	Mittler et al. (2006)
	Crisper/Cas9	*SAPK2*	–	Drought sensitive	Lou et al. (2017)
		Ghd2	–	Drought	Liu et al. (2016)

osmolyte such as proline and trehalose also with the higher transcripts of the stress-inducible genes. Garg et al. (2002) also observed the same phenomenon by overexpressing *Escherichia coli's* trehalose biosynthetic gene(s) (*otsA* and *otsB*) in rice.

2.1.2 Molecular Chaperones

Molecular chaperones are a vast class of protein comprising proteins which play a crucial role in protecting protein conformation during stress. Molecular chaperones maintain protein conformation thereby maintaining its native state along with serving other proteins in synthesis, degradation, and cellular compartmentalization (Boston et al. 1996). One of such molecular chaperones are DHNs (dehydrin) that belongs to LEAII (late embryogenesis group II) and has been reported to involve in osmotic stress tolerance caused by water deficit and salinity stress. Kumar et al. (2014) had stated that *OsDhn1* harboring transgenic rice lines impart drought and salinity tolerance due to an efficient ROS scavenging system. Recently, our group had studied the novel aspect of DHNs to combat drought stress. Besides chaperones, *OsDhn-Rab16D* acts as a positive transcriptional co-regulator to mediate the drought tolerance in transgenic rice through the ABA-mediated signaling pathway (Tiwari et al. 2019). The group II *LEA* gene from rice, *Rab16A* has been found to confer salt tolerance when overexpressed in transgenic tobacco and rice (Roychoudhury et al. 2007; Ganguly et al. 2012).

Heat shock protein (HSP) is also invariably a vital class of chaperones especially involved in imparting abiotic stress tolerance. For instance, transgenic rice overexpressing *OsHSP50.2* which belongs to HSP90 family experience reduced water loss and hence were tolerant to drought stress (Xiang et al. 2018). Similarly, transgenic rice overexpressing *OsHsfA7* exhibited drought and salinity tolerance through efficient ROS scavenging machinery (Liu et al. 2013).

2.1.3 Transporters and Ion Channels

Transporters and membrane channels do have an essential role in maintaining cellular homeostasis during abiotic stress by keeping a higher K^+/Na^+ ratio (Afzal et al. 2016). In two different studies, when the vacuolar Na^+/H^+ antiporter gene *AgNHX1* from *Atriplex gmelini* and *OsNHX1* from rice is overexpressed in rice, it resulted in enhanced salt tolerance of the transgenic lines (Ohta et al. 2002; Fukuda et al. 2004). Literature is available depicting the importance of potassium transporters in enhancing drought tolerance also. For example, Ahmad et al. (2016) proved rice AKT1 potassium channel involved in drought tolerance and supported it with experimental evidence. Likewise, *OsHAK1*, the high-affinity potassium transporter, improves dehydration tolerance through positively regulating the expression of stress-responsive genes and other ion channels (Chen et al. 2017). Abiotic stress responsive high-affinity potassium (K^+) transporters *ApKUPs* from *Alternanthera philoxeroides* also improves water dehydration stress through maintaining intracellular K^+ concentration (Song et al. 2014).

2.1.4 Transcription Factors (TFs)

Transcription factors (TFs) are the key regulators that indulge in the regulation of gene expression under normal and stressed conditions. Therefore, an extensive study on overexpression of TFs provides better adaptability of transgenic lines under drought and salinity stress (Banerjee and Roychoudhury 2015, 2017). Here we discuss a few examples of MYB, AP2/ERF, bZIP, Zn finger, WRKY, and NAC TFs whose overexpression imparts abiotic stress tolerance particularly drought and salinity (Riechmann and Meyerowitz 1998). Rice transgenic over-expressing *OsMYB-R1* gene endure drought stress due to auxin and salicylic acid cross-talk and improvement in its root architecture (Tiwari et al. 2020). A transgenic approach utilizing *DREB2A* and *DREB2B* TFs enhance the drought tolerance of rice harboring *DREB2A* and *DREB2B* (Cui et al. 2011; Mizoi et al. 2013). Similarly, *OsABF1* from rice (*Oryza sativa*) has been reported to impart drought and salt tolerance (Hossain et al. 2010). Further, homologous ectopic expression of *OsbZIP23* in rice augmented the salinity and drought tolerance (Xiang et al. 2008), while *OsbZIP46* and *OsbZIP16* overexpression impart only water stress tolerance (Tang et al. 2012; Chen et al. 2012). Huang et al. (2013) reported that constitutive expression of Zn finger *ZFP182* from rice enables transgenic rice to grow well in arid and saline conditions as compared to wild type. NAM, ATAF1-2, and CUC2 (NAC) transcription factors are known to be linked with various abiotic stress tolerances. Numerous instances supported the fact that NAC TFs are involved in drought and salinity stress like *OsNAC2/6*, *OsNAC10*, *OsNAC6*, and *ONAC045* (Nakashima et al. 2007, 2009; Jeong et al. 2010; Zheng et al. 2009). Overexpression of *OsNAC14* confers only drought tolerance to transgenic rice (Shim et al. 2018).

2.1.5 Signaling Molecules

Signaling molecules perceive the signals that plant transmits on exposure to any type of stress that a plant experience. These molecules initiate the signaling cascades that ultimately trigger stress response helping the plant to recover from the adverse conditions. Depending upon the stress, signaling pathway may be specific or non-specific and ABA dependent or independent (Roychoudhury et al. 2013). Ca^{2+} and MAPK are the two vital secondary messengers in plants involved in response to various abiotic stress stimuli including drought and salt (Harper et al. 2004; Andreasson and Ellis 2010; Wu et al. 2011). It was evident that Na^+/H^+ exchanger is regulated by the Ca^{2+} activates the SOS (Salt Overly Sensitive) protein complex to impart the salinity tolerance (Qiu et al. 2002; Sanchez-Barrena et al. 2007). One of the rice calcium-dependent protein kinases (CDPKs) *OsCDPK21* results in transgenic rice resistant to salt stress (Asano et al. 2011). Similarly, *OsCDPK7* confers salt and drought tolerance to transgenic rice (Saijo et al. 2000). However, MAPK like *OsMAPK33* overexpressing transgenic rice is found to be sensitive as compared to wild type (Lee et al. 2011; Roychoudhury and Banerjee 2017).

2.2 Gene Silencing Approach

2.2.1 MicroRNAs and Small Interfering RNAs

In the last two decades, the gene silencing approach gained tremendous interest among researchers for crop improvement. siRNA (small interfering RNAs) or miRNA (microRNAs) is the most common technique to be utilized by the molecular biologists to inhibit the specific gene expression through post-transcriptional gene silencing (PTGS) (Younis et al. 2014; Banerjee et al. 2016). Manipulation of target gene involved its identification, RNAi constructs preparation and transformation, and maintenance of transgenic lines till screening and assessment of required traits. It is advantageous over antisense technology because of its efficiency, stability, and precision in molecular engineering-based modifications. During salinity and drought stress expression of endogenous salt and drought, responsive miRNA has been detected in *Oryza sativa* and *Oryza rufipogon*, respectively (Mittal et al. 2016; Zhang et al. 2016). Before that Li et al. (2009) had generated the knockdown lines of the receptor for activated C-kinase 1 (*RACK1*) through RNAi gene silencing which shows augmented drought tolerance in transgenic rice. Similarly, RNAi-mediated disruption of the squalene synthase (SQS) which is a catalytic enzyme in sterol biosynthesis leads to dehydration tolerance at the early and late developmental stages of rice (Manavalan et al. 2011). Similar drought tolerance was witnessed in RNAi transgenic rice of RING finger E3 ligase gene-*OsDSG1* and C3HC4 RING finger E3 ligase (Park et al. 2010; Kamthan et al. 2015). Interestingly, Zn finger TFs *Zat10* RNAi mutants were more tolerant to drought and salinity stress as compared to WT (Mittler et al. 2006).

2.2.2 Genome Editing Using Crisper/Cas9

Genome editing gained the new momentum in generating mutants developed through targeted mutagenesis of the genome. Genome editing can be done in four ways, namely, zinc finger nucleases (ZFNs), the transcription activator-like effector nucleases (TALENs), the meganucleases, and the CRISPR/Cas RNA-guided nucleases (Carroll 2014; Curtin et al. 2012). However, ZFNs and TALENs have similar mutation frequency as CRISPR/Cas9, but CRISPR/Cas9 is more straightforward, and its ability to cleave methylated DNA is advantageous over other platforms (Curtin et al. 2012). Among three classified CRISPR/Cas systems (I, II, and III), type II derived from *Streptococcus pyogenes* is recently being used for genome manipulation (Makarova et al. 2011; Ran et al. 2013).

Targeted genome editing is precise with the rare case of background noise, resulted in a choice for researchers to utilize this technique for the advancement of crop improvement. For instance, *SAPK2* which is a primary mediator of ABA signaling was characterized through loss-of-function using CRISPR/Cas9 system (Lou et al. 2017). The sapk2 mutants were found to be sensitive as compared to wild type indicating the importance of *SAPK2* in ABA-mediated drought tolerance in rice. Conversely, Ghd2-CRISPR lines showed improved drought resistance as compared to overexpressing lines and wild type at seedling and later stages of rice development (Liu et al. 2016). Crisper/Cas9 technique is very recent; therefore it needs to be explored for illustration in improving salinity-tolerant rice variety as well.

3 Molecular Breeding Approach to Develop Superior Varieties

To avoid unwanted pleiotropic effects of classical genetic engineering approaches to overcome abiotic stress by overexpression of genes, marker-assisted breeding or molecular breeding comes in the spotlight. Overexpression of the gene(s) and its nonspecific integration into plant genome result in growth and developmental defects, hence invariably reduce the plant yield which is an uncompromisable parameter. Development of salt- and drought-tolerant variety through the breeding process was slow due to limited genetics knowledge governing tolerance and inadequate screening techniques, while recent technological advancement allows the researcher even to identify individual genes controlling the traits of interest on a large scale in a short period.

3.1 QTL Mapping/Gene Discovery to Find Out the Drought and Salt-Related Loci

Quantitative traits locus (QTLs) is the portion of DNA in the genome of an organism linked with a particular trait. QTLs are mapped by identifying which molecular markers correspond with an observed trait. Previously, QTLs for agronomic and domestication traits (Furuta et al. 2014; Subudhi et al. 2015), yield, and morphological traits (Thomson et al. 2003; Septiningsih et al. 2003; Tian et al. 2006) were transferred in rice to develop several introgression line populations. Hence, QTLs related to salt and drought tolerance associated with a particular trait may play an inevitable role in generating stress-tolerant plants. Recent genetic and biotechnology advancement enables the researcher to utilize microarray-based transcriptional profiling of differential gene expression, genetic mapping, and expression profiling to identify genes underlying QTLs (Salvi and Tuberosa 2005; Sahi et al. 2006; Walia et al. 2007; Marino et al. 2009; Pandit et al. 2010). In 2017, Wang et al. identified four DEGs located in the QTL regions using integrated RNA sequencing and QTL mapping that might be associated with salt tolerance and can be utilized to developed salt-resistant rice variety. Recently, Sabar et al. (2019) identified drought-related QTL by crossing IR55419-04 and Super Basmati. The QTLs relating to drought tolerance, root morphological trait, and water uptake were found on chromosome 1, chromosome 3, and qTWU3.1, respectively. These newly identified QTLs can be used to develop salt- and drought-tolerant rice varieties for marker-assisted breeding. Further, the functional relationship between QTLs and phenotypic traits could be established using molecular mapping.

3.2 Marker-Assisted Selection

Techniques like polymerase chain reaction (PCR)-based marker-assisted selection (MAS) gain momentum as it can be used to refine mapping using near-isogenic lines to develop various stress tolerance crops. MAS (marker-assisted selection) increases the rate of conventional breeding as the desired trait can be easily linked with the molecular markers. Further allelic variation in the interest gene offers both a fresh source of genetic information for MAS and can also be used to develop a simple, inexpensive molecular marker, thus offering an ideal marker for a characteristic that contributes to salt or drought tolerance. Recently, genotyping is also used to spot salt-tolerant germplasm lines which allow rapid, reproducible, easy, and affordable screening techniques for successful breeding. For instance, Rahman et al. (2016) utilize 376 single nucleotide polymorphism (SNP) markers to screen 107 rice germplasm accessions in parallel characterized the allelic diversity at the significant QTL Saltol. Seven such landraces, which are salt-tolerant varieties, have been traced by them as they produce fewer Na^+ and comparatively more K^+, retaining a reduced leaf N^+/K^+ proportion. In the same year, Tiwari et al. (2016) had identified salt tolerance QTLs in rice using bulked segregant analysis of biparental recombinant inbred lines (RIL). Mutational breeding also contributed to developing high-yielding salt- and drought-tolerant varieties. For instance, rice mutants rst1 were found to be salt-tolerant as they have elevated shoot biomass and chlorophyll content but reduced lipid peroxidation and electrolyte leakage under salinity stress (Deng et al. 2015). Further microarray analysis of salt-tolerant rice mutant rst1 revealed its role in multiple abiotic stress tolerance (Jangam et al. 2016).

4 Functional Genomics

Functional genomics technologies, such as transcriptomics and proteomics, helped a lot to identify a large number of stress-responsive genes (Agrawal et al. 2016). Recently, due to higher sensitivity and wider application, next-generation sequencing (NGS) is used extensively for extracting putative abiotic stress responsible genes (Bansal et al. 2014).

4.1 Transcriptomics and Proteomics-Based Analysis

Drought and salt resistance/tolerance are vital features of rice breeding strategy for the development of perspective variants. Genetic complexity and related environmental diversity hampered such efforts in many ways, thereby identification of key responsible genes and associated networks involved in drought and salt tolerance always plays a key role in the development of superior genotypes under drought and salt stresses (Cushman and Bohnert 2000). Previously, genomic approaches including microarray analysis as well as transcriptome analysis have been carried out among stress-sensitive and stress-tolerant rice varieties for identification of possible variations at the molecular level (Walia et al. 2005; Lenka et al. 2011; Ray et al. 2011). These reports suggested significant transcriptional modulation under salt, drought, and water-deficit conditions. Later on, RNA sequencing-based analysis has been proved to be an efficient technique for whole transcriptomic based analysis. Several RNA-seq studies explored the modulation of transcriptomes in several plants including rice under abiotic stress conditions (Shankar et al. 2016; Diray-Arce et al. 2015; Niroula et al. 2012). Recently Tiwari et al. (2019) reported the role of rice dehydrin and FK506-binding protein complex under PEG (polyethylene glycol) mediated drought stress. Under control and stress conditions, Rama Shankar et al. (2016) recorded varying transcriptomic modification of drought-tolerant (Nagina 22) and salinity-tolerant (Pokkali) plant cultivar with vulnerable cultivar (IR64). They reported modulation of several members of C2H2, bHLH, and NACTF families (Shankar et al. 2016). Basu and Roychoudhury (2014) also observed differential expression of stress-inducible genes in salt-sensitive and salt-tolerant rice varieties. Some reports about proteomic analysis under drought and salt stress are also available in some plants. For instance, Wang et al. (2015) reported the comparative proteomic analysis of two contrasting Tibetan wild and cultivated genotypes for drought tolerance. Among the 38 identified proteins, they found 6 proteins to be upregulated under drought stress in one genotype compared to others (Wang et al. 2015). Wu et al. (2016) proposed a decreased abundance of photosynthetic machinery and enhanced abundance of ClpD1 protease in drought tolerance characteristics using comparative proteomic assessment of two tolerant contrasting droughts (IAC1131) and drought susceptible crop varieties (Nipponbare). Chaperone protein ClpD1 plays a key role under heat and osmotic stress. Xu et al. (2017), using proteomic analysis, identified 14 proteins to be involved in seed imbibition under salt stress in rice. They further explain that the majority of proteins were overrepresented with energy supply and storage proteins.

4.2 Molecular Signaling Approaches Using Protein-Protein or Protein-DNA Interaction

Protein-protein or protein-DNA interaction triggers a series of stress responses in plants by targeting stress-responsive genes which enable them to adapt in arid and saline conditions. Various in-depth molecular studies proposed the idea that not a single gene but the cascade of events occurs in plants to reach the common objective to protect the plant from adverse conditions. Schmidt et al. (2013) proposed that *Salt-Responsive ERF1* (*SERF1*) activates the MAPK cascade signaling by interacting with promoters of stress-responsive genes such as *MAP3K6*, *MAPK5*, *DREB2A*, and *ZFP179* to provide salinity tolerance in rice. Recently, Das et al. (2019) demonstrated the binding efficiency of biotic and abiotic stress-related transcription factor binding sites in the promoter regions of rice germin-like protein genes. They revealed *OsGLP8-11* and *OsGLP5-1* have the highest and least TFbs, respectively, on investigating 40 diverse *OsGLP* gene promoters for the presence of NAC, WRKY, bHLH, bZIP, MYB, and AP2/ERF TFbs in it. Additionally, protein-protein complex of OsDhnRab16D-OsFKBP was also found to be involved in the drought tolerance mechanism in rice through ABA-mediated pathway (Tiwari et al. 2019). Seo et al. (2011) tried to study the rice stress response interactome using protein-protein interaction assays and co-expression studies to identify novel proteins regulating the rice stress response. Interestingly, they identified ten novel proteins regulating the rice stress response including *OsRac1* which interact with *HSP90* (heat shock protein 90), *OsRBOHB* (rice respiratory burst oxidase homolog B), and *OsMPK1* involved in rice abiotic stress tolerance. The research of Lee et al. (2015) showed the stress-responsive protein-protein interaction of OsSKIP-OsCYP18-2 playing a crucial role in the regulatory machinery of stress-related genes to augment the drought stress tolerance in rice.

5 Epigenomics

Epigenetic control affects genome expression through histone variations, post-translational histone changes, and DNA methylation. These changes in DNA and histone play an important part under various environmental pressures in gene expression and crop growth (Table 2). These modifications may be unstable or stable and can be inherited from generation to generation as "stress memory."

Table 2 Tabular representation of selected studies depicting abiotic stress-related histone modifications and gene expression in rice

Stress	Epigenetic regulators	Functions	References
Salinity	ADA2b	Transcriptional adaptor	Kaldis et al. (2011)
	HDACs (HD2C, HDA6, HDA19)	Histone deacetylase	Luo et al. (2012)
	SWI3B	Chromatin remodeler	Yuan et al. (2013)
	AtROS1	Demethylase	Bharti et al. (2015)
	HDT701	Histone deacetylase	Zhao et al. (2015)
	HDA705	Histone deacetylase	Zhao et al. (2016)
	IDS1	Histone acetyltransferase	Cheng et al. (2018)
Drought	MSI1	Silencer	Hennig et al. (2005)
	AtHD2C	Histone deacetylase	Sridha and Wu (2006)
	CHR12	Chromatin remodeler	Mlynárová et al. (2007)
	MYST, ELP3, GCN5	Histone acetyltransferase	Papaefthimiou et al. (2010)
	ATX1	Histone methyltransferase	Ding et al. (2011)
	BRM	Chromatin remodeler	Han et al. (2012)
	OsHATs	Histone methyltransferase	Fang et al. (2014)
	OsJMJ703	Histone demethylase	Song et al. (2018)

Epigenetic stress memory helps the progeny to cope up with stress conditions efficiently, but the mechanism of mitotic or meiotic heritability is still undeciphered. However, stress memory has a possible snag of negatively affecting plant from growing to its full capacity (Banerjee and Roychoudhury 2018).

5.1 Histone Modifications

Euchromatin is less packed in than heterochromatin and therefore available to the transcriptional machinery for replication and transcription. The significant effective characteristics of euchromatin are post-translational histone modifications, which include chemical changes through acetylation, methylation, phosphorylation, and the ubiquitination of various amino acids such as lysines and arginines in the N-terminal histone tails (Bergmüller et al. 2007; Zhang et al. 2007; Acevedo et al. 2009; Roudier et al. 2011; Sequeira-Mendes et al. 2014; Mahrez et al. 2016). Among them, histone acetylation is a crucial modifier of chromatin structure in the gene expression regulation during different abiotic stresses in plants. Histone acetylation homeostasis is preserved within the nucleus through two main proteins: histone acetyltransferases (HATs) and histone deacetylases (HDACs). Hyperacetylation of histones contributes to the relaxation of DNA and transcription activation, while low acetylation leads to chromatin compaction and transcriptional suppression (Struhl 1998). In rice, four families (CBP, GNAT, MYST, and TAFII250 family) of HATs are known to consist of eight HATs. One of the studies explored the association between drought stress and expression pattern of HATs in rice (Fang et al. 2014). And they found the remarkable increase in the OsHATs transcription and the acetylation of H3 and H4 histone of lysine, suggesting the role of OsHATs in water-deficit stress responses in rice. Another group tried to decipher the genome-wide analysis of stress-related modifications especially in rice under drought stress (Zong et al. 2013). ChIP-Seq and RNA-Seq were used to evaluate histone H3 lysine 4 trimethylation (H3K4me3) and pattern association with rice's entire gene expression models under drought stress. This investigation showed that the rates of H3K4me3 alteration were favorably linked with the pattern of expression of drought-responsive genes. These outcomes suggest that stress regulation in plants is closely linked with chromatin modification and further unknown factors, and their role can be determined using chromatin remodeling.

5.2 Methylation

Gene expression in the plant's responses to environmental stresses is also regulated by another epigenetic mechanism called DNA methylation. Different techniques are available to identify DNA methylation pattern which includes methylation-sensitive amplification polymorphism (MSAP), bisulfite method, bisulfite coupled with RNA sequencing, and methylation DNA immunoprecipitation. Using MSAP, a group of researchers identified DNA methylation patterns in four genotypes of rice under salinity stress (Karan et al. 2012). They reported that methylation patterns and genotype with different range of salt tolerance are not "directed." And the methylation pattern variation in each salt-tolerant rice genotype would be induced by the abiotic stress and then get accumulated through the natural selection process. In contrast, Garg et al. (2015) reported the correlation between DNA methylation and abiotic stress (drought and salt stress) tolerance in three rice cultivar using integrated whole-genome bisulfite sequencing and RNA sequencing. They identified differentially methylated regions (DMRs) among different cultivars associated with the differential expression of genes vital for abiotic stress tolerance. DNA cytosine methylation is related to the inactivity of the gene which represses the gene transcription by methylating the promoter of the gene through both asymmetric (mCpHpH)-methylation and symmetric (mCpG and mCpHpG)-methylation, of CpG islands. Initially, methyltransferases DRM1 (domains rearranged methylase 1) and DRM2 were discovered to catalyze de novo cytosine methylation, while DNMT1-like enzymes MET1 and chromomethylase 3 (CMT3) maintain symmetric CG and CHG methylation (Lister et al. 2008). However, it was also proposed that even DRM1 and DRM2 are crucial for maintaining symmetric methylation and that MET1 and CMT3 can catalyze de novo methylation (Steward et al. 2002; Zhu 2008). High-throughput sequencing of DNA immunoprecipitated with the 5-methylcytosine antibody (MeDIP-Seq) has recently been used by Ferreira et al. (2019) to explore the genome-wide DNA methylation of a salt-tolerant rice variety under salinity stress. They identified higher events of demethylation in salt-treated rice as compared to control along with DMRs influencing the gene expression of nearby genes. It suggests that small regions as DMRs may alter the function of numerous genes enabling rice to cope up in saline conditions. Better perception of epigenetic regulation in reaction to environmental stress and plant development can create new heritable crop enhancement varieties.

6 Concluding Remark and Future Outlook

Environmental challenges are unpredictable, and therefore water deficit due to salinity or drought stress is a critical constraint that affects rice production in different countries. Drought and salinity stress leads to cataclysmic crop failure. In the past, efforts had been made to detect and understand the quantitative trait loci (QTL) involved in dehydration and salinity tolerance of irrigated and lowland rice. Overexpressing and knockdown study generates transgenic/cisgenic rice where desired gene inculcation or shutdown enhanced drought and salinity tolerance (Fig. 1). Still, a functional and molecular aspect of both the stresses in rice needs to be deciphered to inculcate improved strategies for crop development to avoid crop

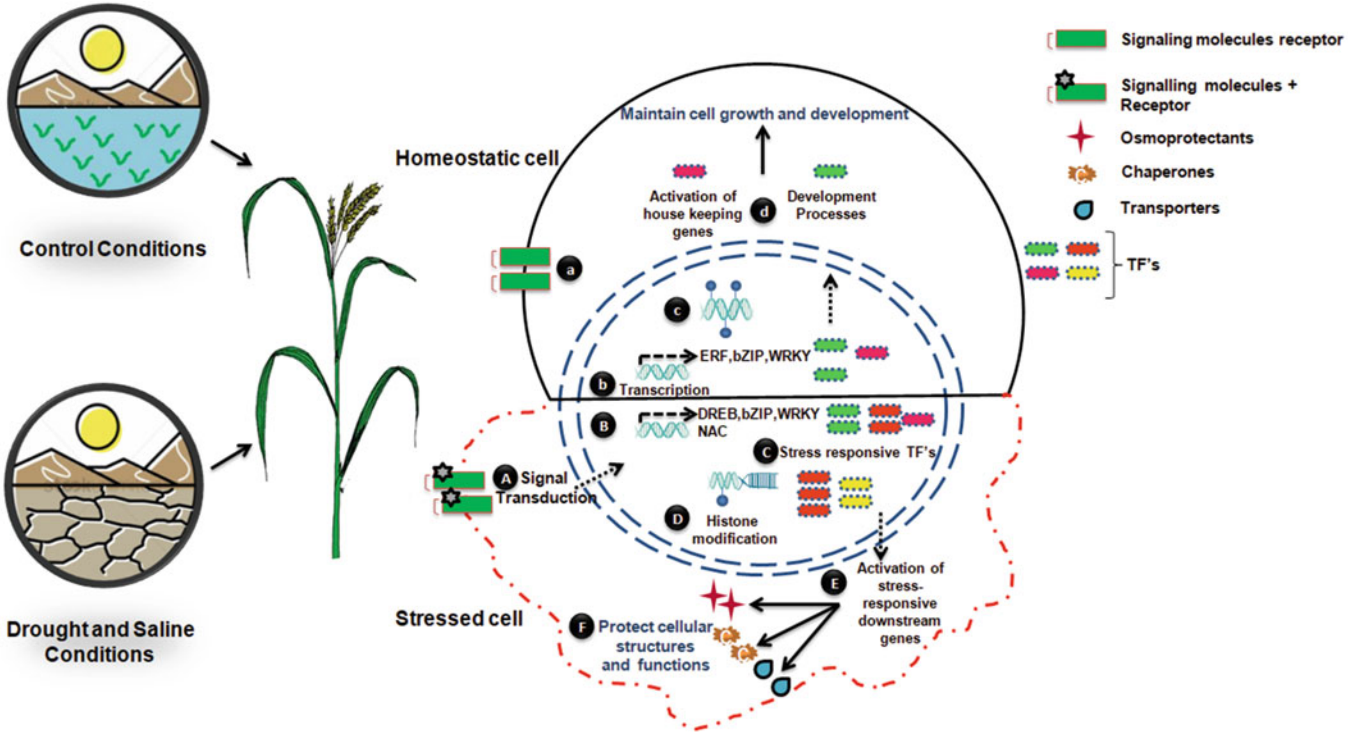

Fig. 1 Comparative pictorial depiction of the molecular events occurring in the control and stressed cell of rice plants. Under control conditions, the cell performs all the fundamental programmed activities and utilizes all its energy for the growth and development of the plant. However, when the plant suffers from water-deficit stress either due to water scarcity or high salinity, even in wild-type rice cascades of events occurred in the cell to maintain the cellular balance to an extent. But after a limit, these WT plants could not resist the insensitive environmental stresses. Therefore, researchers utilized these events to understand the role of the signaling molecule, TFs, and stress-responsive genes either by overexpressing or suppressing the gene function. (A) Signaling molecules like H_2O_2, NO, and Ca^{2+} receive the signals and activate the plant defense system. (B and C) It triggers the transcriptional machinery to activate the stress-responsive TFs. (D) In some cases, chromatin modification occurs which leads to conformation changes in genomic DNA which now allow non-accessible DNA to be transcribed, thus playing a significant functional role in regulating the stress responses. (E and F) Activated TFs with other regulatory protein enables the activation of several others stress-responsive downstream genes to provide the first line of defense to transgenic rice growing in arid and saline conditions

failure during environmental challenges. Consequently, there is an urgent need to refine our understanding of an adaptive mechanism for drought and salinity tolerance in rice to have unceasing rice production to meet the demands of expanding the global population.

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An Integrated Approach for Drought Tolerance Improvement in Rice

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Abstract

Drought is a major threat to agriculture globally and improving crop yield under drought conditions is a major challenge of plant breeding. Many QTLs have been identified for drought stress response, and researchers are striving hard to comprehend and dissect plant tolerance mechanisms related to drought stress. Unravelling the biochemical regulation of drought tolerance and molecular breeding and transgenic approaches can help us manage drought stress in plants. Recent advances achieved regarding genomic tools and genetic techniques in addition to precise phenotyping and advanced breeding methodologies will enable exhibiting metabolic pathways and candidate genes underlying drought tolerance in rice. Taken altogether, new horizons have been opened for the breeders to utilize markers for QTLs, signaling cascade, hormonal cross talk, or gene transformation in plants to develop a drought resistant genotype.

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1 Introduction

Among cereal crops, rice (*Oryza sativa*) is a highly significant crop included in the family of grasses called poaceae, and after maize production, it is the second most cultivated crop worldwide. The tremendously increasing population has enhanced the need for rice yields worldwide (Liang et al. 2014), and rice demand will increase by up to 50% by 2025 (Khush 2001). Rainfed ecosystems provide only 25% of the global water sources. Rice is critically influenced by drought stress because it can reduce production by 15–50% (Srividhya et al. 2011). At the time of reproduction, rice is particularly sensitive to drought stress, leading to decreased grain production (Venuprasad et al. 2008).

Rice (*Oryza sativa*) can be categorized into three subspecies, specifically *japonica, javanica, and indica* (Mae 1997). Because of its adaptability and extensive distribution, rice serves as a tremendous model structure for investigating evolutionary plant genomics in a wide series of developmental, physiological, and morphological varieties located in both rice and its extensively distributed wild lines, *Oryza nivara* and *Oryza rufipogon* (Zhao et al. 2010).

For drought tolerance, conventional breeding, including the selection of diversified landraces under conditions of drought, has facilitated crop improvement (Kumar et al. 2008). Similarly, molecular regulation of candidate genes, viz., regulatory genes and transcription factors, and their expression analysis have helped us to develop drought tolerant plants (Todaka et al. 2015).

2 Mechanisms of Drought Resistance

Drought resistance is described as the capacity of a plant to produce its highest commercial product under water stress conditions and its relation to water control free management. It is a complex feature whose influence depends on the actions and relations regarding various biochemical, physiological, and morphological responses (Kooyers 2015). Under drought stress, rice mostly responds by stomata closure, leaf rolling, and enhanced abscisic acid (ABA) production to reduce the water deficit (Price et al. 2002). Research regarding physiology recommends that drought resistance in rice mostly relies upon water use efficiency (WUE) that tolerates the lowest utilization of water for the highest production and osmotic adjustment that allows plants to conserve the meristem and sustain turgor (Nguyen et al. 2004).

There are several processes for the adjustment of plants under drought stress, including drought tolerance, escape, and avoidance; they are modified based on the cause of their morphological, physiological, and molecular alteration (Fukai and Cooper 1995).

2.1 Drought Escape

Drought escape is defined as the capacity of a plant to accomplish its life cycle before the advancement of critical water deficiency in soil. It includes two distinct processes: plasticity development and quick phenological improvement. In quick phenological enhancement, plants are competent to produce flowers with the lowest vegetative development allowing them to make seeds with an inadequate amount of water. In plasticity development, plants show plenty of vegetative growth, flowers, and seeds during abundant rain. This allows the desert ephemerals to drought escape and survive for long periods without rain. In drought susceptible zones of the southern US, drought escape is a significant process that enables rice to produce seeds regardless of restricted water accessibility (Kumar et al. 2008).

2.2 Drought Avoidance

Drought avoidance is described as the capacity of plants to sustain maximum water contents in tissue in spite of a soil humidity deficiency. The tissue of plants has two possibilities to retain a maximum water status under maximum needs of evaporation and improving water deficiency in soil, to either decrease the loss of water or sustain the water supply. Rice cultivars that can sustain water potential through biosynthesis of ABA, further biochemical processes, or modified root structure arise under different processes of drought avoidance. These cultivars are competent to reduce the loss of yield in drought stress (Singh et al. 2012). Methods exist for increasing water use efficiency (WUE) and minimizing loss of water to achieve drought avoidance. Rice varieties that drought escape generally have bristle-like and deep roots with a maximum capacity for penetration in soil and branching, maximum root to shoot proportion, early stomata closure, and maximum resistance in cuticle and leaf rolling elasticity (Wang et al. 2006).

2.3 Drought Tolerance

Drought tolerance is the capacity of plants to live with minimum water potential in tissue (Fleury et al. 2010). In genetically modified rice, drought tolerance is a composite attribute that is regulated by polygenic impact and includes complicated physiological and morphological processes (Li et al. 2017); for instance, balancing of turgor pressure by osmotic adaptation, cell size reduction, improved cell elasticity, and dehydration tolerance via protoplasmic resistance. The reaction of plants regarding water contents in tissue control their status under drought tolerance (Mitra 2001) and the qualities linked with such processes are studied as secondary attributes.

Secondary attributes, including RWC, osmotic adaptation, stomatal conductivity, and leaf rolling, have been utilized for selection (Kato et al. 2006).

3 Assessing Drought Tolerance Performance

Plant efficiency is an important constituent that needs to be measured while abiotic stress resistance rice is cultivated. Plants efficiency is extremely disturbed with drought stress in the field, which fluctuates in harsh periods, and is also supplemented by synchronized stresses such as low and high temperature. Therefore, analyses of drought tolerance attained from glasshouse conditions is not necessarily equivalent to field conditions. Thus, experiments in the field are essential for the appropriate assessment of drought stress challenges.

Drought stress has been considered in lowland and upland conditions with various levels of stress, such as moderate, severe, and mild environment stress. In the field, drought stress measurement is completed by applying unceasing stress for 10 or more days before proceeding, the resulting International Rice Research Institute's typical measures include observing modifications in soil humidity and diffusion resistance in a stressed field via penetrometer and gravimetric methods (Bengough et al. 2011). RWC is measured after leaf necrosis and rolling have initiated (Jeong et al. 2012; Raman et al. 2012).

The plant reactions under drought stress are further categorized into moderate, severe, and mild depending on the reduction of yield percentage contrasted with grain yield from four non-stress trials (Kumar et al. 2008). In lowland conditions, stress trials revealing a 30% or less reduction of yield are called mild stress, reductions of 31–65% are called moderate stress, and above 65% reduction of yield are denoted as severe stress. At the time of a non-stress upland part of this trial, the reduction percentage of yield contrasted with the average yield of a non-stress lowland trial was utilized to categorize the trial as mild stress upland treatments (Dixit et al. 2012).

The field plots are watered by sprayers two times a week during the development and premature vegetative phase (Kumar et al. 2014). After this period, drought stress is applied via cover-up irrigation and the field is watered only while the water stretches in soil exits under 50 kPa at 30 cm depth of soil. At this water content in soil, the maximum number of rice plants wither and show leaf desiccation. This kind of repeated stress is measured to be competent regarding rice population selection for drought tolerant containing varieties that have the highest growth in this period (Lafitte et al. 2004), and it confirms the capability of the entire plant to tolerate stress during reproductive growth. Similar agricultural trials are useful in non-stress upland field plots, excluding when irrigation is constant two times a week more than 10 days before yield. In the lowland field conditions, uprooted field plots are pumped out after 30 days of uprooting and irrigation is pending to impose drought stress during the reproductive period (Kumar et al. 2014). Drought stress is constant till severe leaf rolling. For the plant's continued existence, irrigation is supplied subsequently through flooding, and water is pumped out after 24 h to apply the next rotation of drought stress. The water table penetration is calculated by installing a 1.1 m polyvinyl chloride (PVC) pipeline in the field plots to ensure constant time intervals. Pipelines are generally installed to a depth of 1.0 m, and 10 cm of pipeline is leftover above the soil surface. Reduction in the water table is constantly calculated through a meter ruler every day after the beginning of the stress (Xiao et al. 2009; Wang et al. 2014).

4 Quantitative Trait Loci for Drought Tolerance in Rice

The process for drought tolerance is complicated, due todifferences in plant phenology and measured by numerous quantitative trait loci (QTLs). Plant reactions are complex and difficult to realize without a detailed study of physiological and molecular sources of how these reactions are managed. Neither conventional breeding nor advanced genetics can successfully expand the drought tolerance in plants if the genetic phenomena associated with production of seed strength are not understood (Lisei-de-Sá et al. 2017). Advances in systematic plant phenotyping, plant physiology, and genomics lead to innovative findings in drought tolerant crops. Consequently, yield breeders will be capable of boosting yields of crops utilizing the modern gene system data and approaches for plant development (Tuberosa 2012). Advancing plant physiology improves our information regarding the complicated system of drought tolerance and its link with various traits, molecular biology, and effective genomics tools, and improved choice productivity will result in the recognition of candidate QTLs and genes associated with these qualities. Tools for molecular breeding can be utilized to develop QTLs for chop enhancement; therefore, candidate genes are a major focus of genetic engineering and production of genetically modified crops (Varshney et al. 2011).

In various abiotic stresses, the recognition of candidate genes reliable for plant resistance, laterally with the utilization of the most appropriate promoters linked with these procedures, is important to produce genetically modified crops with increasing drought tolerance (Varshney et al. 2011). While tools for genetic engineering include high regulatory events and adverse public observations for biosafety, targeted induced local lesions in genome (TILLING) and molecular breeding crops are broadly identified (Varshney et al. 2011). The qualitative traits loci are normally fragments of linkage mapping or linkage analysis-based QTL mapping (Appels et al. 2018). Conventional QTLs mapping contains populations mapping in which qualities associated with tolerance of drought are separating; classifying polymorphic markers that are used for genotyping mapping populations; making genetic maps; correctly phenotyping depends on the drought tolerance associated qualities; and ultimately, according to phenotyping and genotyping QTL mapping data. For drought tolerance in many crops, numerous linkage mapping considerations have been accomplished (Fleury et al. 2010).

Due to intrinsic restrictions connected to populations mapping, linkage analysis-based QTL mapping cannot suggest comprehensive data related to QTLs. These restrictions comprise the following: the recognized QTLs are generally connected with large chromosomal fragments or genomic areas because of insufficient time for hybridization, insufficient phenotypic difference connected to occurring qualities in populations mapping, and the separation of diverse QTLs connected with identical qualities in various populations mapping (Liu et al. 2012).

Linkage disequilibrium (LD) depends on association mapping (AM), which has been utilized in human inheritances, and is recommended as a QTL mapping tool to affect some of these limitations in different crop species (Bolger et al. 2014). The AM technique contains five phases: choosing many distinct panels or groups from a wild pool of the population or germplasm; obtaining perfect phenotypic information on every panel; fascinating candidates genes have high-density sequencing or the markers for a genotyping panel; observing the genetic variation level among panels in the specific population and understanding the factors between distinct pairs in the population; and evaluating the suggestion mapping consequences corresponding to information attained regarding population assembly, relationship, and the association of phenotypic and haplotypic or genotypic information. There are several benefits of the mapping method over biparental mapping linkage, as follows: advanced determination due to use of all recombination actions during the evolutionary history of a particular crop species; avoiding the growth of a specific population mapping and the facility of a wild germplasm selection for a particular crop to decrease the mandatory period for QTL recording; utilizing similar genotyping information and panel of AM for various mapping qualities, assembling it into a practical tool; removing casually hybrid hereditary lines which express a deficient agronomic category from the system of populations; and being competent to model and nearby several alleles for each locus comparative for mapping linkage. In brief, in rice, several QTLs associated with drought tolerance have been recognized. Although, to date, only some QTL reports have been described regarding the influence of grain quality on drought; for instance, a main QTL (*qDTY1.1*), which has stabilizer influence on grain yield (GY) at the reproductive phase in drought stress, was identified on rice chromosomal 1 and lined through RM431 and RM11943 in three populations: N22/IR64, N22/MTU1010, and N22/Swarna (Vikram et al. 2015). Maximum QTLs have been reported based on an extensive series of significant characteristics, including (1) mechanisms of yield, (2) physiological reactions, for example, osmotic adjustment, osmotic potential, RWC, chlorophyll contents, index of rolling flag leaf, osmotic potential of leaf, ratio of carbon isotope, water soluble carbohydrates, and different isotopes of carbon in grain, and (3) root characteristics.

5 Identification of Candidate Drought Tolerance Genes in Model Plant Systems

For drought tolerance, a substantial number of candidate genes can be recognized by significant developments in traditional plant species. Thus far, genomes of several classical plants and the main species of plants have been sequenced (Feuillet et al. 2011). Functional genomics, molecular physiology, and annotation of genome reports have been accomplished in numerous main and model crops to detect candidate genes included under drought tolerance. Under drought stress, these candidate genes contain a large family of excluded genes. Various protein expressions via drought stress linked candidate genes carry out important functions regarding (1) protection of cells, for instance, modification in structure, osmotic adjustment, detoxification, degradation, and repair and (2) positive relations with additional transcription factors and proteins, for example, MYB, protein kinases, DREB, and bZIP that are included in the reaction of plant under drought conditions via controlling further reactive genes, for instance, those which participated in the protection of cells, to survive under drought stress (Basu and Roychoudhury 2014; Banerjee and Roychoudhury 2017). For various main and model plants, detecting drought tolerance (DT) genes is important to consider the practical role of the DT system and its utilization downstream, incorporating proof by MAS over molecular breeding. From distinct plant species, various DT candidate genes involved in transcriptome reactions have been estimated and categorized.

Candidate genes have been verified using various methods; for instance, qRT-PCR, QTL maps penetration, expression analysis, TILLING, linkage mapping, mining of allele, and utilization of these methods have been previously studied (Bevan et al. 2017). Recently, several functional genomic and transcriptomic reports have been accomplished to recognize the stress processes in various crop plants. Prevalent methods that efficiently separate candidate genes associated with drought stress under drought resistant varieties produce expressed sequence tags (ESTs) from complementary DNA libraries of tissues accumulated in drought. To date, various reactive genes for drought have been recognized from numerous crop species, including from seedlings of rice-controlled cDNA libraries to the classification of several genes related to drought tolerance, which were extremely expressed in drought (Hadiarto and Tran 2010).

Another method to detect the candidate genes is transcriptional profiling, and it contains the variance expression of genes in plant tissues during various periods after the beginning of drought stress, as well as between drought tolerant and drought prone cultivars (Hampton et al. 2010). In spite of this, choosing the accurate tissue category, tissue phase, and stress action, such as strength and effectiveness, are important to regulate the minimal drought stress necessary to extract RNA for transcriptomics reports (Du et al. 2017). For hereditary materials, near-isogenic lines (NILs) are the system; they differ only in the particular attributes between cultivars with different genetic conditions. Therefore, concentrating on NILs can offer maximum declaration consequences that are particular and are attained from differentially expressed genes associated with the focus on qualities. Furthermore, miRNAs have been established to be involved in the reaction to drought, and tolerance in several crops (rice and soybean) can enhance drought tolerance (Kulcheski et al. 2011).

For transcriptional profiling, there are several programs which can be used, including cDNA-AFLP (Ridout and Donini 1999), DDRT-PCR (Liang and Pardee 1992), cDNA and SSH (Sahebi et al. 2015), cDNA and microarrays oligonucleotide (Sreenivasulu et al. 2010), and EST depends on digital expression analysis (Raju et al. 2010). Super-SAGE is another method that can be effectively utilized in various crops under stress conditions (Matsumura et al. 2010). Because next generation sequencing (NGS) tools offer accurate real time and digital study of sequences dependent on transcriptomes, other approaches, for example, microarrays, will have a tendency to be replaced with NGS in upcoming years. In analysis of gene expression, the utilization of NGS has contributed to different methods, for instance, RNA-seq (Nagalakshmi et al. 2008), DGE-TAG (Moore 2015) and Deep SAGE (Nielsen et al. 2006).

Using RNA-Seq with NGS tools has numerous benefits in the analysis of transcriptome assembly, for instance, the detection of particular expression of allele and unit intersections (Malone and Oliver 2011), while NGS may offer maximum sequencing quantity results directly from RNA of stress faced tissues from various cultivars. Drought sensitive and drought tolerant transcriptional profiling can recognize candidate genes associated with drought tolerance besides in grouping with genetic/QTL maps and can play a role in markers that are used for molecular genetics (Hiremath et al. 2011).

Numerous candidate genes recognized by the above methods may be correlated with QTLs used for drought tolerance characteristics. Consequently, a genomic and inheritance technique that permits the measurable study of transcriptional profiling may segregate the QTLs (expression QTLs) used for drought tolerance characteristics (Varshney et al. 2005). Therefore, in cis situations, if QTLs expression are identified, the targets genes depending on molecular markers will play a role as screening markers for specific traits (Potokina et al. 2008). Transcriptional profiling depending on NGS tools will likely be utilized to detect candidate DT genes from most important crop species and then applied in genomic, genetic, and molecular breeding.

6 The Morphological Responses of Rice Under Drought Stress

During drought stress, plants suffer maximum transcription rates and deficiency of required water adjacent to the roots. Extensive drought harms the development, yield, and growth of rice. When water is deficient, normally rice growth is reduced or ceased (Zhu et al. 2002). Reduction in development and growth of rice under drought stress have demonstrated a selection of premature morphological modifications in rice (Wakim et al. 2008). Cell growth is reduced by an imbalance in turgor under drought stress (Saddique et al. 2018). Drought effects both elongation and expansion growth (Miller et al. 2010) and hinders increasing cell division (Jaleel et al. 2009). Furthermore, it inhibits the germination of rice seedlings (Swain et al. 2014) and reduces the quantity of tillers (Muhammad 2012) and height of the plant (Sokoto and Muhammad 2014). Drought stress greatly reduces biomass production (Farooq et al. 2010). Numerous reports have discovered a decrease in the fresh and dry biomass of shoots and roots during drought stress. In rice, there is a reduction of photosynthetic and biochemical mechanisms due to the decreasing length and height of the fresh biomass of shoots and roots (Basu et al. 2010a, b; Usman et al. 2013).

7 Drought Stress Omics

Under drought stress, a dataset involving modifications in gene expression, metabolites, and profiling of proteins discovered in plants should be obtained via genomics techniques. Under abiotic stress, dicot and monocot plants utilize similar transcription factors (Yamaguchi-Shinozaki and Shinozaki 2006). In drought tolerant plants, the molecular tools involve (1) a signal transduction force monitored via transcriptional regulation and initiation, (2) protein fortification with support of several proteins, for instance, dehydrin for late embryogenesis or chaperones such as HS proteins (Banerjee and Roychoudhury 2016), (3) increasing of osmolytes, comprising glycine, myoinositol, proline, mannitol, trehalose, and betaine (Roychoudhury et al. 2015), (4) the initiation of antioxidants, for example, ascorbic acid and glutathione (Roychoudhury and Basu 2012), and (5) decreasing of ROS toxicity through glutathione *S*-transferase and superoxide dismutase. Various processes may include numerous identical genes that can be recognized by transcriptomic trials on rice families cultivated under standard and drought conditions. Numerous regulatory methods for drought tolerance occur in several species; however, the molecular reaction under drought stress has been analytically exposed (Aprile et al. 2009) to be less consistent due to alterations in growing stage, tissue analysis, and stress dynamics.

8 The Role of Photosynthesis and Photosynthetic Pigments in the Drought Response

For several metabolic mechanisms, photosynthesis is an important complicated mechanism under water stress. The most significant aspects that control photosynthesis are diffusional limitation in carbon dioxide due to reduction in photosynthesis, early stomata closure, and the reduced productivity of PSII photochemical development and the biochemical mechanisms associated with triose phosphate production. The photosynthetic rate is disturbed by changes in any of these processes. During drought stress, uptake of carbon dioxide is reduced in stomatal conductance (gs) and mesophyll conductance (gm) (Chakhchar et al. 2017; Banerjee and Roychoudhury 2018). Therefore, during drought stress, the gm value exhibits the tolerance in rice under water deficit (Lauteri et al. 2014). Energy and ATP are provided by the activity of PSII. If PSII movement increases, an extreme reduction in ETC in the photosynthetic mechanism may occur, thus promoting ROS production. Therefore, an equilibrium between the need for photochemical and photo assimilates activity should be essential. In rice flag leaves, the PSII activity is damaged expressively by drought stress (Pieters and El Souki 2005). This mechanism occurs because drought stimulates the D1 polypeptide degradation, triggering the inactivation of the reaction center of PSII. Photosynthesis is limited by the drought stress because the efficiency of the enzyme present in the Calvin cycle and the performance of Rubisco have been reduced (Zhou et al. 2007). However, the quantity of Rubisco activase stimulates conformational modification in ATP-dependent, releases Rubisco positions from dead-end blockage, and can enhance the defensive process in drought stress. At this time, enzymes included in photosynthesis of C_4 plants have been initiated in rice crops to change the photosynthesis rate and increase plant efficiency against the stress reaction. Enzymes that are required for photosynthesis of C_4 overexpressed in genetically modified rice crops, for instance, pyruvate orthophosphate dikinase and phosphoenolpyruvate carboxylase, are extremely drought tolerant (Gu et al. 2013). Under the metabolic role, different modifications occur owing to drought in which the most significant is loss and decrease of synthesis of pigments in photosynthesis, thus decreasing energy and light harvesting, which play important roles in photosynthesis.

Yield and biomass have been closely related to alteration in the quantity of photosynthetic pigments (Miller et al. 2010).

Chlorophyll uptake the energy and translocate it into the reaction center of photosynthesis. In higher plants, chlorophyll a and b are generally located and are related to soil dehydration. Furthermore, supplementary pigments such as carotenoids have several roles in the chloroplast photosystem structure, photoprotection, and light harvesting and can moderately stimulate resistance in crops against drought. $F\text{v}/F\text{m}$ (PSII quantum yield) increases and reductions in chlorophyll content have been identified in numerous reports in rice crop against drought stress (Hà 2014). Reductions in chlorophyll content and $F\text{v}/F\text{m}$ have been detected lowest in autotetraploid families compared to diploid lineages in drought stress; therefore, it is suggested that autotetraploid rice is significantly tolerant of drought stress (Yang et al. 2014). Reductions in chlorophyll contents due to the degradation of biosynthesis systems of pigments resulting from stress and the concentration of lipid peroxidation are enhanced and the membrane of chloroplast is degraded.

9 The Biochemical Response of Rice Under Drought

In cytosol the osmotic potential is decreased due to the deposition of organic and inorganic solutes that balance the turgor in drought stress (Rhodes and Samaras 1994). The biochemical method is a kind of osmotic adjustment that is significantly based on water deficit potential. Osmotic adjustment occurs by the deposition of proline, sucrose, and glycine betaine, and in cytoplasm, other solutes stimulate the absorption of water during water shortage. Amino acids such as proline have been extensively studied because they have many roles in alleviating stress under harsh conditions. Deposition of carbohydrates are also induced by water deficiency (Shehab et al. 2010).

9.1 The Role of Proline Under Drought

As declared above, in plants under different unfavorable conditions, the proline works as an osmolyte (Verbruggen and Hermans 2008). In 1954, MacPherson and Kemble presented the first report on the function of proline after their work on rye grass where they observed the deposition of free proline reduced stress (Kemble and Macpherson 1954). In rice, the deposition of proline in stress and non-stress conditions have been investigated (Lum et al. 2014). Furthermore, proline reveals three major function under stress conditions, i.e., as antioxidant responsive molecules, metal chelators, and signaling molecules (Hayat et al. 2012; Roychoudhury et al. 2015). In drought stress, the deposition of proline may help restore the destruction via enhancing the activity of antioxidants (Fahramand et al. 2014). Therefore, the contents of proline can be utilized as a marker to select the rice genotypes for drought tolerance.

9.2 The Role of Antioxidants Under Drought

The difference between reducing and production of reactive oxygen species is the general process in drought stress (Faize et al. 2011). The ROS peroxide and protein are denatured by them, and they cause mutations in DNA, imbalance homeostasis in cells, oxidative cell damage, and lipid peroxidation. The antioxidant mechanism is complicated, comprising non-enzymatic and enzymatic molecules that defend plants alongside the unfavorable result of ROS. Ascorbate (AsA) and glutathione (GSH) serve as nonenzymatic antioxidants in the cell. Enzymatic antioxidants contain glutathione reductase (GR), catalase (CAT), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), superoxide dismutase (SOD), dehydro ascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) (Noctor and Foyer 1998; Das and Roychoudhury 2014). In crops, these antioxidants have crucial reducing ROS mechanisms, and in rice, their regulations are enhanced for drought tolerance (Wang et al. 2005). In rice, due to enhanced drought stress, these antioxidants molecules have also increased AsA, APX, GR, SOD, phenylalanine ammonia lyase, CAT, MDHAR, DHAR, and GSH (Shehab et al. 2010). The tendency of these antioxidant protection enzymes to enhance their activity reveals their defensive action to respond to the oxidative damage stimulated by drought stress in rice. The actions of SOD, CAT, and POD can efficiently reduce ROS, and thus reduce the adverse effect of drought on rice (Lum et al. 2014; Yang et al. 2014).

9.3 The Role of Polyamines Under Drought

Polyamines (PAs) are partially positively charged molecules (Takahashi and Kakehi 2009) that play a role in the reaction of drought tolerance in plants (Calzadilla et al. 2014). In plants, the PAs contains putrescine (Put), spermidine (Spd), and spermine (Spm). Polyamines can intermingle with various signaling systems. Moreover, they control osmotic potential, membranes stabilization, and ionic homeostasis. In plants, enhanced PA concentration during drought is directly associated with reduced loss of water, enhanced limits of photosynthesis, and upgraded osmotic adjustment and detoxification (Roychoudhury and Das 2014), although the full mechanisms are poorly recognized. The functions of PAs include regulating gene expression by upholding ion equilibrium, helping the binding of transcription factors with DNA, stabilizing membranes, scavenging radicals, and inhibiting senescence by conformational change in protein phosphorylation and DNA (Martin-Tanguy 2001). A current report has confirmed that rice can stimulate the biosynthesis of PA, particularly Spd and Spm, and in leaves, unite them into an insoluble state before suppression in drought stress (Yang et al. 2007). Spray of PAs can decrease drought stress. Their utilization enhances WUE, accumulation of free proline, water potential in leaves, soluble phenolics, anthocyanins, net photosynthesis, and reduces oxidative destruction in cell membranes (Farooq et al. 2009).

10 Potential Solutions for Enhanced Yield Stability Under Drought Stress

Increasing productivity is an important part of rice cultivation. Previously, scientists determined that the helpful relations central to crop production and tolerance of drought in drought stress conditions may affects the maximum yield production (Guan et al. 2010). Additionally, several processes respond to numerous drought conditions by adaptive stress approaches, which are the important cause of differences for screening in drought stress (Kamoshita et al. 2008). For specific adaptation, plants need water accessibility, and it is suggested that escape from drought conditions is essential to minimize lethal stress and increase yield from the vegetative to reproductive stages (Blum 2005).

Formerly, studies have shown that plants display the morphology of desiccation avoidance in addition to a short leaf area and reduction of plant size because of the direct selection for yield under stress conditions and the plant ability is significantly reduced (Gu et al. 2012). The enhancement in productivity of yield and production of breeding tools is hindered due to complications regarding GY caused by genetic regulation and many other main features such as raceme, number of tillers, and grains reproductivity (Dixit et al. 2014).

11 Molecular Analysis of the DRO1 Gene for Improved Root Traits

The ability of *Oryza sativa* to originate under stress conditions is possible because of deep root expansion that enables a method for the water supplies and avoidance processes. The population for breeding is attained via the cross of shallow roots of IR64 and deep roots of Kinandang Patong, which have the ability to hold under drought stress, and deeper rooting 1 (DRO1) QTL is detected in the population (Uga et al. 2013). In the DRO1 gene, the 1 bps altered at the four-coding sequence can change into an antisense codon and result in smaller protein synthesis, diminishing the root angle; hence, contradictory to the Kinandang Patong allele, resulting in the descending growth expansion via DRO1kp in harsh drought conditions. The improvement in the yield implementation and sensitive characteristics of roots can be demonstrated as supportive to enhance the drought avoidance via the cross between DRO1-kp and IR64 genes in contrast to inheritor parents in drought conditions. The auxin stimulates the root angel, root elongation, and gravitropism for maximum yield, while the DRO1 gene is too adversely regulated by auxin.

12 Genetic Engineering for Drought Tolerance

Genetic engineering can successfully improve yield by genome editing rice to enhance drought tolerant mechanisms. The genetically modified rice and transformation of external genes in rice and promoters for efficient productivity are mentioned in Table 1. The target genes were identified by studying the response meantime of various stress conditions, recognized as possible genes that play a role in tolerance under stress during flowering.

12.1 AP2/ERF Transcription Factors

In *Oryza sativa*, the photosynthetic process has a multifaceted influence on yield enhancement under stress conditions because it depends on three important steps of resistance: the layer of mesophyll, the stomata (Lauteri et al. 2014), and the ability of the plant to use sunlight. The system for regulation of genes and the biochemistry of the photosynthetic carbon process are associated with transcriptional factors such as maximum rice yield and AP2/ERF. The photosynthetic process can be maximum due to additional expression of the HYR gene in nippobare because of regulation via CaMV355 promoters to locate the physiological and structural processes that also play a part in improving grain yield under harsh conditions. The essential role of the HYR gene is identified as the activation of gene and highly impacts on PSII, which is connected to the carbon metabolic processes (Ambavaram et al. 2014). The additional AP2/ERF and OSP37 gene expression stimulates drought tolerance in genetically modified plants in vegetative stages. Transgenic plants compared to wild plants are more effective at displaying the maximum yield after displaying a deficiency of water conditions; essentially, this is due to improved grain filling. Consequently, it is suggested that grain yield can be improved by OSAP37 during the period of stress and there is no risk of undesirable expression of growth (Oh et al. 2009). DREB/CBF are identified as the transcriptional factors that switch the stress stimulating genes according to the support of CRT/DRE factors and also regulate the minimum disturbance in photo-oxidative and maximum accumulation of ions that control the osmotic potential resulting from overexpression under drought tolerance (Datta et al. 2012).

Table 1 List of genes involved in drought tolerance in rice

Gene	Reference
OsAKT1	Ahmad et al. (2016)
OsAHL1	Zhou et al. (2016)
OsASR5	Li et al. (2016)
OsbZIP23	Srivastava et al. (2017)
OsNAC14	Shim et al. (2018)
OsLG3	Xiong et al. (2018)
OsSAPK2	Lou et al. (2017)
WRKY11	Lee et al. (2018)
OsERF71	Lee et al. (2016)
ONAC022	Hong et al. (2016)
ONAC095	Huang et al. (2016)
OsDRAP1	Huang et al. (2018)
OsbZIP71	Liu et al. (2013)
OsNAC6	Lee et al. (2017)
OsHAK1	Chen et al. (2017)
OsEm1	Yu et al. (2016a, b)
RGA1	Ferrero-Serrano and Assmann (2016)
OsJAZ1	Fu et al. (2017)
OsbZIP46	Tang et al. (2016)
OsERF109	Yu et al. (2016a, b)
RSOsPR10	Takeuchi et al. (2016)
OsHSP50.2	Xiang et al. (2018)
OsMADS26	Khong et al. (2015)
PYL5	Kim et al. (2014)
AmRosea1	Dou et al. (2016)
WRKY58	Cai et al. (2014)
DUF1645	Cui et al. (2016)
OsbZIP66	Yoon et al. (2017)
OsIAA6	Jung et al. (2015)
SNAC1	You et al. (2014)
AtWRKY57	Jiang et al. (2016)
AtDREB1A	Ravikumar et al. (2014)
PDH47	Singha et al. (2017)
OsbZIP12	Joo et al. (2014)
OsETOL1	Du et al. (2014)
AtMYB44	Joo et al. (2017)
OsLEA4	Hu et al. (2016)
OsGRAS23	Xu et al. (2015)
OsNF-YA7	Lee et al. (2015a, b)
OsABA8ox3	Cai et al. (2015)
OsRab7	El-Esawi and Alayafi (2019)
OsNAC10	Jeong et al. (2010)
TSRF1	Quan et al. (2010)

(continued)

Table 1 (continued)

Gene	Reference
OsCYP18-2	Lee et al. (2015a, b)
OsCML4	Yin et al. (2015)
OsMYB48-1	Xiong et al. (2014)
OsSDIR1	Gao et al. (2011)
OsNAR2.1	Chen et al. (2019)

The overexpression of DREBIA of genetically modified plants of Arabidopsis and rice displays maximum drought resistance at the vegetative phase with maximum efficiency spikelet in contrast with non-transgenic crop (Datta et al. 2012; Ravikumar et al. 2014). A1DREBIC transgenic plants show maximum drought tolerance at both reproductive and vegetative phases and have maximum sink ability and improve yield productivity compared to normal drought treated plants (Ishizaki et al. 2013).

12.2 bZIP Transcription Factors

Studies have exposed that binding of responsive factors such as AREB/ABRE/ABI with bZIP transcriptional factors seriously influences ABA biosynthesis under drought tolerance (Fukao and Xiong 2013; Banerjee and Roychoudhury 2017). In genetically modified rice, the OSb21P23 overexpression gene is a maximum functional for ABA biosynthesis, it stimulates the drought tolerance during all the reproductive and vegetative phases under ideal conditions, and the GY/plant was maximum compared to transformed and non-mutant plants. The microarray method releases maximum consecutive genes alongside their role, approximately 30, which have a putative mission associated with dehydrins and transcriptional factors (Xiang et al. 2008). OsbZIP46 is an important transcriptional factor that has consecutive ranges of amino acids such as 122–129 residues that ultimately influences challenging the genes of activation at various locus. Drought tolerance was observed along with GY by eliminating one more gene from over expression in transgenic rice than local. ABF is phosphorylated in Arabidopsis with SnRK2 and Osb21p46 (Todaka et al. 2015). OsbZ1023 and Osb21p46 play vital roles as positive regulators in the ABA pathway. Over expression of Osb21p71 leads to enhanced drought tolerance in CaMV355 along with better seed setting. Furthermore, drought tolerance can also be achieved by gene stacking of COR413-IMI, OSNHX1, and OsMYB4 (Liu et al. 2014). Research reveals that bZIP transcriptional factors could be the best choice to obtain high yield by gene editing under drought stress conditions. The mechanism of tolerance is regulated by signaling of ABA, which is exhibited by the maximum deposition of ABRE and ABA at the promotor positions of responsive genes (Maruyama et al. 2011, 2014).

12.3 NAC Transcription Factors

NAC is studied as a restricted class of transcriptional factors taking the various c-terminal positions that have been identified as carrying out the vital role in stress conditions to control the molecular and metabolism mechanisms (Nuruzzaman et al. 2010). The overexpression of SNACI in genetically modified rice displays improvement under drought tolerance at reproductive and vegetative phases and displays maximum spikelet and seeds productivity compared with non-mutant plants having no yield. Expression factor OSROIc is utilized to produce the transformed plant that has maximum resilience under drought conditions and to protect the loss of water by stomata closure, but the photosynthetic mechanism remains constant (You et al. 2012). Under ideal conditions, the production of maximum yield and resistance under drought stress at the sexual stage is only supported by some factors such as OSNAC5, OSNAC9, and OSNAC10 overexpression in rice having promotors for particular roots. The microarray method described the consecutive function of gene encoding associated with p450 zinc-finger, transporting Ca-ATPase, and non-regulated HAK5 and NCED (Jeong et al. 2010; Redillas et al. 2012). Another transcriptional factor of NAC is OSNAP that transports due to inorganic components such as stress, ABA, and salt. In rice, overexpression stimulates drought resistance, reduces the loss of water and growth at the vegetative stage, and improves yield and increases the number of spikelets at reproductive stages. OSNAP functions similar to the other transcriptional factors such as OSAP37, OSMYB2, and OSDREBIA and some dissimilar genes, for example, OSPP2C68 and OSPP2C66/OSAB12 (Chen et al. 2014).

12.4 Other Transcription Factors

Studies of transcriptional factors in mutant rice plants have shown improved maximum drought resistance and maximum yield potentials. Transcription factor homodomain leucine is coded by the EDT1/HDG11 gene, and in rice, the gene id is transformed by the Arabidopsis gene, resulting in its overexpression of genes exhibiting maximum drought resistance. Drought tolerance is related to efficient root systems, improvement in WUE, and minimal stomatal opening to accommodate the water deficit conditions. The enhancement of grain yield in transformed plants is only probable via the healthier seed formation and maximum rate of photosynthesis (Yu et al. 2013). The Zhengsheng 97B and IRAT109 upland are hybridized to grow QTL drought resistant in the fourth position of the chromosome that remains on OSGRAS23, which is related to the GRAS family (Zou et al. 2005). Inorganic aspects such as temperature and salt stress are related to the expression of OSGRA523 and support reducing the accumulation of hydrogen peroxide. The transformed plants exhibited the maximum horizon rate of seed and enhanced the yield under drought stress (Xu et al. 2015). OSWRKY47 transforms under drought resistant conditions and increases the harvest. OSNRKY47 expression also controls the status of drought resistance through the support of controlling genes CaMBP and CRRSP (Raineri et al. 2015).

13 Drought Protective Genes

13.1 Protein Kinases

In the relationship of the CIPK class with OSCIPK23, a portion of CBL most perspicuous in the pollen and ovule is promoted by the support of hormones such as ABA and abiotic factors (Pandey et al. 2015; Banerjee and Roychoudhury 2017). RNAi regulate the OSCIPK23 in rice and show the prone response under drought conditions at the vegetative growth stage and reduce the formation of seeds at the reproductive stage, exhibiting an important function under fertilization and drought tolerance (Yang et al. 2008). Reporter enzyme GUDK works in cytosol for phosphorylation and is identified from the estimation of drought sensitive mutant genes that show the prone response in inorganic stress. GUDK can be the main reason for reduced biomass and photosynthesis at the proliferation stage. It also reduces the quantity of yield at the reproductive stage under drought stress.

According to phosphoproteome analysis, GUDK is the main regulator of OSAP37, which is responsible for the GY parameter in rice (Ramegowda et al. 2014). Mitogen is triggered by the enzyme, which is reflected to be an essential factor in the plant growth adjustment and consequences of stress (Sinha et al. 2011). DSMI-RNA lineages are recognized as considerably functional in drought during the development of flowering contributing to a lateral decrease in the number of spikelets and consequently a yield reduction. Scavenging and ROS are utilized to develop the overexpression lineages of maximum drought resistance (Ning et al. 2010). The estimated T-DNA transformed are utilized to recognize the salt receptive genes that code for inositol 1,3,4 triphosphate 516 kinase. Under drought conditions, accumulation of osmotic ions, productivity of spikelets, and yield is reduced because of transformed genes. Identical expressions have been discovered associated with the overexpression lineages. To determine the entire result for the essential drought tolerance, the precise expression conditions are required (Du et al. 2011).

13.2 LEA Protein

Late embryogenesis abundant protein constitutes a significant portion of hydrophilic proteins that are involved in functions during stress conditions (Todaka et al. 2015). The OSEA 3-1 gene exhibited maximum expression in rice whilst drought tolerance hinders its regulation under stress inducing factors. It also improves the amount of yield and spikelet efficiency compared to non-mutant plants (Xiao et al. 2007).

13.3 Osmoprotectant Synthesis

The drought producing radicals can be countered with the support of osmolytes and antioxidants. For instance, proline is accumulated as a defensive mechanism as the consequence of loss due to initiation of stress. OSOAT, a kind of pyridoxal-5′-phosphate, is dependent on the biochemistry of arginine and proline. Abiotic factors and plant hormones can trigger the production of OSOAT. An extreme quantity of OSOAT results in extreme tolerance regarding storing water and oxidative stress and improves the seed setting. A significant portion of the GY attribute in transformed plants is not protected from drought stress. OSAT is targeted by SNAC2. The scavenging of ROS and improvement of proline and antioxidant enzymes lead to overexpression of OSOAT (You et al. 2012). APX is the antioxidant that helps to maintain ROS and triggers the dismutation regarding water and oxygen. OSAPX overexpression increases productivity in rice in contrast to the native plant under stress conditions (Zhang et al. 2013).

13.4 Other Genes

At the posttranslational level, Famesy transferase contains two subdivisions as a result of improvement in protein with the support of stimulating the femesyle group at the thiol S–H group of cysteine. The synthesis of isoprenoid based on SOS occurs in ER and helps to trigger the development of catabolism where 2-femsyle pyrophosphate is transformed into squalene, which directly contributes to the production of sterol. RNAi bridges the gap between maize SOS and rice SOS and boosts the yields over natural plants. Drought tolerance might be improved by excluding loss of water and fewer open stomata (Manavalan et al. 2011). Lipid transferase triggers the mechanism of phospholipid transferring in membrane and has some significance concerning the protection of plant and formation of embryo (Kader 1996). LIP OSDIL have more expression characteristics that display maximum tolerance during proliferation stages with the decline of alternative nutritious tissues at the reproductive stage where ABA decreases the enzymes that stimulate the synthesis of ABA and tolerate the level of self-expression for downregulating the OSDIL gene (Guo et al. 2013). SKIP proteins are formed from the crossing of onco (cancer) and yeast genes, while V-SKI exhibits the traits such as fragments of spliceosome and supports the regulation of transcription OSKIPa. In humans, SKIP proteins are similar to rice proteins. This protein is competently relevant to alter the hormones of plant and stress initiating features. During the production of seeds, the plant shows maximum self-expression of OSKIPa, with enhanced spikelet efficiency and amount of yield at reproductive stage. In transformed rice, OSKIPs influences the drought tolerance that is related to tremendous ROS scavenged mechanisms and stressed regulated genes (Hou et al. 2009). In rice crop, the SAP protein has domain A20/ANI that significantly contributes to drought tolerance. OSAP8 protein exhibits its expression to cause drought resistance during the production of flowering lacking any reduction of yield in contrast to non-stressed transformed plants (Kanneganti and Gupta 2008).

The plants exhibit enhanced response in drought stress due to degradation or synthesis of RNA by regulating post transcriptional activities.

The role of RBP during the stress tolerance process and modification of the protein is identified by the AtGRP2 gene overexpression, and transformed plants have the sequence of protein for maximum RBP glycine. The genes that are sensitive to stress are DB21, DIP, and OSE2, where DIP controls improvement in seeds via the ATGRP overexpression in mutant plants (Yang et al. 2014). Seven genes are responsible for promoting the stress resistance in which kinase enzymes are coded by the transcriptional factors SOS2, CBF3/DREBIA, and AP2ERF, while synthesis of ABA occurs by LOS5 and NCED2 genes. Kinase enzymes are also coded by NPK1 and ZAT10 that work similar to a Zn-transcriptional factor. Overexpression of NHX1 antiporters Na^+/H^+ are regulated by the promotors that are stress inducers. The spikelet efficiency and maximum yield are displayed by the PVC tubes in transformed plants. The transformed plants both in field and PVC tubes demonstrate better overexpression of ZAT10 and LOS5 in contrast with the seven genes (Xiao et al. 2009).

14 Plant Hormones Affecting Drought Tolerance

In plants, the signals that transmit the stimulus for better growth and response against abiotic stress are regulated by phytohormones that effectively create a complex network structure and establish signaling molecules (Kohli et al. 2013). In drought stress, different hormones such as GAs, CKs, ethylene, and ABA have a significant role in plants (Wilkinson et al. 2012). In plant body, different mechanisms take place such as elongation of shoot and root during the vegetative stage, morphology, translating phytohormone signals, modification in gases, and relation of water contents, which have been described with detailed methods (Peleg et al. 2011). Moreover, the plant hormones are limited for controlling the traits that are related to yield. The study impact on various hormones, relationships, and protections during the period of sexual stress is a maximum developing trend at this time; mostly, GY is inclined. Drought can induce sterility in pollen at the pre-flowering phase, which can be terminated, and post-flowering stage sink-source association influences the filling of grain (Xing and Zhang 2010).

14.1 Abscisic Acid

Closing and opening of stomata are regulated by ABA hormones, which help to store water in drought stress during the vegetative stage (Schroeder et al. 2001). Under drought stress, the amount of ABA is increased, and it can inhibit the transcription by stomata closure that induces the complication at sexual stage stress (Roychoudhury and Paul 2012). To achieve the improvement in the GY via ABA remains a challenge because stomata closure reduces the uptake of C and ABA causes abortion and sterility (Ji et al. 2011). In wheat, the concentration of ABA was shown to remain at anthers during sexual stress. The species of wheat that have the enhancing enzyme that degrades the ABA effect resulting in minimum accumulation of ABA in anther show the first feedback in drought stress (Ji et al. 2011).

Still, maximum production of ABA may inhibit the production of invertase enzymes that are essential to maintain the tendency of sink resulting in the spoiling of pollens. In sexual stress, ABA can be exploited to improve the GY, except controlling of stomata. For instance, ABA can alter the process of sap in plant by improving the aquaporin competences, and osmotic adjustment measures the sustained growth in the propagative stress (Tardieu et al. 2010; Travaglia et al. 2010). However, the ABA play a significant role in the flowering developing stage. Earlier reports investigate whether grain and ABA are directly linked to each other. The applications of ABA expose that there is a similarity between the ABA and drought stimulating the storage of C in seeds and filling of grain (Yang et al. 2004). Deposition of ABA in leaves and roots is connected with the reprocess of deposited carbon in drought (Yang and Zhang 2010). More studies are required to improve the drought resistant value with the support of ABA maintenance through making some adjustments in ABA metabolism; however, thus far the researchers have not yet been able to produce the maximum improvement in plants that directly influence GY. For instance, ABA regulated genes OSNAC5 and OSNAP and falling of leaves produce some improvement in drought at the anthesizing phase (Chen et al. 2014; Liang et al. 2014). Overexpression is increased in some genes such as OSPP2C68, OSPP2C06/OSAP12, and OSPP09, which are responsible for signaling of ABA. Signaling of ABA can improve the interaction between source and sink in the period of grain stuffing. It is expected that sequences of hydrolases of B-carotene are coded by the DSM gene, which controls the synthesis of zeaxanthin, which is a precursor of biogenesis of ABA. Mutant T-DNA shows the sensitivity against drought stress due to its minimum photosynthesis and reductions in yield in stress. DSM2 shows overexpression to offer the interference for maximum drought tolerance by boosting the pigment of xanthophyll in plants for improvement of spikelet efficiency and seed arrangement (Du et al. 2010).

14.2 Cytokinin

Cytokinin is another phytohormone, which is responsible for differentiation and division of cells in plants. Additionally, it has improved the time of maturity. Under water deficit, it increases the range of aging and death in young leaves. This quality is more efficient in filling of grain and reproductive stress. Concentration of cytokines improves the rice growth for a short time during the filling in grain phase (Yang et al. 2001). The worth of transformed rice cultivars and improvement in GY are increased by the spray of 6-benzylaminopurine, a type of cytokinin (Pan et al. 2013). When biogenesis of CKs occur, the plant becomes more resistant to stress (Kuppu et al. 2013). Expression of IPT in drought and promotors MI improve the GY after and before stress of flowering because it increases the feedback of stress (Peleg and Blumwald 2011). Transformed plants displayed the maximum expression of brassinosteroids (BRs) in consistent gene and reduced expression of gene in JA jasmonate (JA). When expressed, cytokine alleviates the adverse effect of jasmonate in maximum yield productivity.

14.3 Gibberellic Acid

Under drought stress conditions, development of plants is maintained by GA (Colebrook et al. 2014), and DELLl protein releases severe signaling of GA (Davière and Achard 2013). It is accepted that in the pre-filling of grain stage there is a maximum deposition of GA in contrast with the post-filling stage. This promotes the healthy development of embryo, which explains the important function of GA in the development of embryo. In addition, a deficit of water significantly reduced the synthesis of GA. There is an adverse relationship between components of GA and filling of grain (Yang et al. 2004). SNPs have been utilized to examine the adverse relationship between filling of grain and components of GA in propagative stress by utilizing the crossed lines (IR64 × Cabacu) that describe the position adjacent to the sd1 locus and functional sd1 gene GA 20-oxidase influence on GA and GY (Trijatmiko et al. 2014). Through the support of drought resilient alleles, the partial short green rotation with the sd1-inherited structure is formed and positions of qDTY1.1 regarding GY and QTL under drought conditions resist and collapse via hybridization (Vikram et al. 2015).

14.4 Auxin

Auxin is classified as an essential hormone involved in plant body development, including shoots and roots, along with the CKs phytohormone (Su et al. 2011). Meanwhile, in rice, filling of grain depends on the production of auxin, where maximum auxin leads to early filling of grain, while minimum production of auxin leads to less filling of grain; therefore, it is recommended that the concentration of auxin should be maximum early in the filling of grain phase to maintain its quantity (Tamaki et al. 2015). Under drought conditions, at the time of biogenesis, auxin downregulation is linked with genes significances in the minimum assembly of auxin constituents (Du et al. 2011). During the signaling, several genes are convoluted for auxin biosynthesis and are supposed to connect under stress, but its influence on GY remains unclear (Peleg and Blumwald 2011; Zhang et al. 2012). The genes that regulate root elongation are not associated with the progressive regulation by auxin, while it is predictable at the DRO1/QTL position. DRO1 controls drought avoidance in fewer root crops and high yield productivity under stress conditions (Uga et al. 2013).

14.5 Ethylene

Ethylene is an extremely important phytohormone that is responsible for falling of leaves, maturity, and response in waterlogging conditions (Perata and Voesenek 2007). Additionally, during drought stress, root elongation will be stopped by the maximum production of ethylene, and it will also reduce leaf enlargement and other physiology mechanisms such as photosynthesis (Sharp 2002). Excess concentration of ethylene promotes the abortion of embryo and reduces the degree of grain contents (Tamaki et al. 2015). During the early grain stuffing stage, ethylene can promote improvement in rice seeds (Yang et al. 2004). The ethylene displays an inverse relation to grain filling percentage. It is proposed that ABA and ethylene both have an antagonistic function regarding each other, and their effects depend on equilibrium between production of ethylene and ABA to advance the grain stuffing percentage (Zhu et al. 2011).

14.6 Jasmonic Acid

This plant hormone has implications associated with development and defense reactions (Turner et al. 2002). However, its functions and physiognomies are not recognized against stress. Endogenous of JA may be increased in stress (Du et al. 2011). Under propagative stress, the MeJA level is upgraded 19-fold in rice and produces a minimum GY in rice (Kim et al. 2009). The AtJMT gene is related to the advancement of the MeJA level in flowering bunches in Arabidopsis. Lower spikelet efficiency and filling percentage stimulate the reduction in Ubil:AtJMT via GY. Transformed plants comprise the number of spikelets relating to the lower part of flowers, ovule and anther. All these features are the replication of the maximum MeJA level, and it reduces the development of spikelet.

15 Future Trends in Research

Accomplishing improvements in drought resilient crops is a very complex achievement that requires detailed information regarding the formation of phenotype, biochemical, physiological, morphological characteristics, as well as the genotype of plants. A number of drought tolerant crops are produced for survival under drought stress; water status should influence profound rooting, moderation in metabolism should occur, and vital antioxidant and osmotic ions are essential. Undesirable signals are interfered with by signaling mechanisms of phytohormones. Therefore, an optimum and sustained yield is the chief aim of investigations to produce drought resistant crops. However, the integrative plan to link physiology, quantitative genetics, and omics, as recommended above, has been followed by only a few research programs. Rice cultivars are selected for their current sensitive characteristics to produce maximum drought resistance from current QTL to help identify gene areas associated with drought tolerance under stress conditions. Exact phenotyping is very critical to identify the superior collections/core mapping populations to identify the accurate QTLs and isolate genes of interest to use in plant breeding. Detecting the yield productivity under stress is a complex phenomenon, which would be the consequence of several characteristics and it would also be obliged to exhibit its self-expression to identify the primary provider involved in selecting the position under stress conditions; yet it is not a suitable method for production of maximum yield of drought resistance crops. Under several environmental conditions, QTL mapping effects on GY have been realized through molecular methods results. However, the molecular environment is also being utilized, with the editing of genome and functional genes being exposed in the highest production of crops under stress conditions. To recognize the various reaction mechanisms and control field yields, the studies should be sustained on a widespread level. Recent advancements in marker development genome analysis sequencing have motivated the rethinking of the mechanisms of creating populations suitable for, as well as to challenge the precise players in, drought tolerance. Consequently, to obtain the maximum yield production and information regarding the plant breeding section, genotype and physiology at the genetic level is essential. Hence, development of cheap and rapid measures to explore drought response characteristics will effectively enhance genetic resolution by integrating molecular genetics, crop physiology, and breeding approaches.

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Genomics and Genetic Engineering to Develop Metal/Metalloid Stress-Tolerant Rice

Subhabrata Paul, Swarnali Dey, and Rita Kundu

Abstract

Rapid industrialization and urbanization gradually shrink the cultivable land worldwide. To meet the growing demand for food, excessive application of fertilizer/pesticide and irrigation with sewage/industry effluent result in contamination of arable land with heavy metals. Rice, a major staple food used worldwide specially in the Asian countries, is also affected by heavy metal (HM) toxicity. These HMs affect plant growth and metabolism negatively and decrease crop quality and productivity. They are also able to enter the food chain and affect human health. Lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd), chromium (Cr), mercury (Hg), arsenic (As), and antimony (Sb) are some of the metals and metalloids found in contaminated soil. Both the essential and nonessential elements are taken up by the plants through different metal transporters. As more HMs are being transported into the plants, deficiencies of essential elements and oxidative stress may occur. As a defense mechanism, plants employ different strategies to mitigate the oxidative stress. Genetically engineered plants are developed in such a way that they are equipped with the production of enhanced stress tolerance protein/metal sequestration mechanism/efficient metal efflux system/modified metal transporters, which can make them more adaptable in HM stress condition. Cultivation of tolerant/genetically engineered genotypes can help to eliminate metal toxicity and accumulation in grains. Rice grown in As/Cd-contaminated sites causes accumulation of these metals in grains, which is deleterious for health. Transgenic rice engineered with As(III)-*S*-adenosyl methyl

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transferase (arsM) decreased As accumulation in rice grains, and by gene manipulation, Cd entry in rice grain can also be blocked.

Keywords

Heavy metals · Antioxidants · Transporters · Non-protein thiols · Efflux pumps · Reactive oxygen species

1 Introduction

Rice is the main staple food crop consumed across the globe; the consumption rate is more mainly in the African and Asian countries. Though lesser in amount, per capita rice consumption is increasing among the European and American population. It feeds nearly half of the world's population providing 19% of global human per capita energy and 13% per capita protein (IRRI 2009). Being rich in nutrients, vitamins, and minerals, more than 3.5 billion people all over the world depend on rice for more than 20% of their daily caloric supply, and 520 million people in Asia take almost 50% calorie daily from rice (IRRI 2009).

With the increasing population, the global rice demand is estimated to reach 555 million tons in 2035 from 496 million tons in 2020. Natural calamities and rapid urbanization, causing shrinkage of arable land throughout the world, poses a big challenge to meet this ever-increasing food demand. At the same time, contamination of arable lands with various pollutants (mostly heavy metals released from the industries) reduces crop yield and, at the same time, interferes with the uptake of other beneficial macro- and micronutrients to the grains, resulting nutrient-poor crops. These crops may serve the purpose to feed the population, but as they lack the proper nutritional quotient, they may not be as beneficial as crops from uncontaminated lands, resulting in "hidden hunger." Heavy metals are defined as metals usually with high densities and atomic number or weight and metalloids (some of them are considered as heavy metals, such as arsenic) are defined as chemical elements having properties lying between metals and solid non-metals (Roychoudhury et al. 2012a). Trace amount of such HMs and metalloids like Fe, Cu, Zn, Co, Mn, and Se are required for certain biological processes specially in plants (Nieboer and Richardson 1978; Emsley 2011). Though they are supposed to be scarce on earth's crust, anthropogenic activities have accelerated slowly occurring geochemical cycle of metals that eventually lead to the accumulation of HM in soil. This has not only altered soil composition but has also posed detrimental effects on plants, animals, and human beings which have created turmoil in the ecological balance (Wuana and Okieimen 2011). Contamination of soil by heavy metals and metalloids may occur by several reasons, mainly through emissions from the industries, disposal of heavy metal contaminated wastes, coal combustion, spillage of petrochemicals, mine tailings, and use of leaded gasoline and paints in the urban sectors. In the agriculture sectors, uncontrolled application of fertilizers and pesticides, animal manures, sewage sludge, and wastewater irrigation in the arable land increases the heavy metal burdens (Ali et al. 2013; Zhang et al. 2010a).

Crop plants such as rice are affected most due to soil contamination by HMs as it adversely hinders the morphological, physiological, and biochemical properties of the plants, crop yield, and quality. Besides, such HMs are subjected to biomagnification when they enter into the food chain through initial accumulation in rice grains followed by transfer to animals or human through diet. Thus, their effects are not only restricted to plants but rapidly spreading among animals and humans culminating in various disorders and health hazards. Rice can neither be designated as a potential HM accumulator nor as a complete extruder. The ability of HM uptake or accumulation varies among several varieties of rice depending on their genotype.

Several methods, like soil turnover and dilution technique, crop rotation, use of tolerant varieties, and use of hybrid varieties capable of accumulating heavy metals, have been employed to mitigate this problem of heavy metal contamination of arable land over the last few decades (Hseu et al. 2010). The role of polyamines in mitigating heavy metal stress has been documented (Paul et al. 2018). With the advancement of biotechnology and genetic engineering, a novel approach has been undertaken to ameliorate the effects of HM-contaminated soil on rice plants and prevent their accumulation within the grains. Through genetic engineering, it is possible to manipulate or modify a gene to nullify or reduce the harmful effects of HM contaminated soils on crop plants. This strategy can be adopted in agriculture to modify certain genes in rice to raise tolerant varieties and minimize accumulation of HMs in grains. Though scientists across the globe have demonstrated different approaches and techniques, all have showed a common aim to mitigate HM toxicity and raise tolerant varieties. This chapter includes various HMs and metalloids that are common soil contaminants and highlights the genes and various genetic engineering techniques that have been adopted by scientists across the globe in order to solve the foresaid issues. The toxicity of various metals and metalloids (both essential and inessential, such as cadmium, chromium, arsenic, manganese, iron, and copper) in rice, their effects and responses to mitigate the toxicity are discussed below (Fig. 1).

2 Cadmium (Cd)

Cadmium (Cd) is a soft bluish white divalent metal with atomic number 48. The average concentration of cadmium on Earth's crust is between 0.1 and 0.5 parts per million (ICdA). Sources of Cd in the environment are natural sources such as volcanic activities, weathering of rocks, and soil erosion (Adamu and Nganje 2010; WHO 1992). Rocks that are mined for phosphate fertilizers contain varying amounts of cadmium, resulting in a cadmium concentration of about 300 mg/kg (Soler and Rovira 1996) of fertilizers which ultimately causes high cadmium content in agricultural soils. Anthropogenic factors include burning of coal, smelters of nickel–Cd batteries, automobile exhaust, electroplating (Schoeters et al. 2006), and agricultural practices such as application of Cd containing fungicides, super

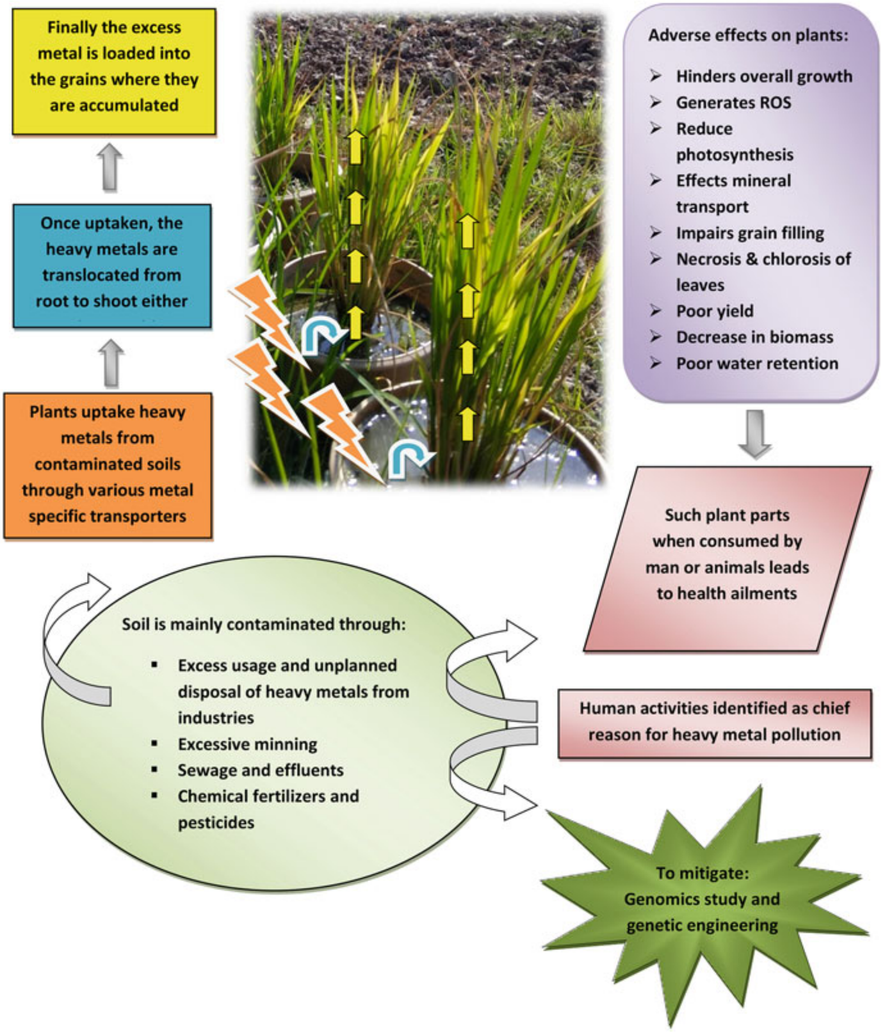


Fig. 1 Diagram showing metal uptake and deleterious effects in plant and animal health

phosphate fertilizers, and Cd containing sewage sludge (WHO 1992; Wang et al. 2009) also adding Cd to the atmosphere as well as transferring Cd into soil. Cadmium in soil can be absorbed by crops such as rice, wheat, starchy roots/tubers/leafy vegetables which then enters in human body through diet (Baize 2009; Figueroa 2008; Flick et al. 1971). Mining operations contaminated the Jinzū River in Japan with cadmium and traces of other toxic metals during 1950s. As a consequence, of which cadmium accumulated in the rice crops that were cultivated along the riverbanks downstream of the mines. Consumption of such contaminated rice developed itai-itai disease which became a worldwide concern (Nogawa et al. 1975; Roychoudhury and Ghosh 2013). Other health hazards include kidney malfunctions, early atherosclerosis, hypertension, cardiovascular diseases, and even cancer. According to the EFSA Panel on Contaminants in the Food Chain, 2.5 µg of Cd per kg body weight is a tolerable weekly intake for humans. Rice, one of the major food crops of the world, is under severe threat of Cd-contaminated soil that hampers the rice quality (Hu et al. 2009), and Cd-contaminated rice poses health risk on mankind. Thus, it becomes utmost necessity to check Cd contamination and find out ways to deal with it.

2.1 Translocation of Cd in Rice Plants

Cd is absorbed by the roots and immediately translocated to the shoot via xylem, OsHMA2, and OsHMA3, taking major role in this process (Sasaki et al. 2014; Satoh-Nagasawa et al. 2012, 2013; Takahashi et al. 2012). OsLCT1 (low-affinity cation transporter 1) encodes a Cd efflux transporter on the plasma membrane (Uraguchi and Fujiwara 2012). After transfer to phloem at nodes, Cd is eventually transported to the panicle instead of leaves (Uraguchi et al. 2011). Cd can either be directly transported to the developing grains via xylem or to the transpiring parts like rachis, culms, flag leaves, and the outer parts of panicles, followed by rapid remobilization into grains through phloem (Rodda et al. 2011) (Fig. 2).

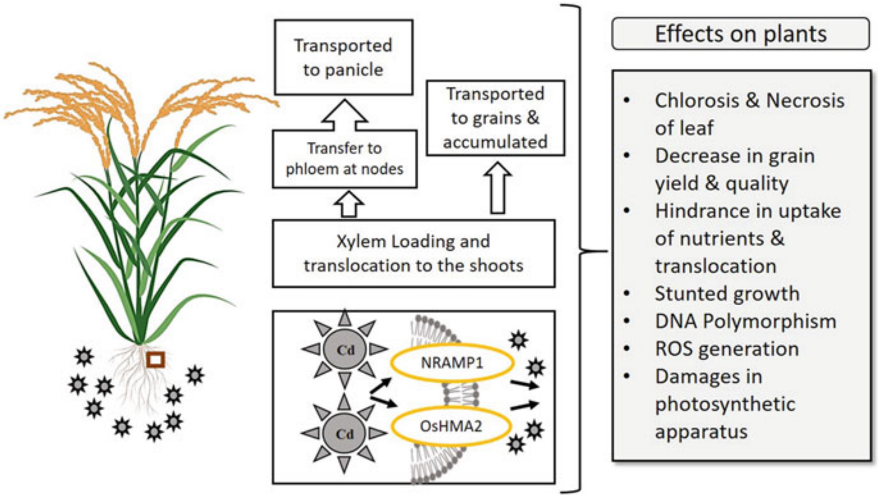


Fig. 2 Diagrammatic representation of Cd uptake and transport in rice plants

2.2 Effects of Cd on Rice

Cd stress hinders rice growth (in terms of root and shoot length, number, and area), affects biomass, reduces seed germination (Ahsan et al. 2007), and causes leaf chlorosis and necrosis (Liu et al. 2003a, b). Under high dose of Cd, decrease in photosynthesis and gas exchange characteristics (chlorophyll a, chlorophyll b, carotenoids, net photosynthetic rate, stomatal conductance, transpiration rate, and water use efficiency) as well as structural damages to photosynthetic apparatus of rice has been reported (Rascio et al. 2008; Roychoudhury et al. 2012b; Wang et al. 2014). Cadmium toxicity also affects the uptake and translocation of essential nutrients (Liu et al. 2008). Studies revealed that Cd transfer to rice grains had decreased grain yield, quality, and nutrient uptake (Arao and Ae 2003; He et al. 2006; Liu et al. 2007; Rodda et al. 2011; Li et al. 2012a, b). Besides, Cd toxicity increases oxidative stress in rice seedlings by the generation of reactive oxygen species (ROS), e.g., hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), that culminate to electrolyte leakage (EL) (Shah et al. 2001; Hassan et al. 2005; Wu et al. 2006; Yu et al. 2013; Srivastava et al. 2014). Cadmium toxicity has been found to induce DNA polymorphism (Aina et al. 2007) and also up or down regulate several proteins (Lee et al. 2010). Paul and Roychoudhury (2018) also showed transcriptome profiling of stress-inducible genes in rice in response to cadmium toxicity.

2.3 Traditional Practices to Minimize Cd Toxicity

Agronomic practices such as soil amendments (Guo et al. 2006), water management (Honma et al. 2016), tillage management (Yu et al. 2014), fertilizer management (Yan et al. 2015), and microbial remediation (Dixit et al. 2015a, b) were key ways to overcome Cd stress. Application of biochar, use of compost, phytoextraction, and use of tolerant rice cultivars are some other practices to deal with Cd toxicity.

2.4 Genetic Engineering Strategies

Intensive research has been going on in the last few years in order to generate Cd-tolerant rice varieties, some of which are summed up as follows:

1. *OsSUV3* gene that codes for a DNA and RNA helicase protein has been found to be upregulated under Cd stress. A transgenic rice variety IR64, overexpressing *OsSUV3* is reported to be Cd stress tolerant. *OsSUV3* was earlier reported to have role in salinity tolerance, but this recent finding suggests it to be responsible for multi-stress tolerance (Sahoo et al. 2014).
2. Novel rice gene low cadmium (LCD) is involved in Cd accumulation and tolerance (Shimo et al. 2011). Knockout of LCD reduced Cd accumulation and

increased growth. It is not a membrane transporter and may be a protein related to Cd homeostasis (Kuramata et al. 2009).

3. Till date, many countries do not allow commercialization of transgenic rice. Ishikawa in 2012 introduced non-transgenic rice mutants by ion beam irradiation. Irradiation of seeds of japonica variety and screening of low Cd mutants revealed three mutant lines, viz. lcd-kmt1, lcd-kmt2, and lcd-kmt3. Sequence analysis revealed that each line had a different mutation [i.e., a transposon (mPingA1) insertion, a single base pair deletion, and a large deletion] in the same gene *OsNRAMP5*, involved in Mn, Fe, and Cd transport in rice roots, which is located near *OsNRAMP1*. While lcd-kmt 1 (with a transposon insertion in *OsNRAM5*) and lcd-kmt 2 (with a single base pair deletion on *OsNRAM5*) mutants did not show significant changes in plant or grain morphology or quality of grain and straw yield, lcd-kmt3 showed early heading and smaller plant size than wild-type probably due to large deletions in *OsNRAM5*.
4. Natural resistance-associated macrophage proteins (NRAMPs) comprise a large family of membrane proteins that help in metal transportation (Fe, Cd, Zn) across the membrane. Takahashi et al. (2011) found that knock down of *OsNRAM5* can remarkably reduce Cd uptake by plants.
5. RNA interference (RNAi)-based gene silencing techniques and critical analysis of role of micro-RNA (miRNA) in plant stress tolerance has set forth a new approach to help combat heavy metal stress and enhance tolerance in rice plants. Some of the instances published till date are mentioned as follows:
   (a) miRNA 166 was reported to be a master regulator of plant development as it targets transcripts of class III homeodomain leucine zipper (HD Zip) transcription factors in plants including rice (Itoh et al. 2008). Recent studies highlight the role of miRNA166 in abiotic stress response of crop plants like rice. It has been revealed from functional analysis of rice miR166 and its target genes that Cd stress strongly downregulates the expression of miRNA166 but upregulates the expression of its target gene *OsHB4*. On the other hand, overexpression of miR166 was found to downregulate *OsHB4* expression and decrease Cd-induced oxidative stress and Cd accumulation in grains, whereas overexpression of *OsHb4* increased Cd sensitivity and accumulation and silencing it by RNAi-enhanced Cd tolerance (Ding et al. 2018). All these findings bring forward the important role of miR166 in Cd tolerance and accumulation through the regulation of HD Zip target genes.
   (b) Ding et al. (2016) also reported another important micro-RNA that serves a vital role in growth, development, and stress tolerance in rice plants—miR390. According to their findings, under Cd stress, miR390 was critically downregulated, whereas target gene of miR390, *Oryza sativa* stress responsive leucine-rich repeat receptor like kinase (*OsSRK*) was induced. Transgenic plants that were overexpressing miR390 was recorded to show less Cd tolerance and higher rates of accumulation of Cd in grains than wild-type of plants. Also, 35S:MIR390 plants showed less expression of *OsSRK* than wild-type. miR390 was thus concluded to be a negative regulator in Cd stress tolerance.

(c) *OsHMA3* gene has been reported to be responsible for decreasing Cd accumulation in rice. RNAi-mediated knockdown of *OsHMA3* gene led to enhanced Cd translocation from root to shoot, but overexpression of the same was found to reduce shoot Cd accumulation. This finding thus suggests the functional role of root cell *OsHMA3* in vacuolar compartmentation of Cd that reduces xylem loading and hence accumulation of Cd in shoots (Ueno et al. 2010).

3 Arsenic (As)

Arsenic (As) is a dreadful health threat worldwide. It is a natural metalloid present in low amounts in environment. Its main sources are geogenic and anthropogenic. It mainly contaminates the ground water which in turn affects both plants and animals (Banerjee et al. 2013). Arsenic poisoning is one of the deadliest environmental hazards. Plants accumulate As from contaminated irrigation water, and it enters in the food chain. Based on the availability of As in the soil and the physiology of the plant, the extent of As accumulation varies (Santra et al. 2013). It exists in different forms in soil. Oxidized arsenate (As^{5+}) form is predominant in aerobic soil in contrary to the presence of reduced arsenite form (As^{3+}) in anerobic paddy soil (Takahashi et al. 2004). As^{3+} form is predominantly present in xylem sap of rice, tomato, etc. As(V) utilizes phosphate transporter (Meharg and Macnair 1992) for its uptake and competes with phosphate. Inter-conversion of different forms of As takes place for its mobilization between different kinds of tissues. As^{3+} is one of the most phytotoxic forms as it is easily taken up by plants (Mandal and Suzuki 2002; Banerjee and Roychoudhury 2018).

3.1 Translocation and Accumulation of As in Rice Plants

Different rice genotypes accumulate As differently (Norton et al. 2010). Organic matter content, pH, texture of soil, etc. control intake and mobilization of As (Mitra et al. 2017). By techniques like plasma mass spectroscopy coupled with X-ray absorption, near-edge spectroscopy helped to measure As in fresh grains (Carey et al. 2009). This study suggested that both xylem and phloem contribute to As unloading in the grain.

In rice, uptake of the different As species takes place at different rates. Rice uptake As in the following order: $As^{3+} > MMA > As^{5+} > DMA$ (Marin et al. 1992). As uptake in rice is found to be influenced by microbe-mediated redox changes of As in the rhizosphere (Jia et al. 2014).

Lsi1, an aquaporin, is a bi-directional As(III) transporter found in rice involved in efflux of As(III). About one fifth of the total As(III) efflux was done by this method (Zhao et al. 2010). After uptake, reduction of intracellular As(V) to As(III) takes place by As reductase, ACR2, (Dhankher 2005; Bleeker et al. 2006). *OsACR2.1* and

OsACR2.2 are identified in rice having similar functions of the yeast *ACR2* genes. *OsACR2.1* is much active than *OsACR2.2* for arsenate reduction (Duan et al. 2007). The severity of As accumulation is higher in rice plants than other crops. TF for As is 0.8 in rice which is much higher than those for wheat (0.1) and barley (0.2).

3.2 Effects of As on Rice

Rice accumulates As in grains more than other cereal crops like wheat and barley (Williams et al. 2007; Su et al. 2010). This accumulation is governed by several environmental and geochemical factors. These influence solubility of As in water which in turn affects its bioavailability (Fitz and Wenzel 2002). As generates oxidative stress and inhibits ATP production which leads to lesser grain yield (Panaullah et al. 2009). Several symptoms like brown spots and stunted growth are often found with As toxicity (Khan et al. 2010).

3.3 Traditional Practices to Minimize As Toxicity

Natural As detoxification mechanisms are present. By proteomic study of rice roots, involvement of putative detoxification mechanisms of As stress were found (Ahsan et al. 2008). Through induction of antioxidant defense system and upregulating cysteine, proline content in some rice varieties (PB1) can detoxify As(V) but some (IR64) could not (Gupta and Anwar 2014).

Under As stress, differential expression of rice lambda class GST subfamily was found in As sensitive and tolerant genotypes (Kumar et al. 2013a, b).

3.4 Genetic Engineering Strategies

1. Transgenic rice having *O. sativa* As(III)-*S*-adenosyl methyl transferase (arsM) decreased As in rice grain (Meng et al. 2011).
2. Another approach is to utilize the bacterial *ArsM* gene that encodes AsIII-*S*-adenosyl methionine methyl transferase to make the transgenic rice. Such method is helpful as the gene product may produce MMA and DMA to bind inorganic arsenic by methylation and subsequent production of volatile trimethylarsine (TMA) (Chen et al. 2017).
3. Inclusion of bacterial AsIII-*S*-adenosyl methyl transferase (ArsM) from (*Rhodopseudomonas palustris*) decreased the accumulation of arsenic in rice grain (Chen et al. 2017).
4. Incorporation of phosphate transporter (Pht1;8) or phosphate starvation response 2 (PHR2) increases phosphate and As(V) uptake and translocation (Wu et al. 2011).

5. It has also been observed that incorporation of yeast arsenate reductase (ACR3) increased As(III) efflux and decreased As accumulation in rice grain (Duan et al. 2012).
6. Mutation of *OsACR2.1* and *OsACR2.2* genes led to decreased arsenate reduction in root, decreased arsenite efflux, and increased As accumulation in root and grain (Duan et al. 2007).
7. Overexpression of *OsPIP2;4*, *OsPIP2;6*, and *OsPIP2;7* had increased As^{3+} tolerance in transgenic *Arabidopsis* during short-term exposure (Mosa et al. 2012). Similar effects were expected in rice.
8. As efflux genes such as *HAC1* can be important in developing rice containing low grain As content.
9. *Arabidopsis* plant mutant for auxin transporters have been found to be more sensitive to As^{3+} stress. Application of exogenous auxin imparts tolerance to As^{3+} in such mutants (Krishnamurthy and Rathinasabapathi 2013). This hints at a possible role of auxin in ameliorating As stress in rice; however, the molecular basis of the mechanism needs to be tested.
10. The transport of As-PC (phytochelatin) complexes to the vacuoles is mediated by the AtABCC1 and AtABCC2 transporters in *A. thaliana* (Song et al. 2010). Such ABC-type transporters are supposed to help in the transport of As-PC complexes in different plant species.
11. Overexpression of phytochelatin synthase gene (PCS) for the production of GSH and PC like chelators used to detoxify arsenic in plants (Sharma et al. 2014).
12. Controlling the expression of transport mediator gene like *OsLsi1* and *OsLsi2* by AMF (Chen et al. 2012) also helps in the mitigation of As toxicity. Using high doses of sulfur reduced the activity of AS transporters, OsLsi1 and OsLsi2 (Dixit et al. 2015a, b).

4 Chromium (Cr)

Chromium (Cr), the first element of group 6 in the periodic table with atomic number 24, is a lustrous, steel gray transition metal (Brandes et al. 1956). It is the 13th most abundant element in Earth's crust (Emsley 2001a), and in nature, it exists in two different stable oxidation states: trivalent (CrIII) and hexavalent (CrVI) differing in terms of bioavailability, toxicity, and mobility (Panda and Patra 1997). Cr is being extensively used in industries such as leather tanning, steel manufacture, electroplating, catalyst application, pigment manufacture, metal corrosion inhibitors, textile (Dixit et al. 2002); thus, it is present in industrial effluents and municipal sewage which in turn results in deposition of the metal in soil and agricultural lands (Zayed and Terry 2003). Besides anthropogenic sources, there are some natural sources of Cr in the environment like rocks, animals, plants, soil, volcanic dust, and gases (Kotas and Stasicka 2000).

Among the two prevalent forms of chromium in nature, the hexavalent form is biologically toxic having no potential biological roles in plants (Von Burg and Liu

1993). However, Cr(III) is less toxic, less mobile, and mainly found coalesced to organic matter in soil and aquatic environment (Becquer et al. 2003) and is believed to have biological role in insulin signaling pathways in animals (Vincent 2011). Chromium is a nonessential metal for plant, having no significant role in its development or growth. Hence, high levels of Cr in soil may lead to several malfunctions in plant as well as animals when it enters in their body through diet.

4.1 Translocation of Cr in Plants

Chromium is not an essential plant nutrient, so plants have not developed any specific mechanism for the uptake of Cr. It is through other heavy metal transporters that Cr enters the plant body through active or passive mechanism depending on the oxidation state of Cr. Cr(VI) transport is an active mechanism that involves carrier of essential ions like sulfate, whereas uptake of Cr(III) seems to be passive (Barcelo and Poschenrieder 1997). Cr moves through xylem once inside the plant body. It is generally stored in root vacuoles in Cr III form and not translocated to shoot which is a natural toxicity response of plants, but Cr VI reducing enzymes are mostly absent in higher vascular plants (Cervantes et al. 2001) thus enhancing toxicity.

4.2 Effects of Cr Toxicity in Plant

Cr toxicity affects several processes in plants including growth and development, germination, decrease total dry matter (DM), production, yield, and metabolic processes such as photosynthesis, water relation, and nutrient uptake, leading to reduced growth, stunting, and eventually plant death (Mcgrath 1982; Shanker et al. 2009). In addition, it might result in degrading pigment status, nutrient balance, antioxidant enzymes, and induction of oxidative stress in plants (Panda 2003).

4.3 Practices to Reduce Cr Stress

To mitigate Cr toxicity, tolerant cultivars are generally cultivated in heavily contaminated soils. Besides, phytoremediation is another technique to deal with this problem. Recently, it has been reported that application of silicon can ameliorate the toxic effect of Cr (Zeng et al. 2011) as also seed priming with salicylic acid has been proven to induce tolerance against Cr toxicity (Shinwari et al. 2015).

4.4 Genetic Approaches

CDPK and NADPH oxidase are activated in response to Cr(VI) via the accumulation of calcium and generation of ROS, respectively (Trinh et al. 2014). Overexpression of *OsGSTU30* had enhanced tolerance to chromium stress in *Arabidopsis* and accumulates more cadmium than the WT plants (Srivastava et al. 2018).

5 Aluminum (Al)

Aluminum (Al), a silvery-white, soft, nonmagnetic, ductile metal in the boron group makes up about 8% of the Earth's crust and stands as the third most abundant element only after oxygen and silicon (Shakhashiri 2008). Al covers 1.59% of the entire Earth's crust by mass (McDonough 2001). As a major nonferrous metal, Al has a wide range of uses. The major uses can be summed up as: manufacture of automobiles, beverage cans, foils, frames, in building and constructions, in electrical wiring as alloys, and also a variety of house hold items including kitchen utensils to furniture. Aluminum toxicity has been a major concern in crop productivity especially in the acidic soils worldwide predominantly in the tropics (Barceló and Poschenrieder 2002). Al does not exist freely in nature. It is always found to be associated with other elements to form compounds. Two such predominantly existing compounds are potassium aluminum sulfate ($KAl(SO_4).12H_2O$) and aluminum oxide (Al_2O_3).

5.1 Translocation in Plants

Al does not usually interfere with the plants till the pH of the soil becomes acidic (below 4.5) (Rosseland et al. 1990). Acidic pH facilitates Al movement into the plants via roots as it coverts Al into a soluble or exchangeable form. Al has been reported to accumulate in the root meristem more, compared to other plant parts, thereby hindering the normal root growth. It is generally stored in the cell wall region and also in the nucleus in some instances (Rincón and Gonzales 1992; Ryan et al. 1993). The plasma membrane bound transporters that are associated in Al uptake have not been well characterized yet, though role of Nrat1 (Nramp aluminum transporter 1) specific for trivalent Al ion is mention worthy (Xia et al. 2010).

5.2 Toxic Effects in Plants

The root system of plants is the primary and chief target of Al toxicity. Inhibition of root elongation, restricting absorption of mineral elements, and water (Slaski 1994) leads to overall reduced growth and mineral deficiencies in shoots and leaves of the rice plants (Foy Charles 2008). Al has also been reported to interfere with the uptake and transport of essential elements such as Ca, Mg, P, K, and Fe. The ability to maintain proper concentrations of macro- and micronutrient cations in the root and shoot is also lost due to Al toxicity (Foy Charles 2008). Other phytotoxic effects of Al are disruption of Ca^{2+} homeostasis in the cytoplasm (Rengel 2004) and damaged respiration in the mitochondria (Yamamoto et al. 2002). Callose deposition at the plasmodesmata in roots has also been reported (Sivaguru et al. 2000) (Fig. 3).

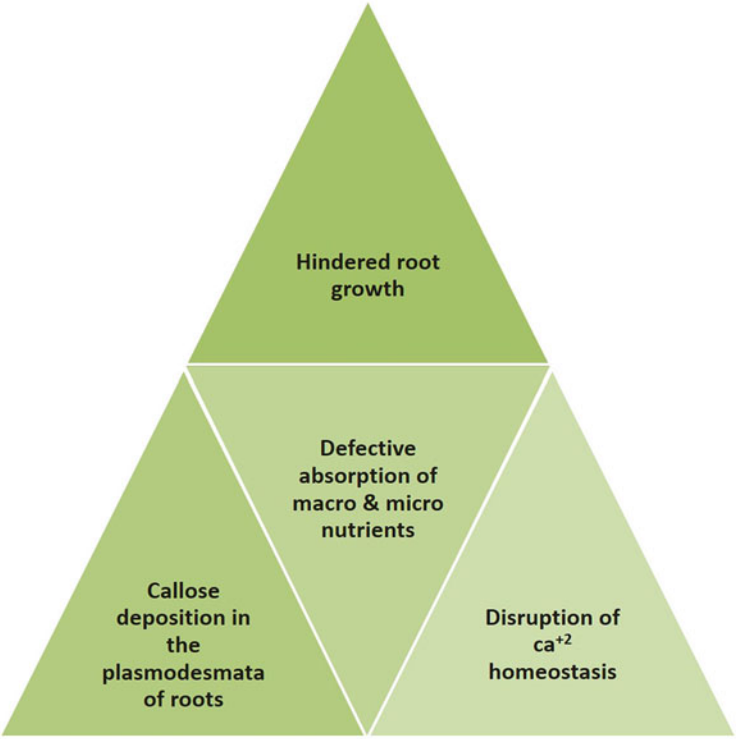


Fig. 3 Effects of aluminum toxicity on rice

5.3 Practices to Reduce Al Toxicity

To prevent crop loss due to Al toxicity, the most accepted method has been selection of resistant or tolerant genotypes that occur naturally or have been raised through breeding techniques. Plants have developed multiple ways to mitigate Al toxicity. The chief techniques being detoxification of Al inside the cell are thereby inhibiting the deposition of Al in the apoplastic region or reduction in Al transport from the environment to inside of the cell. To alleviate damage from ROS produced due to increased levels of intracellular Al, plants tend to increase the activities of the antioxidant enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), and catalase (CAT) (Wang and Yang 2005; Zhang et al. 2010b). Plants like rice, maize, and soybean that are tolerant to Al stress usually practice exudation of citrate from roots (Silva et al. 2001; Ma et al. 2002; Pineros et al. 2002). Vacuolar compartmentalizing of Al is another important way to remediate Al inside the plant (Shen et al. 2002).

5.4 Genetic Approaches

1. Rice has been identified as one of the most resistant crops under field conditions against Al stress (Foy Charles 2008). This tolerance is mainly imparted by Aluminum Responsive Genes in rice (Tsutsui et al. 2012). Aluminum stress-induced genes involve *STAR1* and *STAR2* which encode an ATP binding and a transmembrane domain protein, respectively. STAR1–STAR2 complex transports UDP glucose which is a substrate that helps to modify the cell wall and mask aluminum-binding sites (Huang et al. 2009).
2. ART1, a transcription factor, has been identified as an important component of Al responsive gene expression (Yamaji et al. 2009).
3. Abscisic acid stress and ripening (ASR) proteins are low molecular weight, hydrophilic plant-specific proteins. Studies figured out *ASR5* transcript level hike in response to Al in both root and shoot, whereas *ASR5*-silenced plants became very sensitive to Al (Arenhart et al. 2013).
4. *STAR1* (*ABC* gene) transcript level increased in Al-treated plants (non-transgenic) but decreased in *ASR5*-RNAi plants, thereby revealing that *STAR1* is a down-stream target of ASR5 transcription factor (Arenhart et al. 2014).
5. Genes like *LRR*, *PP2A*, *PTL*, *AT*, *ATPS*, *APR3*, *SR*, *CYS1*, *CYS3*, and *GLO* are either Al stress-induced genes or constitutively expressed in Al-resistant crops compared to the susceptible ones (Zhang et al. 2007).
6. *OsFRDL4* is an Al-induced citrate transporter in the plasma membrane of root cells of rice. *OsFRDL4* is a multidrug and toxic compound extrusion (MATE) family gene that has very low expression in rice roots in the absence of Al, but a brief exposure of Al immediately triggers its expression. *OsFRDL4* is under the regulation of transcription factor ART1 (a C2H2 type 2n Zn finger transcription factor). *OsFRDL4* knockout is sensitive to Al. Thus, *OsFDRL4* can be believed to have a role in Al tolerance (Yokosho et al. 2016).
7. *Nrat1* is a gene that encodes an Al transporter whose substitution decreased *Nrat1* expression and thereby Al uptake and tolerance but enhanced Al binding to the cell wall (Xia et al. 2011).

6 Manganese (Mn)

Manganese (Mn), the 12th most abundant element of the Earth's crust, having atomic number 25 is not generally found free in nature but mostly occurs in minerals in combination with iron. It comprises about 0.1% of the Earth's crust (Emsley 2001b, c). Commonly occurring oxidation states of manganese includes +2, +3, +4, +6, and +7, though the most stable oxidation state for manganese is +2 that has got a pale pink color. Manganese(II) ions are important in biological systems as they have a wide range of functions from serving as cofactors for a large variety of enzymes essential for detoxification of superoxide free radicals that deals with elemental oxygen, to functions in the oxygen-evolving complex of photosynthesizing plants. Growth of plants was found to be inhibited when the tissue manganese level goes below 20 mg/kg (Mengel and Kirkby 1987). Trace amount of Mn is essential for all living beings but Mn in higher doses can act as a potential neurotoxin (Roth et al. 2013; Holleman and Wiberg 1985). Mn is extensively used in iron and steel production owing to its sulfur-fixing, deoxidizing, and alloying abilities. It is also used in the manufacture of aluminum alloys, in alkaline batteries, etc. Increasing level of Mn has marked it as a potent environmental pollutant.

6.1 Translocation in Rice

Mn concentrations in water-logged rice fields are usually very high due to the reducing environment and rice stands as one of the most tolerant crops to Mn stress. Rice plants generally transport accumulated Mn from the root cells to the shoot cells in order to prevent high accumulation of Mn in root cell cytoplasm (Tsunemitsu et al. 2018). For normal growth, rice takes up Mn from the soil via transporter NRAMP5, located at the distal side of exodermis and endodermis of the roots, responsible for the transport of Mn from the soil into the cells (Ishimaru et al. 2012; Sasaki et al. 2012). Recent studies have revealed the role of NRAMP3, a plasma membrane-localized transporter, in manganese distribution control inside the cell (Yamaji et al. 2013). YSL2 (yellow stripelike 2), a transporter of manganese(II)-NA complex, is expressed in the phloem companion cells that helps in long-distance transport of manganese especially into the grain (Koike et al. 2004; Ishimaru et al. 2010). Metal tolerance protein 9 (MTP9), a member of cation diffusion facilitator (CDF), has been identified as the transporter responsible for extrusion of Mn into the stellar cells which is localized at the proximal side of exodermis and endodermis just opposite to Nramp5 in rice root cells (Ueno et al. 2015). Mn is translocated into the shoot, unloaded from xylem at nodes and distributed to the older tissues when excess in plant (Yamaji et al. 2013).

6.2 Genomics

Not much is known about the fact that how rice plants combat Mn stress. A recent investigation however reveals the role of two cation diffusion facilitators, MTP8.1 and MTP8.2, in Mn tolerance in rice roots by sequestration of Mn into vacuoles. MTP8.1 and MTP8.2 are expressed constitutively at high levels in older tissues compared to the younger ones although the level of MTP8.1 is higher than that of MTP8.2. Under high Mn concentration, MTP8.1 knockout shows retarded growth and chlorosis and necrosis of leaf tissues, whereas MTP8.2 knockout did not show any such toxicity. When both MTP8.1 and MTP8.2 were knocked out, plants showed maximum sensitivity to Mn toxicity, thus highlighting the role of vacuolar sequestration of Mn as an important mechanism to withstand Mn stress (Tsunemitsu et al. 2018). Tsunemitsu and coworkers have hypothesized that, by reducing Mn uptake, rice responded to excess Mn showing enhanced Mn tolerance (Fig. 4).

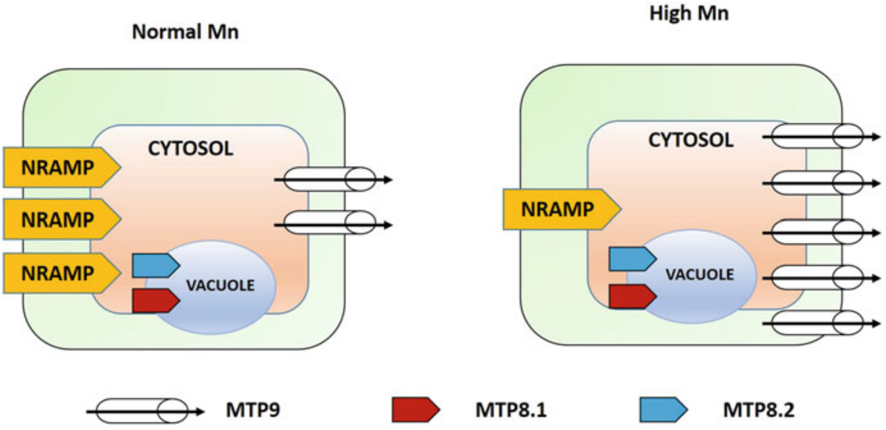
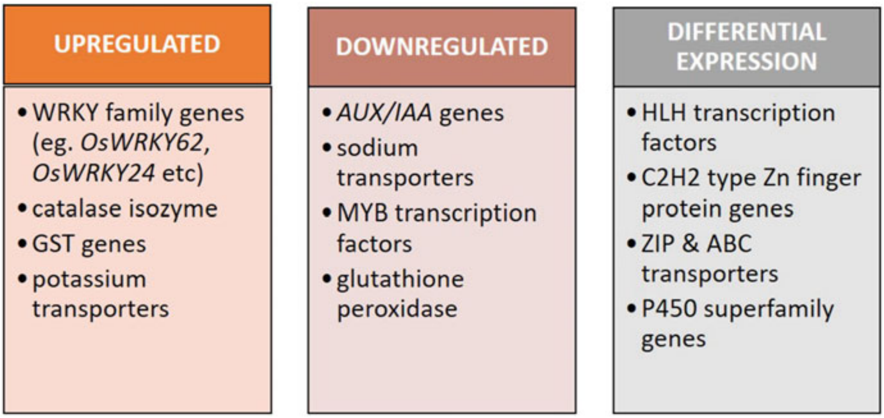


Fig. 4 Rice reduces Mn uptake in response to Mn stress. (Adapted from Tsunemitsu et al. 2018)

A transcriptome analysis by Li et al. (2017) has revealed that Mn resistance in rice is very complex and is a consequence of coordinated interplay among the expression of a large number of genes. While nearly about 1336 genes were reported to be upregulated under abnormal Mn concentrations, 1495 genes were found to be downregulated. Besides, several genes showed differential expression owing to high Mn concentrations. These genes mostly code for several transcription factors, transferase and catalytic proteins, transporter proteins, etc. that are mostly involved in primary and secondary metabolism. Brief account for the genes that are either upregulated or downregulated or being differentially expressed is given in Fig. 5.

7 Lead (Pb)

Lead (Pb) belongs to group IV, period 6, with an atomic number 82. This element is a naturally occurring bluish grey metal, found on the Earth's crust (10–30 mg/kg) in association with sulfur (as PbS, $PbSO_4$). It is the second most harmful pollutant after arsenic (Pourrut et al. 2011), affecting plant metabolism and crop productivity badly (Sharma and Dubey 2005; Ashraf et al. 2015). Pb contamination of soil mainly occurs through mining, smelting, and anthropogenic activities (use of leaded fuels, etc.). It is a nonessential element for biological function, rather, when present, interact with nitrogen and sulfur in amino acid side chains (Clemens 2006). Due to its nature, in human Pb can be accumulated within the body organs (brain) and causes toxicity. Children exposed to high concentration of Pb show impaired development, low IQ, shortened attention span, hyperactivity, and mental retardation, while adults show symptoms like nausea, insomnia, anorexia, weakness of bone joints, and loss of memory. Severe lead toxicity can cause death (NSC 2009).



UPREGULATED

- WRKY family genes (eg. *OsWRKY62, OsWRKY24* etc)
- catalase isozyme
- GST genes
- potassium transporters

DOWNREGULATED

- *AUX/IAA* genes
- sodium transporters
- MYB transcription factors
- glutathione peroxidase

DIFFERENTIAL EXPRESSION

- HLH transcription factors
- C2H2 type Zn finger protein genes
- ZIP & ABC transporters
- P450 superfamily genes

Fig. 5 List of some differentially expressed genes under Mn stress

7.1 Toxicity

Pb toxicity can result in growth retardation, deformation of cellular structure, ion homeostasis, reduced chlorophyll biosynthesis, and overproduction of ROS in plants (Shahid et al. 2011; Kumar et al. 2012). One way to combat Pb stress is the production of antioxidants (both enzymatic and nonenzymatic) by the plants, as a result, enzymatic components, such as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), and nonenzymatic components, e.g., tocoferols, ascorbic acid, carotenoid, glutathione, and cysteine content, increase in tolerant plants (Verma and Dubey 2003). Plants being sessile in nature generally try to undertake homeostatic mechanism to regulate uptake, accumulation, transport, and detoxification of heavy metals (Clemens 2006).

7.2 Transporters and Genes in Rice

In dicot plants, P1B-type ATPase play an important role in metal detoxification via efflux mechanism (Lee et al. 2007). In rice, only few reports are available regarding the genomics of Pb toxicity and tolerance. P1B-type ATPase is a family of nine proteins in rice (OsHMA1–OsHMA9) (Baxter et al. 2003). Lee et al. (2007) tried to characterize the role of P1B-type ATPase in heavy metal toxicity (Cu, Cd, Zn, and Pb) in rice. They have treated 7 day old seedlings of rice (*Oryza sativa* Dongjin) with increasing concentrations of Cu/Zn/Cd and Pb for 3–6 h. It was observed that increasing concentration of Pb showed no effect in OsHMA9 expression, while others (OsHMA4/5/6/7/8) showed enhanced expression in response to high concentration of Cu, Zn, and Cd. Through GUS analysis, it was shown that they are mainly expressed in the vascular bundles, mesophyll cells, and anthers. OsHMA9:GFP fusion proteins were found to localize on the plasma membrane. Two null mutants (oshma9-1, oshma9-2) were generated and tested in the presence of high Pb concentrations. Knockout plants showed retarded growth and reduced height, biomass, and photosynthetic pigment content, at the same time they were found to accumulate more Pb than their wild counterpart. This clearly indicates that OsHMA9 acts as an efflux protein.

8 Iron (Fe)

Iron (Fe), a solid, shiny, grayish metal, is the fourth most abundant element on Earth's crust (7000–550,000 mg/kg) and belongs to group VIII, period 4 element, block-d, with an atomic number 26. It is one of the important essential elements needed for both the plants and animals. About 10–18 mg of Fe is needed every day by human.

Iron is one of the most important micronutrient needed for plant growth and metabolism. But in excess, Fe can cause severe toxicity to plants. It is one of the most important soil problem in West African wetlands and considered as a major impediment for the farmers, as rice production is greatly reduced (10–90% loss is reported) (Audebert and Fofana 2009; Wan et al. 2005; Gridley et al. 2006). High amount of reducible Fe, low pH, low redox potential, low CEC, and low exchangeable potassium ions along with poor drainage are the major causes of Fe toxicity in soil. Fe can be present in soil in very high quantity (2000 mg/l). At low pH, iron is more soluble than at higher pH.

8.1 Toxicity

It generally interferes with the absorption of other nutrients (Ca, Mg, K, and P) into the plants causing bronzing of the leaves, growth retardation, and yield retardation. Onyango and coworkers, while working with four low land rice cultivars, have observed that several morpho-physiological and biochemical parameters (photosynthesis rate, stomatal conductance, RWC, membrane stability, tissue Fe content, soluble sugars, protein, and starch content) varied depending on the tolerance potential. Tolerant cultivars showed more lateral roots, photosynthetic pigment content, and membrane stability (Onyango et al. 2018). Enzymatic oxidation of the root surfaces forming a plaque-like structure hinders the entry of iron by forming a physical barrier (Wu et al. 2014). Limited Fe^{2+} uptake, along with exclusion of Fe^{2+} by root surface, and strong activity of phytoferritin are the markers of tolerant rice cultivars (Saikia and Baruah 2012). In response to Fe toxicity, antioxidative defense system upregulates (SOD, POD, GR) in the tolerant cultivars (Mahender et al. 2019).

8.2 Transporters and Genes in Rice

Fe can be taken by the plants in either Fe^{3+} or Fe^{2+} form. Phytosiderophores form a complex with Fe^{3+} and transport it through xylem and phloem with the help of a group of transporters present (Banakar et al. 2017). Fe uptake and transport from soil to the aerial parts of rice plants are mediated by several transporters (OsYSL1, OsYSL15, OsTOM1, OsIRO2, OsIRT2, and OsNRAMP1) and genes (*OsNAS1-3*, *OsNAAT1*, *OsDMAS1*) (Bashir et al. 2010, 2014; Li et al. 2017; Kim and Guerinot 2007; Connorton et al. 2017; Dotaniya et al. 2013). OSIRT1 is a functional transporter for Fe and also transports Zn and Cd (Lee and An 2009), another transporter OSYSL2 is a rice metal transporter (mostly for Fe^{2+} + DNA conjugate) which transports Fe to phloem and Fe and Mn into the grains (Koike et al. 2004). Involvement of a putative vacuolar ATPase transporter gene was considered for subcellular distribution of Fe in rice under Fe toxic condition. Expression of *OSNAS3* increases tolerance to Fe and Zn deficiencies and Zn, Cu, and Ni toxicity due to excess amount (Lee and An 2009). Under excess Fe, expression levels of ferritin genes, *OSFER1* and *OSFER2*, was reported to increase in leaves (Stein et al. 2009). FRO3 and FRO8 are two mitochondrial Fe transporters, which reduce Fe^{3+} to Fe^{2+} and transport cytosolic Fe into mitochondria (Jain and Connolly 2013). Under excess iron, *YSL1*, *FRO 8*, *ZIP*, and ferritin genes and transporters help to maintain iron homeostasis in cells and tissues. In the presence of excess iron, WRKY, a transcription factor superfamily, represses iron translocation from roots to shoots (Viana et al. 2017). Zang and coworkers (2017a, b) identified 29 QTL, detected on chromosome number 1, 2, 3, 5, 6, and 12, for enhanced Fe accumulation in tissues in the tolerant cultivars, in case of Fe toxicity.

8.3 MicroRNAs

The recent findings of involvement of microRNA (miRNAs) was observed in Fe acquisition in roots under excess iron conditions. Several miRNAs were reported to be involved in Fe homeostasis and toxicity responses (Mendoza-Soto et al. 2012; Li et al. 2012a, b; Paul et al. 2016; Waters et al. 2012). miR156, miR162, miR167, miR168, miR171, miR172, and miR398 were found to be responsible for Fe homeostasis and showed different expression in root and shoot.

9 Zinc (Zn)

Zinc (Zn) is a group IIB, period 4 element with an atomic number of 30 and occurs naturally in soil (70 mg/kg). It is an essential micronutrient and indispensable part of several metallo-enzymes (oxidoreductases, transferases, and hydrolases). It also acts as a component of DNA–RNA binding protein (Zn finger domain of transcription factor). Among the various oxidation states, Zn^{2+} is the most prevalent form found in soil. Concentration above 150–300 mg/kg in soil is considered to be highly toxic. Zn toxicity depends on total Zn content of soil, soil pH, nature of clay, presence of organic matter and presence of other heavy metals in soil. Generally, excess Zn inhibits the absorption of other nutrients into the plants, causing deficiencies of iron, phosphorus, and manganese. As a result, plants show chlorosis and growth retardation. Necrotic spotting between the veins of the leaf blades and inward rolling of leaf margins are tale-tale signs of Zn toxicity.

9.1 Transporters and Genes

Os IRT1 and OsNAS3 impart increased tolerance to Zn and Fe in the presence of excess metal (Lee and An 2009).

9.2 Genetic Approaches

To generate tolerant cultivars, breeding is the only sustainable solution. A total of 222 indica rice accession were grown under excess Zn concentration (200 mg/L) for 21 days. Thirty-one QTL were detected for zinc toxicity, among them ten QTL were detected on chromosome numbers 1, 2, 3, 4, 5, 6, and 12, and two QTL were co-localized in the genomic region of chromosome 5 (Zhang et al. 2017a, b). They were associated with increased accumulation of Zn in shoots in the tolerant lines.

10 Copper (Cu)

Copper (Cu) is a group IB, period 4 element, with atomic number 29. It is an essential element for growth and development, in animals it is needed for the production of blood. High concentration of Cu may cause anemia, liver and kidney damage, and stomach and intestinal disturbances (Wuana and Okieimen 2011).

Copper (Cu) is an import trace element needed for plant growth and metabolism. It catalyzes redox reactions in mitochondria, chloroplast, and cytosol of the plant cells and during respiration acts as electron carrier. It exists in many states in the soil; plants mainly absorb the Cu^{2+} form, but in most cases, it was observed that the maximum amount of Cu was mainly deposited/remain accumulated in the roots, with only a fraction of it being translocated to the aerial parts. Approximately 2–250 μg/g of Cu can be present in soil, and plants can absorb 20–30 μg/g dry weight of Cu. Phytoavailability of Cu depends on several factors. Phytoavailability increases as soil pH increases.

10.1 Toxicity

Due to Cu toxicity, root elongation and growth are hampered. Leaf discoloration, chlorosis, and necrosis are the common symptoms of Cu toxicity. Due to free radical generation, lipid peroxidation occurs leading to membrane damage, an excess Cu is to plants (Cu being redox active metal, catalyze ROS, such as O_2^-, H_2O_2, $HO^\cdot$ via Fenton's reaction).

10.2 Transporters and Genes

Under varying concentration of Cu^{+2} (10, 45, and 130 μM $CuCl_2$) treatment for 24–30 h in *O. sativa* cv. Nipponbane (6/7 week), it was observed that 146 genes were unregulated (Sudo et al. 2008) as studied by 22k Rice Oligo Micro Array kit (Agilent Tech). Among the upregulated genes, the significant ones are related to plant defense. Genes of PAL pathway, synthesizing flavonoid, phytoalexin, and lignin are worth mentioning. Flavonoids act as ROS scavenger.

Peroxidase precursor, glutathione-*S*-transferase, and metallothionine-like protein-1 indicated the activation of defense mechanism. At the same time, Cu toxicity also induced the upregulation of lipoxygenase, Jasmonate-induced protein, universal stress protein, salt stress-induced protein, and PDR-like ABC transporters. Due to Cu toxicity genes for Nuclease PA3, pre-mRNA splicing factor, recA protein, ribonuclease 3, and cytochrome P450 87A3 also get upregulated.

Zhang et al. (2017a, b) have shown that under Cu stress, expression of Rice *MT*/*OsMT2C*/*OsMT-1-2b* and Cu/Zn SOD is induced, as observed by qPCR studies. Rice MT expression blocks the production of H_2O_2 in rice suspension cells under Cu stress and helps to survive them. HMP-type HMA5 and HMA4 were reported to be involved in xylem loading of Cu in rice (Deng et al. 2013; Huang et al. 2016).

11 Conclusion

Contamination of arable land is a problem worldwide, affecting crop quality and productivity. Rice being the major cereal played the most important role in feeding more than three billion people (20% of the world population). In this chapter, we have discussed at length how the heavy metals affect plant growth, development, and metabolism. Heavy metals not only negatively regulate plant growth, by reducing photosynthesis, biomass, plant height, and interfering with the uptake and absorption of other useful micronutrients, but also pose dietary risks to the consumers, as some part of the metals get translocated and accumulated to the grains. Tolerance to heavy metal stress is an innate character and varies among the different genotypes of rice. It was observed that African rice varieties (e.g., Suakoko 8 and WITA 4) are more tolerant to iron stress.

Plants generally try to avoid the transport and accumulation of harmful metals, and for this, they utilize cellular mechanism for metal homeostasis. Several antioxidants (both enzymatic and nonenzymatic) become active, and transporters also act to impart tolerance to the plants. In order to generate tolerant varieties, the scientists have two main approaches, one approach targets to increase the defense mechanism of the plants by overexpressing the genes responsible for antioxidants

(SOD, CAT, POD, phytochelatins, and metallothionines), and the other is to modify its transporter system (OsHMAs, OsNRAMPs, ZIPs, and IRTs) so that uptake and translocation of the excess metals can be prevented. In recent times, roles of transcription factors and microRNAs in regulating metal uptake and tolerance are reported from several studies, which can also be utilized in development of metal-tolerant varieties. Genome-Wide Association Studies (GWAS) and QTL studies are also helping to understand the roles of genes in metal tolerance, which can also help to develop tolerant allele donors in future.

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Potential Biotechnological Strategies to Improve Quality and Productivity of Rice Under Arsenic Stress

Shikha Verma, Pankaj Kumar Verma, and Debasis Chakrabarty

Abstract

Rice (*Oryza sativa* L.) is a widely cultivated staple food crop feeds half of the world's population. However, rice accumulates higher amount of heavy metals like arsenic and serves as a prominent source of arsenic exposure to humans. Arsenic is a pervasive metalloid, and its contamination in soil and water has been reported globally. In an aerobic environment, arsenic is mainly present as arsenate (AsV), while under anaerobic conditions like flooded paddy soil, it primarily exists in the reduced form as arsenite (AsIII). Because of the extensive incidence of arsenic in paddy fields, a substantially high amount of arsenic gets accumulated in grains and causes severe human health risks. Therefore, it is necessary to reduce arsenic toxicity with suitable approaches and mechanisms. Several biotechnological strategies may offer an effective approach to reduce arsenic accumulation in rice grains. Many key processes can be targeted to regulate its accumulation in rice grains. These include arsenic uptake, AsIII efflux, AsV reduction, AsIII sequestration, and arsenic methylation and volatilization. The combination of modern biotechnology with conventional agricultural practices in a sustainable manner may help clear up arsenic contamination in soil and water and decrease its accumulation in grains. Presently, we emphasize on biotechnological approaches and strategies for reducing arsenic accumulation in rice. These strategies can help to achieve food security for the present and future generations.

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Arsenic · Bioremediation · Detoxification · Rice · Transgenic

1 Introduction

Rice is a major food crop for more than half of the world's population. It is an important constituent in the global food security program as it provides 40–70% of the total food calories to the world's population. Various biotic and abiotic stresses severely constrain rice production. Abiotic stresses such as water stress, salt stress, and heavy metal stress affect crop production as well as grain nutrition quality. Among the different heavy metals (As, Cr, Cd, Pb), arsenic causes severe loss in crop production as well as the higher arsenic accumulation in edible parts poses health hazards to humans. Further, rice is the most severely affected crop by arsenic compared with other crops; therefore, there is an imperative need for effective management to reduce arsenic accumulation in rice (Banerjee et al. 2013).

Arsenic contamination of soil and water has been reported across the civilized world, particularly in South and Southeast Asian countries. The eastern part of India and Bangladesh are severely affected by arsenic contamination (Mandal and Suzuki 2002). Arsenic is an element (^{33}As) that exists in three forms: elemental, inorganic, and organic forms. Among these, inorganic forms are incredibly toxic to both plants and animals. Inorganic forms exist in three forms as alloy-like intermetallic compounds: arsenides (As^{3-}), arsenite (As^{3+}, AsIII), and arsenate (As^{5+}, AsV). AsIII and AsV are the dominant forms naturally present in soil and water. In an aerobic environment, arsenic is predominantly present as AsV, whereas under anaerobic conditions like flooded paddy fields, it primarily exists as AsIII (Tripathi et al. 2007). Flooding of soil causes the rapid reduction of AsV to the more toxic and mobile AsIII. The majority of rice crops cultivated in flooded lands where AsIII is predominantly present, thus have excessive arsenic accumulation in grains, making rice the most affected crop. Rice can accumulate much higher (more than tenfold) arsenic than other crops (Su et al. 2010). Rice grains can accumulate up to 2 mg kg^{-1} arsenic (Joseph et al. 2015), which is considerably higher than the tolerable limit. The maximum tolerable daily intake limit of arsenic for humans is 2 μg kg^{-1} body weight (WHO 2011). Thus, if a human weighs 60 kg and consumes 300 g of rice per day, then the safe limit of arsenic concentration in rice grains should be 0.40 μg kg^{-1} dry weight. So, rice can accumulate about 1000 times more arsenic than the tolerable limit.

Rice is a major staple crop worldwide, arsenic accumulation in rice making a global issue of concern (Table 1). The situation is very critical in countries where rice consumption rate is very high, ranging from 250 to 650 g of rice per day per person. Since a large number of populations are being affected by arsenic toxicity, there is an urgent need to develop strategies to reduce arsenic accumulation in rice. To lower arsenic accumulation, various studies have been performed, that revealed several mechanisms to deal with arsenic toxicity and accumulation in plants, including rice. In this context, biotechnological methods could represent a useful and practical approach to reduce arsenic accumulation in rice grains. It can be achieved by

Table 1 Arsenic concentration in rice grains of different countries

Country	Arsenic in rice grains (mg kg^{-1})	Reference
Korea	0.24–0.72	Lee et al. (2008)
USA	0.2–0.46	Meharg and Rahman (2003)
Taiwan	0.1–0.63	Lin et al. (2004)
Spain	0.09–0.40	Torres-Escribano et al. (2008)
Italy	0.09–0.39	Sommella et al. (2013)
Australia	0.09–0.22	Fransisca et al. (2015)
Japan	0.07–0.42	Meharg et al. (2009)
Bangladesh	0.05–1.85	Meharg and Rahman (2003)
India	0.05–0.58	Bhattacharya et al. (2010)
China	0.02–0.11	Fang et al. (2014)
Canada	0.02	Williams et al. (2006)
Thailand	0.02–0.37	Nookabkaew et al. (2013)
Egypt	0.01–0.58	Meharg et al. (2009)
Ghana	0.01–0.15	Adomako et al. (2011)

modulating the expression of arsenic metabolism-related genes controlling arsenic uptake, transformation, and translocation in plants. In this chapter, we discuss the recent progress in biotechnological approaches, bioremediation practices, and molecular breeding techniques to produce grains with low arsenic concentration.

2 Arsenic Transport and Detoxification in Rice

2.1 Uptake and Transport of Inorganic Arsenic Species

Arsenate is the dominant arsenic species in aerobic soil, but it accounts for a minor amount of the total arsenic in flooded paddy soils (Jia et al. 2014). Arsenate is a phosphate analog taken up and transported by phosphate transporters with a high affinity toward phosphate. Rice phosphate transporter gene family consists of 13 *OsPT* genes (*OsPT1–OsPT13*) encoding the transporters (Paszkowski et al. 2002). Various studies have uncovered the role of *OsPT1* and *OsPT8* in arsenic transport. OsPT1 mediates root to shoot AsV transport, whereas OsPT8 is a key transporter protein for AsV uptake into rice roots and inhibits root elongation. Additionally, overexpression of *OsPT8* resulted in enhanced arsenic accumulation in plants (Kamiya et al. 2013; Wang et al. 2016). *OsPT4* (*OsPHT1;4*) knockout (KO) in rice resulted in reduced AsV uptake and reduced arsenic accumulation in grains (Cao et al. 2017). Besides these transporters, other proteins such as OsPHF1 (phosphate transporter traffic facilitator 1) have also regulated AsV uptake and root to shoot arsenic translocation (Wu et al. 2011). Few transcription factors have equally been involved in the regulation of AsV uptake in rice. Among them, transcription factor *OsPHR2* (for phosphate starvation response 2) is involved in AsV uptake and translocation (Wu et al. 2011). Other transcription factors such as *WRKY* gene family have also been shown to be involved in AsV influx and efflux.

OsWRKY28 performs a significant role in AsV accumulation and in root architecture and fertility in rice (Wang et al. 2018).

Arsenite is undoubtedly a toxic ion, and the flooding of the paddy field leads to the mobilization of AsIII (Zhao et al. 2009). It enters plants via nodulin 26-like intrinsic proteins (NIPs, a subfamily of the aquaporin family) (Ma et al. 2008). In rice, a major silicic acid influx transporter, *Lsi1* (Low silicon rice 1, *OsNIP2;1*), is mainly responsible for AsIII uptake, while a silicon efflux transporter, *Lsi2* (Low silicon rice 2), mediates AsIII efflux towards xylem (Zhao et al. 2010, 2009). Both transporters are localized in the plasma membrane of exodermal and endodermal root cells; *Lsi1* localizes at the distal part and *Lsi2* at the proximal side of the cell (Ma et al. 2007). These transporters are prominently involved in the efficient flow of Si but also transport AsIII across the endodermis and toward the xylem. A KO mutation of *lsi1* showed less arsenic accumulation in straw, but no significant difference was observed in the seed. On contrary, *lsi2* KO significantly decreases the rate of AsIII transport to the xylem, thus reducing arsenic concentrations in both rice straw and grains (Ma et al. 2008). These independent reports indicated that *Lsi2* plays a more crucial role than *Lsi1* in arsenic transportation to the grains, but KO of *lsi2* too disrupts Si uptake, which inhibits rice growth and also decreases yield (Ma et al. 2007). Apart from these aquaporins, *OsNIP1;1*, *OsNIP2;2*, *OsNIP3;1*, and *OsNIP3;2* can also efficiently transport AsIII in a bidirectional manner (Bienert et al. 2008; Ma et al. 2008). Besides these, OsPIP2;4, OsPIP2;6, and OsPIP2;7, belonging to the plasma membrane intrinsic proteins (PIPs, a subfamily of aquaporin family), have a role in effective AsIII transportation (Mosa et al. 2012). Additionally, the expression of *OsNRAMP1* (Natural Resistance Associated Macrophage Protein) transporter enhances arsenic accumulation in roots and shoots of *Arabidopsis*. It was also demonstrated that *OsNRAMP1* localizes in the plasma membrane of the endodermis and pericycle cells that may facilitate AsIII xylem loading for root to shoot arsenic translocation (Tiwari et al. 2014). Although *OsNRAMP1* has been extensively studied in *Arabidopsis*, its specific function in rice still requires further investigation.

2.2 Uptake and Transport of Organic Arsenic Species

Several methylated arsenic species have been detected in a paddy field; among them, dimethylarsinic acid (DMA) and monomethylarsonic acid (MMA) are the most common (Zhao et al. 2013; Lomax et al. 2012). In a reducing environment, organic arsenic is reduced to volatile arsines such as monomethylarsine (MMAIII), dimethylarsine (DMAIII), and trimethylarsine (TMAIII) (Jia et al. 2013). The uptake mechanisms of methylated species are not broadly examined as those of inorganic arsenic species. Methylated arsenicals are less efficiently taken up by roots than inorganic arsenic, but the root to shoot translocation is higher than that in inorganic arsenicals. Methylated arsenic species form a complex with the ligands (glutathione [GSH]/phytochelatin), which might be the reason for the better translocation of methylated arsenic species (Raab et al. 2007). The methylated arsenic species are possibly taken up through the nodulin 26-like intrinsic protein in rice. Earlier studies discovered that rice aquaporin *Lsi1* mediates the uptake of undissociated pentavalent MMA and DMA (Li et al. 2009) and that rice *lsi1* mutant loses 80% and 50% of the uptake capacity for MMA and DMA, respectively, compared with the wild-type rice. However, rice *Lsi2* is not involved in the efflux of MMA or DMA toward the stele, possibly because most MMA and DMA are dissociated at the cytoplasmic pH. Methylated arsenic species detected in rice grains are most likely taken up from soil as rice lacks functional arsenic methyltransferase enzyme, which converts inorganic arsenic into its organic form (Lomax et al. 2012). Differences also exist among various genotypes in response to arsenic exposure and accumulation in different organs. Various studies have observed significant genotypic effects on arsenic speciation in rice grains. In rice grains, AsIII and DMA are the dominant species with lower concentrations of AsV and MMA. An investigative report suggested that DMAV is more mobile and translocates with ten times higher efficiency than inorganic species in both phloem and xylem transport (Carey et al. 2010). Furthermore, studies have also shown that inorganic arsenic is poorly remobilized from flag leaves to grain through phloem transport, while DMA (V) and MMA(V) are efficiently retranslocated (Carey et al. 2011). This indicates that inorganic arsenic is predominantly translocated via the phloem, while DMA is transferred via both the phloem and xylem (Carey et al. 2010).

2.3 Arsenic Detoxification/Accumulation in Rice

Arsenate detoxification occurs via the reduction of AsV to AsIII. Arsenate can be reduced either enzymatically by arsenate reductase, which uses GSH as a reductant, or nonenzymatically by binding with reducing equivalents such as GSH. In rice, *OsHAC1;1* and *OsHAC1;2* function as AsV reductase (Shi et al. 2016). Recently, another group member, *OsHAC4* was reported to function as AsV reductase. Mutation of *OsHAC4* decreases AsV reduction, thus lowering AsIII efflux, which leads to an increase in arsenic accumulation (Xu et al. 2017). In previous studies, we reported that rice glutaredoxin (*OsGrx*) possessed arsenate reductase activity and maintained GSH pool, which helps in AsIII efflux (Verma et al. 2016a, b). After the reduction of AsV to AsIII, further detoxification of AsIII occurs in vacuoles via vacuolar sequestration. Arsenite has a higher affinity for sulfhydryl (-SH), a group of peptide thiols such as GSH and phytochelatins (PCs); thus, AsIII is sequestered in vacuoles by complexing with GSH or PCs in plants. The synthesis of GSH is catalyzed by a rate-limiting γ-glutamylcysteine (γ-EC) synthetase, followed by glutathione synthetase to conjugate Gly and γ-EC (Dhankher et al. 2002). PCs are synthesized non-translationally by phytochelatin synthase. In rice, two phytochelatin synthase enzymes, OsPCS1 and OsPCS2, have been reported, and *OsPCS2* RNAi lines of rice have been found to be more sensitive to AsIII stress with a significant decrease in PC synthesis and higher content of GSH in comparison to the wild-type plant (Hayashi et al. 2017; Das et al. 2017; Uraguchi et al. 2017). These PCs form a complex with AsIII, which can be transported by ATP-binding cassette (ABC) type transporters.

For example, plant C-type ABC transporters (ABCC) transport AsIII after chelating with PC into vacuoles (Song et al. 2014, 2010; Briat 2010). In rice, OsABCC1 sequesters AsIII-PC in vacuoles, hence reducing arsenic accumulation in grains (Song et al. 2014). A member of ABC transporter family, YCF1, can transport the GSH conjugate of As [As(GS)$_3$] for vacuolar sequestration (Song et al. 2014; Li et al. 1997). Apart from these, some ABC type transporters also transport As-GSH conjugates of [As(GS)$_3$] to the extracellular space. Another group of metal-binding proteins, metallothioneins (OsMTs), also have a role in arsenic detoxification by binding AsIII to their cysteine residues (Gautam et al. 2012). Besides these, many studies also uncovered the role of iron plaque formation in arsenic detoxification. Iron plaque formation occurs on plant roots by oxidation of Fe^{2+} to Fe^{3+}, which was done by microbial activities. These plaques are involved in arsenic detoxification by adsorbing arsenic on the root surface of plants. It can adsorb and/or co-precipitate arsenic, thus sequestering arsenic on roots and reducing arsenic uptake and accumulation in grains (Mei et al. 2009; Colmer 2003).

3 Biotechnological Strategies for Mitigating Arsenic Accumulation in Rice Grains

3.1 Genetic Engineering

Innovative approaches should be focussed on genetic manipulation to reduce arsenic content in rice grains. These approaches may include increased arsenic tolerance to cope with arsenic-contaminated environment, decreased arsenic uptake followed by increased arsenic extrusion, and finally increased arsenic volatilization for improved food safety. Enhanced PC production in roots may be one of the ways to restrict arsenic translocation to shoots through AsIII-PC complexation and vacuolar sequestration in roots. A previous study demonstrated that arsenic tolerance increased in transgenic rice overexpressing *OsPCS1* gene and that significant expression of PC gene lowers arsenic accumulation in grains (Hayashi et al. 2017). Another study showed that overexpression of *PCs* from *Ceratophyllum demersum* in rice leads to more arsenic concentration in roots and so lesser arsenic accumulation in grains and husk (Shri et al. 2014). Another strategy is to reduce AsIII accumulation by overexpressing rice glutaredoxins, *OsGrx_C7* and *OsGrx_C2.1*. These genes are involved in AsV reduction to AsIII and its efflux by altering the expression and function of aquaglyceroporin in yeast, *Arabidopsis*, and rice. These genes are also involved in maintaining cellular redox status mediated by the Grx/GSH system, thereby protecting the cell under arsenic stress (Verma et al. 2016a, b, 2020). Further, a study suggested that *OsHAC4* gene encodes a rhodanase-like protein that acts as AsV reductase, followed by AsIII efflux, for the management of arsenic accumulation in rice. Arsenate reduction, followed by AsIII efflux, is an important mechanism of AsV detoxification (Xu et al. 2017). In yeast, AsIII is effluxed outside the cell by AsIII efflux transporter. Arsenical Compounds Resistance protein 3 (ACR3), and overexpression of *Acr3p* in rice resulted in 30% lesser arsenic accumulation in roots and shoots in comparison to the wild-type plant (Duan et al. 2011). Therefore, in the future, these genes could be manipulated to reduce arsenic accumulation in rice grains.

For plants, it is not possible to block the entry of AsV or AsIII because of their shared transport systems with essential or beneficial elements. However, it could be possible to identify variants of phosphate transporters, NIP aquaporins, or *Lsi2* that are more discriminatory against arsenic. In rice, numerous studies have been reported for the modulation of important transporters and other metabolism-related genes and their effect on arsenic accumulation, especially in grains. The roles of phosphate transporter *OsPT4* (*OsPht1;4*) have been explored for reduced AsV uptake and accumulation in rice grains. *OsPT4* demonstrates a high affinity for AsV but lesser for Pi and is also involved in the xylem loading of AsV. Mutation in *OsPT4* resulted in increased AsV tolerance and lower arsenic accumulation in grains (Ye et al. 2017). The plasma membrane transporter *OsNIP3;2* is expressed in roots and involved in AsIII uptake. The mutant lines showed decreased arsenic accumulation in the roots, whereas there was no significant change in arsenic accumulation found in any above-ground parts, including grains (Chen et al. 2017). Other *OsNIP1;1* and *OsNIP3;3* transporters are involved in AsIII uptake from soil to root and its loading to the xylem. Mutation of these transporters did not alter arsenic accumulation in the roots and shoots; however, overexpression resulted in decreased arsenic accumulation in above-ground parts (Sun et al. 2018). Vacuolar membrane transporters, ABCC1 transporters (ABC transporters), have a crucial role in sequestering AsIII-PC complexes into vacuoles. A KO mutation of rice *OsABCC1* transporter leads to more excessive arsenic accumulation in grains and decreased accumulation in nodes I and II, which indicated its importance in arsenic detoxification in rice (Deng et al. 2018). Another plastid-localized transporter, *OsCLT1* [CRT (Chloroquine-Resistance Transporter)-Like transporter], has also been shown to be essential for reducing arsenic accumulation in rice. *OsCLT1* maintains GSH homeostasis by exporting γ-EC and GSH to the cytosol for GSH biosynthesis. The mutation leads to reduced levels of γ-EC and GSH, ultimately lowering the rate of PC biosynthesis; in this manner, arsenic accumulation decreases in root (Yang et al. 2016). Glutaredoxin also maintained the GSH pool, consequently enhanced arsenic accumulation in the root, thereby decreasing arsenic translocation to the areal parts of the rice plant, particularly grains (Verma et al. 2020). Recently, *OsARM1* (Arsenite-Responsive MYB1 transcription factor) has been shown to regulate the expression of *OsLsi1*, *OsLsi2*, and *OsLsi6* in rice. This in turn regulates AsIII uptake from the soil as well as root to above-ground part translocation. The KO lines of *OsARM1* allowed higher expression of these transporters, consequently increasing arsenic accumulation in rice plants, while contrasting results were found in overexpressing lines (Wang et al. 2017). A further approach of arsenic detoxification would represent a reduction of AsV to AsIII by arsenate reductase followed by AsIII extrusion. In rice, *OsHAC1;1*, *OsHAC1;2*, and *OsHAC4* have AsV reductase activity. Overexpression of these genes increases AsV reduction and decreases arsenic accumulation, while mutant lines had increased arsenic accumulation in rice grains (Shi et al. 2016; Xu et al. 2017).

Another potential strategy to reduce arsenic accumulation in rice grains is the expression of arsenic methyltransferase (*arsM*) gene, which converts inorganic arsenic into volatile arsenicals. In the genome of higher plants, no functional gene encoding *arsM* has been identified, including the rice genome. Hence, the overexpression of *arsM* gene provides a strategy to generate transgenic rice plants that accumulate lesser arsenic in grains. Earlier, Meng et al. overexpressed *arsM* from soil bacterium *Rhodopseudomonas palustris* in japonica rice (*Oryza sativa* L.) cv. 'Nipponbare' and found that transgenic lines accumulate lesser amount of arsenic by methylating arsenic into volatile arsenicals, however their results were not so effective to generate arsenic free rice variety (Meng et al. 2011). For the first time, our group identified a novel arsenic methyltransferase gene (*WaarsM*) from a fungus, *Westerdykella aurantiaca*, and found that genetically engineered yeast expressing *WaarsM* showed arsenic tolerance (Verma et al. 2016c). *WaarsM* overexpressed in *Arabidopsis* showed increased arsenic tolerance and accumulated less arsenic (Verma et al. 2016d). Later, *WaarsM* was further overexpressed in rice and it was found that transgenic rice showed increased arsenic tolerance and decreased arsenic accumulation in grains via bio-volatilization hence, provides a potential strategy to produce arsenic-free rice (Verma et al. 2018).

3.2 Use of Microbes

Bioremediation represents the method of using organisms to neutralize or eliminate contamination from the environment. Several microorganisms (bacteria, fungi, and algae) have been reported to be useful in lowering arsenic concentration in soil, thereby reducing arsenic accumulation in plants. These microbes utilize various mechanisms such as bioavailability, oxidation, reduction, and biotransformation of inorganic arsenic to organic arsenicals to lessen the effect of arsenic contamination. Diverse studies have been reported the successful utilization of plant growth-promoting microorganisms to regulate arsenic accumulation and improve tolerance and growth of rice plants. Several bacteria like *Kocuria flava* and *Bacillus vietnamensis* that have been isolated from the rhizosphere of mangrove plants in Sunderban were screened for arsenic-resistant properties. These strains improved the growth of rice plants and decreased arsenic accumulation under arsenic stress (Mallick et al. 2018). Many arsenic-resistant bacterial strains were isolated from paddy soil, such as *Bacillus altitudinis*, *B. megaterium*, and *Lysinibacillus* sp. strain SS11, which could be used to mitigate arsenic toxicity (Singh et al. 2015). It has also been reported that *Brevundimonas diminuta* NBRI012, a rhizobacterium, displayed improved arsenic tolerance and growth in rice under arsenic stress (Singh et al. 2016a). Similarly, *Pseudomonas* sp. HN-2 (a mesophilic, gram-negative, AsIII-oxidizing and AsV-reducing bacteria) reduces arsenic mobility in both aerobic and anaerobic conditions and could be used in arsenic remediation (Zhang et al. 2016). Another report demonstrated that *Staphylococcus arlettae* (NBRIEAG-6), *Staphylococcus* sp. (NBRIEAG-8), and *Brevibacillus* sp. (NBRIEAG-9) are arsenic-tolerant bacterial strains that also increased rice plant growth during arsenic stress (Singh et al. 2016b). All these studies focused on the enhancement of rice plant growth under arsenic stress and the potential of different bacterial strains as arsenic bioremediation agent. Still, there is a lack of research focused on the effect of these bacterial strains on arsenic accumulation in rice.

Many studies have shown the role of fungi in arsenic tolerance and accumulation in rice. Srivastava and co-workers isolated 15 fungal strains from arsenic-contaminated agricultural soil. These strains are capable of removing arsenic from the media via volatilization and could be utilized as potential bioremediation agents (Srivastava et al. 2011). Other studies also suggested that arbuscular mycorrhizal fungi (AMF) exhibit the ability to suppress the expression of AsIII uptake transporters (*OsLsi1* and *OsLsi2*). Like so, AMF reduces arsenic uptake and translocation in rice plants; hence, they perform a defensive role in arsenic-rich environment (Chen et al. 2013). As a result, AMF increased biomass and grain yield without increasing arsenic accumulation in rice grains. A report showed that three strains of *Glomus* fungi (*Glomus geosporum*, *G. versiforme*, and *G. mosseae*) increased phosphate uptake and reduced arsenic uptake and translocation to rice grains (Wu et al. 2015). Li and co-workers demonstrated the potential of *R. intraradices* for reduced inorganic/organic arsenic ratio in rice grains after inoculation (Li et al. 2016). The endophytic fungus *Piriformo sporaindica* not only protects rice plants from arsenic toxicity by reducing arsenic bioavailability to the plant but also restricts arsenic in colonized roots by immobilization into the insoluble particulate matter; it also modulates antioxidant responses of plants to combat arsenic stress (Mohd et al. 2017). Recently, our study showed that yeast expressing arsenic methyltransferase (*WaarsM*) gene from the soil fungus *Westerdykella aurantiaca* showed arsenic tolerance via bio-volatilization. These genetically engineered yeast cells improve rice plant growth and decrease arsenic accumulation in grains under arsenic stress. This is the first report of the utilization of genetically engineered yeast cells as arsenic bioremediation agents, which simultaneously enhances the growth of rice plants (Verma et al. 2019).

Algal species are capable of decreasing arsenic toxicity by biosorption. The inoculation of two algae, *Chlorella vulgaris* and *Nannochloropsis*, was compared in rice plants under arsenic stress and was able to reduce arsenic accumulation in the root and shoot of rice plants (Upadhyay et al. 2016). *Anabaena* sp. is also capable of improving rice plant growth and reduces arsenic accumulation in the presence of arsenic (Ranjan et al. 2018). The role of *Pseudomonas putida* and *C. vulgaris* consortium in arsenic toxicity was also studied, and it was found that inoculated rice plants showed significantly improved growth and decline in arsenic concentration in root and shoot (Awasthi et al. 2018). However, the utilization of microbes in arsenic stress amelioration in plants is yet largely unexplored.

3.3 Molecular Breeding

Plant breeding is an efficient approach to minimize arsenic uptake, translocation, and accumulation in rice grains. Worldwide, selection of various rice varieties that can naturally regulate arsenic accumulation could help to reduce arsenic accumulation in

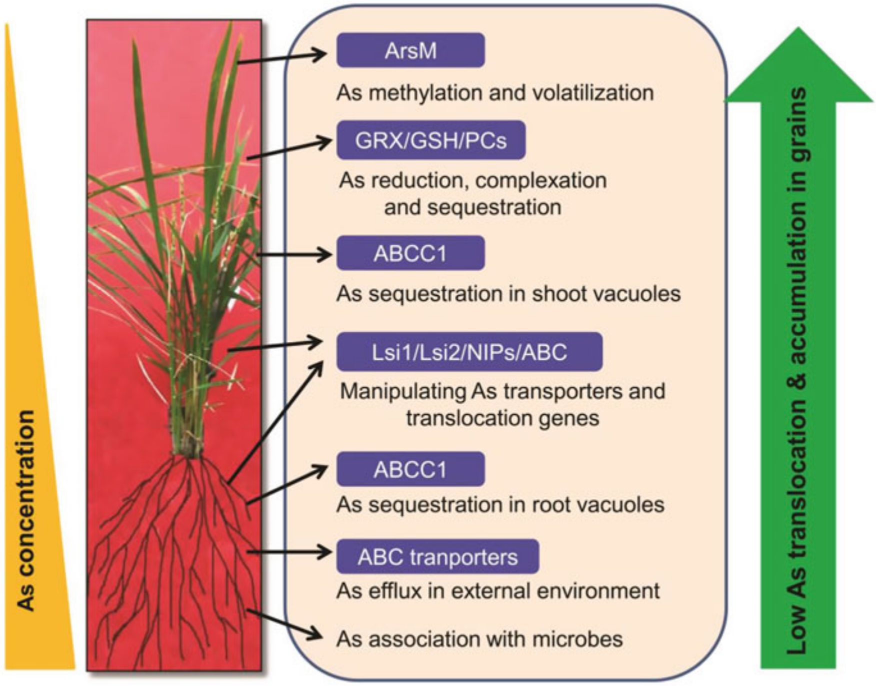

Fig. 1 Potential biotechnological strategies to reduce arsenic (As) accumulation in rice grains

grains. Therefore, additional studies are required to discover the variation in arsenic uptake, translocation, transformation, and accumulation in grains of different rice varieties. The quantitative trait loci (QTLs) linked with arsenic accumulation were mapped in F1 plants obtained from a cross between Japonica cultivar 'CJ06' and an Indica cultivar 'TN1' (*Oryza sativa*) using a doubled haploid population established by anther culture. At the seedling stage, one QTL on chromosome 2 and one on chromosome 3 was mapped for arsenic concentrations in the shoot and root, respectively. While at maturity, two QTLs were found on chromosomes 6 and 8 for arsenic concentrations in grains (Zhang et al. 2008). The previous report demonstrated a large amount of two-loci epistatic interaction for rice grains and leaf and found that there is an independent mechanism of arsenic accumulation in different tissues. By manipulating the large, wide epistasis among chromosomes 1 and 12, arsenic a concentration within grains was reduced (Norton et al. 2010). Kuramata and co-workers discovered two QTLs on chromosome 6 and one QTL on chromosome 8, which control DMA level in rice plants of the F2 population from 'Padi Perak' (a high-DMA accession) and 'Koshihikari' (a low-DMA cultivar) (Kuramata et al. 2013). These studies suggested that it could be a feasible task to reduce arsenic toxicity by developing new rice cultivars using robust QTLs through marker-assisted breeding. Various strategies to reduce arsenic accumulation in rice grains are shown in Fig. 1.

4 Concluding Remarks and Prospects

Arsenic in rice remains a grave concern for human health. Reducing the levels of this ubiquitous carcinogenic element in rice is a primary requirement for public health goals. Arsenic concentration and speciation vary in diverse regions, paddy soils and within different rice cultivars. During the preceding decade, molecular biology research on how plants deal with arsenic has opened up creative opportunities to make rice grains safer by lowering arsenic concentration. Research using transgenic systems can inform plant breeders to select specific genetic markers over others to obtain low arsenic rice varieties. Besides, gene-editing technologies represent recent developments that encourage a researcher in the context of characterizing gene function and improved crop production. Based on the CRISPR/Cas9 (Cluster Regularly Interspaced Short Palindromic Repeats-associated nuclease) of bacteria, precise gene editing is being attempted with an RNA-guided CRISPR/Cas9 system, apart from other technologies like transcription activator-like effector nucleases and zinc-finger nucleases to achieve a precise requirement. The approach from agriculture practices to potential rice plant development and subsequent field implementations are required. Comprehensive multidimensional integrated investigations must provide a sustainable way to remediate arsenic polluted soil and their judicious use to reduce the extent of bioaccumulation in economically important crops to meet human food demand.

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Genetic Engineering: A Powerful Tool to Abrogate the Effect of Metal/Metalloid Toxicity in Rice

Ankur Singh and Aryadeep Roychoudhury

Abstract

Environmental pollution is a matter of growing concern all over the world. The release of elements from industries pollutes the surface water, which when used for irrigation deposit heavy metals in the field. Crops like rice, which is a major food crop of the world, require large amounts of water during their life span. They accumulate toxic elements in their grains when irrigated with contaminated water due to their higher translocation factor. Metal toxicity can be of two types: deficiency of metals, which is the most common problem for metals like iron, and excess of metal present in the surrounding, which is commonly seen for mercury, arsenic, cadmium, etc. Both types of toxicity hamper plant growth and reduce the yield and nutritional content of the grains. To overcome the problem of metal toxicity, several methods have been used. One such method is genetic engineering. In this approach, a gene is introduced in the plants, which enhances the tolerance level of the plants against the metal stress without changing the agronomic trait. Till date, several transgenic rice plants have been introduced, which confer tolerance against higher concentration of heavy metals. This chapter mainly deals with the toxic effects of such metals in rice plants and the genes introduced in the plants, which help them overcome the toxic effects and enhance their survival under stress condition.

Keywords

Heavy metals · Metalloids · Rice · Transgenic approach · Phytoremediation · *Schizosaccharomyces pombe* · *merAB*

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1 Introduction

Anthropogenic activities and dynamic development of industries in the twentieth century have resulted in the release of higher levels of chemical pollutant in the environment. Metals and metalloids constitute most of these pollutants, and due to their ability to form compound with other constituents of the environment, they easily get mixed into the environment, which leads to human health risk. In addition to humans, chemicals also cause serious risk to agricultural land with a total area of about 2.35×10^{12} m^2 being contaminated with metalloids and heavy metals (Bermudez et al. 2012). Metal having density more than five times that of water with high atomic weight is considered a heavy metal, such as mercury (Hg), cadmium (Cd), cobalt (Co), copper (Cu), lead (Pb), iron (Fe), manganese (Mn), and chromium (Cr) (Anjum et al. 2015; Hafiz et al. 2017; Shahzad et al. 2018). Several metals like zinc (Zn), Mn, and nickel (Ni) are required in trace amounts and play a significant role in plant development (Kabata-Pendias 2010). Metalloids are elements that have properties of both metal and non-metal. Most common metalloids include arsenic (As), silicon (Si), boron (B), germanium (Ge), and antimony (Sb).

Rice is one of the staple foods for the world, and due to rise in population, its demand is growing day by day. Higher metal and metalloid level in agricultural land decreases the productivity of field and is also toxic for plants. Metals can be transported to rice kernel from agricultural fields, fertilizers, irrigation with water previously contaminated with heavy metals, and other chemicals like pesticides (Bian et al. 2015). Rice kernel accumulates the toxic chemical from the soil, which poses serious threat to humans as these chemicals are toxic, highly persistent, and non-biodegradable (Song et al. 2014). Toxic effects of heavy metals first appear on leaves, resulting in chlorosis of leaves along with reduced size, tip burn, and necrosis of tips. Cu, Zn, and Pb reduced the growth parameters such as total germination, root and shoot length, and root:shoot ratio of rice seedlings with more pronounced effect shown by Cu toxicity followed by Zn and Pb. Seedlings grown in soil having higher level of Pb showed more accumulation of Cu and Zn in shoots and roots, whereas seedlings harvested from soil contaminated with Cu accumulated more Zn and Pb in their roots (Fahad et al. 2019). Reactive oxygen species (ROS) production is the first response of rice seedlings to heavy metal stress by Haber-Weiss reaction, which results in oxidative stress (Shahzad et al. 2018). Metal stress also hampers the antioxidative system (Srivastava et al. 2004) and nutritional system (Dong et al. 2006), along with the electron transport chain (Qadir et al. 2004). Furthermore, heavy metals also cause membrane alteration, lipid peroxidation, root and leaf disintegration, and higher ROS level, which again enhance the malondialdehyde (MDA) levels and electrolyte leakage in rice seedlings.

To overcome the toxic effect of heavy metals and metalloids, plants have several protective machineries such as chelation, exclusion, and compartmentalization of toxic elements, reducing the effective concentration to a homeotic level (Hall and Williams 2003; Roychoudhury et al. 2012a). Application of exogenous chemicals like Ca and H_2S also mitigated the toxic effect of heavy metals in rice. Ca accumulation in rice due to exogenous application enhances protein kinase activity and calcium-dependent protein kinase level, which inhibit the cell death process induced under Pb toxicity (Huang and Huang 2008). Physiological and biochemical traits of rice were also induced by H_2S under Cd stress, which may be due to higher redox state of H_2S and higher activity of enzymatic anti-oxidant induced by exogenous application of H_2S, which reduces the level of methylglyoxal and ROS (Fahad et al. 2019).

In addition to this, molecular and transgenic approaches are also used to enhance the tolerance level of rice against heavy metal and metalloid stress. Transgenesis is the process of introduction and overexpression of genes that protects plants from stress conditions and controls various processes such as metal sequestration and translocation of metals from the cytosol to the vacuole (Das et al. 2016; Mani and kumar 2014; Shukla et al. 2013). Zhang and Liu (2017) reported the upregulation of *Oryza sativa metal tolerance protein (OsMTP) 11* gene under Cd, Zn, Ni, and Mn stress, which shows that the gene plays an important role in inducing heavy metal tolerance in rice. Mutant *Arabidopsis* plants containing *cytochromes- like proteins (CYP)* from rice locus *Os08g01480* showed enhanced tolerance toward abiotic and heavy metal stress, suggesting that by enhancing the level of *Os08g01480* in rice seedlings, tolerance level of rice seedlings toward heavy metal can be raised further (Rai et al. 2015).

In this chapter, our main focus will be on the toxic effects of several heavy metals and metalloids in rice and transgenic approaches applied to overcome the deleterious effects of these elements.

2 Effect of Toxicity of Element (Mode of Action)

Elements cause toxicity to plants in three different ways: (a) production of ROS by the process of auto-oxidation or Fenton reaction, which causes damage to the cell membrane and other organelles, (b) interaction of heavy metals with proteins, which alters their normal function, and (c) removal of essential elements causing collapse in the structure and function of important metabolites of plants (Hossain et al. 2012).

Elements can be divided into two groups: redox inactive (Zn, Cd, Ni, etc.) and redox active (Fe, Cu, Cr, etc.). Redox-active elements can directly cause oxidative stress by producing O_2^- radical, which is ultimately converted into H_2O_2 via Haber-Weiss and Fenton reaction (Schützendübel and Polle 2002), whereas redox-inactive elements interact with the electron transport chain, lipid membrane, and antioxidative defense system for the production of ROS.

Cofactors are required for the proper function of enzymes. Displacement of one heavy metal ion by other can decrease or permanently damage the function and structure of enzymes. Mg^{2+} present in ribulose-1,5-bisphosphate-carboxylase/oxygenase can be displaced by other divalent cations like Co^{2+}, Zn^{2+}, and Ni^{2+}, which inactivates the enzyme. Again, the removal of Ca^{2+} from calmodulin, an important cellular signaling molecule by Cd^{2+}, leads to the inhibition of phosphodiesterase activity (Hossain et al. 2012).

3 Transgenic Approach to Ameliorate the Toxic Effect of Heavy Metals and Metalloids

3.1 Chromium (Cr)

Chromium belongs to the transition element group and is placed in VI-B in the periodic table. Cr(III), being a less toxic and less mobile form of Cr, is mainly found in bounded form with organic matter in aquatic and terrestrial ecosystems (Becquer et al. 2003). Cr(VI) is considered as the most toxic form and is mainly found as a compound of oxygen, such as chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$). Cr is a toxic and non-essential element for rice growth and development; thus, the specific transporter for Cr is not present in rice. Cr is actively transported by carriers of several essential ions like sulfate (Cervantes et al. 2001). Cr toxicity affects morphological parameters as it decreases germination and reduces the number of roots, shoots, and leaves, which ultimately results in lower biomass of the plants. Cr has a high oxidative potential that ultimately increases the ROS content of plants and causes oxidative stress. Cr toxicity also inactivates the enzymes of the Calvin cycle, inhibits the electron transport system, decreases CO_2 fixation rate, and disorganizes the chloroplast (Shanker et al. 2005).

The detoxification mechanism of Cr(VI) in rice is not well studied. In rice plants, Cr is unable to induce the expression of phytochelatins, which suggests that there exists a different mechanism for the protection of rice plants against Cr(VI) toxicity (Srivastava et al. 2019). Tripathi et al. (2014) identified two tau glutathione S-transferase (GST) in rice, which are specific to roots and help in the sequestration of Cr. On introducing *OsGSTU30* and *OsGSTU41*in *Schizosaccharomyces pombe*, resistance against Cr toxicity was developed. They also observed that yeast overexpressing genes showed enhanced GST activity and higher accumulation of Cr(VI) in the vacuoles. As a continuation of this study, Srivastava et al. (2019) transformed *OsGSTU30* in *Arabidopsis thaliana* and showed the dual action of the gene in conferring resistance against Cr toxicity and drought stress. Overexpressed plants showed higher enzymatic activity of GST and glutathione peroxidase (GPX). Symptoms of Cr toxicity reappear on treating the plants with buthionine sulfoximine, which acts as a potential inhibitor of GST, and shows that GST is conserved in rice for detoxification of Cr. These studies show the major role of *OsGSTU30* in protection against Cr stress and it has further implications in development of rice crop resistance to Cr stress.

3.2 Cadmium (Cd)

Rice growing in Cd-contaminated soil accumulates Cd in the grains, which can be hazardous when consumed (Aziz et al. 2015). Cd is absorbed by the roots from the soil as Cd^{2+}; from there, it is transported to the stele and finally accumulated in the grains through the stem (Song et al. 2013; Miyadate et al. 2011). Cd toxicity decreases the growth and biomass of rice plants. Wang et al. (2014) reported that

Cd stress decreases photosynthetic parameters such as chlorophyll content and gas exchange parameters such as transpiration rate, water exchange efficiency, and stomatal conduction. Excess Cd content of the soil also interferes with the uptake of essential metals from the soil, thus lowering the nutritional content of the plants. Cd interacts synergistically with Cu, Zn, and Fe for its translocation from root to shoot in rice plants (Liu et al. 2003a, b).

To survive in Cd stress, rice plants possess several mechanisms at the biophysical and molecular levels. Excess Cd can be chelated by several non-protein thiol compounds or can be retained by roots leading to lower Cd toxicity in shoot (Zhang et al. 2013). Several antioxidative enzymes increase due to Cd toxicity and then decrease indicating that plants could tolerate Cd toxicity upto a certain concentration (Roychoudhury et al. 2012b; Shah and Nahakpam 2012). Again, several transgenic approaches have been undertaken to increase tolerance level of rice plants against Cd stress. Harada et al. (2001) had shown that transgenic tobacco plants expressing cysteine synthase (CS) from rice are tolerant to the toxic level of Cd stress. They showed that overexpression of *CS1* gene from rice in tobacco plants leads to the accumulation of cysteine and its derivatives, which helps in the detoxification of Cd toxicity. Islam and Khalekuzzaman (2015) transformed rice cultivar (BRRI dhan29) with *yeast cadmium factor 1* (*YCF1*) gene. Transformed plants showed higher resistance to Cd stress than wild type plants. They also showed higher Cd accumulation in plant vacuoles, which ultimately results in lower Cd level in grains, thus decreasing the risk of health hazards. Higher Cd accumulation in vacuoles will also lower the Cd level in soil functioning in the phytoremediation process for decreasing heavy metal toxicity of soil.

3.3 Iron (Fe)

Iron is an essential element required for plants as it acts a co-factor for several enzymes involved in chlorophyll synthesis, mitochondrial respiration, nucleic acid repair and synthesis, photosynthesis, and structure function maintenance of several proteins. Fe deficiency is thus a major concern as compared to its toxicity. Fe deficiency in rice plants may cause interveinal chlorosis in young leaves, which ultimately decreases the chlorophyll content of the leaves, giving a yellowish color to leaves with a green pattern of the veins. Decreased length of root and shoot was also reported along with lower growth, and grain yield (Mori et al. 2016; Curie and Briat 2003; Mengel et al. 2001). Acute Fe deficiency may result in stunted growth and higher cell division in leaves, which ultimately gives a whitish appearance due to the reduced rate of chlorophyll synthesis (Kobayashi et al. 2014; Banakar et al. 2017).

To overcome Fe deficiency, plants take up Fe^{2+} from soil since it is easily available to plants as compared to Fe^{3+}, which remains mostly in the bounded form with other elements. In severe condition, plants have several mechanisms to uptake Fe^{3+} from the soil, such as release of proton from roots in the surrounding environment, which reduces Fe^{3+} to Fe^{2+}, or specific Fe transporter like phytosiderophores are released by the roots that can bind to Fe^{3+} and the whole complex is ultimately transported to plants through the plasma membrane of cells present in roots (Epstein and Bloom 2005).

Several transgenic approaches have been undertaken to overcome Fe deficiency in rice plants. Takahashi et al. (2001) introduced barely genomic DNA having two *naat* genes, which are responsible for the synthesis of enzymes required for phytosiderophores synthesis. Plants having *nicotianamine aminotransferase (naat)* gene showed higher nicotianamine aminotransferase activity, which increases the secretion of phytosiderophores, ultimately chelating Fe^{3+} from the surrounding environment and transporting it to the plants; this results in a higher Fe content of the mutant plant. Similar types of work have also been reported by Suzuki et al. (2008), where they transformed three rice plants with *barley nicotianamine synthase (HvNAS)1* gene, *HvNAS1* along with two *naat* gene, and *Fe-deficiency specific clone no. 3 (IDS3)* genes and tested the transgenic line in calcareous soil. All the three transgenic lines showed higher Fe content than wild type plants. On transferring Fe^{3+}-chelating reductase gene *refre1/372* of yeast in rice plants under the promoter of an Fe transporter *Oryza sativa Fe-regulated transporter (OsIRT) 1*, higher Fe content was noted in grains and symptoms of Fe deficiency were considerable reduced in plants (Ishimaru et al. 2007). Ogo et al. (2006) overexpressed *OsIRO2* under the CaMV35S promoter in rice plants, which showed enhanced tolerance to Fe deficiency. Masuda et al. (2017) developed a double rice mutant plant with *refre1/372* and *OsIRO2* genes. They showed that double-mutant plants were more tolerant and showed fewer symptoms of Fe deficiency than rice plants containing a single-mutant gene. Thus, several transgenic approaches have been undertaken in the past to increase the uptake of Fe in rice plants by enhancing the production of phytosiderophores, which chelate Fe present in the surrounding environment, ultimately making it available for plant uptake. Higher Fe uptake from the soil decreases the symptoms of Fe deficiency and also increases the nutritional value of grains.

3.4 Arsenic (As)

Arsenic, a metalloid and highly toxic element, is ubiquitous in all types of soil (William et al. 2005). As is found in different states in soil, among which the oxidized form arsenate (As^{5+}) is mostly found in aerobic soil, whereas the reduced form arsenite (As^{3+}) is noted in anaerobic soil like paddy fields (Takahashi et al. 2004). Irrigation of rice with As-contaminated water results in higher As accumulation in rice tissues than other crops due to its translocation factor (0.8) (Xu et al. 2008). As per the guidelines of the World Health Organization of July 2004, white rice containing 200 μg/kg and brown rice containing 400 μg/kg of As or below is marked as safe for consumption for humans (Sohn 2014). Arsenic transporter like Low silicon rice (Lsi) 1 and Lsi2 help in the transport of As^{3+} from the surrounding environment in the rice tissues. Accumulated As in the tissues causes deterioration in growth, morphology, and physiology of plants (Abbas et al. 2018). Phytotoxic effect of As in rice includes chlorosis and shrinkage of leaves, necrosis of leaf tips, reduced root length, brown spots, and scorching of leaves (Bakhat et al. 2017).

Researchers and breeders have developed several strategies to ameliorate the toxic effect of As in rice. Shri et al. (2014) transformed the *phytochelatin synthase* gene, *CdPCS1*, in rice from *Ceratophyllum demersum* as a means of detoxification mechanism against As accumulation. The transgenic plants overexpressing *CdPCS1* showed higher As content in roots and shoots, but the seeds were relatively free from As accumulation compared to the wild type. Overexpression of *PCS1* in transgenic plants enhanced the synthesis of phytochelatin, which sequesters As in the roots and shoots of the plants, ultimately reducing its accumulation in grains. Deng et al. (2018) tried a similar approach for minimizing the As content of grains. They overexpressed the genes *ScYCF1* (*Saccharomyces cerevisiae yeast cadmium factor1*) and *OsABCC1* (*Oryza sativa C-type ATP-binding cassette*) responsible for As sequestration in the vacuole intermodal phloem and root cortical cells under RCc3 promoter. Rice plants overexpressing the two genes showed a significant decrease in As content (around 70%) in brown rice grains without showing any change in agronomic traits of the plants. Both the above approaches mainly focused on reducing the As content of grains by chelating the total As in the roots and shoots. As a result, total As level of the plants was increased; consequently, animals consuming these plants accumulated As in their tissues and ultimately biomagnified As entered the human food chain. In order to decrease the total As content of the plants, Xu et al. (2017) overexpressed *Oryza sativa High As Content (OsHAC) 4* gene, which is responsible for the reduction of As(V) and also efflux of As(III) from the cells. *OsHAC4* codes for As(V) reductase, which is responsible for the reduction of As(V) to As(III), an important step for detoxification of As(V). Rice plants which are *Oshac4* mutant showed no significant change in the reduction of As(V), whereas the efflux of As(III) was significantly reduced, thus proving the direct involvement of *OsHAC4* in As detoxification. A wide range of approaches have being undertaken to reduce the As content of the plants and especially grains without jeopardizing the agronomic traits (Banerjee and Roychoudhury 2018).

3.5 Mercury (Hg)

After As, Hg is the most toxic and abundant chemical found in the environment. Hg is present in both organic and inorganic forms. The organic form is the most toxic since it is readily available in soil and can be easily absorbed by plants as compared to the inorganic form, which mostly remains bound with the other compounds present in the soil. Due to their high hydrophobic nature, organic mercury compound moves across the cell membrane and accumulates in cell organelle membranes, which ultimately result in the inhibition of major metabolic activities like photophosphorylation, Hill reaction, electron transport, oxygen evolution, chlorophyll content and fluorescence, and nutritional content and yield of the plants (Patra et al. 2004; Patra and Sharma 2000; Kupper et al. 1996). Several reports have also showed that Hg toxicity leads to oxidative stress (Shiyab et al. 2009) thus enhancing the membrane damage (Moreno-Jimenez et al. 2009).

Rice plants do not possess mechanisms to detoxify or to change Hg in less toxic forms; thus, genetic engineering of the plants is required to make them Hg tolerant. Two important genes involved in the phytoremediation of Hg are *mercuric ion reductase* (*merA*) and *organomercurial lyase* (*merB*) of the bacterial system. *merB* converts organic-Hg compound to Hg^{2+}, which is then reduced to Hg^0 by *merA* and is finally vaporized from the plants. To develop rice plants tolerant to Hg toxicity, Heaton et al. (2003) introduced the *merA* gene in rice by particle gun bombardment. This was the first approach to develop a monocot crop having high tolerance toward Hg. Mutant rice having *merA* gene showed tolerance to higher level of Hg toxicity (250 μM) in comparison to that of wild type, which can resist Hg stress only up to a concentration of 150 μM. Use of *merAB* system in detoxification of rice plants from Hg toxicity is not supported by various groups of researchers, as it involves the release of Hg^0 formed due to the activity of *merA* and *merB* during the conversion of organomercuric compounds into volatile Hg^0 into the atmosphere. Thus, in future, several other initiatives are needed to develop transgenic rice varieties that can sequester the Hg absorbed from the soil in vacuoles of plants or to reduce its absorption from the soil by roots.

4 Conclusion and Future Prospects

Industrial evolution of human leads to the release of more toxic elements into the surrounding and thus polluting them. Heavy metals and metalloids released due to human activities enhance the level of toxic elements in the environment. These elements enter the agricultural field, and they pose serious threat to the growth and yield of the plants. Contamination of paddy field with heavy metals poses serious threat to food security and safety of the environment. Higher translocation factor of paddy enhances the concern of heavy metal pollution, since rice accumulates more toxic elements in their grains compared to other cereals like wheat and barley.

Rice being a staple food crop is widely consumed across the globe. Metal toxicity greatly reduces the growth and yield of plants. Several intrinsic mechanisms like decreasing the absorption and transportation from roots, compartmentalization of metal to vacuoles, and production of compounds that can chelate heavy metals etc. are present in plants, which help them overcome metal stress (Yadav 2010; Hossain et al. 2012). In addition to these intrinsic mechanisms, several other approaches have been adopted by researchers all over the world to overcome the toxic symptoms of metals and metalloids without jeopardizing the agronomic traits. Transgenic approach is a powerful tool to ameliorate the toxic effect of heavy metals and metalloids. In transgenic method, genes that are responsible for providing protection are selected, and then these genes are incorporated in desired cultivars to derive transgenic plants. Rice plants developed by this approach have high resistance against metal toxicity. The presence of several elements in soil positively affects their absorption by the roots, but till date, no work has been carried out to minimize this synergistic uptake of elements from the soil. In near future, further works need to been done that can provide tolerance to the plants against multiple stresses at a time. Again, in case of several elements like As, transgenic approaches have only led to the lower accumulation in grain by enhancing the metal localization in vacuoles or stem, which ultimately enters the human food chain indirectly. Also, for Hg, there is no major mechanism in rice for the conversion of organomercuric compounds to the less toxic Hg^0. Introduction of *merA* gene in rice converts Hg^{2+} to Hg^0, which pollutes the atmosphere. Thus, a proper transgenic approach is required to address these problems.

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Genomics and Genetic Engineering of Rice for Tolerance to Ozone and Anoxia

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Abstract

Rice is one of the dominant staple foods of Southeast Asia; its production has been affected by a number of abiotic and biotic factors. Oxides of nitrogen, carbon monoxide and volatile organic compounds produced from vehicles and industries interact with ultraviolet light and form tropospheric ozone. Excessive ozone in the troposphere and anoxia caused by submergence of plants are two important abiotic stresses causing extensive damage to the rice crop. The physiology and growth of rice is extremely susceptible to ozone stress, which can cause reduction in productivity of the crop. Therefore, it is a call of the time to address these abiotic stresses to safeguard the rice production system. Recent advancements in genomics facilitate the identification of various quantitative trait loci (QTLs) and single nucleotide polymorphisms related with tolerance to both these stressors. It is assumed that the trait is controlled by multiple medium-effect loci rather than by a single large-effect locus. However, the underlying mechanism of stress tolerance is yet to be investigated, or in other words, the target genes that could be engineered in high-yielding varieties are yet to be fished out. The present chapter summarises the available knowledge on molecular physiology of hyper ozone concentration and anoxia tolerance in rice plant.

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Keywords

Rice · Ozone tolerance · Anoxia · Tropospheric ozone · Quantitative trait loci

1 Introduction

Ozone is present in small amounts in the Earth's stratosphere and absorbs ultraviolet radiation. It is used in many industries as an oxidant and disinfecting agent. It has bactericidal effects against a broad array of organisms like gram-positive and -negative bacteria as well as spores and vegetative cells. Ozone is harmful when present in the troposphere and the majority of tropospheric ozone formation occurs when oxides of nitrogen (NOx), carbon monoxide (CO) and volatile organic compounds (VOCs) react in the atmosphere in presence of sunlight. Ozone enters the plant through stomatal opening and causes chlorosis, necrosis and leaf bronzing, which result in low yield from the crop (Felzer et al. 2007). Ozone pollution is one of the most important abiotic factors that affect the rice production system. The photosynthesis process is highly affected by ozone exposure along with decrease in the stomatal conductance and the rate of transpiration (Banerjee and Roychoudhury 2018).

Ozone is quickly degraded to reactive oxygen species (ROS) in the apoplast (Rao and Davis 2001; Baier et al. 2005). These ROS lead to direct necrosis or induce programmed cell death (Kangasjarvi et al. 2005; Frei et al. 2010a). Ozone tolerance can be developed by two ways, with the help of antioxidants or antioxidant enzymes. The response of rice plant to ozone varies from cultivar to cultivar, and the regulation of these traits takes place at gene, transcriptome, proteome and metabolome levels. There are various approaches for the development of ozone-tolerant rice varieties. Rice is also badly affected by environmental stresses such as anoxia and oxygen deficiency. Ethanolic fermentation is the principal pathway of anaerobic carbohydrate catabolism in rice during anoxic condition. Advance functional genomics studies, QTL mapping and genetic engineering approaches can lead to the development of ozone- and anoxia-tolerant rice varieties. The present chapter is an attempt to summarise the molecular mechanisms of ozone and anoxia tolerance and the genetic engineering approach to develop tolerant rice varieties.

2 Ozone Pollution and Mechanism of Hyper Ozone Tolerance

2.1 Ozone and Ozone Pollution

Ozone is the tri-atomic allotrope of oxygen, i.e. three atoms of oxygen (O) form a bent molecule with an O–O–O angle of 116.78°. It is mainly present in the stratosphere of the earth's atmosphere at a range of around 10–50 km from the ground at a concentration of about 8–10 ppm. This region of the earth's atmosphere is known as the ozone layer, and it is the layer that absorbs most of the ultraviolet radiations (wavelength range of 200–315 nm) of the sunlight and protects all life forms from its harmful effects. Due to its highly unstable structure and high oxidisation property, it is used in many industries as an oxidising agent including pharmaceutical and lubricant industries. It reacts with iron and manganese in water and precipitates it, which can be filtered to make water drinkable. It is a highly reactive molecule and has bactericidal property; thus, it is used as a disinfectant.

Despite a wide range of beneficial effects and industrial applications, it has many harmful effects on all life forms including plants, animals and humans. In case of animals, ozone has been found to affect a wide range of metabolic functions, especially lung functions; in all the species including humans, rat, mouse, sheep, pigs, monkeys, and dogs, rapid and shallow breathing develops on exposure to ozone. Ozone exposure also causes inflammation and increases the susceptibility of animals to infections. Apart from this, it has been found to be having DNA mutational effects.

Ozone also causes devastating effects on plants, and it has been claimed that ozone causes more damage to plants than all other air pollutants combined (https://www.ars.usda.gov/southeast-area/raleigh-nc/plant-science-research/docs/climate-changeair-quality-laboratory/ozone-effects-on-plants/). Ozone enters the plants through the stromatal opening and causes several symptoms such as chlorosis, necrosis, flecks, bronzing and reddening, leading to loss of yield.

2.1.1 Sources of Ozone Pollution

Ozone is not directly synthesised or emitted from any natural or anthropogenic activity; rather, it is synthesised as a by-product of the photochemical reaction of many primary pollutants originating from various anthropogenic activities involving oxygen. These primary pollutants include oxides of nitrogen (NOx), methane, carbon monoxide and other VOCs. Fossil fuels are the primary sources of both nitrogen oxides and carbon monoxide. Apart from that, nitrogen oxides are generated from bio-mass burning, lightning, soils and other sources, whereas VOCs are produced from natural vegetation. These gases in the presence of sunlight react with oxygen to produce ozone. Thus, solar radiations play an important role in ozone production and its production is higher in stagnant high pressure systems in summer when the temperature is high (Fig. 1).

Ozone is also transported into a region by local winds and downward from the stratosphere. The different spatial distributions of NOx and VOC production, often result in the largest ozone concentrations downwind of urban centres, rather than in urban areas themselves (Felzer et al. 2007).

2.1.2 Effect of Ozone Pollution on Rice Plants

Rice is the most important staple food worldwide, especially in Asia, and one of the agricultural commodities with the highest production. In the year 2017, about 769 million metric tonne of rice was produced worldwide. China, followed by India, is the top lister among the rice-producing countries. China and India together produced around 382 million tonne of rice, which is approximately 50% of the total

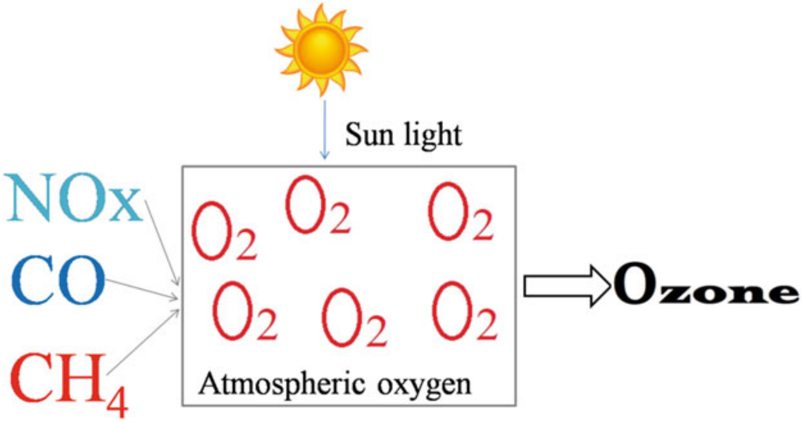

Fig. 1 The process of ozone synthesis in the atmosphere

rice production worldwide. The importance of rice in human nutrition can be understood from the fact that about 20% of the calories consumed by humans worldwide come from rice.

Rice production has grown steadily in the recent years from 538 metric tonne in 1994 to 769 million tonnes in 2017 due to better management practices including water, fertiliser and pest management. However, the rice production system is facing constant challenges, both biotic and abiotic stressors being the foremost challenges. Abiotic stressors include drought, submergence (excessive rain), salinity, and ozone pollution, whereas the abiotic stressors mainly include pests and pathogens.

Ozone pollution is one of the most important abiotic factors that affect the rice production system. It has been found that for an additional day with the ozone concentration of >120 ppb, there could be loss of about 1.12% in rice yield compared to a day with ozone concentration of <60 ppb (https://www.ucdavis.edu/news/surface-ozone-pollution-damages-rice-production-china/).

The physiological effects of ozone pollution on plants include reduced photosynthesis, increased turnover of antioxidant systems, damage to reproductive processes, increased dark respiration and lowered carbon transport to roots (Felzer et al. 2007; Ariyaphanphitak et al. 2005). The physiological aberrations ultimately lead to decline in productivity. Zong-wei have reported 49.1%, 26.1% and 8.2% decrease in grain yield per rice plant when exposed to 200 ppb, 100 ppb and 50 ppb of ozone. Ariyaphanphitak et al. reported a whopping 78% reduction in filled seed per ear in Pathumthani 1 rice cultivar when exposed to 150 ppb of ozone. Similarly, Shi et al. reported yield loss of 17.5% and 15% in SY63 and LYPJ rice varieties of China, respectively, when exposed to 50% more ozone than ambient.

2.1.2.1 Physiological and Molecular Effects on Rice Plants

The effect of high ozone concentration may vary from cultivar to cultivar. Leaf injury leading to severe necrosis and chlorosis is one of the primary visible effects of high ozone exposure. The appearance of brown spots on rice leaves or otherwise called "leaf bronzing" is also observed in ozone-exposed plants. The photosynthesis process is highly affected by ozone exposure as synthesis of many of the proteins related with light harvesting and electron transport systems decline. The stomatal conductance and the rate of transpiration decline, which results in decline in photosynthesis, ultimately leading to chlorosis (Banerjee and Roychoudhury 2018). Along with the photosynthetic activity, there is a decline in grain yield. Even though these two are often simultaneous processes in ozone-exposed rice plants, the degree of visible leaf injury does not correlate well with loss in grain yield (Tsukahara et al. 2015; Sawada and Kohno 2009).

Ozone enters through the stomatal opening and it is quickly degraded to ROS such as the superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) in the apoplast (Rao and Davis 2001; Baier et al. 2005). These ROS can have dual effects; it may lead to direct necrotic tissue damage or may induce programmed cell death (PCD) (Kangasjarvi et al. 2005; Frei et al. 2010a).

On a biochemical level, ozone induces the synthesis of salicylic acid, jasmonic acid, abscisic acid and ethylene, which play important roles in plant defence mechanism including cell death (Rao and Davis 2001). Apart from these, the synthesis of other antioxidative enzymes like superoxide dismutase (SOD), catalases and ascorbate peroxidase have also been found to be increasing (Banerjee and Roychoudhury 2018). These biochemicals act on various interdependent or independent and sometimes antagonistic pathways related to the plant defence mechanism. For instance, salicylic acid has an inhibitory effect on enzymes such as catalase and ascorbate peroxidise. Catalase and ascorbate peroxidase are enzymes that are involved in the metabolism of H_2O_2; when there is increased synthesis of SA, it inhibits the synthesis of catalase and ascorbate peroxidise, leading to cell death of leaves (Rao and Davis 2001). Similarly, jasmonic acid and ethylene act synergistically and may antagonise or synergise the effects of salicylic acid depending upon the stimulus and rice variety. Ozone exposure has been found to induce the biosynthesis of ethylene, which is known to induce organ senescence.

Abscisic acid (ABA) is another plant hormone that has been found to have protective effects against ozone. Lin et al. (2001) have showed that treatment of rice plants with ABA significantly reduced H_2O_2 content in leaves of ozone-exposed plants. It also effectively reduced stomatal conductance and the degree of injury. They have suggested that ABA induced tolerance to ozone could be associated more with stomatal movement than on the modulation of antioxidant enzymes.

2.1.2.2 Effect of Ozone on Rice Yield

Ozone when present in the troposphere is called as bad ozone. This ozone is produced from methane, VOCs and nitric oxides (Ainsworth et al. 2012). Troposphere ozone significantly affects the growth of rice; the number of main stem leaves decrease and there is marked decrease in the plant height of rice. Significant reduction in the percentage of filled spikelet and individual grain mass in rice is due to bad ozone (Shi et al. 2009). Due to the increased concentration of ozone, carbon dioxide assimilation at the leaf level decreases, which significantly affects the yield of crop (Fiscus et al. 2005). High level of ozone leads to stomata closing and affect the yield of rice (Imai and Kobori 2008). Grain yield is reduced significantly due to the exposure of rice to ozone (Pang et al. 2009). Exposure of plant to high concentration of ozone causes oxidative damage that leads to tissue damage, which can be subsequently detected from the damaged leaves (Baier et al. 2005; Fiscus et al. 2005; Rao and Davis 2001).

High concentration of ozone affects the quality of rice grain and straw. In case of grain, starch concentration decreases and protein content increases (Frei et al. 2012a, b; Wang et al. 2012; Zheng et al. 2013). Ozone enhances senescence, so nitrogen remobilisation takes place in the grain. High protein content is a good nutritional property but the quantity of rice grain decreases significantly so protein yield also decreases (Frei et al. 2012a, b; Zheng et al. 2013). It also affects the texture of rice as the grain becomes tougher and chewier (Hamaker 1994; Singh et al. 2011). High level of ozone induces grain chalkiness, which leads to change in the visual appearance of rice grain, ultimately affecting consumers' acceptance (Wang et al. 2014a, b). Frei et al. (2012a, b) found that lipid concentration increases in rice grain but the grain size becomes smaller.

Rice straw contains high amount of lignin and phenolics under ozone exposure than normal grown rice. Lignin and phenolics are antinutrients for ruminants. Straw quality is degraded on exposure to ozone, which is not suitable to feed herbivores (Devendra and Sevilla 2002; Frei et al. 2010b, 2011; Frei 2013; Wang and Frei 2011).

Ozone at a molecular level causes toxicity. It produces ROS like hydrogen peroxide, superoxide radicals and hydroxyl radicals. These radicals damage the lipids and proteins of the cell membrane. Membrane function and fluidity changes as a consequence of lipid damage caused due to ozone toxicity (Luwe et al. 1993). ROS also degrades proteins and DNA (Sharma et al. 2012). Ozone along with primary ROS produces secondary ROS, which degrades biomolecules (Choudhury et al. 2013). High ozone concentration induces chlorosis and necrotic lesions at a cellular level (Schlagnhaufer et al. 1995; Rao et al. 2000, 2002).

2.2 Mechanism of Ozone Tolerance

Ozone produces ROS in the apoplast region of plant. The ozone signalling mechanism in plants and its possible pathways are depicted in Fig. 2.

Mechanism of ozone tolerance is associated with the scavenging of ROS generated by oxidative stress. ROS detoxification involves antioxidants and antioxidant enzymes (Mittler 2002; Neill et al. 2002; Dietz 2003). Antioxidants are mainly ascorbic acid, glutathione and polyphenols. Antioxidant enzymes are SOD, catalase and peroxidase (Blokhina et al. 2003). The ascorbate-glutathione cycle works with the help of enzymes that use ascorbic acid and glutathione as substrate. Scavenging of ROS takes place and the reduced state of antioxidants is restored (Noctor and Foyer 1998). Ascorbic acid level and its reduced state are a critical factors for ozone detoxification (Conklin and Barth 2004). Polyamines and putrescine help in the

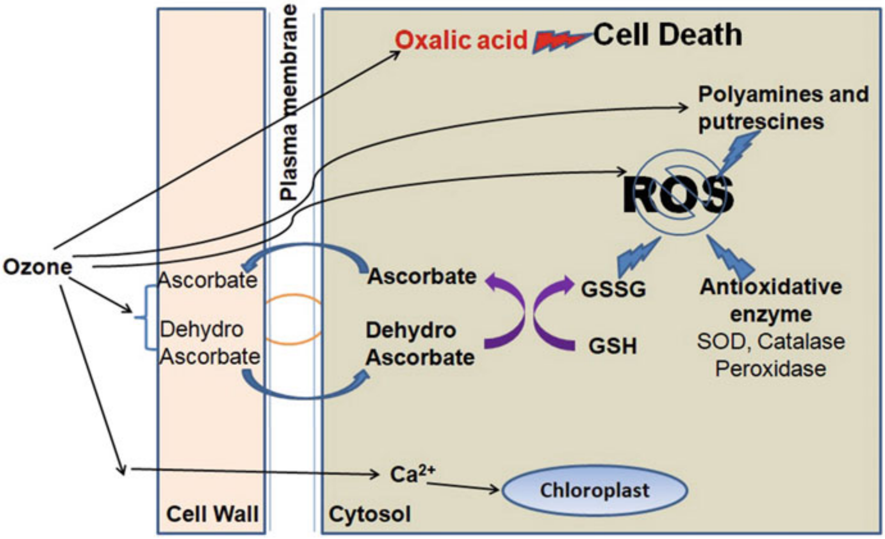

Fig. 2 Ozone signalling mechanism in plants

scavenging of intercellular ROS (Langebartels et al. 1991; Navakoudis et al. 2003; Nali et al. 2006; Bussotti et al. 2007). Similarly, isoprene has significant role in scavenging hydrogen peroxides and revert the effect of lipid peroxidation of membranes (Loreto et al. 2001; Loreto and Velikova 2001). SOD and monodehydroascorbate reductase play important roles in the protection of plants from ozone damage and provide tolerance to ozone (Eltayeb et al. 2007; Van Camp et al. 1994).

2.2.1 Molecular Responses of Rice Plant Against Ozone Toxicity

The response of rice plant to ozone varies from cultivar to cultivar; some of the varieties are tolerant, whereas others have been found to be susceptible. So, it is obvious to expect the regulation of these traits at the gene, transcriptome, proteome and metabolome level. There have been numerous studies on the mechanism of hyper ozone tolerance in plants; these studies are not limited to rice. In fact, only few studies have been carried out in rice. Some of such studies are discussed here.

Cho et al. (2008) have extensively studied the transcriptomic, proteomic and metabolomic alterations in rice plants to survey ozone response in leaves of rice seedling. Microarray-based transcriptomics study has, shown upregulation of 1266 genes and suppression of 267 genes involved in a wide range of pathways including photosynthesis, MAPK cascades, and jasmonic acid, ethylene and tryptophan biosynthesis. These results were also corroborated by proteomic and metabolomic investigations. One of the most highly represented genes were those of the WRKY domain proteins. OsWRKY1, 11, 24, 26, 28, 42, 68, 69, 71, 45, 55, 72, 76 and

77 were among the WRKY genes that were upregulated in one or other time points of ozone exposure. Many of these WRKY proteins have antagonising effects. For instance, OsWRKY72 and -77 activate the abscisic acid-inducible HVA22 promoter, whereas OsWRKY24 and 45 repress it. This study puts light on the molecular mechanism of the ozone-induced enhancement on the abscisic acid synthesis as discussed in the previous section. Further, the findings of the study suggested that ABA signalling is controlled by the competitive mechanism of positive regulators and negative regulators.

A number of previous studies have determined genetic factors associated with ozone tolerance in rice. Rice is among the very few crop plants for which putative QTLs for ozone tolerance have been identified (Frei et al. 2010a). It has been suggested that ozone tolerance is controlled by multiple medium-effect loci rather than by a single large-effect locus (Frei 2013; Ueda et al. 2015). Frei et al. (2008) have identified two QTLs for ozone tolerance through screening 23 rice varieties with a leaf bronzing score (leaf bronzing is one of the symptoms of ozone toxicity). Out of the 23 varieties, the indica variety 'Kasalath' showed no visible symptoms, whereas the japonica variety 'Nipponbare' was found to be moderately susceptible. Subsequent backcrosses identified two QTLs associated with leaf bronzing, i.e. OzT3 and OzT9. These two QTLs have been found to be having contrasting effects; while OzT3, located at 2 cM on chromosome 3, has been found to be enhancing LBS, the OzT9 has been found to be reducing it. Further, they characterised the QTLs through comparative gene expression analysis, which revealed that these genes are involved in PCD and antioxidative ROS generation synthesis (Frei et al. 2008).

Wang et al. (2014a, b) have shown that pyramiding the two QTLs OzT9 and OzT8 provides improved tolerance to ozone exposure. They created lines with tolerance alleles at both loci by crossing two chromosome segment substitution lines containing individual ozone tolerance QTLs *OzT8* and *OzT9*. It was found that the lines containing both the QTLs performed better than the Nipponbare parents SL46 (line with *OzT8*) and SL41 (line with *OzT9*) in terms of leaf bronzing, lipid peroxidation, chlorophyll level and stomatal conductance. These physiological advantages also led to better yield. It was observed that pyramiding of both the QTLs provided better yield as compared to a single QTL (Wang et al. 2014a, b).

An apoplastic protein, namely Ozone-Responsive Apoplastic Protein1 (OsORAP1), has been found to induce cell death in ozone-stressed rice plants. This gene is associated with the QTL *OzT9* and has a sequence similar to ascorbate oxidase. A knockout rice line of OsORAP1 has been found to have enhanced tolerance to ozone stress as evidenced from less formation of visible leaf symptoms (i.e. cell death), less lipid peroxidation and lower NADPH oxidase activity, which indicates reduced production of ROS (Ueda et al. 2015).

2.3 Genetic Engineering for Ozone-Tolerant Rice Variety

Given the emission of greenhouse gases from a wide range of anthropogenic activities, the atmospheric ozone concentration will surely increase in days to come. This would adversely affect all crop plants, especially rice plants, and thus, there is an urgent need to look for mitigation strategies. Even though multiple QTLs for ozone tolerance have been identified, there are speculations regarding their stable expression after the incorporation of these QTLs into high-yielding varieties through conventional breeding. Incorporation of novel genes through genetic engineering is one of the feasible strategies that could be employed to develop ozone-tolerant rice varieties.

Even though the molecular pathways involved in the process of ozone tolerance have not been extensively studied, there are some report on the transcriptomic and proteomic alterations in tolerant rice varieties in response to ozone. These studies have shown that the antioxidative enzyme systems and the pathways involved in ABA, JA, SA and ethylene metabolism play important role in ozone tolerance. Thus, genes involved in these metabolism processes are possible targets that could be engineered and overexpressed for providing ozone tolerance. For example, the wrky genes have been found to play an important role in ozone tolerance. The wrky gene identified in rice, OsWRKY89, known for its ROS scavenging activity, could possibly be overexpressed for ozone tolerance. Similarly, gene encoding enzymes associated with the AsA-GSH cycle like APX, dehydroascorbate reductase, monodehydroascorbate reductase and GR can be overexpressed to promote O_3 tolerance (Frei et al. 2012a, b). Downstream proteins like late embryogenesis abundant proteins can also be expressed to confer O_3 tolerance (Banerjee and Roychoudhury 2018).

3 Anoxia and Mechanism of Anoxia Tolerance

Plants usually face anoxia as an ecological challenge during their life cycle due to certain natural conditions like amalgamation of freezing and flooding stresses including spring floods and ice encasement. Aquatic plants have developed many adaptive metabolic structures to combat hypoxia or anoxia (Blokhina et al. 2003). Energy crisis is the major concern during anoxia so anoxia-tolerant plants develop mechanisms to adapt to energy crisis. Plant tissues enhance carbohydrate catabolism during the initial period and downregulate the rate of carbohydrate catabolism with time (Gibbs and Greenway 2003). Energy production is totally through glycolysis concurrent to ethanolic fermentation during the initial 3–4 h of anoxia in rice (Menegus et al. 1991; Ricard et al. 1994). There are reports that supply of high concentrations of exogenous glucose improves anoxia tolerance in rice coleoptile tips, as they do not lose K^+, P and Cl^- even up to 120 h of anoxia, indicating that these tips did not suffer injury. Energy-dependent solute transport also has priority during an energy crisis. During anoxia, the energy distribution in rice coleoptiles favours increase in fresh weight instead of protein synthesis (Alpi and Beevers 1983).

Rice coleoptiles reacted to anoxia considerably at the level of changed metabolite pools. A set of enzymes, i.e. alcohol dehydrogenase (*ADH*), phosphoenolpyruvate carboxykinase (*PCK*), pyruvate decarboxylase (*PDC*) and pyruvate orthophosphate dikinase (*PPDK*), showed increased abundance in anoxic rice. There are reports of accumulation of certain amino acids such as Ser, Gly and Ala in anoxic rice coleoptiles. Many genes have been found to be downregulated under anoxia, especially those genes that code for enzymes requiring oxygen for their activity, indicating the existence of an energy-saving strategy in the regulation of gene expression. Similarly, various genes coding for signal transduction components (Baxter-Burrell et al. 2002), transcription factors (Liu et al. 2005), nitrogen metabolism (Mattana et al. 1994), nonsymbiotic haemoglobin (Dordas et al. 2004), ethylene biosynthesis (Vriezen et al. 1999), and cell wall loosening (Saab and Sachs 1996) were upregulated at low oxygen concentration. Anaerobic proteins like enzymes involved in sugar metabolism, glycolysis and fermentation pathways are overexpressed during anoxia (Huang et al. 2005).

3.1 Genetic Engineering for Anoxia-Tolerant Rice Varieties

Rice (*Oryza sativa* L.) is extremely sensitive to anoxia during germination and early growth of the embryo (Yang et al. 2019). Genetic engineering of novel genes into intolerant rice varieties could successfully resolve the limitations of anaerobic germination. Siangliw et al. (2003) reported that the *Sub1* locus strongly affects the survival of rice flooding tolerance varieties by synthesising ethylene response factors. Incorporation of the *Sub1* locus into the ‘japonica’ (intolerant; Toojinda et al. 2003) significantly increased its flooding tolerance (Fukao et al. 2006).

Very few papers have been published on the genetic engineering of anoxia-tolerant recombinant lines. Quimio et al. (2000) reported overexpression of PDC (*pdc1*) in the rice cultivar ‘Taipei 309’ that improved anoxia tolerance as a result of an increase in alcohol metabolism. Kretzschmar et al. (2015) explained that the expression of trehalose-6-phosphate (T6P) phosphatase gene (*OsTPP7*) increases starch mobilisation and the elongation of coleoptile, as a result of enhanced anoxia tolerance. However, the precise mechanism of anoxia tolerance is still not clear and merits further investigation. QTL mapping and genome-wide association studies were recently used to identify some QTLs associated with anoxia tolerance. Even though numerous tolerance loci have been identified, only one QTL (*qAG-9-2*) has been fine mapped and cloned as *OsTPP*7.

4 Conclusion

The chapter summarises the molecular mechanisms developed by rice to cope with ozone and anoxia. Recent developments in molecular biology and genetics have enhanced our understanding of mechanisms adapted by rice plant to survive under anoxia and ozone stress conditions. Further efforts are needed to develop transgenic rice varieties that can solve the global food demand in the era of climate change.

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Physiological and Genetic Basis of Submergence Tolerance in Rice

Aditya Banerjee and Aryadeep Roychoudhury

Abstract

Submergence or flash flooding is one of the most prevalent types of abiotic stress in the Indian subcontinent and it largely affects fragile ecosystems. The staple food crop rice is greatly affected by such submergence stress, especially during its reproductive stage. This leads to uncontrolled production loss and crop death due to cellular necrosis under prolonged anoxia. Several physiological, transcriptomic and metabolomic studies have been undertaken to isolate a flood-tolerant genotype among rice cultivars. Upon approval of FR13A as a submergence-tolerant rice cultivar, several mega yielding rice varieties have been introgressed with the submergence-tolerant allele *Sub1*. This has led to the development of high yielding submergence-tolerant rice varieties. The aim of the manuscript is to detail out the requirement of submergence tolerance and also to illustrate its ultimate utilization under real field conditions. Thus, the current chapter states the physiological and genetic basis of submergence tolerance in rice, its mechanistic action and the overall effects on enhancing crop yield.

Keywords

Submergence · Flash flooding · Tolerance · Rice · Yield · Omics · QTLs · *Sub1*

1 Introduction

Rice is the staple food crop of the world, with Asia alone accounting for 90% of worldwide rice production (Banerjee et al. 2019a, b). India is the second largest producer of rice grains just behind China. The reports of the Central Rice Research Institute (CRRI), Cuttack, India, show that by 2030, India will be the most populous

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country in the world and it shall require a massive production of 120 million tons of rice grains to feed its one and a half billion plus population (CRRI Vision Document 2011). This would only be possible when extensive lands and water resources can be seamlessly devoted towards agricultural pursuits with no hindrance from abiotic or biotic factors. Such economic miracle was promoted in the 1960s by the Green Revolution in Asia (Zeigler et al. 2006).

Submergence is considered as the third-most widespread abiotic constraint for rice production among the 42 types of stresses (Sarkar and Mohanty 2010). The extent of flood-affected areas has increased by more than two folds from 5% (19 million hectares) to 12% (40 million hectares) of Indian topology in the last five decades (World Bank 2008). Flash floods resulting in submergence most readily affect rain-fed lowlands which act as fragile ecosystems. Submergence stress leads to anoxia or loss of oxygen for the plant species, resulting in impaired respiration and physiological metabolism. Rice seedlings can withstand anaerobiosis (Banerjee and Roychoudhury 2019; Banerjee et al. 2018). However, excessive or prolonged flooding leads to partial or complete submergence of the seedlings. The quality of water responsible for submergence also determines the actual effects on rice seedlings, and hence, the effects of flooding or the ability of rice to tolerate submergence at one site cannot be extrapolated as a universal truth for all other sites (Setter et al. 1998). Less crop damage is incurred by flooding due to rainwater (clear water) compared to that by silted or turbid water (Sarkar et al. 2014). The quality of water actually affects the intensity of light reaching the crops and stimulates shading responses. Turbid water ensures that a small fraction of solar radiation reaches the crop canopy and hence negatively affects photosynthesis (Sarkar et al. 2006). Due to the production of O_2 and utilization of dissolved CO_2 during photosynthesis, the pH of water is raised (Das et al. 2009). Such increase in alkalinity is detrimental for overall rice seedling growth and grain production. Submergence due to flash flooding thus leads to large-scale loss in rice grain yield and is a problem encountered annually by farmers. The current chapter effectively discusses the detrimental consequences of submergence stress in rice cultivation and also briefly describes the advancements in the generation of flood-tolerant rice genotypes.

2 Biophysical, Anatomical and Morphological Alterations Occurring During Submergence Stress

Temperature plays an important role in determining rice survival during submergence stress. Das et al. (2009) reported that rice seedlings coped better with submergence stress during winter compared with summer. Compared with winter, the average temperature is almost 4.8 °C higher in rice fields during summer. At such elevated temperatures, even intact rice plants experience O_2 shortage in the roots, thereby stimulating ethanolic fermentation (Sarkar et al. 2014). This promotes higher mortality in rice seedlings subjected to submergence at higher temperatures.

Higher accumulation of O_2 during flash floods reportedly increases the mortality rate of rice seedlings. The O_2 concentration in the flooding water steadily increased beyond 0.125 mol m^{-3}, which is considered as the threshold limit to support respiration in germinating seeds, coleoptiles and embryos of rice (Sarkar et al. 2014). O_2 concentration beyond the threshold limit gradually imposes oxidative stress in rice tissues by accelerating the production of reactive oxygen species (ROS) responsible for membrane lipid peroxidation, protein misfolding and chromatin damage (Banerjee and Roychoudhury 2018a). Sarkar et al. (2014) performed field-based study on the effects of CO_2 and pH on rice plants subjected to submergence stress. It was observed that when the pH was 5.0 along with high CO_2 bioavailability, 100% and 62% survival rates were recorded for the tolerant rice cultivar 'Flood Resistant 13A' ('FR13A') and the susceptible rice cultivar 'IR-42', respectively, under submergence stress. However, after prolonged submergence when the pH increased to 8.0 along with reduced CO_2 availability, the 'IR-42' survival rate dropped to about 17%. However, the tolerant 'FR13A' exhibited 100% survival under these conditions as well (Sarkar et al. 2014). Such tremendous effect of pH on CO_2 and O_2 bioavailability largely limits rice agriculture during submergence stress. This phenomenon has been recorded in low-lying rice fields of Bihar, which are usually flooded with alkaline water with a pH above 8.0 (Ramakrishnayya et al. 1999).

Due to the presence of aerenchyma tissues, rice seedlings are better adapted to water logging. Das et al. (2005) showed faster formation of aerenchyma in the roots of the tolerant cultivar 'FR13A' subjected to short-term flooding compared with that in 'IR-42'. Compared with the flood-tolerant cultivars, submergence triggers several morphological modifications in the flood-susceptible cultivars like elongation of leaf sheath and lamina along with reduction in the leaf mass and area. Sarkar et al. (2014) concluded that rice cultivars exhibiting least divergence in morphology during submergence stress are more likely to be tolerant to flash flooding.

3 Physiological Plasticity Required to Tolerate Submergence

Rice cultivars belonging to the lowland and deep water ecosystems exhibit significant photosensitivity. These cultivars require optimum maintenance of the photoperiod during flowering, and submergence at this stage even for a few days leads to the formation of sterile spikelets with empty grains (IRRI n.d.). Survival rate of rice seedlings subjected to submergence stress was positively correlated with the endogenous carbohydrate content (Das et al. 2005). It was found that the ability of the cultivar to maintain high energy reserves (carbohydrates) throughout the period of submergence dictated its post-flooding survival. Das et al. (2009) reported that due to higher retention and efficient maintenance of non-structural carbohydrates, the rice cultivar 'Gangasiuli' showed higher survival rate than the rice variety 'Raghukunwar'. A correlation between nitrogen content and submergence tolerance has been investigated. Sarkar et al. (2014) reviewed that application of nitrogen to flood-susceptible 'IR-42' substantially increased their survival even under submergence. Generation of robust seedlings capable of tolerating submergence has been proposed by nursery management. This includes sowing of good quality seeds on a seedbed supplemented with 15, 40 and 20 kg ha^{-1} nitrogen, phosphorus and potassium respectively (Sarkar et al. 2014).

Prolonged submergence negatively regulates cellular metabolism and inhibits antioxidant synthesis. Thus, during re-oxygenation in the post-submergence period, cells experience excessive oxidative stress (Banerjee and Roychoudhury 2018b, c). Rice plants activate anaerobic fermentation by stimulating the activity of alcohol dehydrogenase, which involves the formation of H_2O_2. Das et al. (2004) verified that rice cultivars accumulating higher H_2O_2 better tolerates submergence stress in spite of the fact that H_2O_2 is a toxic ROS. Activity of catalase (CAT) and superoxide dismutase (SOD) decreased in susceptible and tolerant rice cultivars exposed to submergence. The tolerant cultivars were found to be efficiently operating the ascorbate-glutathione (AsA-GSH) cycle chiefly responsible for ROS scavenging (Panda and Sarkar 2013). Pucciariello and Perata (2017) highlighted that ROS and gasotransmitter nitric oxide (NO) act together in regulating submergence stress tolerance in plants. Huang et al. (2019) reported that pre-treatment of rice seeds with the ethylene precursor 1-aminocyclopropane-1-carboxylic acid triggered ROS generation, which activated the antioxidant machinery. This phenomenon enabled the pre-treated seeds to germinate and tolerate complete submergence stress (Huang et al. 2019). Xuan and Khang (2018) showed that exogenous application of protocatechuic acid and vanillic acid improved rice tolerance against submergence stress by increasing the endogenous phenolic content and triggering the activity of multiple antioxidant enzymes.

4 Omics Approach to Decipher Submergence Tolerance in Rice

Submergence 1A (*Sub1A*) is the genetic loci identified for conferring tolerance to submergence stress. The protein SUB1A encoded by the gene is an ethylene-responsive transcription factor (TF) (Locke et al. 2018). Transcriptomic and metabolomic analyses of the *Sub1A*-overexpressing lines (subjected to sublethal submergence) revealed constraints in the remodeling of cellular and metabolic activity linked to growth and development. It was observed that SUB1A influenced the expression of about 1400 genes and also the content of amino acids, glucose and sucrose during the post-stress period of recovery (Locke et al. 2018). The metabolic homeostasis was recovered during the post-stress period. Thus, convergent signaling pathways were identified to be regulated by SUB1A in mediating submergence stress tolerance in rice (Locke et al. 2018). Similarly, Pena-Castro et al. (2011) reported abscisic acid (ABA) hypersensitivity and gibberellic acid (GA) insensitivity in the *Sub1A*-overexpressing *Arabidopsis* lines. The plants also exhibited delayed flowering, which was found to be a component of quiescence survival strategy in rice seedlings exposed to submergence (Pena-Castro et al. 2011). Deep transcriptome sequencing of wild halophyte rice *Porteresia coarctata* led to the identification of

15,158 genes associated with submergence and/or salinity (Garg et al. 2014). Annotation of the differentially expressed genes (DEGs) showed that several of them belonged to stress-responsive TF families like MYB, bHLH, AP2-EREBP, WRKY, bZIP and NAC (Garg et al. 2014).

Xiang et al. (2017) analyzed the effects of GA and its biosynthetic inhibitor paclobutrazol (PB) in submergence stressed rice seedlings by using RNA-Seq technology. Analysis of the DEGs indicated that application of PB promoted submergence tolerance in rice seedlings by enhancing the photosynthetic capacity and nutrient metabolism. Compared with the control and GA-treated seedlings, the PB-treated plants retained higher endogenous chlorophyll content and activity of alcohol dehydrogenase during submergence (Xiang et al. 2017). Hussain et al. (2016) performed transcriptome analyses of 4-day-old selenium (Se)-, salicylic acid (SA)- and non-primed rice seedlings subjected to submergence stress. Overrepresentation of genes regulating secondary metabolism, development, transport and protein synthesis was observed in the primed seedlings. Induction of genes involved in carbohydrate metabolism, metabolic biosynthesis, nitrogen metabolism and oxidative stress regulation was also reported in the sets primed with Se and/or SA (Hussain et al. 2016).

5 Role of *Sub1* Locus in Regulating Submergence Tolerance in Rice

Submergence tolerance in rice is controlled by the *Sub1* locus, which in turn mediates ethylene- and GA-dependent responses within the system. This leads to reduced utilization of carbohydrates and restriction in shoot elongation during submergence (Xiong and Li 2010). The *Sub1* gene was taken from the highly submergence-tolerant indica rice cultivar ‘FR13A’ for breeding programs. It was observed that the ‘FR13A’, ‘Khoda’, ‘CR Dhan 300’, ‘Savitri Sub1’, ‘IR-64 Sub1’, ‘IC-568009’ and ‘IC-568842’ genotypes were tolerant to submergence (Pradhan et al. 2015). In order to generate rice varieties tolerant to both submergence and drought stress, and that can be easily cultivated in the fragile ecosystems of India, seven quantitative trait loci (QTLs) for yield under drought stress, viz., qDTY1.1, qDTY2.1, qDTY2.2, qDTY3.1, qDTY3.2, qDTY9.1 and qDTY12.1 have been introgressed into submergence-tolerant rice mega-varieties like Swarna-Sub1, Samba Mahsuri-Sub1 and IR-64 Sub1 (Singh et al. 2016). Afrin et al. (2018) reported the use of *Sub1* as a simple sequence repeat marker for identifying submergence-tolerant genotypes of rice. Niroula et al. (2012) screened 109 *Oryza* genotypes for identification of flood tolerance. The flood-tolerant accessions identified from *O. nivara*, *O. rufipogon*, *O. rhizomatis* and *O. eichingeri* interestingly did not contain SUB1A. Interestingly, these genotypes possessed a SUB1 orthologue that was highly similar to the SUB1C of *O. sativa* (Niroula et al. 2012). The results also verified that SUB1A-1 was not quintessential in the wild rice *O. rhizomatis* to promote submergence tolerance under flash flood situations (Niroula et al. 2012).

Sharma et al. (2018) reported that the DEGs observed in case of IR-64 and IR-64 Sub1 lines mostly encoded TFs like MYB, NAC, TOFY and Zn-fingers. These TF-encoding genes were mainly downregulated upon submergence stress. The DEGs were also found to be associated with GA, ABA and jasmonic acid (JA)-responsive signaling pathways (Sharma et al. 2018). Sandhu et al. (2019) showed the utility of pyramiding yield-associated QTLs along with *Sub1* to promote normal reproduction under submergence as well as drought stress. Singh and Sinha (2016) reported that SUB1A activated the mitogen activated protein kinase 3 (MAPK3) during submergence. In turn, MAPK3 physically interacted with SUB1A and phosphorylated the latter. It was also found that the tolerant allele SUB1A1 associated with the promoter of *MAPK3* and controlled its expression via a positive regulatory loop during submergence stress, thus illustrating the mechanistic regulation of submergence stress signaling in rice (Singh and Sinha 2016).

6 Conclusion

Submergence or flash flooding is one of the most prevalent types of abiotic stress responsible for large-scale rice yield loss in the Indian subcontinent. Rice can usually resist anoxic conditions; however, it is susceptible to prolonged floods. The quality of water responsible for flash flooding along with the pH and gaseous availability in water also determines the ability of the crop to tolerate submergence stress. Exhaustive screening studies have identified the Indian landrace ‘FR13A’ as a submergence-tolerant genotype. Transcriptomic and metabolic studies have shown the *Sub1* locus to be responsible for generating flood tolerance. Thus, several breeding approaches have been undertaken to introgress the *Sub1* allele within the susceptible rice genotypes in order to generate tolerant lines, which can then be used in field trials. Several rice mega-varieties like ‘Swarna-Sub1’ and ‘IR-64 Sub1’ Sub1 have been designed with the *Sub1* allele incorporated in their respective genomes. The mechanism of SUB1 to operate submergence stress tolerance in rice occurs via multiple phytohormone-dependent signaling pathways and also by MAPK3.

7 Future Perspectives

The field of research on generating submergence tolerance in rice is gradually expanding due to its potential impacts. Future perspectives include further screening of wild rice varieties that are tolerant to submergence and identification of novel QTLs responsible for prolonged flood tolerance. Global warming has led to the occurrence of drought, salinity and flooding at the same place under different climatic influence. In order to combat such situations, breeders and genetic engineers should aim at designing high yielding rice cultivars that are tolerant to multiple abiotic stresses. This can be achieved by QTL pyramiding. Exhaustive metabolomic and proteomic studies should be performed to understand the negative impacts of submergence stress in rice cellular metabolism. The aspect of epigenetics during submergence stress in rice is almost unknown. Thus, future studies might also include the reporting of epigenomic data generated from rice seedlings subjected to short- and long-term submergence stress.

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Genetic Engineering to Enhance Rice Survival in Nutrient-Deficient Soil

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Abstract

Nutrient deficiency occurs in plants either by the inadequacy of nutrients in the soil or by their unavailability in plant-absorbable forms. Nutrient deficiency is a very old issue caused by increased food demand by humans, which has resulted in the development of varieties with enhanced nutrient-uptake. Inadequacy of nutrients in the soil is a major threat to food crops affecting their yield. After the Green Revolution, extensive agriculture, which was praised as a boon for humans, has become the multifaceted anathema in the form of exhaustion of natural soil minerals, expensive farm inputs, and destruction of the environment. Nutrient depletion in the soil directly causes enormous stress to the plant and hinders the normal physiological processes, endangering survival. Rice, which is a staple food of more than half of the population of the world, is especially affected negatively by all these above problems, particularly by nutrient deficiency in soil, which may worsen in the near future. The only workable way out to minimize the after-effects of high inputs of chemical fertilizer on plant and environment is to reduce their use, which decreases farm production. Therefore, reduced nutrient input should be practiced with such varieties that can better

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perform under a low supply of nutrients. Rice gene pool is rich with variabilities for nutrient response including low nutrient tolerance, which can be used to develop low nutrient-tolerant varieties. In the recent past, various experiments to enhance the expression of genes related to metal chelators and transporters phytase and iron storage proteins have been carried out successfully. Many genes have been found to successfully induce nutrient deficiency tolerance in rice (*Oryza Sativa* L.). Here, we will review the deficiency of major nutrients in rice soils, all possible mechanisms to combat their deficiency and the progress made in developing nutrient starvation-tolerant rice crops using genetic engineering techniques.

Keywords

Rice · Nutrient-deficient soils · Genetic engineering · Low nutrient stress · Breeding

1 Introduction

Rice (*Oryza sativa* L.) is a staple cereal crop and requires a significant input of important mineral nutrients for its cultivation to feed the increasing world population. It is cultivated in >150 million ha of land globally, which is one-third of the area used in cereal cultivation. This area is mostly confined to the tropics where water availability and climate have a great influence on soil nutrients and their availability to plants (Fageria et al. 2011a, b; Semenchuk et al. 2015; Hou et al. 2018; Srivastava et al. 2018). Various systems are used to cultivate rice, and 55% of harvested area globally is under wetland rice with 75% of rice production in the world. While upland and lowland (wetland) rice together occupy 80% of the global area with 90% production worldwide, large area of this land is deficient in one or more than one essential nutrients (Haefele et al. 2014). Lands deficient in both macronutrients [nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca)] and micronutrients [zinc (Zn), copper (Cu), boron (B), and molybdenum (Mo)] resulted in reduced growth and yield of rice under particular soils and environments (Ismail et al. 2007; Sarker et al. 2018; Husnain et al. 2019). Globally, N, P, and Zn are among the major nutrient deficiencies that constrain growth and yield of rice (Dobermann and Fairhurst 2000; Ismail et al. 2007; Haefele et al. 2014). With the advent of the Green Revolution, these nutrient deficiencies have been fulfilled by the application of chemical fertilizers along with the introduction of high-yielding modern varieties (HYMVs) that are far better in their response to chemical fertilizers compared with the traditional varieties (Taylaran et al. 2016; Naher et al. 2019). The introduction of HYMVs enhanced fertilizer input and upgraded the irrigation facilities; this was collectively labeled as “Green Revolution package,” which has produced enough food for the population growth and was thus responsible in avoiding large-scale food shortage (Otsuka and Kalirajan 2006; Pingali 2012). Moreover, modern varieties have shorter duration due to high cropping intensity of irrigated rice and produce 10^{-15} t year^{-1} ha^{-1} grain in comparison to other grain crops (Dobermann and Fairhurst 2000). Meanwhile, high cropping intensity depletes nutrient reserves of the soil faster, which results in the requirement of more fertilizers for nutrient input; however, tremendous use of fertilizers may deteriorate sustainable agriculture goals (Sithole et al. 2019; Yadav and Sarkar 2019).

Increase in rice production is today's need because of the demand of more grain by the increasing world population. With increasing nutrient demand, the use of fertilizers has become unaffordable for poor rice farmers. These marginal farmers dependent on subsistence farming cannot buy these expensive fertilizers. In 1960, rice semi-dwarf varieties were introduced, taking rice production to a new astonishing level by adopting best management strategies including enough fertilizer use. As breeders were thoughtful to introduce high-yielding varieties, they selected such varieties that responded to high fertilization. For continuous cropping, these varieties require high fertilization as they take significant amount of nutrients from soil for biomass and grain production (Taylaran et al. 2016; Naher et al. 2019). In developing countries, farmers lack knowledge of balanced use of fertilizer or have financial issues, which force them to indiscriminately use fertilizers, ultimately resulting in nutrient starvation.

Nutrient starvation is a serious problem requiring agronomists' and breeder's attention for sustainable rice production even in the future. Presently, a rigorous worldwide research is focused on improving nutrient use efficiency to at least partly reduce the economic and environment prices of fertilization. Several studies have reported that nutrient uptake by plant roots, transport in the xylem, and translocation in tissues of plants are regulated genetically (Orsel et al. 2002; Hammond et al. 2004; Ning et al. 2015). Hence, conventional breeding techniques as well as genetic engineering approaches can be applied to modify the plants genetically to enhance nutrient uptake, translocation, redistribution, and assimilation of mineral nutrients. Specifically, genetic engineering is a powerful tool that can be exploited in numerous ways, such as overexpressing or silencing of genes to change amount and/or activity of particular proteins, introgression of novel genes, manipulation of regulatory pathways used in nutrient uptake, and assimilation and disruption of inhibitors of nutrient absorption/assimilation (Johnson et al. 2011; Abhishek et al. 2015). Therefore, in this chapter, we have attempted to summarize the advances made through genetic engineering in rice plants to improve nutrient uptake, translocation, redistribution, and assimilation as a sustainable way to enhance the quality and productivity of rice under nutrient-deficient soils.

2 Sustaining Rice Mineral Nutrition

Sixteen nutrients including primary, secondary, and micronutrients are required for healthy rice growth and production. Their main source is soil, except carbon (C), oxygen (O), and hydrogen (H), which are non-mineral major nutrients. N, P, and K are the major nutrients whose requirements are high, and their source is fertilizers, while sulfur (S), magnesium (Mg), and Ca are secondary nutrients required in smaller quantities, and supplementary fertilizers are a source of their soil supply. Moreover, not all other micronutrients such as chlorine (Cl), Mo, iron (Fe), Zn, B, manganese (Mn), and Cu are supplied through any fertilizer because their requirement is very little, and it is considered that enough is present in soil. Although these nutrients are required in a very small amount, their deficiencies are reported in many areas. Rice requires all nutrients for its metabolic reactions and functions in order to complete life cycle. Although quantity requirement varies, all the nutrients are essentially required for the rice plant to carry out various metabolic functions to complete its life cycle (Dobermann and Fairhurst 2000). If any nutrient is limited, deficiency symptoms appear as the result of metabolic impairments. Moreover, mineral deficiency makes plants sensitive and vulnerable to various abiotic and biotic attacks, which lead to yield loss, morbidity, and mortality (Dordas 2008; Banerjee and Roychoudhury 2018; Yasin et al. 2018; Awan et al. 2019; Ahanger and Ahmad 2019). In grain crops, nutrient cycling is partially non-cyclic because with every harvest, a significant nutrient quantum is removed without their return to the soil. Grains, which are a major part of the food chain, take maximum portion of this nutrient loss, especially through wastes of both animal and humans and at the end become a part of sewage (Cordell et al. 2009; Drangert et al. 2018; Gülser et al. 2019). Although the Green Revolution had multiple benefits including poverty alleviation and increasing food production, input nutrient demand was more and its counter effect was loss of nutrients through harvested grains and soil drainage.

Significant quantities of N and P followed by S, Mg, P, and Ca are removed from soil through rice crop. Dobermann and Fairhurst (2000) reported nutrient loss through grains after harvesting. They recorded harvested grain nutrient removal as 67% P, 15% K, and 60% N. Earlier, rice straw retained balance because its significant quantity was recycled through soil incorporation and cattle feeding in soil, but now straw is disposed by burning in open fields, so nutrient recycling is decreasing (Jain et al. 2014). Moreover, there are other methods of straw disposal including their use in paper industries (El-Kassasa and Mourad 2013; Nassar et al. 2015; Jani et al. 2016) and for biofuel generation for pyrolysis, gasification, and aerobic digestion (Silalertruksa and Gheewala 2013; Nygaard et al. 2016). All these practices are responsible for depleting the soil nutrient reserves and deteriorating soil health.

Nutritional sustenance regarding rice production is indispensable, and several methods, especially those related to genetic interventions and agro-management, are used to achieve this goal. Sole dependence on excessive use of fertilizers is not a good remedy as these deplete soil organic and inorganic resources (Magdoff 2013; Sithole et al. 2019; Yadav and Sarkar 2019). Nutrient drainage must be reduced, and unavailable nutrients should be solubilized to make them available to plants to prolong soil nutrient status. Many researchers explained composting as one of the best strategies to reduce nutrient loss in rice after harvesting (Saha et al. 2012; Gosal et al. 2018). In order to reduce nutrient losses through grains and making lost nutrients a part of the nutrient cycle again, sewer solids are being recycled for struvite production, which can possibly be an alternative to P and N fertilizers (Liu et al. 2011; Morales et al. 2013; Kacprzak et al. 2017).

In intensely cultivated areas in Asia, organic wastes such as animal waste as well as crop residues having no use in manufacturing and for fuel are recycled (Smil 2000). These management practices have no significant effect on nutrient input reduction. Genetic engineering of rice is an emerging and effective strategy to tackle the problem of high nutrient input demand. By introducing genes into crops, several parameters that are dependent on nutrient use can be improved such as crop growth under low fertilization. This can be obtained by improvement in nutrient foraging, microbial biomass, nutrient mobilization, solubilization ability, and yield production efficiency. Even such varieties can be developed that will prevent nutrient loss by exporting less of them to grains. Hence, nutrient loss by unsolicited means can be prevented (Rose et al. 2013b; Morales et al. 2013; Fang et al. 2018; Latha et al. 2019; Lau and Latif 2019).

3 Deficiency of Major Nutrients

Ammonium and nitrate, which are predominant forms of soil N, are water soluble and highly mobile forms of N in rice-grown areas (Li et al. 2014), which may cause losses due to leaching. In addition, denitrification processes may cause N loss as N oxides or atmospheric N are volatile forms (Galloway et al. 2003; Ward et al. 2009; Xia et al. 2017). Wetland rice occupies >55% of the total land area under rice cultivation, and this wetland rice prefers to take N in the form of ammonium because ammonium assimilates easily in amino acids with less energy requirement than nitrate. Although ammonia losses are more when soil conditions are alkaline, in rice soils under flooded conditions when soil is slightly to moderately acidic, ammonia volatilization occurs. In flooded water, CO_2 is utilized by aquatic biota and algae, which results in increasing water pH and consequently ammonia volatilization, causing substantial N loss (Gill et al. 1998; Nurulhuda et al. 2018). Urea fertilizers do not decompose easily. They dissociate into NH^{4+} ions after catalytic hydrolysis while ammonia fertilizers easily decompose to NH^{4+}. There is a loss of ammonium ions in the interaction with water molecules, so they are converted to ammonia (non-ionized) rapidly, which escapes as gas (Choudhary and Kennedy 2005). Numerous factors affect ammonia volatilization such as floodwater temperature, crop growth, floodwater pH, soil properties, algal aquatic growth, and weed growth (Choudhary and Kennedy 2005; Pelster et al. 2019; Van Andel et al. 2017). According to Xing and Zhu (2000) under flooded conditions, applied N losses through ammonia volatilization are almost 60%. Coarse textured acidic soils having low organic matter (<0.5%) and calcareous and alkaline soils with low organic carbon can easily become N-deficient. While soils having less N supply naturally such as saline soils, P-deficient and acidic sulfate soils, and wetland soils with poor drainage where biological N fixation and N mineralization is lacking, are always N-deficient (Zhang et al. 2016; Barker and Bryson 2016).

P is a macronutrient essential for the development of plant growth. Although agricultural soils have high P-content, only 0.1% is available to plants (Richardson and Simpson 2011; Dey et al. 2017). So, the application of P fertilizers is of utmost importance to maintain soil P-balance, but they degrade the environment and are costly. Additionally, 75–90% of these fertilizers complex with Fe^{3+} and Al^{3+} when soil conditions are acidic or calcareous with Ca^{2+} and gets immobilized (Islam and Hossain 2012), resulting in available P-shortage and affecting plant nutrition (Merbach et al. 2010).

HPO_4^{2-} and $H_2PO_4^{-}$ are two plant-available forms of P (Raghothama and Karthikeyan 2005). These are assimilated in plants as phosphoric-anhydride bonds containing adenosine tri- and di-phosphates and Pi monoesters. However, period of P availability in soil is very short due to its fixation with polyvalent cations such as Al^{3+}, Fe^{3+}, and Ca^{2+}, which makes them insoluble. As P deficiency is a prevailing problem in rice-cultivated areas (Fageria 2013), this problem is severe for lowland soils because of high P fixation capacity. P-supplementation is required in soils having low supplying capacity for P; otherwise, deficiency occurs (Leinweber et al. 2018).

In upland soils, erosion losses are the reason for P deficiency. Soils with P deficiency include coarse textured sandy soils with less organic matter as well as minimal reserves of P; sodic and calcareous soils; peat soils; lowland degraded soils; volcanic soils; and clayey, acid upland and highly weathered soils having high capacity for P fixation, such as oxisols and ultisols, and acidic soils with active Fe and Al, which results in insoluble complex formation. At low soil pH, P deficiency is usually due to Fe toxicity and at high soil pH, it is associated with Fe and Zn deficiency and salinity (Dobermann and Fairhurst 2000; Devau et al. 2011). N fertilization and application of excess amount of liming along with less P-application also result in P immobilization.

K plays a crucial role as an essential nutrient in numerous physiological and biochemical processes affecting plant growth (Amrutha et al. 2007; Yang et al. 2009; Jaiswal et al. 2017). According to Rengel and Damon (2008), K availability to plants is limited due to low mobility, fixation with soil particles, and strong adsorption (such as in China, 3/4 of the paddy soil and in Australia, 2/3 of the wheat belt). Therefore, there is a need to increase K availability to plants to improve agricultural production.

K limitation in rice-grown ecosystem is rare (Gierth and Maser 2007) and its deficiency is only due to K-fixation in soil and ultimately K-unavailability to rice crop. Jia et al. (2008) reported that K deficiency is increasing in rice-grown soils around the world. Sandy soils, upland acid soils, organic well-drained soils, and lowland degraded clayey soils become K-deficient because of poor reserves of K. So, K-fertilization becomes the need of the hour. Clayey soils also become K-inhibited due to the presence of 2:1 type of clay minerals, which fix K. Similarly, (Ca + Mg)/K wide ratio soils have most K adsorbed on their cation exchange sites, so its release to the soil solution is little. Acidic soils having high leaching capacity may also be K-deficient (Yadav and Sidhu 2016).

4 Deficiency of Secondary Nutrients/Micronutrients

Deficiency of micro and secondary nutrients is not considered a serious problem regarding rice soils in the world. But in some regions, there may be deficiency of these nutrients. In Bangladesh, Indonesia, and India, sulfur deficiency has been reported (Papademetriou 2000; Oo et al. 2007; Singh et al. 2012) and S-fertilizers are added to address that problem. In lowland irrigated soils, sulfur deficiency may not be a serious problem but well-drained coarse sandy soils, low organic matter containing soils, Fe-oxide-rich weathered soils, and Al silicate clay rich soils may suffer from S-deficiency (Dobermann and Fairhurst 2000).

Zn is an important essential micronutrient for submerged rice. Application of Zn fertilizers together with NPK is necessary to increase grain yield in rice because its deficiency is a prevailing disorder in lowland rice (Fageria et al. 2011a, b). According to Wissuwa et al. (2006), after P- and N deficiency, Zn deficiency has been placed as a micronutrient major deficiency while considering rice lowland soils. To alleviate Zn deficiency, either fertilizers are applied or rice crop is engineered genetically to improve Zn uptake under Zn-limited conditions (Rose et al. 2013a). Bicarbonate-rich calcareous and neutral soils are deficient in Zn and S, which ultimately affects rice at different phenological stages. Zn deficiency is due to complex formation with organic or inorganic ions or due to its precipitation as zinc sulfide or zinc hydroxide, which are sparingly soluble (Singh et al. 2012). Other soils that are mostly Zn-deficient are sandy soils, peat soils, acidic soils, sodic, and saline soils. While talking about crops, Zn deficiency has been observed in lowland rice (Rattan et al. 2009; Fageria et al. 2011a, b; Kandali et al. 2015).

All other micronutrients including Mn, Mg, Cu, and Ca may be deficient in weathered and highly leached acidic soils or coarse highly drained sandy soils on upland or lowland. Excessive drainage due to leaching or crop removal through intense cultivation limits the availability of micronutrients in soil and is the main reason for micronutrient deficiency. Fe toxicity disrupts rice crop physiology. Fe deficiency is seen in aerobic, high pH, and upland soils, while its toxicity affects lowland rice. Fe concentration in plant is dependent on plant's nutritional status. In soil, Fe contents range from 1 to 20% with an average of 3.2%, while in plants, normal Fe concentration is 0.005%. Fe plays a key role in chlorophyll formation. Its deficiency causes interveinal chlorosis in leaves, and these symptoms first appear on young leaves (Zu et al. 2012).

Mg deficiency is due to Mg absorption, which decreases Mg uptake. Excessive fertilization causes Ca imbalance with all other nutrients, affecting its uptake, and hence, Ca-deficiency occurs, while Mn-deficiency is due to ammonium present in soil solution. Cu-deficiency is due to adsorption or competition with another nutrient such as Zn. NPK fertilization removes soil Cu by increasing vegetative growth.

5 Strategies to Combat Nutrient Deficiency Stress

Less fertilizer application increases nutrient stress problem. According to previous studies, plants adopt different strategies to cope with nutrient deficiency. According to Lee (1993), P, S, and N uptake in barley is dependent on plant nutrient pool and their efflux does not have significant effect on nutrient uptake regulation. In *Arabidopsis*, Mg, K, P, and N deficiencies have different effect on sugar and carbohydrate translocation (Hermans et al. 2006). Similarly, N- and P-deficiencies in soil caused buildup of starch and soluble sugars in rice leaves, and deficiency of either K and/or Mg caused accumulation of the other cations in rice shoots (Cai et al. 2012).

When N is deficient, root biomass increases, which results in increased root surface area and increased carbohydrate accumulation in shoots as compared to other plant parts when plenty of nutrients are available. P deficiency also affects plants the same way as N deficiency, especially in *Arabidopsis*. However, K deficiency does not bring specific structural changes as there is no increase in plant root biomass and less carbohydrates accumulate in shoots than in other plant parts under P and N deficiency (Hermans et al. 2006). Under nutrient deficiency, although plants make different physiological changes, Hermans et al. (2006) performed microarray analysis, which showed that under nutrient-deficient conditions, downregulation and overexpression of primary metabolic genes occur. Especially, expression of sucrose synthesis and photosynthesis-related genes changes under P-deficient conditions in plants. Sucrose plays a vital role in controlling gene expression. Usually, it acts as signaling molecule and affects plant development. Meanwhile, for long distant transport, sucrose is the main carbohydrate in rice (Li et al. 2003; Gibson 2005; Cai et al. 2012).

5.1 Need to Develop Nutrient Starvation Tolerance

Nutrient deficiency prevails because of continuous practices of crop removal in modern agriculture. Fertilizer application is standard practice to ameliorate soil nutrients. Increasing world population has placed high pressure on fertilizer use and fertilizer-responsive-cultivars because of increased demand for food. Meanwhile, availability of fertilizers is decreasing due to finite resources of fertilizer production. Therefore, cost of fertilizers is increasing, which results in unaffordability for poor rice farmers to buy these fertilizers. Apart from that, indiscriminate fertilizer use has environmental hazardous effects (Vinod and Heuer 2012; Sithole et al. 2019; Yadav and Sarkar 2019). According to all mentioned issues, usually three factors are responsible for reducing fertilizer input in agricultural systems. These include

- Depletion of fertilizer natural reserves.
- Low fertilizer use efficiency (FUE).
- Environmental degradation.

Although crops themselves adapt to stressed conditions and try to combat as much as they can to develop nutrient deficiency tolerance, breeders are also trying their best. Regarding major and some essential nutrients, there are a number of possible ways to tackle this stressed condition as follows:

5.1.1 N Deficiency Tolerance

N use efficiency (NUE) of rice is 30–50% as loss of applied N from soil is 50–70% (People et al. 1995). This loss occurs through leaching, volatilization, or any other means. If NUE is increased, N loss from soils as well as its input requirement will reduce. When N is deficient, genotypic responses in rice take place including root modifications such as change in root surface area, volume, density, root-shoot ratio, and root distribution (Fan et al. 2010; Ogawa et al. 2014), which accelerates N uptake and internal recycling (Tabuchi et al. 2007; Sales et al. 2011). Basically, both internal and external signaling are involved in N uptake and before panicle initiation, internal buildup of N reservoir is necessary (Vinod and Heuer 2012). When enough N accumulates for internal homeostasis, N assimilation is the next step for optimal yield and growth (Witcombe et al. 2008; Xu et al. 2012).

In rice, N metabolism depends on several genes, such as secondary and primary N assimilating genes, ammonium and nitrate transporters, transporters of amino acids, and urea and transcription factors (Nishcal et al. 2012; Lea and Miflin 2011). Mostly, these genes are self-regulatory, which means there must be some genes that are expressed under N deficiency and trigger different mechanisms as intensive and extensive foraging (Pathak et al. 2008). This may involve those genes that provide an early signal to the N deficiency in the seedling stage itself, accelerating mechanisms for the development of extended and deeper root system while provisioning enough energy to support the signaling and early development mechanisms. This early seedling development is a crucial factor in identifying low N-efficient genotypes. Apart from that, photosynthetic efficiency along with photosynthate translocation are other traits of interest under N-deficient conditions, which maintain canopy longevity and delaying senescence of leaf (Vinod and Heuer 2012). Such genotypes are selected by providing reduced N conditions. Identification of specific genes that help crop to survive by uptake and assimilation of N (Li et al. 2009a; Goel and Singh 2015) and QTLs that increase NUE (Vinod and Heuer 2012) represent new hope to breeders for successful QTL introgression such as *saltol* for salt tolerance and *Sub1* for submergence tolerance (Septiningsih et al. 2009).

5.1.2 P Deficiency Tolerance

Modern rice genotypes typically have soil P recovery efficiency less than 20% (Fageria 2013) much less than other major nutrients, N and K. Internal use efficiency for P is totally in contrast to it and higher than that for N and K. Rice grains have higher P-content than straw, resulting in high (almost 72%) P harvest index of rice. This data indicates that to improve overall P use efficiency of rice, it is necessary to improve P uptake rather than internal P utilization. The paradox of high Pi mobility within the rice plant system while growing in a pool of immobile soil P warrants the need for the solubilization of soil P before being mobilized into plants.

Rice elicits various genotypic changes including physiological, metabolic and morphological adaptations under P-deficient conditions. This response is known as Pi starvation response (Plaxton and Tran 2011). P starvation response causes various adaptive changes, such as root system changes and increased production of root exudates to gain access to P (Heuer et al. 2013). According to recent reports, QTL *Pup 1* (*Phosphorous uptake 1*) functionality to tolerate P deficiency suggests that *PSTOL1* improves root growth to tolerate P starvation (Gamuyao et al. 2012; Chin et al. 2011; Heuer et al. 2009). Rice roots discharge various compounds including membrane proteins, organic acids, ribonucleases (RNases) and acid phosphatases under P-deficient conditions. Soil P solubilization is enhanced by organic acids present in root exudates, such as malates, oxalates and citrates (Rose et al. 2011; Hocking 2001). These low-molecular-weight organic acids (LMOAs) lower activities of P-fixing cations, including Al^{3+}, Ca^{2+} and Fe^{3+} because of their higher chelation capacity (Meena et al. 2018).

Activities of proton pumps and H^+-ATPase are enhanced under P-deficient conditions, contributing to rice root rhizospheric acidification (Zhang et al. 2011a). Rhizosphere acidification occurs by the proton release from roots, as a primary process of H^+-ATPase activity in the plasmalemma of root cells. The mechanism of H^+ release balances excess intake of cations over anions by acting as a primary proton pump, thereby creating pH and electric potential differences across the plasmalemma. Activity of H^+-ATPase enhances the electrochemical gradient of protons, which improves P nutrition by ion transport across membrane. Another adaptation to P starvation may be LMOA efflux coupled with H^+-ATPase activity to improve soil P mobilization. In rice, P deficiency also results in acid phosphatase root secretion (Li et al. 2009b). Acid phosphatases mobilize and release soil P from its organic complexes through hydrolysis.

Rice increases acid phosphatases as well as intercellular phosphatase secretion under P-deficient conditions to mobilize soil P (Tian et al. 2012). RNases are also produced significantly along with acid phosphatases in rice under P starvation (Plaxton and Tran 2011). RNase secretion decays organic matter and degrades nucleic acids to mobilize soil P and to increase P acquisition (Fang et al. 2009). Arbuscular mycorrhizal association (AM), basically a symbiotic association, is another mechanism of P acquisition (Vallino et al. 2009). In rice, anaerobic conditions prevail while arbuscular mycorrhizae are usually aerobic symbionts. However, according to various evidences, these associations may exist in rice to contribute to P uptake under submerged soil conditions (Hajiboland et al. 2009). According to Ipsilantis and Sylvia (2007), in wetlands, this association is probably maintained by supplying oxygen through the aerenchyma.

P transporter gene family, which falls under high and low-affinity transport systems, regulates P uptake. Some P transporters have a complex role to uptake P because of their involvement in arbuscular mycorrhizae symbiosis under P starvation (Heuer et al. 2013; Vinod and Heuer 2012). Moreover, several genes including those coding for protein and DNA degradation enzymes, genes responsive to stress as metallo-thioneins and peroxidases, MYB transcription factors and glycolytic enzymes get unregulated when P is deficient in the roots of rice crop (Li et al. 2009b).

Table 1 Phosphate deficiency-inducible genes

MSU-Locus	Gene symbol	Objective	References
LOC_Os10g25310	OsSPX3	Phosphorous starvation response	Wang et al. (2009a, b)
LOC_Os06g40120	SPK1 (SYG1)	Pi-dependent inhibitor of phosphorous starvation response regulator 2 (PHR2)	Wang et al. (2014)
LOC_Os02g10780	SPK2 (SYG2)	Pi-dependent inhibitor of phosphorous starvation response regulator 2 (PHR)	Wang et al. (2014)

Gho et al. (2018) also reported genes that are activated under P-deficient conditions and increase rice P use efficiency. Although 820 genes were recognized, performing multiple functions but these were performing against phosphorous starvation (Table 1).

5.1.3 K Deficiency Tolerance

K is an abundant cation and constitutes 10% dry weight of plants (Very and Sentenac 2003). K use efficiency is higher than N and P use efficiency because K is not lost due to soil fixation and volatilization. K is immobile in soils and recycled during crop rotations. Recovery of K from soil ranges at 20–60% (Roberts 2008). Besides, in Asian rice systems, K depletion is higher (Dobermann and Cassman 2002), which is threatening to rice ecosystems. K input will reduce in future due to breeding of K-efficient varieties. Although in rice significant genotypic variations have been recorded to make K use efficient (Yang et al. 2003, 2004), insufficient information is available to assess the breeding potential of rice varieties having low K tolerance. Rice absorbs K as monovalent cation K^+ and drives by biphasic system of transport at plasmalemma. K^+ and micronutrient diffusion across membranes (lipid bilayer) require specific transporter proteins (Eide 2005). Under K-deficient conditions, ATPases and K^+/Na^+ symporters/uniporters of gene family HKT regulate K^+/Na^+ uptake depending upon ionic conditions of the external environment (Hauser and Horie 2010) and play an important role in plant K^+ loading (Szczerbab et al. 2009). The presence of Ca^{2+} ions activates K^+ channels and increases its uptake (Lebaudy et al. 2007).

Rice crop is dominant under saline tropic conditions so induced K deficiency is important to study under these conditions (Vinod et al. 2013). Na^+ ions interfere and compete with K^+ under saline soil conditions, which results in K deficiency and Na toxicity in plants. K^+ transporters are salinity sensitive (Fuchs et al. 2005) but are not NH^{4+} sensitive (Szczerba et al. 2008). K^+ uptake increases in presence of Ca^{2+}in soil because under saline conditions, K^+ channels are regulated by Ca^{2+} but K^+ channels gets inactivated in the presence of Na^+ ions. In rice crops, several other genetic factors are regulated under K-deficient conditions such as G proteins (guanine nucleotide-binding protein) (Urano et al. 2013), aquaporins (Maurel et al. 2008), signals of ion-sensing (Szczerbab et al. 2009), tonoplast proteins (Banuelos et al. 2002) and ion channels (vacuolar) (Isayenkov et al. 2011).

5.1.4 Zn Deficiency Tolerance

Zn uptake as Zn^{2+} ions gets affected due to its precipitation with sulfides in wetland soil conditions (Hafeez et al. 2013). Zn precipitates in the presence of Fe and Mn oxides, forming franklinite ($ZnFe_2O_4$) or sesquioxides under submerged soil conditions (Alvarez 2010). Many reviews are taking it as a topic of discussion (Arnold et al. 2010; Rose et al. 2013b; Yu et al. 2012; Bashir et al. 2012; Impa et al. 2013). Different mechanisms are involved in Zn uptake such as root sensing of Zn sufficiency that controls ligand molecules including phyto-siderophores (PS) root excretion, translocation in shoots and re-translocation in grains and metal-binding transporters expression.

Several genes control Zn uptake, accumulation, translocation and remobilization. Many belong to large families of genes such as Fe-regulated transporters (IRT), Zn-regulated transporters (ZRT) and Nicotine-amine synthases (NAS). Besides gene involvement in PS synthesis (Arnold et al. 2010), transporters of PS and metal ion-PS complex (Nozoye et al. 2011) are also involved in Zn uptake (Murata et al. 2006). Two ZIP genes including *OsZIP3* and *OsZIP1* control Zn uptake (Ramesh et al. 2003) and *OsZIP4, OsZIP8* and *Oszip5* control translocation in shoots (Lee et al. 2010a, b).

PS are non-protein, low-molecular-weight amino acids that form complexes with Mn, Cu and Zn micronutrients and Fe^{3+} cations, which are soluble and aid in cation mobilization in plants. Phyto-siderophores mugineic acid family along with nicotinamine is involved in Fe^{3+} transport in plants (Bashir et al. 2012). Nicotianamine (NA) is Fe^{2+} and Zn^{2+} chelator and is biosynthesized through NA synthase from S-adenosyl methionine (Nozoye 2018). All plants produce NA while graminaceous plants produce mugineic acid (MA). Deoxy-mugineic acid (DMA) is one of the early species of MA produced in graminaceous plants, which is synthesized from NA by NA aminotransferase (NAAT) and DMA synthase (DMAS) in a conserved pathway (Bashir et al. 2012). Numerous modeling studies implicate DMA in Zn uptake in rice crops along with its mobilization (Ptashnyk et al. 2011; Arnold et al. 2010). According to latest evidences, Zn-NA predominates in the sap of phloem (Nishiyama et al. 2012), which indicates that Zn-NA complex is preferable for Zn transport than the Zn-DMA complex in rice. However, for Zn-DMA and Zn-NA complexes, effective transporter needs to be identified in rice.

5.1.5 Sulfur Deficiency Tolerance

Sulfur, which is a secondary essential nutrient, is involved in different metabolic processes and is a constituent of biomolecules, specifically of amino acids containing sulfur such as methionine and cysteine (Saito 2000). SO_4^{2-} is the form of sulfur taken by rice and gets converted into organic sulfides (Smith et al. 1995). Many intensely crop cultivated areas have S-deficiency, and S-tolerant genotypes can be an advantage. Proton-SO_4^{2-} symporters mediate S uptake in rice (Takahashi et al. 2000) and SO_4^{2-} is transported through roots by following the symplast pathway (Godwin et al. 2003). After loading in xylem, SO_4^{2-} ions are delivered to the chloroplast where ions are adenylated and finally reduced by an enzyme sulfite reductase firstly to sulfite then sulfide before they get incorporated into biomolecules in plants (Davidian and Kopriva 2010).

Different internal genetic signals regulate S assimilation. Under S-deficient conditions, S uptake in roots is under biphasic control where transporters have the major role (El Kassis et al. 2007). SO_4^{2-} limitation stimuli upregulate these transporters under S-deficient conditions (Yoshimoto et al. 2007). Apart from S-transporters, several genes, micro-RNAs, transcriptional regulators as *S limitation 1 (SLIM1)* and *S-responsive element (SURE)* and post-transcriptional regulators regulate sulfur uptake and ultimately its translocation (Davidian and Kopriva 2010). To develop tolerance to S starvation, S metabolism complicated system works in a coordinated manner to improve plant sulfur use efficiency (Davidian and Kopriva 2010).

5.1.5.1 Genetic Engineering of Rice

Although different strategies including crops genes are involved in combating nutrient-deficient conditions, genetic engineering of crops especially rice is an emerging science to enhance survival under stressed environment. Genetic engineering is also called genetic modification. It is used to manipulate genome through biotechnology (Kuzma 2016).

Now, genetic modification of crops, especially rice, for nutrient deficiency tolerance is an emerging field of interest.

5.2 Genetic Engineering of Rice and Nutrient Starvation

Rice yield loss is basically due to abiotic stresses including drought, salinity and nutrient deficiency in soil. Multiple genes that can induce stress tolerance have been recognized. Expression of *CBF3/DREB1A* induces stress tolerance by accumulation of stress-responsive proteins. Latha et al. (2019) reported *AtDREB1A* gene that induces stress tolerance in rice. To develop nutrient use efficiency, genetic manipulation in rice is limited to experimental validation of candidate genes. Several reports are available on genes having varying influence on parameters of nutrient use in all crops including rice (Ramaekers et al. 2010; Rose et al. 2011, 2013a, b; Vinod and Heuer 2012;Veneklaas et al. 2012; McAllister et al. 2012; Zhang et al. 2014).

5.2.1 Engineering to Tolerate N Starvation

While talking about N, Yang et al. (2015) tried to find a gene that is activated in N starvation, and he reported 1650 genes in rice crop expressed after 12 h of N starvation. The response was confirmed by RT-PCR and GUS assays. To develop plants having higher NUE, transgenic rice with reduced contents of Rubisco was developed by transforming Rubisco with small subunit anti-sense gene *OsRbcS* under *OsRbcS* promoter control using anti-sense technology, which resulted in N-use-efficient rice under high irradiance and saturated CO_2 conditions (Makino 2011). While tiller numbers were reduced due to *OsrbcS1* overexpression and regarding photosynthesis, there was no significant change in multiple transgenic lines (Morita et al. 2014).

Table 2 Genes that improve nitrogen use efficiency (NUE) in rice

Genes	Crop	Strategies used	Effects	References
OsNPF8.20	Rice	Ubi1 promoter	Increase in NH^{4+} uptake as well as grain yield	Fang et al. (2013)
OsNPF7.3	Rice	Ubi promoter	Increase in growth at N supply different ranges	Fan et al. (2014)
OsNPF6.5	Rice	CaMV 35S/native promoter	Increase in NUE and grain yield	Hu et al. (2015)
OsNRT2.3b	Rice	CaMV 35S/Ubi promoter	Increase in NUE, growth and yield	Fan et al. (2016)
OsAMT1.1	Rice	Ubi1 promoter	Increase in NH^{4+} uptake and grain yield	Ranathunge et al. (2014)
OsAlaAT	Rice	*OsANT1* (root-specific promoter)	Increase in grain yield, tiller number and plant nitrogen	Beatty et al. (2009)
OsRDD1	Rice	Maize Ubi1 promoter	Increase in nutrient uptake and N responsiveness	Iwamoto and Tagiri (2016)

Upon transgenic rice screening containing glutamine synthetase cytosolic genes of rice (*OsGS1:1* and *OsGS1:2*) along with *E. coli(glnA)*, an increase in GS activities of leaf and amino acids and soluble concentration of protein and N contents of plant were recorded. However, a significant increase in yield was not recorded. Even the concentration of amino acids in grains was lower in these transgenic lines (Cai et al. 2009). Transgenic rice line (*OsGS1:2OX*), driven by ubiquitin promoter, was showing great utilization efficiency with better spikelet fertility, grain number and harvest index than wild types. While transgenics showed no significant advantages under N-limited conditions than N sufficiency (Brauer et al. 2011) (Table 2).

When rice was grown under optimal and suboptimal N conditions, *OsAMT1:1* (an NH^{4+} transporter gene) was overexpressed to indicate higher permeability for NH^{4+} and higher NH^{4+} accumulation in transgenic rice, which results in increasing N assimilation, chlorophyll, sugars, starch contents and grain yield (Ranathunge et al. 2014). A rice transgenic line with higher grain yield and biomass was produced by Shrawat et al. (2008) upon introducing barley alanine aminotransferase (*AlaAT*) cDNA, which was driven by a specific promoter of rice tissue (*OsAnt1*). These transgenic lines had specific changes in N contents and key metabolites, indicating an increase in NUE. Upon further testing at various N levels, Beatty et al. (2013) recorded an improvement in NUE at high and medium N supply.

Transgenic plants containing nodulin gene *OsENOD93-1* showed positive effects for NUE, which resulted in increasing seed yield and short dry mass. Under N-deficient conditions, an increase in amino acid concentration in xylem sap was also recorded (Bi et al. 2009). Guevara et al. (2014) recorded 23% increase in yield and 16% in biomass in *OsENOD93-1OX* transgenic rice driven by ubiquitin promoter, in comparison to wild plants when grown under N starvation. During seedling development, arginine plays an important role in N transport and its storage. By introducing an enzyme of arginine hydrolysis (*OsARG*) involves in arginine catabolism, an increase in grain number in *OsARGOX* line was recorded by Ma et al. (2013)

under N-deficient conditions with strong expression of *OsARG* in panicles. Under N starvation, high-affinity transporter of urea *OsDUR3* was upregulated in roots of rice crop, which resulted in improved growth under control (low urea) conditions and increasing urea uptake in roots (Wang et al. 2012).

Introduction of Ca^{2+}-dependent protein kinase *OsCDPK12* transgenic resulted in improving plant N contents along with dry weight under N-deficient conditions (Asano et al. 2010). An improvement in growth, increase in C and N accumulation accompanied by enhanced N distribution to roots, shoot-root ratio modification and a significant increase in root biomass were recorded for rice transgenic lines having *ZmDof1* (maize transcription factor) under N-deficient conditions (Kurai et al. 2011). In *Arabidopsis*, *OsDof25* (rice transcription factor) introduction resulted in increasing expression of low- and high-affinity NH^{4+} transporters *(AtAMT2.1* and *AtAMT1.1*) and repressing high-affinity NO^{3-} transporters (*AtNRT2-1*). This resulted in an increase in enzyme level and amino acid content, suggesting that *OsDof25* plays a crucial role in metabolism of organic acid and uptake of NH^{4+} in plants (Santos et al. 2012).

5.2.2 Engineering to Tolerate P Starvation

Rice ecosystems mostly suffer from P deficiency. *Pup1* QTL was analyzed by transgenic expression and cloning of kinase gene of serine threonine protein *OsPSTOLI,* driven by a promoter 35S, to check P deficiency tolerance. An enhancement in root growth at an early stage of development in rice was recorded, which resulted in increased surface area of root, consequently enabling plants to explore larger soil area to take more nutrients, specifically P (Gamuyao et al. 2012).

To check P deficiency tolerance, various transcription factors have been implicated. Enhancement in P starvation tolerance was observed due to transgenic OX of transcription factor of rice (*OsPTF1*), which may be involved in P deficiency response in primary roots, lateral roots and leaf phloem cells in rice lines. A concomitant enhancement in rice proton-translocating pyrophosphatase (H^+-PPase) expression was recorded upon microarray data of *OsPTFOX* transgenic line (Yi et al. 2005). These H^+-PPases respond to all abiotic stresses including drought and salinity and are conserved sequences of plant genomes (Gaxiola et al. 2001). They are also responsive to P deficiency (Yi et al. 2005). Under P starvation, rice lines that were overexpressed *Arabidopsis* H^+-pyrophosphatase gene *(AtAVP1)*, H^+-PPase *type1*, showed sustained growth of shoots while control exhibited poor growth in comparison (Yang et al. 2007). Increase in rhizosphere acidification and root hair density was observed, and robust root systems were developed in these rice lines than in control under both P-deficient and P-sufficient conditions (Gaxiola et al. 2011).

AtPHR1 (Arabidopsis phosphate starvation response regulator 1) is an MYB transcription factor known as *PIBS* (PHR1-binding sequences), which binds to cis-element motif *GnATATnC* and plays a significant role in P starvation signaling (Rubio et al. 2001). Improvement in root architecture and P starvation tolerance were observed in OsMYBP-1OX lines by R2R3 MYB transcription factor *OsMYB2P-1* under P-deficient conditions. There are two *AtPHR1* rice orthologs including *OsPHR1* and *OsPHR2*. Under P-replete conditions, lines with *OsPHR2* were characterized by P toxicity and excess accumulation of P (Wang et al. 2009a; Zhou et al. 2008). Recent advances show that transgenic rice line *OsPHR2OX* responds to P starvation by activating P starvation-induced genes (*PSI*) (Wu et al. 2013). Overexpression of *OsPHR2* and exposure to Pi starvation were also found to upregulate *PAP* (purple acid phosphatase) genes, resulting in increase in activities of acid phosphatase in roots of rice crop (Zhang et al. 2011b). On the other hand, *OsSPX1*, which is an SPX domain gene (SYG/PHO81/XPR1), acts as an *OsPHR2*-negative regulator in rice (Wang et al. 2009a).

Transgene analysis shows that *OsSPX1* decreases excess shoot P accumulation and suppresses *OsPT2* expression by *OsPHR2* (Liu et al. 2010). Auxin-responsive genes are regulated by auxin responsive 19 and 16 factors (*OsARF19* and *OsARF16*), which is another transcription factor group in rice and under P starvation, lateral root growth increases (Wang et al. 2014; Shen et al. 2012). In P uptake in rice crop, leaf tip necrosis (*OsLTN1*) acts as a negative regulator and plays a significant role in P signaling (Hu et al. 2011). Multiple reports are present on micro-RNAs in rice crop, which are associated with P deficiency response (Kuo and Chiou 2011). Under Pi starvation, analysis of upregulated miR827 showed target gene *OsSPX-MFS2* and *OsSPX-MFS1* regulation, which encode SPX-MFS (SPX-major facilitator superfamily) proteins involved in sensing along with transport of P (Lin et al. 2010).

P transporter such as OsPht1:8 effect by OX as well as repression by RNAi was analyzed by Jia et al. (2011). A change in P uptake was recorded along with reduction in size and number of panicles with spikelet sterility of >80%. OsPht1:1-overexpressing rice showed high accumulation of P in leaves when conditions were P-deficient (Sun et al. 2012). In another previous investigation, only few of the transgenic rice plants overexpressing the tobacco transporter NtPT1 were found to outperform the controls, only to yield less than the controls on an average (Park et al. 2010). Expression of a P transporter OsPT11 that is specifically induced during arbuscular mycorrhizal (AM) symbiosis in yeast knocked down mutants for high affinity Pi transporter PhO1 was found to complement the defect in phosphate uptake in the mutants (Paszkowski et al. 2002).

RNAi-mediated slicing of specifically OsMIPS (myo-inositol-3-phosphate synthase gene), which catalyzes the biosynthesis of phytic acid in rice seed, was used to reduce phytate contents in grain to prevent P loss (Feng and Yoshida 2004). Aleurone layer as well as embryo of transgenic plants had reduced contents of phytate, which were driven by *Ole18* (Oleosin18) promoter. Their effects on myo-inositol metabolism in seed were undesirable (Qu and Takaiwa 2004). Ali et al. (2013) silenced inositol 1,3,4,5,6-pentakisphosphate 2-kinase gene (*OsIPK1*), which is involved in the catalysis of the last step of phytic acid biosynthesis, using the techniques of RNAi with no effect on initial steps. Hence, reduced contents of grain phytate and increased inorganic phosphate and Fe were observed in rice lines with silenced *OsIPK1*.

5.2.3 Engineering to Tolerate K Starvation

Some genes such as *OSHAK 16* get activated under K starvation conditions for rice crop survival. Chen et al. (2015) reported that K deficiency caused reduction in root growth, which led to the reduced uptake of K in rice plants (Cai et al. 2012). Emergence and growth of adventitious roots are stimulated by *WOX11*; however, genetically modified rice produced extensive root system but poorly developed shoots. Thus, few genetically modified rice plant expired before the production of seeds (Zhao et al. 2009). To combat the impact of K deficiency on the growth of root system and, thereby, increase the tolerance of rice to K starvation, they manipulated the expression of *WOX11* by modifying the *HAK16* promoter: *WOX11* construct into rice. The *HAK16p: WOX11* resulted in total K uptake by up to 110 and 35% at 0.1 and 1 mM K supply level (Chen et al. 2015). It is strange to note that the expression of *OsHAK5, OsHAK1* and *OsAKT1* was strongly upregulated by the expression of *WOX11*.

When rice crop is genetically engineered with HtNHX2 and grown under low K^+ contents. This potassium deficiency doesn't affect rice crop nutrition and survival. As Zeng et al. (2018) showed that in K-deficient soil, improved straw K^+ contents, enhanced harvest index and up to 30% increase in grain yield were recorded in genetically modified lines expressing HtNHX2. HtNHX1 does not help in rice survival under K starvation. A larger portion of K uptake in *Arabidopsis*, K^+ channel *AKT1* facilitates K^+ uptake over a wide range of external K^+ concentrations (above 10 mM) and is primarily controlled at post-translational level rather than at transcriptional level (Geiger et al. 2009; Wang et al. 2010). The Ca sensors *CBL1/9* regulate *AKT1*, which mediates the activity of the K^+ uptake and their interacting protein kinase *CIPK23* (Xu et al. 2006). Overexpression of *CIPK23, CBL1* or *CBL9* or all improve the tolerance in *Arabidopsis* to survive in K starvation (Xu et al. 2006). The similar regulatory mechanism has been frequently described in several crop species, i.e., in maize (Geiger et al. 2009), barley (Boscari et al. 2009) and particularly rice (Li et al. 2014). Therefore, it shows that regulation of AKT1-like channels through a *CIPK-CBL* complex might be a universal mechanism in various plant species. Therefore, genetic modification of *CBL* and *CIPK* gene expressions in crops may be a prospective approach to enhance the efficiency of K uptake in the crop.

5.2.4 Engineering to Tolerate Iron and Zn Starvation

Iron deficiency is also a serious problem, and for crops like rice, it is difficult to survive under Fe deficiency conditions. Ferritin is a ubiquitous protein for Fe accumulation and accumulates about 4000 Fe atoms in a complex (Theil 2003). Boonyaves et al. (2017) developed transgenic rice plants that expressed the soybean ferritin gene *SoyferH1* in the endosperm using the endosperm-specific 1.3-kb *GluB1* rice promoter; the transformants exhibited higher Fe buildup in brown rice seeds (Boonyaves et al. 2017). A few studies have reported the production of Fe biofortification rice by endosperm-specific expression of ferritin (Lucca et al. 2002; Qu et al. 2005). Similarly, Qu et al. (2005) expressed *SoyferH1* under the control of both the *OsGlb1* promoter and 1.3-kb GluB1 promoter to enhance seed Fe content. The other technique uses the overexpression of NAS genes responsible for increased transportation of Fe within the plant. Nicotinamine (NA) is a chelator of metal cations such as Fe(II) and Zn(II), and it is biosynthesized from S-adenosyl methionine via NA synthase (NAS). Furthermore, another approach involves the increase of Fe content in seeds by increasing the expression of mugineic acid synthase gene in plants. In graminaceous plants, NA is the precursor of mugineic acid family phyto-siderophores (MAs), which are natural Fe(III) chelators used in Fe uptake from the rhizosphere (Masuda et al. 2012). Graminaceous plants use TOM1 transporter to release synthesized MAs into the rhizosphere (Nozoye et al. 2011). They make Fe(III)–MAs complexes, which are absorbed into the root by means of YS1 and YSL transporters (Curie et al. 2001, 2009). Rice biosynthesizes 29-DMA, which eases Fe acquisition and internal transport (Kobayashi and Nishizawa 2008). These engineering techniques can increase Fe uptake even under Fe deficiency conditions and make their survival possible.

Masuda et al. (2012) observed that concentration of bioavailable Fe in rice is increased when it was manipulated with multiple genes, including ferritin, under the control of endosperm-specific promoters, *NAS* overexpression, and *OsSUT1* and *OsGlb1* promoter-driven *OsYSL2* expression and help in combating the deficiency of iron in soil.

Tiong et al. (2015) recorded uptake of Zn and its transportation from root to shoot in barley plants by recording the expression of the ZIP (*ZRT/IRT*-like protein) gene family under Zn-deficient soil. After that, 0.5 μM Zn was resupplied to soil and the transcripts of 13 *HvZIP* genes were quantified by the authors. Under Zn deficiency, uptake as well as root to shoot transportation were increased; this increase continued for several days after that. Six different *HvZIP* genes were observed to be induced in the roots of Zn-deficient plants and their interacting proteins were transported to the plasma membrane of root cells. Further, these ZIP genes can be examined to find how plants react to changing levels of Zn in the soil. The information reviewed above can be used to devise new strategies to improve Zn uptake in cereal crops, as well as to enhance tolerance to Zn deficiency (Tables 3).

Kappara et al. (2018) identified two alleles of the *OsHMA7* transporter gene in rice using QTL and analyzing lines that store high and low levels of Fe and Zn. It was observed that increased expression of the allele 284 in transgenic rice lines enhanced the uptake of Fe and Zn and enhance the tolerance in rice to tolerate Fe and Zn starvation. Five-time increase in *OsHMA7* transcript levels was recorded in the modified lines. These alterations in the transcript levels also changed Fe and Zn homeostasis in the plants by modifying the expression of the Fe-responsive gene. The authors conferred that their identified gene is a novel gene that can transport heavy metals and thus improve grain yield as well as Fe and Zn concentration, leading to an improved line of rice for nutrient-deficient soils.

6 Conclusion

Globally in rice-growing areas, nutrient deficiency occurs in agricultural soils either by nutrient exhaustion due to intensive farming or naturally in nutrient-poor and marginal lands. Nutrient constraint along with the need for enhanced food production imposes a demand for chemical fertilizer application to soil. However, natural reserves of the chemical fertilizer sources are diminishing speedily all around the

Table 3 Different genes involved in mineral biofortification of rice

Gene	Promoter	Cultivar	Fold increase in Fe/Zn	References
AtNAS1+, Pvferritin+, Afphytase	CaMV 35S, Glb-1, Glb-1	*Japonica cv. Taipei 309*	6.3/1.6	Wirth et al. (2009)
HvNAS1	Actin	*Japonica cv. Tsukinohikari*	3.4/2.3	Masuda et al. (2009)
HvNAS1, HVNAS1 + HvNAAT, IDS3	Genomic fragments	*Japonica cv. Tsukinohikari*	1.0/1.0	Masuda et al. (2008)
OsNAS1, OsNAS2, OsNAS3	CaMV 35S	*Japonica cv. Nipponbare*	2.2/1.4	Johnson et al. (2011)
OsNAS2	Activation tagging	*Japonica cv. Dongjin*	3/2.7	Lee et al. (2011)
OsNAS3	Activation tagging	*Japonica cv. Dongjin*	/2.2	Li et al. (2009b)
OsNAS1	GluB1	*Japonica cv. Xiushui 110*	1.0/1.3	Zheng et al. (2010)
PvFerritin + rgMT	Glb-1	*Japonica cv. Taipei 309*	2.0	Lucca et al. (2001)
SoyFer	Glu-B1	*Indica cv. IR68144*	3.7/1.4	Vasconcelos et al. (2003)
SoyFer	Glu-B1; Glb-1	*Japonica cv. Kitaake*	3.0/1.1	Qu et al. (2005)
OsFer2	OsGluA2	*Basmati rice*	2.1/1.4	Paul et al. (2012)
TOM1	CaMV 35S	*Japonica cv. Tsukinohikari*	1.2/1.6	Nozoye et al. (2011)
OsYSL2	OsSUT1	*Japonica cv. Tsukinohikari*	4.4	Ishimaru et al. (2010)

world, increasing the cost and sociopolitical divides. Moreover, areas with continuous rice cultivation get large amounts of fertilizers that result in environmental deterioration. A sustainable single solution that will reduce use of chemical fertilizers is required, and its feasibility requires development of rice varieties, which can be friendly to low nutrient conditions.

Rice gene pool harbors enough variability to nutrient use that has already been successfully exploited in developing high-yielding varieties using conventional approaches. In order to sustain internal homeostasis of nutrients, different factors stimulate responses under nutrient-deficient conditions such as enhanced uptake. Promising genes, usually responsive to nutrient deficiency, are selected from wild varieties, not modern ones. A model approach to develop starvation-tolerant varieties will be merging of older varieties having higher uptake efficiencies with modern varieties having high-yielding ability and internal consumption efficiency. Responses to nutrient starvation are very complex, and the breeding objective itself is a reversal to what has been done towards the development of modern HYVs. These molecular approaches can be suited to develop nutrient starvation response in old genetic backgrounds. In rice crop, introgression of QTLs specifically related to tolerance against abiotic and biotic stress has been successfully established. Moreover, genome that may quicken the development of targeted trait-based crop improvement should be selected. With proper identification functioning of every gene and rice crop morphology, breeders can easily genetically engineer rice crop and make its survival easier in nutrient-deficient soils. So, even under nutrient-deficient conditions, rice crop can provide good yield.

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Genetic Engineering of Rice to Survive in Nutrient-Deficient Soil

Fazal Akbar, Atta Ur Rahman, and Abdul Rehman

Abstract

Rice (*Oryza sativa* L.) is one of the most important crops in the world, providing staple food for approximately half of the world's population. Globally, the widely cultivated varieties of rice are *Oryza glaberrima* (Steudel) and *Oryza sativa* (L.). Asia produces approximately 90% of the total world's rice (nearly 640 million tons) where the major contributors are China, India, and Pakistan. Approximately 3.5 billion people rely on rice for 20% of their daily calorie intake. The major obstacles to wheat and rice cultivations include various physical and chemical parameters including excessive soil tillage; soils with low carbon (C), zinc (Zn), potassium (K); and inefficient use of soil nitrogen (N). Problems such as iron, phosphorus, and zinc deficiency or excess aluminum, iron, and salts in the soil have limited rice land in Asia by about 50 M ha. Modern agricultural systems use various plant varieties developed by the conventional breeding system, which has resulted in a significant increase in crop yields, specifically cereals. Development of effective gene transfer mechanisms in combination with mixed "omics" technologies has promoted knowledge of plant physiology and biochemistry for adaptive responses to adverse environmental conditions by recognizing the main molecular players regulating these responses. This chapter discusses how genetic engineering in rice has made it possible to survive in nutrient-deficient soil, with high yield.

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Keywords

Rice · Nutrient-deficiency · Soil · Breeding · Genetic engineering

1 Introduction

Rice (*Oryza sativa* L.) is one of the most important crops in the world, providing staple food for approximately half of the world's population (FAO 2004). Rice has great economic importance and is a member of the genus *Oryza* (family *Poaceae*), which has 22 recognized species (Bajaj and Mohanty 2005). Globally, the widely cultivated varieties of rice are *Oryza glaberrima* (Steudel) and *Oryza sativa* (L.) (Lu 1999). Asia produces approximately 90% of the total world's rice (nearly 640 million tons) where the major contributors are China, India, and Pakistan. Approximately 3.5 billion people rely on rice for 20% of their daily calorie intake (IRRI 2010).

Many essential rice nutrients come from the soil and crop residue, but high rice yields still require additional nutrients (Buresh 2007). However, inappropriate farming methods, overgrazing, and deforestation cause degradation of soil, such as organic matter loss and depletion of nutrients, chemical contamination, salinization, and acidification, as well as deterioration of physical properties, such as wind and water erosion. About 2000 million hectare (M ha) of global cultivable land is adversely affected due to such practices. Degradation of soil is a serious food security threat and may become a big constraint for future food production (Fahad et al. 2019). Plants suffer from nutrient deficiency stress when the supply of nutrients in soil (and/or the amount of nutrients taken from soil) is lower than needed to support cellular processes at a specific stage of growth. Nutrient insufficiency can result from: (1) inherently low nutrient levels in the soil, (2) low versatility of nutrients in soil, and (3) weak solubility of chemical types of nutrients (Rengel and Marschner 2005).

The major obstacles to wheat and rice cultivations include various physical and chemical parameters including excessive soil tillage; soils with low carbon (C), zinc (Zn), potassium (K); and inefficient use of soil nitrogen (N) (Adnan et al. 2018; Joshi et al. 2007; Chatrath et al. 2007). Such restrictions generally lead to low productivity in wheat and rice cultivation systems (Nagarajan 2005; Ladha et al. 2003, 2007). Rice yield shows a decreasing trend due to the negative balance of nutrient and poor management of soil and crops (Von Uexkull and Beaton 1992).

Problems such as iron, phosphorus, and zinc deficiency or excess aluminum, iron, and salts in the soil have limited rice land in Asia by about 50 M ha (Neue and Lantin 1994). Similarly, due to least solubility of Fe in reduced form, the deficiency of Fe is a serious problem in the production of rice (IRRI 1977), and it may cause up to 65% yield reduction in susceptible rice cultivars (IRRI 1979). Likewise, deficiencies of other nutrients also reduce the production of rice.

Modern agricultural systems use various plant varieties developed by the conventional breeding system, which has resulted in a significant increase in crop yields, specifically cereals (López-Arredondo et al. 2015). Conventional rice breeding is based on the introduction of new desirable traits into breeding lines through intercrossing of related species. Despite significant progress, this strategy is unlikely to yield enough to satisfy the needs of the increasing global population, particularly in developing countries (Conway 1998; FAO 2004). Furthermore, losses caused by insects, weeds, and pathogens can all reduce production, reducing the long-term stability of high-yielding rice production. Conventional rice-breeding approaches therefore need to be augmented which will satisfy the current needs of increasing global populations by new biotechnological tools. Genetic engineering was used to separate desired genes from other species and use them in different varieties of rice to address the inherent limitations of traditional rice-breeding programs. It is possible to identify, alter, and insert useful genes into rice genomes for better expression from bacteria, viruses, fungi, unrelated crops, and others organisms (including human beings), and artificial genes can also be created (Bajaj and Mohanty 2005).

Development of effective gene transfer mechanisms in combination with mixed "omics" technologies have promoted knowledge of plant physiology and biochemistry for adaptive responses to adverse environmental conditions by recognizing the main molecular players regulating these responses. Rice is the first plant to have sequenced genome (Sasaki et al. 2002; Delseny 2003; Yu et al. 2002; Goff et al. 2002; Feng et al. 2002; Sasaki and Burr 2000; Barry 2001). Many genes, which encode metabolic enzymes, transporters, and transcription factors (TFs) and have the ability to improve crops, have been identified. Transgenic crops have shown to be successful and competitive, because they have many advantages for farmers (e.g. 37% less pesticides used, 22% higher production, and 68% more gain). Globally, genetically modified plants are cultivated in over 180 million hectares of land (López-Arredondo et al. 2015).

This chapter discusses how genetic engineering in rice has made it possible to survive in nutrient- deficient soil, with high yield.

2 Green Revolution Package and Rice Production

The combination of high-yielding varieties, extended and improved irrigation services, increased fertilizer inputs, and new cultivation methods are collectively called the 'Green revolution package'. Green revolution replaced the old agricultural practices of the 1960s, and rice production was extended to meet the world food demands and prevent future food shortages (Otsuka and Kalirajan 2006; Pingali 2012). Rice was one of the main contributing crops with higher yields in the green revolution package.

Deficiencies of both micronutrients, such as copper (Cu), boron (B), zinc (Zn), and molybdenum (Mo), and macronutrients, such as phosphorus (P), nitrogen (N), calcium (Ca), and potassium (K), can affect growth of rice in particular environments and soils (Ismail et al. 2007). Particularly, the deficiency of N, P, and Zn largely limits the worldwide rice production (Doberman and Fairhurst 2000; Ismail et al. 2007; Haefele et al. 2014). Since the beginning of the Green Revolution, the use of modern fertilizer along with the use of high-yield modern plants have been far more effective than conventional fertilizers and plant varieties (Khush 1995).

Though the green revolution's phenomenal achievements in providing security of food is overwhelming, its dependence on fertilizer has created environmental hazards, such as enhanced eutrophication of waterway due to P-rich sediments erosion and N deposition in watercourses (Carpenter et al. 1998). The nutrients problems occur mainly due to the poor recovery of P, N, or Zn by crop because of soil fixation (Zn, P) or leaching (upland cultivation) for N and gaseous losses (lowland crops) (Wissuwa et al. 2006; Linquist et al. 2013; Fageria and Filho 2007). In fertilizers, rice plants recover as little as 20–40% N, while majority of the N losses due to volatilization to NH3 or in flood systems denitrification to N2 (Linquist et al. 2013). In terms of environmental harm, denitrification (N2) to atmosphere may be insignificant; however, accumulation of NH3 in later rain may result in increased level of N in watercourses, subsequently causing problems of eutrophication (Carpenter et al. 1998). Therefore, creating plants with higher efficiency of nutrients is an important consideration for sustainable and eco-friendly agriculture.

Cost and access are secondary problems linked to fertilizers. Poor transport facility to distant inland areas contributes significantly to fertilizer costs, especially in Africa, wherein compared to international prices, the rate of local fertilizer can be twice as high (Otsuka and Kalirajan 2006). It reduces agricultural profits and effectively prevents most poor farmers from accessing fertilizers (Ismail et al. 2007). More rises in fertilizer cost is expected, because the rock phosphate is a non-renewable resource in most P fertilizers, and N fertilizer prices tend to rise along with petroleum prices (Vinod and Heuer 2012; Cordell et al. 2009). There are therefore powerful environmental and financial reasons for improving the efficacy of fertilizer use in agriculture in general, particularly in rice, due to the amount of poor farmers who depend on rice cultivation for their survival (Cassman and Pingali 1995).

The green revolution package was usually implemented in favorable settings, especially those with a reliable supply of water (Otsuka and Kalirajan 2006), which represents just approximately 50% of the region where rice is grown (Doberman and Fairhurst 2000). The rest of rice is grown under less favorable conditions, such as drought, flood-prone or upland rice ecologies, where excess water, lowland rainfed, low overall soil fertility or some other issues related to soil, or a mix of these factors could restrict rice production (Haefele et al. 2014). While high-yielding modern varieties (HYMVs) claim to perform well in varied environments (Khush 1995), this is not because of any particular adaptation to infertile or stressful environments but can be due to their high harvest index (Rose and Wissuwa 2012). P-deficient soil screening experiments have apparently shown that some traditional varieties performed better than the modern rice varieties (Wissuwa and Ae 2001a, b; Tyagi et al. 2012) or sorghum (Leiser et al. 2012). Because HYMVs have been chosen on breeders' stations under conditions of high-input, it is possible that particular traits that confer benefits under conditions of low input have been lost, especially if these traits come with a cost of yield under conditions of high input. The *PSTOL1* gene that gives P deficiency tolerance is a fascinating example because most of the breeding materials created for the irrigated highlands by the International Rice Research Institute (IRRI) lack this gene but many breeding lines aimed at unfavorable environments have this gene (Chin et al. 2010).

3 Rice as a Model Monocot System

Rice is considered as a model monocot system (Shimamoto and Kyozuka 2002; Goff 1999) because of its small genome (~430 Mb) that has been completely sequenced (Sasaki et al. 2005), well-developed genetics, easy transformation, molecular markers, and dense physical map availability (Wu et al. 2002; Chen et al. 2002). It is used to study various basic issues related to plant growth, development, and physiology, ranging from functional analysis of single gene to engineering of the entire metabolic pathway (Bajaj and Mohanty 2005). Additionally, among other cereals, rice shares widespread synteny, thus increasing the usefulness of this system (Devos and Gale 2000). These, along with ~28,000 full length cDNAs availability (Kikuchi et al. 2003), an enormous amount of expressed sequence tags, bacterial artificial chromosomes, artificial yeast chromosomes, P1-derived artificial chromosomes libraries and rich forward-and-reverse genetic assets (Hirochika et al. 2004) have made rice a worthy precursor among plants, particularly cereals.

4 Discovery of Genes Underlying Rice Root Structural and Functional Traits

The plant root system is clearly vital for growth of plants and provides a broad range of tasks such as anchorage, water and nutrients acquisition, and symbiosis in soil with useful microflora to improve the effectiveness of nutrient acquisition (Hodge et al. 2009). High-yield irrigated rice breeding favored shallow root system phenotypes which capture nutrients easily in the topsoil layer, while breeding for low input upland conditions has, conversely, favored deep root systems with strong capability of drilling and ability to extract nutrients from poor soil layers. The variety of rice that adapts to varying soil conditions and water regimes has favored identification of genes and quantitative trait loci (QTLs) underlying resource uptake traits and root development (Hill et al. 2013). Analysis of QTL is an effective method for analyzing genetic variation and control of genetically modified root structure and transport characteristics in response to deprivation of nutrients. Recently, enormous research on molecular, genetic, and physiological regulations for root structure related to nutrient efficiency of plant has been published. There are a number of genes identified in soybean (Guo et al. 2011), rice (Wu and Wang 2008), maize (Li et al. 2011; Lin et al. 2013), and *Arabidopsis* (Młodzińska et al. 2015), which are capable of altering structure of root to promote improved acquisition of nutrient. In addition, some proteins (Araya et al. 2014), micro RNAs (Vidal et al. 2010; Meng et al. 2010), and regulators including transcription factors (Dai et al. 2012; Miura et al. 2011; Devaiah et al. 2007), have also been shown to be involved in regulatory networks connecting root structure to nutrient efficiency.

In recent years, the rice research community has experienced major breakthroughs in discovering genes underlying root stress response. *PUP1* is a root QTL for uptake of phosphorus (Gamuyao et al. 2012) and *DRO1*, a root depth QTL (Uga et al. 2013), have been cloned, and fine mapping of other QTLs (*DRO2, DRO3*, *CHR9*) responsible for rice root/stress response is ongoing (Ahmadi et al. 2014). Recent advances in genome-wide association mapping approaches (GWAS) and MetaQTL analyses can decrease confidence intervals of QTL and can aid in increasing the accuracy of the position and number of detected QTLs (Courtois et al. 2009, 2013).

5 Methods of Transformation for the Production of Transgenic Rice

5.1 Polyethylene Glycol (PEG) Treatment

The PEG was the first method used in rice to achieve genetic transformation. PEG method was effective only when embryogenic cell suspensions were used for isolation of protoplasts. The first transgenic rice was produced by direct DNA transfer into protoplasts via PEG (Zhang and Wu 1988; Toriyama et al. 1988). Although Zhang and Wu (1988) regenerated rice plants for GUS-positive plants without selection and testing. Nevertheless, the cultivation of PEG-treated transgenic crop was often restricted to some genotypes of japonica, in which the process of protoplast regeneration is well known. This process was also used to endeavor the transformation of indica rice with the enhancement of plant regeneration from protoplasts. Transgenic indica rice plants recovery from protoplasts have been recorded (Peng et al. 1992; Datta et al. 1990). The regeneration of fertile transgenic rice plants has been accomplished. Explant collection is often limited to the suspension of embryogenic cells for protoplast isolation. Such cultures are very problematic to initiate and upkeep, and with increasing age of cultures, the capacity of these cultures to regenerate has been shown to gradually decrease (Jähne et al. 1995). Additionally, protoplast plant regeneration is time-consuming, inefficient, labor-intensive, and highly dependent on genotype. However, several elite indica cultivars from protoplasts are difficult to regenerate, and the fertility of the regenerated plants is too small.

5.2 Electroporation

Some researchers prefer to use electroporation for gene delivery over PEG (Zhang and Wu 1988), as it has been shown to be more effective. Shimamoto et al. (1989) were the first to report electroporation recovery of fertile transgenic rice plants (Shimamoto et al. 1989). The introduced genes were *hph* and *gus*, and the presence of transgenes in the progeny was confirmed. Subsequently, in both japonica and indica cultivars, several scientists documented the transgenic crop recovery using this DNA delivery method. Co-inheritance of the selectable marker gene and reporter gene (hygromycin phosphotransferase and ß-glucuronidase) has also been reported with no important variations in morphology and seed size between non-transformed and transgenic plants (Goto et al. 1993). However, like PEG technique, this technique also suffers from the same disadvantages as this also relies primarily on the use of protoplasts to introduce foreign genes. Recently, a new method of electroporation delivery of DNA to intact cells in cereals has been developed, and transient expression of the introduced gene (gus) has been achieved in suspension cultures (Chaudhury et al. 1994), leaf (Dekeyser et al. 1990), and embryos of rice seed (Chaudhury et al. 1995).

5.3 Particle Bombardment/Biolistic Method

Particle bombardment is also known as biolistic method or particle acceleration of gene transformation. The biolistic method was used enthusiastically in cereals, including rice, soon after its development as a gene delivery method in tissues and complete cells (Christou 1997). The approach is based on bombardment of plant cells with high-speed DNA coated with microprojectiles (gold or tungsten) accelerated by release of gun powder or helium or electrical current. Christou et al. (1991) was the first to obtain DNA-delivered transgenic rice plants using ACCELL® technology in several commercially important rice japonica and indica cultivars' immature embryos. The development of transgenic rice crops with embryogenic cell suspensions using the PDS1000/He BiolisticTM method was reported later by Cao et al. (1992), which was further enhanced by applying selection pressure after 2 weeks of DNA transmission and continuous selection of resistant clusters of cells and regenerated tissue (Li et al. 1993). Since then this process has been commonly used in the production of transgenic rice plants. The reason for the success of this process is that it is possible to obtain transgenic plants from any regenerative tissue, eradicating the need for protoplast isolation and regeneration. In addition, tissue culture aberrations that are so common in protoplast-mediated transformation are reduced as particle gun-mediated transformation can be accomplished from direct regeneration explants or with limited callusing phase. More specifically, this technique is said to be genotype-independent with more than 40 already transformed rice varieties (Datta et al. 1998; Christou 1995), and in some cases, transformation frequency has been documented to be as high as in dicots. However, it is observed that different researchers reported variable frequency of transformation. In rice, many ergonomically significant genes were introduced using the particle gun method. These include those which are tolerant to water and salt stress, insect, and herbicide. In reality, herbicide-resistant transgenic rice has completed field trials successfully (Oard et al. 1996). Like other techniques, there are also drawbacks to the biolistic technique. In some cases, there is a high number of copies and rearrangement of the inserted DNA, making transgene susceptible to gene silencing and inducing genomic changes. Nevertheless, new information does not support the worries of genetic integrity (Arencibia et al. 1998) and silencing (Qu et al. 1996). In addition, in a single transgenic locus, numerous copies can be integrated (Kohli et al. 1998) to avoid segregation issue during breeding programs. The other significant problem is the limited availability of machinery due to its high price. However, given appropriate resources and transfer of technology between well-established labs and others, the most versatile and successful approach seems to be the particle gun approach (Christou 1995, 1997).

5.4 *Agrobacterium*-Mediated Gene Transfer

The advance of effective systems for *Agrobacterium tumefaciens*-mediated rice transformation has produced remarkable progress. It has been tried to raise and regenerate transgenic calli following Agrobacterium-mediated transformation (Raineri et al. 1990). Chan et al. (1992, 1993) showed root explants and inactive embryos to regenerate from Agrobacterium-transformed calli (Chan et al. 1992, 1993). This strategy has already transformed about 40 different genotypes of javanica japonica and indica rice (Shrawat and Lörz 2006). The efficacy of *Agrobacterium tumefaciens*-mediated transformation has been affected by several factors, including Ti plasmid type (Cheng et al. 1998; Hiei et al. 1994), wide-ranging strains of bacteria (Aldemita and Hodges 1996; Dong et al. 1996; Hiei et al. 1994, 1997), pre- and during inoculation conditions (Mohanty et al. 1999; Aldemita and Hodges 1996), and exogenously inserted acetosyringone activation of the T-DNA transmission system (Khanna and Raina 1999; Rashid et al. 1996; Aldemita and Hodges 1996). Scutellum-derived calli are among the numerous explants used as the product of choice for effective rice transformation (Kant et al. 2001; Hiei et al. 1994). Indica rice genetic background exerts a strong impact on the frequency of transformation (Mohanty et al. 1999; Aldemita and Hodges 1996), while three different Japonica cultivars do not exhibit the same (Hiei et al. 1994). Use of high-performance techniques for agrobacterium-mediated transformation, 1000 stable transformants were obtained in japonica rice from only 150 calli derived from seeds (Terada et al. 2004). The genotypical effect is frequently overcome by altering the nutrient medium (Lin and Zhang 2005; Rachmawati et al. 2004) or transformation conditions, because for all varieties, the same nutrient medium is not suitable (Sridevi et al. 2005; Ge et al. 2006).

The backbone of the vector has a major effect on the process of transformation as the dual binary vector (Komari et al. 1996) and additional *vir* genes (Vain et al. 2004) have increased the effectiveness of transformation. In order to effectively eliminate *Agrobacterium* after transformation, in the selectable hygromycin resistance marker gene, a castor bean CAT1 intron was inserted, suppressing gene expression in Agrobacterium and making it vulnerable to hygromycin (Wang et al. 1997). Agrobacterium has developed many useful features in rice, including genes for biotic stress tolerance, abiotic stress tolerance, increased photosynthesis, nutritional enhancement and herbicide tolerance (Table 1).

Table 1 Genes used for enhancing nutrient deficiency tolerance in transgenic rice

Gene/transcription factor	Gives deficiency tolerance to	References
OsAMT1.1	N	Kumar et al. (2006)
Alanine aminotransferase (AlaAT)	N	Shrawat et al. (2008)
ZmDof1	N	Kurai et al. (2011)
Glutamine synthetase genes GS1 and GS2	N	Sun et al. (2005)
TOND1	N	Zhang et al. (2015)
OsPTF1	P	Yi et al. (2005)
Phosphorus uptake1 (Pup1)	P	Chin et al. (2010)
PSTOL1	P	Gamuyao et al. (2012)
OsWRKY74	P	Dai et al. (2015)
OsGDPD2	P	Mehra et al. (2019)
OsMYB2P-1	P	Dai et al. (2012)
OsPHF1	P	Chen et al. (2011)
HvNAAT-A and *HvNAAT B*	Fe	Takahashi et al. (2003)
HvNAS1	Fe	Suzuki et al. (2008)
refre1/372	Fe	Ishimaru et al. (2007)
OsIRO2	Fe	Ogo et al. (2011)
FRO2	Fe	Connolly et al. (2003)
HvYS1	Fe	Banakar et al. (2017)
OsIRT1	Fe	Lee and An (2009)
MxIRT1	Fe, Zn	Tan et al. (2015)
HvNAS1	Zn	Masuda et al. (2009)
AtNAS1	Zn	Wirth et al. (2009)
OsNAS1, OsNAS2 and OsNAS3	Zn	Johnson et al. (2011)

5.5 Other Methods of Transformation

In addition to the above-mentioned standard and commonly used strategies, certain reports of genetic transformation are made by utilizing new methods based on pollen tube pathways (Luo and Wu 1989), WHISKERS™ (Matsushita et al. 1999), imbibition of seeds or embryo in the presence of DNA (Yoo and Jung 1995), and light amplification by stimulated emission of radiation (LASER) (Guo et al. 1995). In order to avoid the need for sterile environments and tissue culture, the process of planta transformation (Supartana et al. 2005) relies on a needle dipped into Agrobacterium culture to prick the embryonic portion of the seed, which then develops into a plant and sets transgenic seeds. This could be a step in the development of a more effective method as good as floral dip in *Arabidopsis* (Clough and Bent 1998).

6 Nitrogen

Nitrogen (N) has a prominent position in the scheme of plant metabolism. Protein is associated with all vital processes in plants, and nitrogen is an essential component of protein. Consequently, the implementation of nitrogen is indispensable in order to obtain more crop production. In agriculture, nitrogen plays a main role by increasing crop yields and thus improves the quality of life (Massignam et al. 2009; Ullah et al. 2010). Optimum level of N in the soil improves processes of plant photosynthesis, leaf area production, leaf area duration and net rate of assimilation (Ahmad et al. 2009). The highest leaf area and total leaf biomass are the determinants of higher crop production (Rafiq et al. 2010). Due to the high use of N and proper management practices, the yield of different plants has risen worldwide since the past 50 years (Smil 2004).

Nitrogen acts as a major element of plant food and is main constituent of chlorophyll and protein present in most of the major parts of plant body. Nitrogen plays a major role in different events of plant physiology. It gives dark-green color to plants, improves growth and development of the stem, leaves and other vegetative parts. In addition, it also boosts root growth and development. N also enhances fast early growth, improves fruit quality, increases protein content of fodder plants, maximizes the growth of leafy vegetables, promotes the absorption and use of certain nutrients such as K, P, and regulates overall crop growth (Bloom 2015; Hemerly 2016).

Nitrate and ammonium are the two most widely present inorganic compounds of nitrogen in cultivable land. Nitrate reductase (NR) and Nitrite Reductase (NiR) combined activities convert nitrogen to ammonium after nitrate has been taken from the soil through different transporters. Ammonium, which could also be derived from photorespiration and from the degradation of organic molecules and proteins responsible for transport of nitrogen, is then processed into glutamine (Gln) and glutamate (Glu) through cyclic reaction catalyzed by glutamine synthetase (GS) and glutamate synthase (GOGAT) (Andrews et al. 2004). A lot of amino-transferases can transmit the Glu amino group into amino acids (Lam et al. 1996).

The productivity of four main crop crops; wheat, rice, cotton and sugar cane is greatly improved by N. Nitrogen at a rate of 120 kg ha^{-1} displayed notable results for rice tillers numbers, dry weight, height of plant, length of panicle, panicle number/filled grains, harvest index, yield of straw, benefit cost ratio and yield of grain (Malik et al. 2014). N deficiency causes decreased growth, chlorosis (change of green leaf color to yellow color), appearance of purple and red spots on the leaves and restricting growth of lateral bud (from which branches, stems and leaves develop) (Malik et al. 2014). Commonly, the symptoms of N deficiency appear first on older leaves (Bianco et al. 2015), and then leaf senescence begins. Excessive use of nitrogen has negative effects on growth of plant, boosts extra dark-green color on the leaves, makes the whole crop succulent and facilitates less fruit quantity with lower quality. Plants take up nitrogen only in a useful way, plants do not use nitrogen directly from the air because nitrogen itself is unreactive, most plants take nitrogen in the form of nitrate, but in some soils, such as submerged, it is not functional, whereas

NH_{4+} is most stable and appropriate for rice (King et al. 1992). Leaching is one of the main issues among nitrogen-limiting factors. When Nitrogen is applied to crops, it is dissolved in irrigation water and then leached to the lower part of the soil from the top.

6.1 Nitrogen Deficiency Tolerance via Genetic Engineering

Various strategies have been developed in a variety of plants using genetic engineering to maximize N assimilation. These strategies include:

6.1.1 Transgenic Rice Overexpressing *OsAMT1.1*

OsAMT1.1 is the most N-responsive rice gene (*Oryza sativa* L.) responsible for $NH4^+$ high-affinity transport (HATS). Kumar et al. (2006) measured the plant biomass and $NH4^+$ influx in overexpressing transgenic lines and two wild type (WT) rice cultivars, Taipei and Jarrah, with single or multiple copies of *OsAMT1.1*. When grown at 10 μM external concentration of $NH4^+$, influx of $NH4^+$ was higher for the Jarrah overexpression lines but not for Taipei's overexpression lines. Jarrah lines 75-4 and 77-1, grown at 2 mM concentration of external $NH4^+$ displayed an improved influx for seedlings; however, two overexpressed Taipei lines of displayed decreased influx rates. Transgenic lines biomass displayed either decreased or displayed no statistically significant differences compared to WT lines, when grown at low and high external $NH4^+$ levels. While the flow of $NH4^+$ into the roots of Jarrah line 75-4 grown at 10 μM concentration of external $NH4^+$ was considerably greater than in the wild form, there were no variations in the NH4 efflux measurements, resulting in greater net $NH4^+$ uptake in this line of overexpression (Kumar et al. 2006).

6.1.2 Transgenic Rice Expressing *Alanine Aminotransferase*

Alanine aminotransferase (AlaAT) has a key role in N metabolism and carbon fixation because it produces alanine and oxoglutarate by catalyzing the reversible reaction of pyruvate and glutamate (Miller et al. 2007). Shrawat et al. (2008) reported that in transgenic rice, significantly higher biomass and grain yields results from overexpression of AlaAT controlled by *OsAnt1* (a tissue-specific promoter), which in the epidermis of root directs strong expression. Most importantly, transgenic AlaAT expressing rice plants display major improvements in main metabolites, particularly glutamine (Gln) and glutamate (Glu), as well as an increase in total nitrogen content compared to controls (Shrawat et al. 2008).

6.1.3 Transgenic Rice Expressing *ZmDof1*

Maize Dof1 (ZmDof1) is a transcription factor specific to plants that are found to enhance assimilation of nitrogen even under nitrogen-deficient conditions in *Arabidopsis thaliana* (Arabidopsis). *ZmDof1* gene influence on rice assimilation of nitrogen and carbon has also been investigated (Kurai et al. 2011). In transient protoplast assays, *ZmDof1* induced expression of *phosphoenolpyruvate carboxylase*

(PEPC) genes and transactivated PEPC promoters in transgenic rice plants, displaying similar results in rice as in Arabidopsis. *ZmDof1* expressing transgenic rice grown in the presence of 360 μM (nitrogen-sufficient) or 90 μM (nitrogen-deficient) nitrogen concentrations displayed modification in the content of metabolite and expression of gene related with the anaplerotic pathway for Krebs cycle, indicating an increased flow of carbon towards assimilation of nitrogen. In addition, an increase in the amount of nitrogen and carbon per seedling was observed in Dof1 rice grown under deficiency of nitrogen.

6.1.4 Transgenic Rice Expressing *Glutamine Synthetase* Genes, *GS1* and *GS2*

Glutamine synthetase (GS, EC6.3.1.2) is a main enzyme in plants for the assimilation of ammonia. It plays a key role in the effective use of sources of nitrogen and metabolism of nitrogen. In higher plants, two groups of GS isoenzymes were identified, cytosolic (GS1) and plastidic (GS2). Sun et al. (2005) for the first time constructed plant expression vector p2GS which harbors GS1 controlled by rice actin 1 (Act1) and GS2 controlled by maize ubiquitin (Ubi). The constructed p2GS was successfully introduced by Agrobacterium-mediated transfer method into rice. Northern blot analysis confirmed the transcription of GS1-GS2 genes in the transformants. The transgenic rice plants obtained could grow effectively in MS medium where nitrogen supply is substituted for $(NH_{(4)}(_{2})SO_{(4)})$, the transformant's fresh weight was considerably higher than that of the control plants. The results indicate that p2GS expression makes nitrogen deficiency tolerance in transgenic rice plants.

6.1.5 Transgenic Rice Expressing *TOND1* Confers Tolerance to Nitrogen Deficiency

Zhang et al. (2015) identified an important quantitative locus on rice chromosome 12, known as Nitrogen Deficiency Tolerance 1 (TOND1), which gives N deficiency tolerance in indica cultivar Teqing. They reported that sequence verification of 75 japonica cultivars and 75 indica cultivars from different regions of 18 countries showed that *TOND1* is only present in 27.3% of cultivars (41 indica cultivars), while 72.7% of cultivars, including all 75 japonica cultivars and the remaining 34 indica cultivars lack the *TOND1* locus. In *TOND1*-deficient cultivars, the over-expression of TOND1 increased tolerance to N deficiency. The discovery of TOND1 provides a molecular foundation for breeding rice crops with an increased grain production despite lower inputs of N fertilizers (Zhang et al. 2015).

7 Phosphorus (P)

Like Nitrogen, Phosphorus (P) is also an essential nutrient for plant growth and productivity. Phosphorus has an essential role in a range of cellular processes, such as maintaining structures of membrane, biomolecule synthesis, and formation of high-energy molecules. It also contributes to cell division, inactivation/activation of enzymes, and metabolism (Razaq et al. 2017). It stimulates germination of seeds at the whole plant level; root development, stem and stalk strength; formation of flowers and seeds; yield of crops; and quality. Furthermore, P availability increases leguminous plants' Nitrogen-fixing ability. Phosphorus is therefore vital at all phases of development, from germination to maturity. It is a major component of energy-rich compounds such as adenosine triphosphate (ATP), guanosine triphosphate (GTP), uridine triphosphate (UTP), cytidine triphosphate (CTP), phosphoenol pyruvate and other intermediate phosphorylated compounds. Therefore, it provides energy to drive various cellular endergonic processes. It is vital for reproduction and protein synthesis as it is a major constituent of nucleic acids (RNA, DNA). Plants undergo different biochemical physiological and morphological adaptations to sustain their functions under inorganic phosphate (Pi-deprived conditions). These include changes in root architecture, shooting growth, cluster root formation, exudation of organic acid and alternative glycolytic and respiratory pathways (Vance et al. 2003).

Phosphorous concentration in plant varies from 0.5 to 0.05% of the total dry weight of the plant. Although the soil concentration of P is 2000 times greater than that of the plant, its fixation in the form of aluminum/calcium or iron/magnesium phosphates makes it unsuitable for plant absorption. Therefore, plants frequently encounter the issue of P deficiency in agricultural areas. It is difficult to diagnose its deficiency as plants usually show no early visual symptoms. Its deficiency is often confused with N deficiency, because under both deficiency conditions, the veins of young leave show redness. However, in P-deficient crops, there is no overall chlorosis. Phosphorous deficiency decreases plant growth due to either an increase in investment in energy or a decrease in photosynthesis. Its deficiency has an adverse effect on quality and yield of crop. Phosphorous deficiency has been estimated to reduce crop production on 30–40% of the aerable land of the world (Correll 1998; Smith 2003).

7.1 Phosphorus Deficiency Tolerance via Genetic Engineering

Several strategies using genetic engineering have been developed to optimize P assimilation in plants. These strategies include:

7.1.1 Pi Starvation Induced Transcription Factor 1 OsPTF1

Yi et al. (2005) reported a novel helix-loop-helix transcription factor (*OsPTF1*) in *Oryza sativa* responsible for inorganic phosphate (Pi) starvation tolerance. Northern-blot analysis showed that Pi starvation induces *OsPTF1*'s expression in roots. Pi starvation tolerance in transgenic rice increased with overexpression of *OsPTF1*. Shoot and root biomass, tillering capacity, and plant phosphorus content were about 30% higher in transgenic rice than WT plants in Pi-deficient hydroponic experiments under Pi starvation conditions in transgenic rice. As compared to WT rice, transgenic rice plants displayed considerably higher root surface area and total root length resulting in a higher instantaneous uptake rate of Pi under Pi-deficient conditions (Yi et al. 2005).

7.1.2 Phosphorus Uptake 1 (*Pup 1*)

Phosphorus uptake 1 (*Pup 1*) is an essential QTL associated with soil phosphorus deficiency tolerance and is located on rice chromosome 12 (Wissuwa et al. 1998, 2002). Kasalath, the donor variety of Pup 1, was originally recognized under rain-fed conditions during screening of 30 different genotypes of rice in a soil with P deficiency in Japan. It has experimentally been shown that Pup 1 improves uptake of P (Wissuwa et al. 2002; Wissuwa and Ae 2001a, b) and provides significant yield advantage in pot experiments with different types and environments of P-deficient soil (two to four-times higher grain weight per plant) (Chin et al. 2010).

7.1.3 Protein Kinase Phosphorus Starvation Tolerance 1 (*PSTOL 1*)

Pup1, recognized as an important QTL responsible for tolerance to P deficiency, was identified a decade ago in the traditional aus-type rice variety Kasalath (Wissuwa and Ae 2001a, b; Wissuwa et al. 1998). However, its mechanism of function remained unclear (Gamuyao et al. 2012) till the locus was sequenced, showing a Pup 1-specific protein kinase gene that was termed *PSTOL1*. This gene is absent in the genome of rice and other contemporary phosphorus starvation tolerant varieties (Chin et al. 2010, 2011). Gamuyao et al. (2012) showed that *PSTOL 1* overexpression in these varieties considerably increases grain yield in phosphorus-deficient soil. More analyses by Gamuyao et al. (2012) demonstrates that in early root growth, *PSTOL1* acts as an enhancer, enabling plants to absorb more phosphorus and other nutrients. The lack of PSTOL1 and other genes, including *SUB1A*, in modern rice varieties emphasizes the significance of its preservation and investigation of these genes in traditional germplasm. It is expected that introgression of this gene will significantly improve productivity conditions in locally adapted rice varieties under low phosphorus.

7.1.4 OsWRKY74

WRKY Transcription factors (TFs) is a large family of plant regulatory proteins. At its N-terminal, TFs have WRKY domain which is the most prominent characteristic of the TFs. WRKY domain is a region of 60 amino acids with highly conserved WRKYGQK amino acid sequence, and in its C-terminal, it has a C-CH-H/C zinc finger motif (Eulgem et al. 2000). WRKY proteins are categorized into three distinct groups based on the type of their zinc-finger motif and the number of WRKY domains (Eulgem et al. 2000). The percentage of WRKY gene members which are responsive to biotic stress in comparison to other multigene families of plant TFs is very large suggesting that WRKY proteins have important roles in the control of biotic stress (Ülker and Somssich 2004). Unlike biotic stresses, little data on the roles of WRKY proteins in responses of plant to Pi starvation and abiotic stress is known. For example, WRKY75 acts as a positive regulator for responses to Pi stress, and suppressing WRKY75 with RNAi leads to Arabidopsis impaired Pi starvation (Devaiah et al. 2007).

Dai et al. (2015) showed that OsWRKY74 rice belonging to the WRKY transcription factor family group III was involved in Pi starvation tolerance. OsWRKY74 is located in the nucleus and mostly expressed in leaves and roots. Overexpression of OsWRKY74 considerably improved Pi-starvation tolerance while transgenic lines with OsWRKY74 downregulation were susceptible to Pi-starvation. OsWRKY 74-overexpressing plant was ~16% greater in shoot and root biomass and phosphorous concentration than the WTs crops in Pi-deficient hydroponic solution. In soil pot experiments, OsWRKY74-over-expressing rice plants, >24% increase in P concentration, grain weight and tiller number were noted compared with WT crops when cultivated on P-deficient media. Additionally, OsWRKY74 may also be engaged in response to N and Fe deficiency as well as cold stress in rice.

7.1.5 Glycerophosphodiester Phosphodiesterase

Pi remobilization from membrane phospholipids may be a significant strategy for improving phosphorus use efficiency. Although the majority of photosynthetic membranes are non-Pi-containing glycerolipids, one-third of the plants total cellular organic P are due the polar head groups of Pi-containing phospholipids (Veneklaas et al. 2012; Dörmann and Benning 2002). Deficiency of Pi activates breakdown of these phospholipids, which leads to precursor molecules like DAG (diacylglycerol) being developed for the biosynthesis of sulfolipids (SQDG; sulfoquinovosyldiacylglycerol) and non-Pi containing galactolipide (DGDG; digalactosyldiacylglycerol and MGDG, monogalactosyldiacylglycerol). In plastidial and extra- plastic membranes, non-Pi containing glycerolipids substitutes phospholipids, thereby preserving Pi that can be channeled into essential cellular processes (Tjellström et al. 2008; Russo et al. 2007; Härtel et al. 2000; Andersson et al. 2003). Mehra et al. (2019) reported that OsGDPD2 is an OsPHR2 transcriptionally controlled Pi deficiency responsive gene. *OsGDPD2* confirmed phosphodiesterase activity in enzymatic assays and in silico analysis of active site residues. As compared to WTs, all lines of overexpression showed greater accumulation of biomass, Pi content, GDPD activity, and, root growth. On the other hand, *OsGDPD2* silencing caused a reduction in activity of GDPD and Pi content.

7.1.6 *Oryza sativa* MYB2P-1 (OsMYB2P-1)

Dai et al. (2012) identified *OsMYB2P*-1, A *R2R3 MYB* transcription factor, from microarray analysis data while studying the expression profile of seedling of rice subjected to Pi deficient conditions (Dai et al. 2012). It was indicated that Pi starvation caused the expression of *OsMYB2P-1*. *OsMYB2P-1* is located in the nuclei and show activity of transcription activation. In rice as well as in Arabidopsis, the over expression of *OsMYB2P-1* increased Pi deficiency tolerance, while *OsMYB2P-1* suppression through RNAi in plants increased the sensitivity of transgenic rice to Pi. In addition, under Pi-sufficient conditions, *OsMYB2P-1* overexpressing plants' primary roots were shorter than WT plants, while *OsMYB2P-1* overexpressing plants' primary roots and adventitious roots were longer than WT plants under conditions of Pi deficiency. These findings also suggested that

OsMYB2P-1 could be related to root system architecture regulation. *OsMYB2P-1* overexpression has resulted in higher expression of genes that gives response to Pi such as UDP-sulfoquinovose synthase, *OsmiR399j, OsmiR399a*, *OsIPS1* and *OsPAP10.*

7.1.7 *Oryza sativa* Phosphate Transporter Traffic Facilitator 1 (OsPHF1)

Phosphate transporter traffic facilitator 1 (PHF1) is known to control plasma membrane localization of inorganic phosphate transporter 1-1 (PHT1-1). Arabidopsis high-affinity inorganic phosphate (Pi) transporter, *OsPHF1*, an *AtPHF1* homologous rice gene was isolated (Chen et al. 2011) and observed to regulate the position of Pi transporters with high and low affinity to the plasma membrane. In solution culture with Pi-supplied condition, overexpression of OsPHF1 improved the accumulation of Pi in both shoots and roots. These findings show that function of *OsPHF1* is special in locating high- and low -affinity Pi transports on the plasma membrane of rice and defines the absorption and translocation of Pi in rice.

8 Iron (Fe)

Iron is an important micronutrient for nearly all living organisms because it has crucial functions to play in metabolic processes including DNA replication, photosynthesis and respiration. In addition, iron activates many metabolic pathways and is a prosthetic group of many enzymes (e.g., iron-sulfur [Fe-S] proteins and cytochromes involved in oxidation and reduction reactions). It is also a component of non-heme iron proteins involved in N_2 fixation, photosynthesis and respiration (Rout and Sahoo 2015). Iron deficiency is a major problem for agriculture land, and it damages one-third of the Earth's agricultural land. Iron deficiency induces a decrease in many components of photosynthesis machinery including Fe-S proteins ferredoxin (Fd), which participate in important chloroplastic oxidation pathways (Tognetti et al. 2007). Iron-deficient plants generally develop poor root formation and interveinal chlorotic symptoms in young leaves. In extreme iron-deficient conditions, it can leads to retardation of growth, stasis and plant death (Kobayashi and Nishizawa 2012). Both extremes of pH cause Fe deficiency in agricultural soils. In addition, several factors contribute either separately or in combination with chlorosis development, these includes supply of low Fe, increase level of heavy metals, high light intensities, increase level of phosphate, soil bicarbonate and water logged condition, high or low temperature, poor soil aeration, imbalance in cation ratio, increase level of nitrate nitrogen, addition of certain organic matter to soil, viruses and damage of root by nematodes and other organisms (Embleton et al. 1973).

8.1 Iron Deficiency Tolerance via Genetic Engineering

The following strategies have been developed to tolerate iron deficiency in rice.

8.1.1 FRO2 Ferric Chelate Reductase Overexpression

The *Arabidopsis* gene ferric reduction oxidase 2 (*FRO2*) codes for the low-iron-inducible ferric chelate reductase in the root surface responsible for iron reduction. Iron-regulated transporter 1 (*IRT1*) and *FRO2* are the two transporters having a key role in the high-affinity absorption of Fe from soil. These key transporters are responsible for high-affinity iron absorption from the soil. Both of these transporter genes are coordinately controlled at both transcriptional and post-transcription levels (Connolly et al. 2003). After iron starvation, *IRT1* and *FRO2* are induced together and are coordinately repressed after iron resupply. Zinc and cadmium also coordinate the steady-state mRNA levels of IRT1 and FRO2. In roots epidermal cell, like *IRT1,* mRNA of *FRO2* is detected in accordance with their proposed role in the soil iron uptake. In shoot and roots of *35S-FRO2* transgenic plants, *FRO2* mRNA is observed in a high level. However, *35S-FRO2* crops ferric chelate reductase activity is only elevated under Fe deficient conditions, showing that *FRO2* is subjected to post-transcription control as previously shown for *IRT1* (Connolly et al. 2002). Finally, as compared to wild crops, the *35S-FRO2* plants grow well in Fe-deficient conditions, supporting the idea that the rate-limiting step in absorption of iron is the reduction of ferric iron to ferrous iron.

8.1.2 *HvYS1* Overexpression Increases Fe Uptake in Rice

Some of the previous attempts of overexpressing different metal transporters to extend the Zn and Fe quantity of rice endosperm have led unintentionally to the cadmium (Cd), manganese (Mn) and copper (Cu) accumulation. Barley yellow stripe 1 (HvYS1) is particular to Fe, unlike other metal transporters. Banakar et al. (2017) studied this preference's mechanistic basis by *HvYS1* expression in rice controlled by *ubiquitin1* promoter of maize and compared the loading and mobilization of different metals. HvYS1 expressing plants showed a small increase in absorption of Fe, endosperm loadings, seed accumulation and root-to-shoot translocation, but the selection of Fe was confirmed without change of absorption and root-to-shoot transfer of Mn, Cu or Zn.

8.1.3 *OsIRT1* Overexpression Leads to Increased Iron

Lee and An (2009) produced *OsIRT1* overexpressed transgenic rice plants to assess their functional roles in homeostasis of metal. In the seedling phase, these crops showed increased tolerance to iron deficiency. This overexpression in the paddy field caused alteration in the plant architecture. Moreover, the crops were susceptible to excess Cd and Zn, which indicated that *OsIRT1* transported these metals as well. In the roots, shoots and mature seeds of overexpressing crops, iron and zinc contents were elevated as expected.

8.1.4 Zinc and Iron Content in Rice Seeds Increases with Overexpression of the *MxIRT1* Gene

MxIRT1 is a ferrous transporter screened from *Malus xiaojinensis*, an iron-efficient apple tree genotype. In order to minimize iron deficiency, Tan et al. (2015) produced *MxIRT1*-over expressing transgenic rice lines. These overexpression led in mature plant seed to a triple increase of Fe and Zn. Similar to *OsIRT1* transgenic plants, such transgenic lines also displayed an increased tolerance to iron and zinc deficiency as compared WT rice. Under deficiency of Zn and Fe, these transgenic lines displayed increased tolerance as compared to WT rice lines, which was similar to *OsIRT1* (Lee and An 2009). Sequence of the *MxIRT1* was very similar to the *OsIRT1* series.

9 Zinc (Zn)

Zinc is one of the main micronutrients involved in controlling different biological and physiological processes (as it acts as a co-factor of numerous enzymes and proteins) in plants (Nanda and Wissuwa 2016). Zn was first recognized by Sommer and Lipman as an essential micronutrient for higher plants (Sommer and Lipman 1926). Zinc is involved in a numerous physiological processes of plant metabolism and growth, including metabolism of carbohydrates, lipids, nucleic acids and auxins, enzyme activation, protein synthesis, reproductive development (pollen formation), and gene expression and regulation (Rehman et al. 2012).

Following the deficiency of phosphorus (P) and nitrogen (N), widespread deficiency of Zn was also observed to be responsible for reduction of rice yield (Fageria et al. 2002; Rehman et al. 2012). Zinc deficiency has been recognized as the most widely spread micro-nutritional disorder of wet land rice (Cayton et al. 1985). Zn deficiency in cereal crops, including rice, is a well-known problem causing a worldwide decline in agricultural productivity (Gao et al. 2005). Among different soil constraints, Zn deficiency is most common in the production of crops and pastures, particularly for high-yielding cereal (Alloway 2008). Deficiency of Zn causes stunted growth, bronzing of leaf which occurs 2–3 weeks after transplantation, reduce grain yield, increase plant mortality and it also delays maturity (Neue and Lantin 1994). In antioxidant mechanisms, Zn is recognized as one of the main component, therefore absence of Zn in plants results in bronzing of leaf/death of cell with photo-oxidation and leakage of solutes from roots with peroxidation of membrane (Lee et al. 2017). Zn deficiency in soil is caused by high pH, absorption and precipitation of Zn with other particles of soil as well as by redox potential (Kabir et al. 2014). Deficiency of Zn in plants frequently results in low contents of Zn in grain, which results in malnutrition in children, birth problems in pregnant women and susceptibility to infectious diseases (Graham et al. 2012; Prasad 2009).

9.1 Zinc Deficiency Tolerance in Rice

Due to agronomic and economic factors, such as the relatively high price of fertilizer, fertilization is not always a choice to overcome Zn deficiency. Conversely, exploiting genetic variability to produce staple crops with high Zn efficiency (ZE) could provide a cheap and sustainable approach to overcome problems of Zn deficiency. The ability of a genotype to grow well under conditions of Zn deficiency is termed as ZE, and ZE is typically expressed as the ratio of shoot dry weight under Zn deficiency to that of sufficient Zn supply (Gao et al. 2005).

ZE is an importance trait since Zn-deficient conditions prevail worldwide (Kabir et al. 2017). Mechanisms that control the tolerance to Zn deficiency seem to have many uncertainties. More likely, in any plant species, there is no single mechanism (Gao et al. 2005). In spite of great research work on the investigation of Zn efficiency in plants and high occurrence of Zn-deficient cultivated soil worldwide, the molecular pathways involved in Zn efficiency are poorly understood. In spite of the wide range of research investigating Zn efficiency in crops and the high occurrence of Zn-deficient cultivated soils worldwide, the molecular mechanisms involved in Zn efficiency are still poorly understood. Tolerance to Zn deficiency can usually be accomplished by means of two mechanisms: effective use and translocation of Zn in the plant also known as Zn use efficiency (ZUE) and higher root uptake of Zn (Nanda and Wissuwa 2016).

Under Zn-deficient conditions, plants have evolved complex regulatory mechanisms for absorbing and transporting Zn (Henriques et al. 2012). Several transporter and transporter like protein have been identified that are involved in the uptake and transport of metals in plants, these include members of the 15 Zinc-regulated transporters and Iron-regulated transporter-like Protein (ZIP) gene family (Ishimaru et al. 2005).

9.1.1 Overexpression of Nicotianamine Synthase (NAS) Genes

Nicotianamine (NA) is a transitional metals chelator that plays vital roles in higher plants in the short- and long-distance transport of metal cations, including Fe^{2+} and Fe^{3+} (von Wirén et al. 1999; Takahashi et al. 2003). NA is biosynthesized by S-adenosylmethionine trimerization, a NA synthase (NAS) enzyme catalyzed reaction. Transgenic strategies to increase concentration of NA have frequently focused on everexpression of exogenous NAS genes in crops. Masuda et al. (2009) reported that *HvNAS1* overexpression in rice has resulted in significantly higher concentrations of NA (15 times higher than wild type) as well as 1.5 and 2.3 times higher Zn and Fe concentrations, respectively, in polished grain. A 6.3-fold increase of Fe concentration in rice endosperm was achieved by constitutive expression of the Arabidopsis NAS gene, *AtNAS1*, together with endosperm-specific expression of genes encoding phytase and ferritin (Wirth et al. 2009). Johnson et al. (2011) showed that overexpression of rice NAS genes (*OsNAS1-3*) shows enhanced tolerance to Fe and Zn deficiency.

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Developing C4 Rice for Higher Photosynthetic Efficiency and Environmental Stress Tolerance

Aisha Kamal and Farhan Ahmad

Abstract

Rice is the staple sustenance of over portion of the total populace, and this rice-devouring populace is expanding exponentially. As per FAO estimation, it was calculated that 925 million people agonize from prolonged malnutrition, and the condition is expected to get worse with an expected population increase by 9 billion globally by 2050. Further restrictions will be put on agricultural by environment and ecological stress; water inadequacy will additionally intensify constraints on rice production. Also, rice is a C_3 plant, and in hot dry conditions, CO_2 obsession response is aggressively restrained by O_2, prompting the vivaciously inefficient procedure of photorespiration. In perspective on the yield levelling, change of metabolic pathway of C3–C4 photosynthesis is being considered as one of the potential courses to build efficiency in billions of rice crop. It has likewise been experimentally moved towards that insertion of C4 enzymes directly or indirectly by using photosynthetic machinery into rice has the potential to improve its resistance to stress by improving photosynthetic efficiency. Progression and advancement in genome sequencing, gene discovery and genome altering provide a phenomenal chance to explain the formative and developmental procedures of C4 rice attributes. This seeing needs to incorporate the multifaceted communications among overhang design, the development and advancement of a leaf and the organic chemistry of the photosynthetic mechanical assembly notwithstanding simply joining C4 chemicals in the rice plant. In this chapter, the possibility of converting rice from C3 to C4 photosynthetic pathway is discussed.

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C4 rice · Photorespiration · Kranz anatomy · Thermotolerance · RuBisCO

1 Introduction

Rapid explosion of population is also associated with shortage of nutrition and food, water and land. In modern days, fertile and agricultural land is being debased in the light of the fact that huge zones of farmland are being occupied for industrialization (Tang and Wang 2018). As a consequence of urbanization and excessive interference of human in environment, the situation is getting worsen day by day by irregular climate change coupled with various abiotic stress such as drought, salinity and heavy metal toxicity which further reduce agriculturally viable land and increased inter as well as interspecific competition for survival (Fahad et al. 2016; Ahmad et al. 2019). To make accessible food and nourishment for the exponential growth of world population, rice with adequate nutrient quality and yield should be improved by 60% (FAO stat 2009) which is mainly used as food worldwide. In addition to the above, rice is a C3 plant and grows in hot dry conditions, and CO_2 fixation reaction is comparatively suppressed by O_2, leading to the occurrence of the degenerative and energy-consuming process of photorespiration. This poses an enormous challenge as the expected demand for rice which was 480 million globally will increase by about 291 million tonnes (Fao 2012). By knowing the above fact and situation, an effective strategy or scientific novel approach mitigating the above problem without troubling the environment any more should be applied. In view of the crop yield, conversion of metabolic pathway of C3–C4 photosynthesis is being considered as one of the possible strategies to increase the productivity of rice crop at present and potentially in future warmer environment (Zhu et al. 2010; Karki et al. 2013; McQualter et al. 2016) as C4 pathway is reported to have relatively high photosynthetic energy conversion efficiency (Hanson et al. 2012; Li et al. 2017). Major significant dissimilarity somewhere in the range of C3 and C4 photosynthesis is that C4 photosynthesis has a CO_2-concentrating component, which expands the CO_2 focus around RuBisCO and correspondingly discourages photorespiration and builds the net CO_2 fixation rate (Roychoudhury et al. 2008). Besides these specific variations in biochemical structure and of action between these two modes of photosynthesis, C4 plants have also propelled a profitable metabolite transportation system between the two cell types (Leegood 2002). In this review, we will try to recall the important point of plant photosynthetic mechanism related to developing C4 rice plant having compatibility with C3 anatomical structure; however, it is quite possible that some common metabolism and reaction remain left untouched. An effort has been made to figure out feasible approaches and associated problems in creating C4 rice; specific gene, anatomical structure such as leaf structure and the physiological process must be added into the rice and communicated in a suitable way which is not feasible by the usual plant-reproducing systems. Using rice as a working model to develop C4 photosynthetic pathway is that the first crop whose complete genome sequence equipped with the processed data and findings of genome and genetic information makes it an ideal tool for research in this segment. A significant research and ideas will also help to plant biologist to improve the photosynthetic pathway of rice that gives palatable development of grain effectiveness and high return capability of rice.

2 Mode of Photosynthesis and Associated Challenges

Photosynthesis is the most essential metabolic scheme linked to crop output as carbohydrate (simple and complex form) comprises more than 85% of the dry weight in plants. It is the phenomenon by which green plants and other photosynthetic organism change light energy into chemical vitality. During photosynthesis in green plants, light energy is caught and used to change water, carbon dioxide and minerals into oxygen and energy-rich organic compounds. There are three modes of photosynthesis: C3 pathway, C4 pathway and Crassulacean acid metabolism (CAM). Autotrophic organisms first evolved the C3 pathway of photosynthesis. However, over the geologic period, several other CO_2 concentration mechanisms were developed by plants in response to the decrease in atmospheric CO_2 levels, such as bicarbonate transport system in cyanobacteria, algae and aquatic plants, whereas the C4 pathway and CAM in higher plants. Though the most beneficial yields, for example corn, sorghum and sugarcane, utilize the C4 pathway, still the greater part of the agronomic harvests, for example rice, wheat and potato, utilizes the C3 pathway. Three distinct photosynthetic mechanisms were identified in higher plants, viz. C3, C4 and CAM (Tieszen et al. 1983). The major differences, variation and structural changes in these different modes of photosynthesis have been summarized in Table 1.

The problematic mechanism of C3 plants including rice is that the initial process of CO_2 fixation is carried out by using universally abundant enzyme RuBisCO which catalyse oxidation of ribulose-1,5-bisphosphate which is mainly responsible for photorespiration and reduction in photosynthetic productivity (Sage 2004). At temperature above 30 °C which is the average in tropical rice-producing areas of the world, the excessive oxygenation productions cut down the photosynthetic profitability of C3 plants by up to 40% (Betti et al. 2016). In addition to this, paces of photorespiration increase with temperature and shortage of water reinforce prolonged RuBisCO oxygenation (Walker et al. 2016). Thus, at the end of the process, photosynthesis productivity of rice in the tropics and warm quiet regions ends up in an inefficient way. The C4 plants which have CO_2 concentrating system inside their leaves due to anatomical advantage have reduced the degrees of photorespiration and consequently have progressive features to flourish in hot, dry situations and offer profitable capabilities for harvesting improvement techniques. Rice plants with a C4 photosynthesis system would have expanded photosynthetic effectiveness even though employing infrequent necessary supply of water and compost nitrogen required for sustainable growth (Aubry et al. 2011; Kant et al. 2012). Successively, it will perform well under high temperature and require less

Table 1 Features of C3, C4 and CAM characteristics of higher plants (Modified from Tieszen et al. 1983)

Feature/character	C3	C4	CAM
Leaves	Thin	Thin	Succulent
Anatomy	Mesophyll diffuse	BS Kranz	Mesophyll
Chloroplasts	Monomorphic granal	Dimorphic	Monomorphic
Primary CO_2 enzyme	RuBisCO	PEPC	PEPC
Secondary	None	RuBisCO	RuBisCO
Primary substrate	RUBP	PEP and RUBP	MDH
CO_2 product	3PGA	OAA	OAA
Photorespiration	High	Low	Low
O_2 inhibition	High	Low	–
CO_2 evolution in light	High	Low	–
Mesophyll conductance	Low	High	High
Quantum of yield	High	Independent	Independent
Optimum temperature	15–25	25–35	25–35
K_m (Michaelis–Menten constant) for CO_2	13–26 μm	28–60 μm	
Enzyme size	Large	Small	–
Starch accumulation	Normal in mesophyll	Normal in PEP	
Climatic adaptation	Temperate	Tropical	Arid tropical
Carbonic anhydrase	High in mesophyll	High location uncertain	

water and nitrogen, and C4 rice would introduce favourable circumstances on different sorts of rice situations including rural landscapes. According to research-based fact, even a 5% decline in photorespiration would be worth about $540 million consistently in yield gain for less economical viable countries (Roychoudhury et al. 2012; Walker et al. 2016).

C4 plants are potentially progressively profitable at higher temperatures typically experienced by rice. To exploit this successful photosynthetic mechanistic approach present in nature, and to fulfil nutrition demand and for economic food costs, there are achievements towards inserting the C4 framework, for instance, which is found in maize into rice (Rizal et al. 2012). This epic way to deal with change in the photosynthesis arrangement of rice is a difficult and laborious attempt in the light of the fact that the C4 pathway is confounding, and several mechanisms controlling the system are as yet unclear. In this manner, it requires curiosity and capability of researchers engaged with differing orders, for example natural chemistry, bioinformatics, genomic studies, plant metabolomics and stress biology share a common platform to elucidate all the underlying mechanism. For the equivalent, the C4 rice consortium was conceptualized and developed which began the sensible work of C4 rice structuring since 2009 (http://photosynthome.irri.association/C4rice/).

Considering the examination of the improvement of C4 from C3 species and the related changes, the going with adjustments is essential to set up a viable C4 photosynthetic pathway in rice. Later section will provide an account for necessities to make C4 rice and headway made in the field of innate planning.

3 C4 Photosynthesis and Its Subtype

An adaptive mechanism is well known in C4 plants which regulate the concentration of CO_2 and help in reduced photorespiration and improved net CO_2 fixation rate. The structural organization of bundle sheath and mesophyll cell has been directly linked to CO_2 fixation in C4 plants. In these plants, the underlying CO_2 assimilation is not catalysed by RuBisCO; however rather, CO_2 is changed over to bicarbonate (HCO_3) in the cytosol of mesophyll cells, and fixation is carried out by phosphoenolpyruvate carboxylase which leads to the formation of C4 acids that are immediately transported into bundle sheath where they are decarboxylated back to CO_2. This CO_2 is thusly refixed by RuBisCO arranged in the bundle sheath of the chloroplasts. The high partiality to phosphoenolpyruvate carboxylase to bicarbonate and the low vulnerability of the bundle sheath to mesophyll cell interface lead to a high CO_2 focus in bundle sheath cells. This high CO_2 preoccupation considers a lot of lower RuBisCO oxygenation and photorespiration rates (Furbank et al. 2009; Langdale 2011; Lin et al. 2019). The bundle sheath cells are thickened to outline a Kranz structure which helps in the carbon dioxide fixation. The centralization of RuBisCO is high while PSII development is a very low in these cells; however, in mesophyll cells, the intermingling of PEP carboxylase (PEPC), PSII and PSI practices are kept up at unusual states (Sage 2004).

It is also well known that various mode of C4 photosynthesis has been operated in plant to cope different environmental conditions. However, based on enzymatically catalysed decarboxylation reaction of four carbon acids, they could be categorized into three subtypes. The most common subtype is classified as NAD-dependent malic enzyme (NAD-ME) and NADP-dependent malic enzyme (NADP-ME) which are generally present in all C4 plants while no pure subtype PEP carboxykinase C4 type has been revealed yet (Sage 2004; Rao and Dixon 2016). The specific subtypes and heredities of C4 plants were guessed to have developed to overcome scarcity of nitrogen and water (Bräutigam and Gowik 2016). The first two common subtypes showed remarkable differences in leaf anatomy and vascular system arrangement, for example layering between bundle sheath and vascular system is not present in between them in NADP-ME subtype (Rao and Dixon 2016). Phylogenomics analysis proposed three models describing that NAD-ME and NAPD-ME have been arisen from a common ancestor or two different ancestors or that NAD-ME as a common ancestor of C3–C4 intermediate (Washburn et al. 2015). C4 plants for the most part have an intrinsic higher photosynthetic CO_2 take-up rate and transformation proficiency of solar energy vitality when contrasted with C3 plants (Zhu et al. 2010; Giuliani et al. 2019). Other than higher light use productivity, C4 plants are additionally equipped with better water and nitrogen use effectiveness bringing about better returns significantly under hotter temperatures (Zakaria et al. 2002). These intrinsic qualities of C4 plants emphatically point that communicating a C4 pathway in C3 rice species may turn into a valuable procedure to improve rice efficiency (Leegood 2002).

4 Anatomical Features of C3 and C4 Plants

C3 and C4 plants are metabolically extraordinary as well as they have distinctive anatomical highlights as well. Rice which is a C3 plant has unpredictably orchestrated overwhelming lobed mesophyll cells which are situated between the bundle sheath cells. These mesophyll cells comprise about 66% of the protoplast volume which is involved by the chloroplasts, 97% of which is dominatingly present in the fringe of the cells, which may be useful to boost the dissemination of CO_2 into the stroma (Sage and Sage 2009). While its bundle sheath cells contain less chloroplasts with only 21–52% in the remote zone (Sheehy et al. 2008). In contary to this, leaves of C4 plants are equipped with Kranz anatomy, that is, each vascular group is enveloped by an internal ring of bundle sheath cells and an outside ring of mesophyll cells (Furbank et al. 2009). The mesophyll cells in maize are similarly lobed, anyway not as extensively as in rice (Wang et al. 2017; Rae et al. 2017). The C4 mesophylls have less chloroplasts appeared differently in relation to those of related C3 species, and they do not cover the absolute cell fringe (Stata et al. 2014). While bundle sheath cells of C4 species are greater and contain larger shaped chloroplasts than C3 species, the chloroplast is arranged towards the mesophyll cells (Maai et al. 2011; Königer 2014). Low venial separations and a high proportion of between-group sheath and mesophyll cell volume is the normal trademark for C4 descents (Griffiths et al. 2013; Christin and Osborne 2014).

5 Strategies to Convert C3 Rice to C4

Nowadays various attempts are under process to enhance the rice yield through genetic engineering and capable C4-type photosynthesis into rice (Kajala et al. 2011). Therefore, genes responsible for the regulation of leaf anatomy and other biochemical procedures must be embedded into the rice and communicated suitably through genetic engineering which is not possible through regular plant breeding systems. The outline for the development of C3 rice line with sequential possible approaches has been summarized in Fig. 1. However, the authors will mainly focus on the genetic and biochemical derived mechanistic approach to proceed towards C4 pathway.

The introduction of a multi-genic trait such as C4 photosynthesis into rice, no doubt, possesses a very big challenge. However, one of the main reasons to take this task is the tough altruistic case for expressively accumulative photosynthetically active radiation use efficiency. Several biological reasons exist that paw a pathway

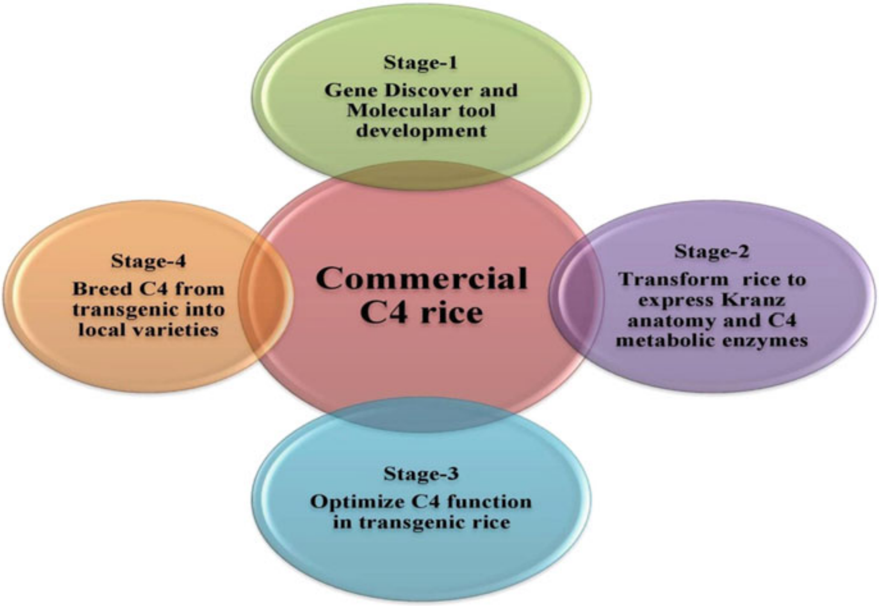

Fig. 1 Stepwise sequential approaches for the development of C4 rice plant

for the development of C4 photosynthesis and may no longer be as tough as it initially seems, due to the following reasons. Firstly, the evolution of C4 photosynthesis had been viewed more than one instances in many species, indicating that C3 species can also in some methods be preconditioned for the C4 pathway (Sage 2004). The most convincing proof to install the feasibility of C4 rice engineering is the transformation of *Eleocharis vivipara,* a species of flowering plant in the sedge household from C3 to C4 photosynthesis in exclusive environmental and hormonal stipulations (Ueno 2001). Secondly, no new genes are required for the C4 pathway, as all the enzymes of the C4 cycle already exist in C3 species, even though they frequently accumulate to low degrees and are notions to fulfil housekeeping features. Shockingly, C3 plants have characteristics of the C4 pathway and are firmly connected with veins of stem and petiole (Hibberd and Quick 2002; Brown et al. 2010). Moreover, the arrangement of four-carbon compound PEPC and obsession C4-type obsession of CO_2 have likewise been accounted for in guard cells (Raines 2006; Yamori et al. 2014). Thirdly, very few supported research studies are available, which present gauge around 3% of the creating leaf transcriptome differentiates between immovably related C3 and C4 species (Bräutigam et al. 2011). Fourthly, it seems, by all accounts, to be possible that a better than average degree of these movements to the leaf transcriptome are a direct result of either assistant effects related to the progressions to fundamental digestion in a C4 leaf or various methods related to phylogenetic partition between these species. This to some degree can be attempted by assessing the impact of isolating an immense portion of the C4 cycle into mesophyll and BS cells of rice. In the long run, progressing propels in sequencing advances, proteomics and the openness of different C4 genomes (Wang et al. 2009; Paterson et al. 2009) have opened the C4 pathway to exploratory dismantling using a structures science approach. Close transcriptomics and proteomics have given encounters into the separating of C4 quality articulation and protein hoarding through the investigation of mesophyll and BS cells in maize (Majeran et al. 2010; Friso et al. 2010; Huang and Brutnell 2016) among mesophyll and group sheath cells of rice (Jiao et al. 2017), along the maize leaf developmental incline (Li et al. 2017), and between both remotely related (Bräutigam et al. 2008) and immovably related C3 and C4 species (Bräutigam et al. 2011). Later on, the structure science approach will be loosened up to different family lines of solidly related C3 and C4 species to reveal centre parts of C4 science. Considering the majority of the above focuses it appears like a reason to begin trying to design the two-celled C4 cycle into the rice. In this segment, we will attempt to examine the potential methodologies to consolidate CO_2-siphoning framework into C3 plants, so the transgenic plants would be relied upon to show exceptionally improved photosynthetic execution and efficiency.

5.1 Development of Engineered Thermotolerant RuBisCO Enzyme

The most abundantly available protein on earth and an important enzyme for CO_2 fixation in photosynthetic process is RuBisCO. C3 plants are known to have low catalytic activity, so RuBisCO performance can be enhanced, which is a recognizable target for increasing both photosynthetic performance and nitrogen usage competence (Yamori et al. 2014). There is an ongoing detailing that the sorghum determined C4-RuBisCO and that insertion of *Rbc* gene within rice effectively delivered chimeric RuBisCO with a more noteworthy synergist turnover pace of RuBisCO (kcat) in the transgenic rice (Ishikawa et al. 2011). To sustain the activity of *RuBisCO activase* under high-temperature conditions, the thermotolerance of RuBisCO activase has to be increased. Kurek et al. (2007) and Kumar et al. (2009) reported that insertion of a thermostable *RuBisCO activase* into *Arabidopsis* provides heat tolerance and enhanced photosynthetic performance at higher degrees of temperature. It was also observed that overexpression of *RuBisCO activase* of maize in rice enhanced the thermal stability of photosynthesis (Yamori et al. 2012). Thus, genetically manipulating RuBisCO activase could be a potential methodology for the incitement of photosynthesis and particularly the development of rice in tropical region.

5.2 Cell-Specific Regulation of Calvin Cycle in Rice

To have high CO_2 absorption in C4, two cell-specific types of cells, the mesophyll cells and bundle sheath cells, play an important role in C4 photosynthesis. Before fixation of CO_2 by RuBisCO, an additional stepwise synthesis of organic acid known as oxaloacetate has been governed by enzyme phosphoenolpyruvate carboxylase in mesophyll cell. This C4 organic acid is transported to BS cell via the formation of different intermediates such as malate and aspartate where decarboxylation process occurs and CO_2 is available for RuBisCO fixing and accomplishment of Calvin cycle in the chloroplast of BS (Majeran et al. 2010; Manandhar-Shrestha et al. 2013). To prompt C4 photosynthetic pathway in rice holding C3 integrity, particular set of photosynthetic enzymes have been included into C4 rice both independently or synergistically (Taniguchi et al. 2008). In this context, tight regulation and coordination of theses specific enzymes, viz. phosphoenolpyruvate carboxylase, C3-specific NADP-malic enzyme, C4-specific pyruvate, orthophosphate dikinase, NADP-malate dehydrogenase, should be achieved in a functional manner (Ku et al. 1999; Taniguchi et al. 2008; Fukayama et al. 2001). The overexpression of PPDK, MDH or ME used to be found no longer to have any enormous effect on the photosynthesis. But in the case of PEPC, there was once a moderate discount in the photosynthesis. This discount precipitated by using the overexpression of PEPC was once overcome by using the mixed overproduction of the three different enzymes, i.e. PPDK, ME and MDH (Taniguchi et al. 2008). The overexpression of all these strong combination of enzyme(s) delivered pronounced photosynthetic efficiency rate even at greater CO_2 fixations, acclaiming that even the overexpression of these component does not alter CO_2 concentration in the chloroplasts (Taniguchi et al. 2008) (Fig. 2).

Most of the modern-day C4 engineering–associated work focuses on expressing an entire NADP-ME-kind C4 metabolism in a C3 crop (Kajala et al. 2011; Miyao et al. 2011; Sage and Zhu 2011). However, it is very fundamental to recognize that whether or not it is fine to engineer such a C4 metabolic cycle into a typical C3 leaf barring, putting off the authentic C3 metabolic approaches and by means of making use of C3 leaf anatomy. The reply to the above is very fundamental due to the fact that it will decide whether or not the present-day C4 engineering work wants the elimination of the current C3 metabolic cycle from C3 leaves and the requirement of modifications in leaf anatomy. von Caemmerer (2003) analysed the efficiency of an engineered single-cell C4-type concentrating mechanism in rice and determined that a single-cell strategy limits the strength of effective photosynthesis. They recommended that the compartmentation of CO_2 decarboxylation in the bundle sheath can also be a long way greater profitable strategy. Wang et al. (2017) tried to increase a 3D reaction-diffusion mannequin that accounts for a two-compartment C4 metabolism being expressed in a C3 background, while additionally accounting for the normal leaf anatomy discovered in rice. This model was once developed with the aid of incorporating an NADP-ME-type C4 metabolism and a C3 biochemical model in a practical geometry representing section of a rice leaf. They advocate that expressing a two-cell C4 metabolism in a C3 rice leaf might also lead to accelerated photosynthetic efficiency. Furthermore, the RuBisCO allocation and energy partitioning play an indispensable function to attain improved photosynthesis in the engineered leaf. To develop efficient C4 rice, understanding the underlying mechanism is required to gain all the essential information and possible strategies to improve effective C4 photosynthesis. The next important steps are to establish

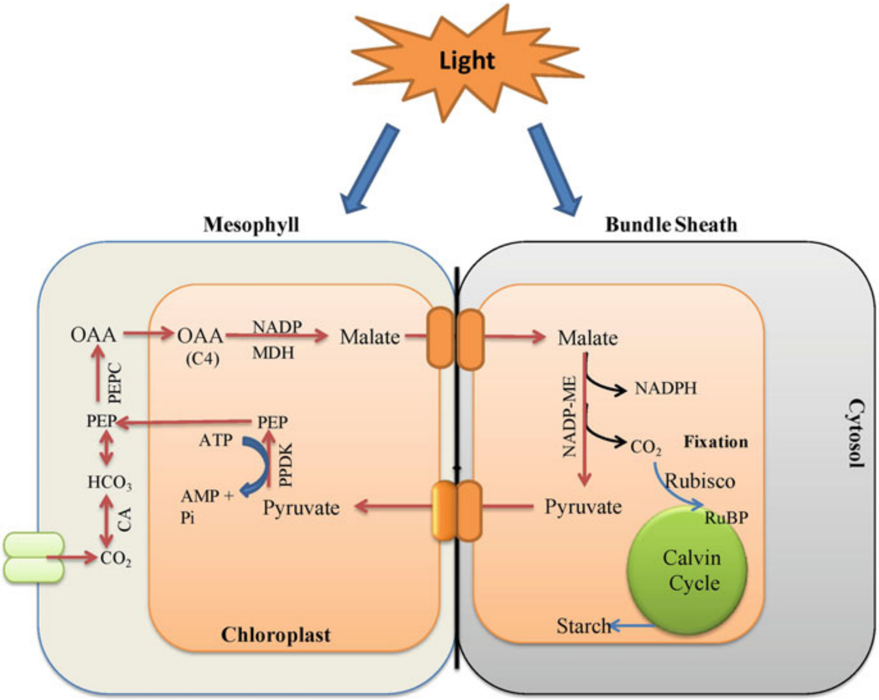

Fig. 2 Graphic representation of designed C4 photosynthesis pathway in rice. *CA* carbonic anhydrase, *NADP-MDH* NADP-malate dehydrogenase, *NADP-ME* NADP-malic enzyme, *PEP* phosphoenolpyruvate, *OAA* oxaloacetate, *PEPC* phosphoenolpyruvate carboxylase, *PPDK* pyruvate orthophosphate (Pi) dikinase, *PPT* phosphoenolpyruvate phosphate translocator. (Detailed process has been discussed in the text)

functional and profitable coordination between engineered mechanisms in essential metabolism without diminishing the integrity of existing C3 fixation mechanism and correspondingly decrease carbon fluxes. At last, the most important feature of C4 accompanying Kranz anatomy is to maintain and retain balance between CO_2 assimilation and photosynthetic efficiency (Wang et al. 2017). All these efforts, even though did not generate the preferred CO_2 concentrating mechanism, then again furnished precious insights for modern-day C4 rice research.

5.3 Modification of Chloroplast Size and Number in BS Cells of Rice

C3 plants have mesophyll cells that records for an excess of 90% of the chloroplasts of the leaf, and subsequently, the greatest rate of photosynthesis happens in it (Yoshimura et al. 2004). Be that as it may, on account of C4 plants, both mesophyll cells and bundle sheath cells have equivalent quantities of chloroplasts in this manner, and the procedure of photosynthesis is sectionalized into both mesophyll and sheath cells. First, the procedure of carbon dioxide obsession begins in the mesophyll cells where arrangement of oxaloacetate happens, and afterward oxaloacetate is changed over to malate which is shipped to bundle sheath cells. Like mesophyll cells of C3 plants, the bundle sheath cells of C4 plants perform similar function of decarboxylation of C4 organic acid of photosynthesis and regulation of C3 cycle intermediates. To play out these procedures, the bundle sheath cells in C4 plants are developed and have more chloroplasts, making them more pronounced and photo synthetically active. In that manner, bundle sheath cells and mesophyll cells are able to develop two sub-variant of photosynthesis in C4 species. To integrate the C4 mechanism in rice, there ought to be progressively photosynthetic chloroplasts in the bundle sheath cells by regulating Golden2-like genes. In *Arabidopsis* and *Zea mays*, these cell-specific genes are well known for coding nucleus transcription factor responsible for providing C4 features to chloroplast (Bravo-Garcia et al. 2009; Liu et al. 2016).

5.4 Alteration in Vein Density and Spacing Rice Leaf

These cells pushes the veins a long way from one another in this manner, expanding the vein dispersing and decreasing the vein density (under six veins for every millimetre in rice) while C4 plants forces in excess of seven veins for each millimetre. This high vein thickness in leaves of the C4 plants prompts about coordinated proportion of the volumes of mesophyll and BS tissues. The rehashing example of vein-BS-M-M-BS-vein frequently frames the inward life structures of a C4 leaf. Mesophyll cells which encompasses the BS cells frame a wreath-like structure. The term utilized for such sort of leaf-life structures is classified ‘Kranz Anatomy’. A nearby contact among mesophyll and pack sheath is profoundly urgent for the proficient working of the C4 pathway. These are firmly interconnected to one another with enormous numbers of plasmodesmata (Dengler et al. 1994; Muhaidat et al. 2007; Wang et al. 2017). Studies on leaf life systems and morphology have uncovered a few qualities answerable for the development, improvement or deformations of cells in leaves (Table 2). So as to have a Kranz life system designing, the SCARECROW/SHORTROOT administrative system has been

Table 2 Specific gene responsible for the development of transgenic lines of C4 rice

Gene	Gene function	References
ACAULIS1	Elongation of leaf cells	Tsukaya et al. (1993)
CURLEY LEAF (CLF)	Curling leaves in *Arabidopsis*	Kim et al. (1998)
Rotunda 1 (RON1)	Venation pattern and rounded leaf structure	Robles et al. (2010)
Scarecrow	Irregular diversity of BS chloroplast, alteration in vein density	Slewinski et al. (2012)

found out to be one of the significant segments. This is on the grounds that the leaves of C3 plants with transformed *Scarecrow* gene was ordinary, while in the C4 plants, transformation in a similar gene harmed the Kranz systems (Slewinski et al. 2012); the C3 leaf life systems can be adjusted by overexpressing the maize Scarecrow quality in leaf tissue (Tolley et al. 2012).

6 Figure Out Transcriptional Factor and Computational Approach

One of the fundamental difficulties of C4 rice that is inquired about is to distinguish the administrative components, which includes translation variables and microRNAs, controlling enhancement of a number of highlights of C4 photosynthesis. The only well-known transcription factors including Golden2-like (GLK1 and GLK2) are members of Myb families regulating nuclear transportation and chloroplast development in plant genes and DOF family proteins whose function is not clear (Chen et al. 2016). In spite of the fact that recognition of administrative elements (counting each of the translation factors and microRNAs) and clarification of the contraptions whereby these variables manipulate C4 development is nonetheless at the early stage, the combo of excessive throughput 'omic' data with quite a number of informatics calculations gives an extraordinary outlook to perceive hereditary manipulation of C4 improvement. In this regard, searching over cell-explicit framework stage transcriptome, proteome and metabolomics studies of main C4 is very well-timed (Nelson et al. 2008). To achieve the result, algorithm studies will help to identify -cis/-trans regulatory components governing a precise biological progression by sequence analysis and gene expression analysis by high-throughput data model (Siddharthan et al. 2005). After both unique transcription components and microRNAs are known by computational methodology, it can be cross checked through transgenic test to validate with their biological function. In addition to figuring out essential regulatory factors, computational algorithms are required to evaluate the coding and regulatory genomic sequence in C3 and C4 species to distinguish adaptable critical motifs associated with C4 progression and assess omics data throughout unique evolving or evolutionary levels or tissue kinds or extraordinary conditions (Zhu et al. 2010). In this regard, the requirement-based modelling holistic approach can be applied and utilized effectively in detecting and designing genetic and molecular studies for better crop improvement.

7 Challenges and Future Prospective

It is unfortunate that development and advancement of molecular biology, system biology and genetic technology fitted out with skilled hand and extensive laborious researchers to find new and ideal strategy to achieve the goal on time. The complicated nature of C4 anatomy exhibits that its integration into C3 plants will require a remarkable trial. The complete insertion of working C4 toolbar having biochemical, molecular and anatomical functionality will be only possible when evolutionary development blueprint and modern approaches applied together. Filling information gaps with scientific data will also modify our planning and strategy of work with every advancement. Existing strategies for hereditary designing are most possibly missing for its establishment, and the constructing task will possibly increment as we distinguish greater gene prototypical to C4. Taking the entirety into account, altering over a C3 harvest to C4 photosynthesis is an extraordinarily difficult aim to preserve up a C4 plant in a handy way to alleviate world appetite. The main hurdle is to develop bundle sheath must be capable of performing C4 decarboxylation and refixing of CO_2 to minimize the photorespiration. To achieve the perquisite goal, skilled hand equipped advanced molecular approach and scientific interpretation of data and knowledge related to physiology, biochemistry, molecular biology and agronomy to date would be required to develop efficient C4 mechanism in rice plants. The extent of our perception of photosynthesis truly shows that ample scope is left for the enchantment and regulation of this ancient and critical biological reaction to gain our dreams of sustainable meals production.

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Rice Tolerance to High Light Intensity and UV Radiation Through Biotechnological Approaches

Samrat Bhattacharyya

Abstract

Global climate change has brought about deleterious consequences like stratospheric ozone depletion, leading to high white light irradiance and unprecedented UV ray exposure which might impact staple crop plants as the most delimiting abiotic factor. Alarming ozone deficiency as much as 4% over the tropical rice-growing continents including Asia can severely affect the agricultural turnover. As a result, a multitude of morphological, physiological and genetic alterations are categorically expected for rice cultivars and agro-ecosystems at expositions of increased irradiative and UV exposure-related stress. On a phenotypic note, visible changes like stunting, leaf blotching, spikelet number alteration and grain count reduction have been observed and compared on varietal rank leading to the qualitative identification of resistant and susceptible lines. Insights over physiological as well as genetic basis of UV and high light intensity stress have revealed mechanisms of injurious responses like photosynthetic suppression, increase in non-photochemical quenching as a consequence of the photoinhibition of PSII, lipid peroxidation and protective accumulation of UV screen guarding compounds, differential expression of proteins involved in detoxification or antioxidation and various other metabolic processes. Understanding of the various common molecular responses against UV/light stress has elucidated categorical genetic and protein-level deformities which can be overbridged by selective breeding-, transcriptomics- or proteomics-based approaches. Several crop improvement programmes have been attempted already with the effort of overexpressing pivotal genes concerned with UV stress resistance. Contrastingly, a comprehensive range of betterment benefits have also been documented for certain rice varieties under UV stress, such as increased accumulation of antioxidant compounds, upheaval of total nitrogen and elevated

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storage protein content in grains. Thus, certainly a plethora of parameters concerning the UV or high light intensity stress amelioration need to be reviewed and analysed for the wholesome quality improvement of rice through biotechnological advancements in the near future.

Keywords

UV/high light stress · UV-susceptible/resistant rice · Photo-protective mechanisms · UV responsive signalling · UV resistance candidate genes · CPD-photolyase · UV-stressed rice nutrients

1 Introduction

1.1 Prime Importance of Rice and Its Vulnerability to Global Climate Change

Changes in the global climate are magnifying in a faster pace which can significantly affect human health, natural aquatic/terrestrial ecosystems, and agricultural standards. Worldwide attention has recently turned to these issues, and scientists from many disciplines and many countries are working to assess the potential magnitude and modes of the changes that are yet to be visualized. If global climate changes anyhow reduce food production, serious long-term consumption shortages and aggravation of socioeconomic balance would disrupt life on this planet. Unambiguously of all the world's crops, rice is the most important one for human consumption due to some prime cause. It is the staple food for over half of mankind, with at least two billion people in Asia alone depending on rice for much of their daily caloric intake. More than 90% of the world's rice production is in Asia, primarily in an area from approximately 40°N latitude to 10°S latitude, including 19 countries. China and India grow the most rice, with 41% and 20% of the Asian production, respectively, in 1985–1987 (IRRI 1989). Nearly three fourths of rice production is from irrigated fields, especially in China. So, whatever climatic adversity might come in future, improvement of rice has to be a central focus in the rice-dependant portions of the world. In this advanced era, enhanced UVB radiation and high light irradiance is a global climate change hazard threatening the tropical rice growing regions. Increases in UVB radiation are accentuating due to the dramatic depletion of stratospheric ozone layer over the recent years. Thus, time has come to concentrate and channelize our knowledge to understand the UV or high light–induced stress biology of rice.

1.2 Consequences of UV/High Light Exposure Observed in Plants

The first and foremost approach should be the field-level identification of UV/high light–stressed rice phenotypes. As a result of UVB radiation, initially bronzing or glazing of leaves are observed which is followed by the development of irregular patches and with prolonged exposure, these patches gets converted into brown necrotic spot and dead patches (Singh et al. 2008). Reduction in leaf area occurs due to destruction of photosynthetic pigments, but to cope up with the situation and to increase photosynthesis, the number of tillers tends to increase in monocots. High irradiance also imparts more or less similar phenotype during prolonged summers. Different degrees of resistance modes existing between different japonica and indica rice cultivars can be studied to improve field selection trials. The molecular response of UV/high light stress mainly includes physiological impairment and cellular or DNA damages. Main physiological targets of UV radiation are photosystem II, D1 and D2 proteins involved in photosynthesis (Fiscus and Booker 1995). According to many previous reports, almost all components from Mn-binding sites to plastoquinone acceptor sites within PS-II on thylakoid membrane are sensitive against UVB. Moreover, some indirect effects of UVB are also observed which alters the rate of photosynthesis, such as stomatal closure and destruction of Rubisco Large subunit in chloroplast. On genetic level, UV induces the formation of cyclobutane pyrimidine dimers (CPDs) and pyrimidine-6-4-pyrimidone photoproducts (64PPs) which damage the DNS and abrogate DNA replication. Further dangerously, UVB stress leads to the production of reactive oxygen species. Rao et al. (1996) proposed that UVB activates membrane-localized NADPH oxidase, which then leads to the generation of ROS. Plants including rice possess intrinsic combatting mechanisms to neutralize UV-induced stresses. Several strategies are applied by plants to acclimatize and metabolize ROS. These include photolyase-mediated DNA repair, active defence systems employing low molecular weight antioxidants such as ascorbic acid, phenols, flavonoids, glutathione, carotenoids, etc. and enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT), etc. (Cervilla et al. 2007).

1.3 Conceptions and Approaches Towards Improvement of Rice

Understanding of all the defence mechanism enable us to get hold of the UV-responsive signalling pathway operating in plants. Further enlightenment can be achieved by studying the UV/high light–induced transcriptomic and proteomic signature. Upregulated mRNA or protein level after experimental UV treatment gives the precise idea about pivotal genes involved in UV stress alleviation. Biotechnologically, this UV-responsive protein characterization approach paves the way for crop improvement. Initially, quantitative trait loci identification and selective marker-assisted breeding started to proximately pay off well in rice improvement programmes. Fascinating enough, the recent trend of stress biology-related research has advanced towards transgenic rice development conferring hyper-resistance against UV/high irradiance. Suitable candidate genes which have so far been tested include CPD photolyase encoded by the qUVR-10 locus (Ueda et al. 2005) and a silicon transporter protein Lsi 1 (Fang et al. 2011). Other promising genes which could soon be used in high irradiance stress combatting large-scale

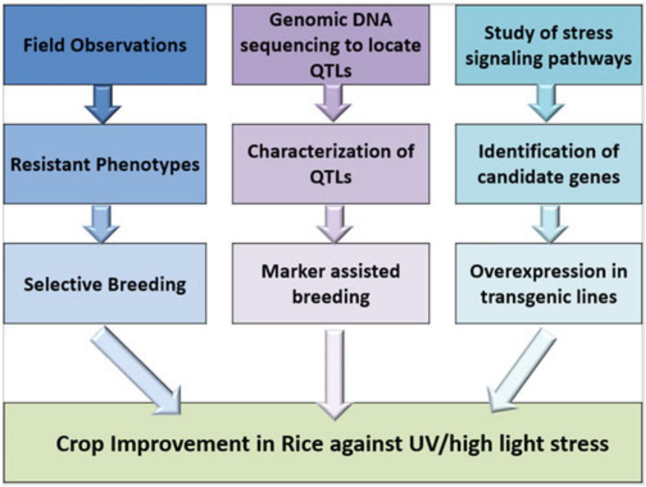

Fig. 1 Different approaches leading to rice improvement against UV/high irradiance

transgenic development include Fibrillin5 (OsFBN5) involved in plastoquinone synthesis (Kim et al. 2017) and geranylgeranyl reductase (OsGGR2) with functions in antioxidant tocopherol biogenesis (Kimura et al. 2018). Preparing for a harsh future, it should also be considered to try making the best out of UV-exposed rice harvest. It has also been experimentally observed that UV stress can hike total nitrogen content and amino acids in rice grains (Hidema et al. 2005). Similar studies on this direction might unravel more wonders leading to the induction of a super-crop in future. Various approaches of UV/high irradiance tolerant rice crop improvement are synoptically furnished in Fig. 1.

2 Ozone Layer Thinning Potentiates UV and High Light Exposure

2.1 Global Ozone Decrease Scenario

Depletion of stratospheric ozone has led to a significant increase in UV radiation reaching the surface of the Earth (McKenzie et al. 2007). This is predicted to continue in the nearest future (Caldwell et al. 2003). The global atmospheric change scenario plays the central role in ozone layer weakening, and the threat is being intensified due to the bulk increase of certain gases. Chief among the effects of these gases is the trapping of solar energy at the Earth's surface in the form of heat. Such gases are often termed 'Greenhouse gases' because they trap solar heat just as greenhouse glass does. The concentrations of these gases have a powerful influence on the average global temperature of the planet. One of such most harmful atmospheric trace gases, the chlorofluorocarbon (CFC), has the most destructive effect because its photodegraded active radical products act to deplete ozone (O_3) in the stratosphere. In addition to CFCs, concentrations of other greenhouse gases such as carbon dioxide (CO_2) and methane (CH_4) have increased significantly in Earth's atmosphere since preindustrial times. Certain notable points about the increment pattern of CO_2 are: (1) A doubling of atmospheric CO_2 concentration to over 600 ppm is predicted within 100 years. (2) The actual rate of CO_2 increase will vary depending on the magnitude of the increase in future emission rates for CO_2 and carbon cycling feedbacks with the atmosphere, oceans and biosphere. (3) Global air temperature generally is predicted to increase by 3–5 °C with a doubling of atmospheric CO_2 (Schneider 1989). Whatever the magnitude of the predicted temperature changes, the pattern of temperature increase would vary over tropical rice-producing areas depending on geographical factors. Relevant data shows that between 1969 and 1987, estimated stratospheric ozone depletion over Asian sky was 1–4% (Watson and Ozone Trends Panel 1989) which is most likely to further hike up.

2.2 Harmful Effects of Peroxy Radicals

Ozone can be destroyed by a number of free radicals such as hydroxyl free radical ($OH^\cdot$), nitric oxide free radical ($NO^\cdot$), chlorine free radical ($Cl^\cdot$), peroxy radical ($HO_2{}^\cdot$) and superoxide radical (${}^\cdot O_2-$), and these are abundant in certain organic compounds especially the CFCs. The Cl and Br atoms are liberated from the parent compounds by the effect of ultraviolet radiation.

$$CFCl_3 + \text{electromagnetic radiation} \rightarrow CFCl_2 + Cl$$

These free radicals can eventually destroy ozone molecules through various reactions. The simplest example of such reactions is a chlorine atom reacting with ozone molecule to form ClO and leaving an oxygen molecule. The newly formed chlorine monoxide (ClO) reacts with the second molecule of ozone to serially form another chlorine atom and two molecules of oxygen (Rai and Agrawal 2017).

$$Cl + O_3 \rightarrow ClO + O_2$$
$$ClO + O_3 \rightarrow Cl + 2O_2$$

2.3 Spectral Categories of Ultraviolet Radiation

The UV radiation spectrum which is directed towards earth can be divided into three regions: UVC (190–280 nm), UVB (280–315 nm) and UVA (315–400 nm). UVC, which comprises highly energetic wavelengths, is eliminated by the stratospheric ozone layer and is not encountered by plants (Pattanaik et al. 2007). UVB radiation reaches the earth's surface. Incident UVB radiation (in particular, the waveband 297–310 nm) is increasing due to the reduction in the stratospheric ozone concentration (Caldwell et al. 2003). The UVA region of the spectrum is not attenuated by ozone, so its radiation will be unaffected by the reduction of the ozone layer.

Special considerations regarding the UV radiation estimates on rice-growing areas are as follows: (a) Under clear-sky conditions, the ambient UVB flux in tropical rice-growing areas is already among the highest on the earth's surface because the stratospheric ozone layer is naturally thinner than higher latitudes. Thus, with stratospheric ozone depletion, the UVB flux in tropical areas is likely to exceed that experienced anywhere in the world. (b) Clouds can reduce UVB transmission through the atmosphere. This way, in the tropics where there is a strong monsoon-driven seasonality in cloud cover, actual UVB radiation during certain times of the year may be lower than that predicted for clear skies. So, climate change–driven monsoonal shortening can detrimentally increase UV exposure.

2.4 Threats of High Light Irradiance

Another emergent problem directly associated with the lessening of stratospheric density is high light irradiance. Although crop plant stress responses to light intensity and quality vary along the latitude and altitude gradients, but uniformly it exposes to simultaneous stresses all over the globe, e.g. excessive light-generated heat shock and drought stress due to gross increase in evaporation of ground water. Sustained changes in irradiance can induce alterations of rice yield massively in the days to come (Banerjee and Roychoudhury 2016). Rice plant requires about 1500 bright sunshine (BSS) hours between the period from field implant to maturity (Bharali et al. 1994). Under synthetic experimental setup, light intensity of 300–500 μmol photons m^{-2} s^{-1} for 14 h photoperiod simulates the ambient light condition. On the other hand, experimentally 700–1000 μmol photons m^{-2} s^{-1} intensity range denotes high light intensity range which is shown to deleteriously affect crop survivability (Huang et al. 2013). Perceived concepts of irradiance stressed damage highlight the facts like: (a) Plant responses to light differ based on the lighting environment, season, genotype and cultivation practices in the tropics (Kozai et al. 2016). (b) The accumulation of reactive oxygen species (ROS) is the result of a disturbance between supply and demand for electron transport end products which is triggered under high light stress (Vranová et al. 2002). (c) Rice as a C3 plant is more vulnerable to light intensity induced than alternative C4 crop because of increased photo-respiratory stress (Li et al. 2009). (d) Carbon assimilation, immobilization and nitrogen metabolism face a great imbalance under prolonged high light exposure (Poolman et al. 2013).

All these parameters need to be accounted and understood in a better way to combat adversities of increasing UV radiation and high light irradiation threat over rice cultivation.

3 Phenotypic Changes of Rice Manifested Under UV Radiation and High Irradiance

The general responses induced by UVB radiation in plants have been reviewed during last few decades by numerous authors. To estimate the UVB effects, in vitro experiments have been conducted within small growth cabinets, greenhouses or in the field supplementing white light with artificial UVB radiation either in a light shut off–turn on way along different time periods or in a sunlight tracking way by supplementing the ambient solar UV radiation with the desired amount of external UVB irradiation.

Laboratory and greenhouse experiments are useful in elucidating UV stress responses and accompanying mechanisms; however, they are less reliable in providing estimates for natural field, where many environmental parameters such as water and mineral stress and/or high sun light irradiances are often interfering. With such artificial experiment setup, it is difficult to maintain the balance between UVB and other spectra and to assess the exact UVB effects on plants. To overcome this drawback, an appropriate system can be employed in which the UVB lamp output is controlled proportionally to the ambient solar UVB irradiation on the field, which would be more realistic (Nouchi and Kobayashi 1995).

On a phenotypic note, manifestation of UVB-caused damage has been critically observed in rice. Different cultivars have shown a wide array of morphological and anatomical changes after being treated with enhanced UV irradiation in experimental conditions. All the UV/high light–incited phenotypes of vulnerable varieties have been presented in Table 1. Details of the observable morphological manifestations are being discussed.

Table 1 Morphological manifestations of UV/high light exposure

Nature of radiation	Phenotypes	Susceptible rice varieties	References
UVB exposure	Stunting of growth	Amarelao, IR45, Norin 1	Barnes et al. (1993), Hidema et al. (1996)
	Elongated internode	Fujisaka 5, Cafuringa 1, ROK 5	Barnes et al. (1993)
	Leaf area reduction	IR45, IR74	Dai et al. (1992)
	Necrotic spots	Calero	Basiouny (1986)
	Leaf browning	Norin 1	Hidema et al. (1996)
	Reduced panicle number	Sasanishiki	Hidema et al. (2005)
	Grain count fall	Sasanishiki	Hidema et al. (2005)
High white light exposure	Increased leaf thickness	pgl, Z802	Gu et al. (2017)
	Constitutive paleness	pgl	Gu et al. (2017)

3.1 Morphological Alteration Caused by UV Exposure

3.1.1 Dwarfing/Alteration of Plant Length

Most frequently observed effects of UVB radiation on seedling growth is stunting or dwarfing due to the reduction in internode length. In monocot cereals, stunting may also be caused by shortening of leaf sheath, since the height of culm (stem morphotype of grass family) depends on the length of sheathing leaf bases that cover the internodes. Vast range of stunting responses have been documented by rice cultivars.

- According to the presentation of Barnes et al. (1993) 9 out of 30 cultivars studied showed stunting with varieties Amarelao and IR45 with the greatest stunting while Fujisaka 5, Cafuringa 1 and ROK5 surprisingly showed increase in plant height.
- Japanese rice Norin 1 cultivar was found highly sensitive to UV radiation–induced damage and showed severe growth stunting (Hidema et al. 1996).

3.1.2 Adaptive Changes/Symptoms in Leaves

Morphologically and anatomically, leaves generally show the following changes: (a) expansion or reduction of leaf area, (b) enhanced mesophyll parenchyma layers beneath upper epidermis and hence rise in specific leaf weight, (c) increased wax content over cuticular layer, etc. Such adaptive changes are found in many crops and monocot cereals along with rice.

- Demonstrations by Barnes et al. (1993) showed both decreases and increases in leaf area in case of 30 rice cultivars under UV stress.
- Leaf dry weight and leaf area of the rice cultivars, IR 45 and IR 74, were significantly decreased after 4 weeks of UVB treatment as documented by Dai et al. (1992).

  On a different aspect, enhanced UV radiation can also derogate leaf homeostasis and might induce symptoms like leaf blotching and chlorosis.
- Basiouny (1986) reported leaf necrosis in the rice cultivar ‘Calero’.
- In the susceptible Japanese cultivar Norin 1, severe leaf browning was reported (Hidema et al. 1996).

3.1.3 Inflorescence and Grain Count

Anomalous flowering responses of plants to UVB irradiance have been exhibited in several crops. Such symptoms in case of rice varieties include: (a) delayed panicle emergence, (b) stunted or reduced panicles, (c) abortion of flowers leading to reduces in grain count or incomplete grain filling, etc.

- No significant ($P < 0.05$) effects of the enhanced UVB were observed at harvest, on growth parameters such as panicle number or grain filling in case of Japonica rice cultivar, ‘Koshihikari’, and two Indica rice cultivars, ‘IR 45’ and ‘IR 74’ (Kim et al. 1996).

- In another study, panicle number was significantly reduced by supplementary UVB radiation in the Japanese variety Sasanishiki. Such reductions were enhanced by unusual climatic conditions such as lower temperature and lesser sunshine. Also, sensitivity to the inhibitory effects of supplementary radiation in a UV-sensitive cultivar was more greatly enhanced by such unusual climatic conditions than in a UV-resistant cultivar (Hidema et al. 2005).
- Notably, supplementary UVB radiation more profoundly affected grain count, and the grain size also decreased significantly in japonica cultivar Sasanishiki (Hidema et al. 2005).

3.2 Phenotypic Change Caused by High Light Exposure

High irradiance stress exerts more or less similar visible changes in plant morphology like that of UV radiation but has a lesser magnitude. Also experimentally high irradiance conditions are easier to set up and apparently closer to the realistic field environment. Typically, high-intensity light can be provided by a fluorescent PL-L tubes, supplemented by low-intensity tungsten filament bulbs maintained with appropriate photoperiod in growth chambers (Murchie et al. 2005). Phenotypic changes under high irradiance in rice include the following.

3.2.1 Changes in the Leaf Morphology/Anatomy

Leaf thickness and architecture get highly influenced if grown in high irradiance due to expansion of mesophyll tissue demanded by higher rates of photosynthesis. In indica rice cultivar genotypes pgl and Z802, leaf thickness was greatly improved with increasing light intensity and elevated leaf thickness also resulted in a corresponding increase in leaf dry matter per unit area (LMA) (Gu et al. 2017). Paleness of leaves due to decreased chlorophyll content has also been shown to be advantageous in terms of photosynthetic acclimation under higher light in rice (Gu et al. 2017). Theoretically, decrease in leaf chlorophyll content is assessed to be evolutionarily advantageous in high-light and high-temperature environments (Tardy et al. 1998).

3.2.2 Changes in Flowering/Grain Yield

Singular factor effect of high irradiance on rice panicle number or emergence and grain yield is not well reported till date. Although low white light irradiance plays limiting role in flowering and grain filling in rice as per several reports (Dutta et al. 2017).

3.3 Comparative Note on Resistant Field Varieties

3.3.1 UV-Resistant Varieties

There is a considerable intra- and interspecific variability in sensitivity of crop plants to UVB radiation, and the sensitivity varies widely among species and cultivars

(Correia et al. 1998). Teramura et al. (1991) have shown that Asian rice cultivars differ in their responses to elevated UV radiation in terms of growth and physiological processes and mentioned that the geographical location where the cultivars had been domesticated might have influence over sensitivity to UVB. Conversely, Barnes et al. (1993) demonstrated that the geographical location where the cultivars had been cultivated had no assertive effect on radiation sensitivity. Sato and Kumagai (1993) examined the vulnerability to UVB radiation of 198 rice cultivars, belonging to five Asian rice ecotype categories under indica group (aus, aman and boro from the Bengal region and tjereh and bulu from Indonesia) and Japanese lowland and upland rice under popular japonica group. They found that sensitivity to UVB radiation varies widely among different cultivars belonging to the same ecotype. Among the Japanese lowland rice cultivars, Sasanishiki is more resistant to UVB radiation, while Norin 1, a progenitor of Sasanishiki, is less resistant (Kumagai and Sato 1992). In addition, they found that rice cultivars originating from regions with higher ambient UVB radiation do not necessarily exhibit higher levels of tolerance. Many rice cultivars belonging to the Japanese lowland rice group, and the Indonesian boro ecotype, are resistant to UVB, while the more sensitive cultivars were found to belong to the aus and aman ecotypes from the Bengal region. It is conventionally speculated that spontaneous mutation of UVB sensitivity in rice has occurred throughout the various regions in which the crop has been cultivated.

3.3.2 High Light Exposure Resistance

When the resistance or acclimation against light intensity stress in concerned, lowering leaf chlorophyll content has been shown to act as a photo-protection mechanism, mitigating the damaging effects of high irradiance and high temperature in wild grasses and cereal landraces adapted to semi-arid environments (Royo et al. 2014). However, in rice, it is unknown whether and to what extent photo-oxidative stress is relieved and solar energy conversion efficiency is improved. High irradiance stress-resistant rice cultivars are quite under-reported except a few prominent instances. As such an example, in a comparison between two closely related indica cultivars, chloroplast development and photosynthesis were reportedly improved in the pgl (pale green leaf) genotype under high light, while an opposite trend was found in Z802 genotype (Gu et al. 2017). So it can be fairly cited that selecting a low chlorophyll rice genotype like the irradiance-resistant pgl might further intrigue the search for similar varieties in future.

4 Molecular Response of UV Exposure and High Light Irradiance in Rice

4.1 Physiological Manifestations of UV and High Light Intensity

The effective reactions of the rice crop to high UVB irradiation and high irradiance in terms of net physiology are complicated and multifaceted, since growth stages, canopy reactions, levels of visible radiation before and during treatment, cultivar differences and related other factors have culpable contribution. Nonetheless, a few in vitro glasshouse-regulated experiments have revealed quite a few plausible physiological alterations. All the injurious or defensive physiological responses with corresponding sensitive and resistant varieties have been arranged in Table 2.

Table 2 Physiological/cellular injurious and protective responses of rice under UV exposure and high irradiance

Nature of radiation	Physiological effects	Sensitive varieties	Resistant/least sensitive varieties	References
UVB exposure	Stomatal resistance	IR74	IR64	Dai (1990)
	Imbalance in CO_2 concentration ratio, reduction in $\delta^{13}C$ value	–	IR45, IR74	Kim et al. (1996)
	Degradation of Rubisco sub-units	Surjamukhi, Sasanishiki	–	Fedina et al. (2010)
	$^{14}CO_2$ fixation rate reduction	Norin 1, Sasanishiki	Surjamukhi	Fedina et al. (2010)
	Reduced PS-II activity	Norin 1, Surjamukhi	Sasanishiki	Fedina et al. (2010)
	Lowered chlorophyll	Koshihikari, IR45, IR74	–	Kim et al. (1996)
	Loading of non-photo synthetic pigments in vacuoles	Norin 1	Purple rice	Fedina et al. (2010), Hada et al. (2003)
	Defective CPD photolyase activity	Norin 1	Surjamukhi, Sasanishiki	Teranishi et al. (2004), Hidema et al. (2005)
	Decimation of IAA, GA1/3	Shan you 63, Nancheum, IR65600-85	–	Lin et al. (2002)
High-ambient white light exposure	Photo-inhibition	Z802	pgl	Gu et al. (2017)
	ROS accumulation	Z802	pgl	Gu et al. (2017)
	Increase in carotenoid pool	NPT	IR72	Murchie et al. (2002)

4.1.1 Stomatal Resistance to UV Stress

Stomatal conductance is a critical and variable factor which governs the responses of plant physiology to UVB. Therefore, intra- and inter-specific variations in the impacts of UVB often correlate with the intrinsic capacity of stomatal conductance; plants with higher rates of stomatal uptake exhibiting greater effects (Mansfield and Freer-Smith 1984). In rice background, research by Dai (1990) pointed out that stomatal resistance increased with enhanced UVB in glasshouse conditions. This is partly the resultant effect of stomatal closure and the collapse of the guard-cell flaccidity especially in UVB-sensitive cultivars. Stomata of rice plants exposed to UVB opened later than those in the control plants when returned to normal conditions. This response may indicate less efficient CO_2 exchange and lower transpiration rates in UVB-sensitive rice plants. With closed stomata, the effective higher leaf temperatures and lower CO_2 decreases the photosynthetic rates and eventual biomass production in rice. Ranking cultivars on the basis of relative changes in total biomass production related to stomatal resistance, between UVB irradiated and control plant, IR74 was found to be the most sensitive and IR64 the least sensitive.

4.1.2 Photosynthetic Rate Alteration by UV Radiation

4.1.2.1 Impairment of Overall Photosynthetic Capacity

UVB radiation potently encroaches most of the aspects of photosynthesis, but the main target of UVB is PS-II (Booker et al. 1992). PS-II is comprised of two proteins, namely D1 and D2 which are very sensitive against UVB. The impairment of PS-II can be measured in terms of decreased oxygen evolution or variable chlorophyll florescence (Rai and Agrawal 2017). It has been vividly reported that photosynthetic capacity is reduced by UVB radiation in many plants (Teramura 1983). However, the work of Kim et al. (1996) suggested no significant effect of the enhanced UVB irradiation on the $\delta^{13}C$ values of the flag leaves in two sensitive indica cultivars, IR45 and IR74. Since the CO_2 concentration in the stomata is affected by the activity of photosynthetic carbon assimilation and/or stomatal aperture, the UVB enhancement under other conditions may not have affected photosynthetic carbon assimilation or stomatal activities.

4.1.2.2 Loss of Main Photosynthetic Enzyme

Decimation of the main photosynthetic enzyme Rubisco is indeed a primary factor in UVB-induced inhibition. Supplementary UVB radiation results in a reduction in the amounts of total leaf nitrogen, soluble protein and Rubisco in fully expanded leaves in rice (Hidema et al. 1996). In another study by Fedina et al. (2010), the UVB-affected photosynthetic response of three rice cultivars (*Oryza sativa* L. cvs. Norin 1, Sasanishiki and Surjamkhi) was documented. The $^{14}CO_2$ fixation rate of Surjamkhi was higher in comparison to Norin 1 and Sasanishiki when the cultivars were subjected to visible radiation alone. UVB treatment moderately reduced the photosynthetic activity of the tested cultivars. The $^{14}CO_2$ fixation declined by 72% in Surjamkhi, by 63% in Norin 1 and by 52% in Sasanishiki. The amounts of Rubisco large (LSU) and small (SSU) subunits were similar in the non-UVB-treated rice cultivars. As a result of UVB irradiation, the strong reduction in Rubisco LSU and SSU contents was observed in Surjamkhi and relatively lower in Sasanishiki. UVB treatment did not influence PS-I activity in cultivar Sasanishiki, since there was no change in far-red induced P700 oxidation. The photochemical activity of PS-II in Surjamkhi decreased as a result of UVB treatment. As the outcome of UVB irradiation, ΦPSII was reduced by 11% and 24% in Norin 1 and Surjamkhi, respectively, while in Sasanishiki it was not affected.

4.1.2.3 Reduction of Chlorophyll

Elevated UVB radiation reduces the chlorophyll content in many plant species; this occurs due to lipid peroxidation in chloroplast membrane. The photo reduction of proto-chlorophyllide to chlorophyllide by proto-chlorophyllide oxidoreductase is one of the possible targets of UVB (Marwood and Greenberg 1996). Because this reaction is light driven, it is possible that UVB can damage this enzyme resulting in lowering rate of chlorophyll accumulation. UVB downregulates the expression of genes, which encodes for chlorophyll a/b binding proteins (Casati and Walbot 2003). It has been reported that chlorophyll content in rice leaves is changed by UVB enhancement, but that the direction and extent of the change varies among cultivars (Nouchi and Kobayashi 1995). Study by Kim et al. (1996) exhibited that the decrease of chlorophyll content was found up to the third leaf in some japonica (Koshihikari) and indica (IR45, IR74) cultivars. This suggests that the effect of UVB irradiation on the biosynthetic pathway of chlorophyll is limited to the early growth stage.

4.1.3 Accumulation of Non-photosynthetic Compounds Under UV Stress

The activation of the genes of phenyl propanoid pathway producing phenolic compounds is a common manifestation of UVB stress in plants. Increase in concentrations of UVB-absorbing compounds including phenolic acid and flavonoids is the most consistent response of plants to elevated UVB radiation. Another class of compounds to be contextually mentioned are the flavonoids. UV radiation–induced flavonoids and flavonoid glycosides include common compounds like quercetin and kaempferol (Rai and Agrawal 2017). Accumulation of flavonoids, in the leaf epidermis, has been suggested as an acclimation policy against UVB radiation. Significant increase in UV-absorbing compounds in several rice cultivars exposed to UVB radiation was found under greenhouse setup (Teramura et al. 1991) and growth chamber experiments (Dai et al. 1992). In a field experiment, the change in concentrations of UV-combatting compounds in the third leaves and the flag leaves of IR 45 and IR74 tended to be higher with the enhanced UVB radiation (Kim et al. 1996). The mechanism of UV and high irradiance defence by accumulated antioxidant compounds will be discussed later in this chapter.

4.1.4 Decimation of Hormones in UV-Treated Rice

Quite limited studies on alterations of phyto-hormone synthesis have surfaced so far in the context of UV treatment, and the work of Lin et al. (2002) deserves solitary mention in this regard. In the said study, Shan You 63 (Sy63), Nancheum (NC) and IR65600-85 were the chosen experimental varieties. The result indicated that the contents of IAA and GA1/3 were significantly reduced in all rice cultivars used for this experiment under enhanced UVB radiation treatments for 7–28 days, in which the contents of IAA and GA1/3 were decreased by 58.92% and 45.48% in the leaves of Sy63, by 43.31% and 56.20% in the leaves of NC, and by 38.60% and 47.33% in the leaves of IR65600-85. On the contrary, endogenous content of ABA was significantly increased by 14.4%, 99.6% and 56.7%, respectively, in the three rice cultivars concerned which led to various degrees of growth suppression.

4.1.5 Photosystem Hindrance Under High Irradiance

4.1.5.1 Photo-Oxidative Stress Under High Light Intensity

The energy of irradiated light is often much higher than the demand for photosynthetic metabolism of NADPH and ATP (Yamori and Shikanai 2016). Excess excitation of plant photosystems results in over excitation of the reaction centre, causing chlorophyll molecules to attain a triple state, 3P680∗ (Hideg et al. 1998). 3P680∗ reacts rapidly with O_2, resulting in the production of deleterious singlet O_2 ($^1O_2$) (Vass and Cser 2009), which oxidatively degrades D1 protein, a PS-II reaction centre protein, inactivating PS-II (Krieger-Liszkay et al. 2008). The PS-II reaction centre is so vulnerable to light-induced damage that it has to be rebuilt approximately once every 30 min (Foyer and Shigeoka 2011). Furthermore, inherent limitations in the capacity for electron transport through the cytochrome b6/f complex lead to the production of photo-excited Chl, 1Chl∗, and its triplet state, 3Chl∗, causing $^1O_2$ accumulation in the thylakoid membranes (Telfer 2014), which stimulates peroxidation and degradation of membrane bilayers.

4.1.5.2 Genotypic Difference in Photo-Inhibition

Observations published by Gu et al. (2017) concerned with light capture and light energy conversion in rice, a genotype with pale green leaves (*pgl*) and a normally pigmented control (Z802) were subjected to high (HL) and low light (LL). *Pgl* had a smaller size of light-harvesting chlorophyll antenna and absorbed less photons than Z802. *NPQ* and the generation of ROS were also low, while photosystem II efficiency and ETR were high, resulting in improved photosynthesis and less photo-inhibition in *pgl* than Z802. Chlorophyll synthesis and solar conversion efficiency were higher in *pgl* under HL compared to LL treatment, while Z802 showed an opposite trend due to the high level of photo-inhibition under HL. In Z802, excessive absorption of solar energy not only increased the generation of ROS and *NPQ*, but also exacerbated the effects of increases in temperature, causing midday depression in photosynthesis.

4.1.6 Photorespiration Under High Light Intensity–Mediated Stress

The induction of the photo-respiratory apparatus of rice leaves in response to high light intensity is physiologically very important because conventionally, rice farming has certain practices like low light germination, transplantation and greening of rice seedlings in the field, which generally avoid high light exposure (De Datta 1981). Photo-respiratory responses under high irradiance show particular physiological reactions. The induction of glycine-dependent photorespiration proteins by light is widely reported in other crop plant species, such as wheat (Lee et al. 2010). Reports on rice photorespiration show that the mitochondrial proteins involved in glycine metabolism, such as GDC subunits and SHMT, are significantly increased in abundance of light. Moreover, GDC-H could be turned over rapidly after its synthesis. Interestingly, the GDC-L subunit which is known to contain cysteine residues sensitive to damage has been found to get reduced under high light. Suggestively, changes in GDC stoichiometry highlight the importance of mitochondrial rate-limiting components under high light stress (Huang et al. 2013).

4.2 Incurrence of Cellular and Genetic Damage

There are two potential primary mechanisms involved in UVB-induced cellular and biochemical damage. DNA lesions such as cyclobutane pyrimidine dimer (CPD) and pyrimidine (6–4) pyrimidone photoproducts interfere with DNA replication and transcription (Britt 1996). The second mechanism is through the modification of proteins by photo-oxidation or by reactive oxygen species (ROS) and free radicals produced during photosensitization (Foyer et al. 1994). Both the mechanisms are represented as follows.

4.2.1 Genomic DNA Damage by CPD After UV Radiation

DNA is one of the major targets of UV damage, and prolonged UVB radiation is capable of directly distorting its structure. The two main photoproducts formed between adjacent pyrimidines on the same strand are the CPDs and the (6–4) photoproducts (Britt 1996). CPDs account for the majority of the DNA damage (~75%); the (6–4) products account for most of the remainder (Mitchell et al. 2004). The yields of CPD and 64PP are highest at around 260 nm, and the action spectra of their formation are in parallel to the absorption spectrum of DNA. UV induces specific types of mutation: base substitutions of cytosine (C) → thymine (T) at di-pyrimidine sites and CC → TT tandem base substitutions, although the latter rarely occur. These two types of mutation are also called UV signature (Brash et al. 1991), and their methodical detection can be done after moderate exposure to UV. In one of the mechanisms of formation of these UV-specific mutations, deamination of cytosine bases in a CPD is thought to be involved (Tessman et al. 1992). It is also known that 64PP can change into an isomeric secondary product, Dewar valence isomer, after subsequent absorption of UVA wavelengths around 325 nm (Matsunaga et al. 1991). These photo-lesions, CPD, 64PP and Dewar, are assumed to cause UV-specific mutations. It has been known that UVB and daylight UV induce CPDs preferentially at 5-methylcytosine (mC)-containing di-pyrimidine sites. This site preference of CPD formation requires the methylation of cytosines (Tommasi et al. 1997) and is not observed for UVC (Monti et al. 1999). Although cells try to excise these photo-lesions by a DNA repair mechanism called nucleotide excision repair (NER), failure in the repair before replication fork passing would lead to a stall and collapse of the fork at the damaged site, which could cause a DNA double-strand break and result in cell death.

4.2.2 Response of Organelles to UV and High Light Intensity

4.2.2.1 Organellar DNA Damage

In addition to the nuclear genome, the cells of higher plants contain two additional genomes, one in chloroplasts and another in mitochondria. The chloroplast and mitochondria contain genes that encode many proteins important for photosynthesis and respiration. Very evidently, UVB radiation also induces the formation of CPDs in these organellar DNAs leading to deleterious consequences (Chen et al. 1996). However, no genes that encode CPD repair proteins have been identified in the chloroplast or mitochondrial genomes of *Arabidopsis* or *Oryza*.

4.2.2.2 Enzymes and Metabolites of Chloroplast

An article by Poolman et al. (2013) precisely adhering to light intensity–mediated alterations of metabolism in rice pointed out following details regarding organellar responses:

- In the chloroplasts, oxidation of pyruvate and malate during photosynthesis, prevention of excessive reduction reactions and increased use of alternative oxidases in support of maximal photosynthesis are an observed trend under high light seen in rice.
- Export of 2-OG to cytoplasm derived from pyruvate and C4 precursors presumably supports nitrogen metabolism and amino acid synthesis is notable.
- Operation of an incomplete TCA cycle and different as well as fluctuating activities of three enzymes, namely succinate dehydrogenase, fumarase and malate dehydrogenase.
- High-irradiance-mediated inhibition of pyruvate dehydrogenase has also been noted. Thus, it is a strong assumption that the stoichiometric constraints of enzymes and metabolites lead to the fall of organellar functions at high light levels.

4.2.2.3 Accumulation Within Vacuoles

The effect of UV radiation on vacuoles have also been worked out. As a mitigating measure, the vacuoles of purple rice were found to accumulate heavy amounts of anthocyanins and other UV-absorbing antioxidant pigments. This way the need of CPD photo-reparatory activity was effectively minimized in the purple rice (Hada et al. 2003). However, some data showed that there was no significant correlation between the degrees of sensitivity to UVB and accumulation of UV-absorbing compounds in three rice cultivars studied: Norin 1, Sasanishiki and Surjamukhi (Fedina et al. 2010).

4.2.3 Reactive Oxygen Species (ROS)–Mediated Damage Under UV and High Irradiance

UVB radiation produces ROS such as superoxide, H_2O_2 and singlet oxygen (Hideg et al. 1998). Karpinski et al. (1997) reported that an increased H_2O_2 level was detected simultaneously with the inhibition of photosynthesis by UVB radiation, suggesting that the UVB-induced oxidative burst of H_2O_2 is associated with the damage and degradation of the thylakoid membrane. Superoxide radicals are also formed within cells under high light intensity. It has been reported that, in chloroplasts under illumination, the large subunit (LSU) of Rubisco is directly fragmented into two polypeptides by ROS (Ishida et al. 1999).

The experimental detection of ROS can be done with dihydroethidium (DHE) staining and DHE is also considered a marker of cell death, showing higher fluorescence signals in the zone around epidermal cells and vascular bundles (Rodríguez-Serrano et al. 2006). The damage by ROS-like activity can be measured by quantifying malondialdehyde (MDA) content which rises after lipid peroxidation in the membrane (Dhindsa et al. 1981). A comparative experiment with two rice genotypes pale green leaf (pgl) and normally pigmented control Z802 showed that in general, O^{-2} radical accumulation was higher in Z802 compared to pgl, suggesting photoinhibitory damage in the leaves of Z802. This is consistent with the content of MDA, the marker of lipid peroxidation. ROS scavenging enzymatic activity increased in both Z802 and pgl under LL compared with HL. Activities of these enzymes clearly indicate deleterious increase of oxidative stress under HL. Except for peroxidase activity, both superoxide dismutase and catalase activities were higher in pgl than Z802 under HL, possibly due to the photo-oxidative damage in Z802 (Gu et al. 2017).

5 Intrinsic Defence of Plants Against UV Radiation and High Light Intensity

The internal mechanism of defence in plants focussed to minimize and cure UVB, and high light–mediated alterations can be thoroughly discussed if we contextualize intrinsic physiological responses like free radical scavenging, accumulation of UV radiation alleviating pigment compounds, DNA repair methods (photo-repair and dark repair) and UV or high light–triggered signal transduction pathways. On a practical point of view, there is a strong potential application of studies on UV-defence mechanisms to elucidate rice cultivar differences in the UVB sensitivity/resistance, which might possibly lead to the selection of UV hyper-resistant cultivars.

5.1 Reparatory Mechanism for Combating Genetic Damage

In order to remove the DNA replication hampering photo-product lesions from DNA, plants possess two major mechanisms known as photo-repair and dark repair (Britt 1999).

5.1.1 Mechanism of Photo-Repair

Photo-repair involves photolyases and removes photoproducts in vast group of organisms except placental mammals (Menck 2002). Photolyases carry the following two types of cofactors. Flavin-adenine dinucleotide (FAD) is excited by blue/UVA light energy and monomerizes the photoproducts, whereas methenyltetrahydrofolate or deazaflavin acts as the photo-antenna (Britt 1999). Photolyases show substrate specificity to either CPDs and hence called CPD photolyases or 6–4 pps, therefore called 6–4 photolyases. Higher eukaryotic organisms including plants have class II CPD photolyase in them which serves a major role in DNA repair (Kanai et al. 1997).

5.1.2 Dark Repair Mechanism

The other type is dark repair in which photoproducts are removed from DNA by the evolutionarily conserved nucleotide excision repair (NER) mechanism which is present in all organisms. The initiation of NER depends on the location of DNA lesions. When the DNA lesions are scattered throughout the genome, the NER pathway called global genomic repair (GGR) plays a role in repairing damage, while when the DNA lesions are in the regions of transcribed genes, the pathway of transcription-coupled repair (TCR) does the task (Thoma 1999). Another dark repair method where oxidized or hydrated base and single-strand breaks are corrected, is called base excision repair (BER). The damaged bases are removed by BER glycosylases, which cleave the glycosylic bound between the specific damaged base and the deoxyribose, with subsequent incision by AP-endonuclease (aprinic/apyrimidic endonuclease) at the resulting abasic site, followed by DNA synthesis and ligation. Bio-informatics-based identification of plant homologues of BER proteins has been undertaken in *Arabidopsis* and rice (Kimura and Sakaguchi 2006).

5.2 CPD Photolyase Activity and Varietal Difference in Rice

Rice CPD photolyase has been experimentally found to be encoded by a single gene and is targeted not only to nuclei but also to chloroplasts and mitochondria mainly functioning to repair UVB-induced CPDs. In other words, a single DNA repair enzyme CPD photolyase is ‘triple targeted’ in rice cells, functioning simultaneously in three different organelles (Takahashi et al. 2011). A wide range of variation exists in the UVB resistance level among rice cultivars. Most of the Japanese varieties, classified as *japonica* subspecies, are more resistant to the enhanced UVB radiation than most of the *indica* varieties. Among *japonica* varieties, cv. ‘Sasanishiki’ shows higher UVB resistance than cv. ‘Norin 1’. This cultivar difference in the UVB resistance was ascribed to the activity of CPD photo-repair and NER (Hidema et al. 1997). Teranishi et al. (2004) examined the correlation between UVB sensitivity and the capacity for CPD photo-repair in 17 japonica rice cultivars (including four ancient strains) that were the progenitors and relatives of the UV-resistant (Sasanishiki) and UV-sensitive (Norin 1) strains. They summarized that the cultivars could be divided into two groups: a UV-resistant group, with higher CPD photo-repair ability, similar to Sasanishiki; and a UV-sensitive group, with lower CPD photo-repair ability, exemplified by varieties like Norin 1. It was further demonstrated that the photolyase–CPD complex in ‘Norin 1’ was highly thermolabile compared with the photolyase in ‘Sasanishiki’ (Fedina et al. 2010). A cDNA clone of CPD photolyase in ‘Sasanishiki’ was isolated and the nucleotide sequence comparison between CPD photolyase genes in these cultivars indicates that an amino acid change at position 126 of CPD photolyase from Gln in ‘Sasanishiki’ to Arg in ‘Norin 1’ is the cause of a lower activity of CPD photolyase in ‘Norin 1’ than that in ‘Sasanishiki’. On the other hand, many UV-sensitive indica rice cultivars belong to the aus ecotype from the Bengal region (Sato and Kumagai 1993). Surjamkhi, a local variety of the aus ecotype, is more sensitive to UVB radiation than Norin 1. The relative rates of CPD photo-repair among three varieties were found as: Sasanishiki $>$ Norin 1 $>$ Surjamkhi (Teranishi et al. 2004).

5.3 Accumulation of Pigment Compounds to Shield the Exposure of UV and High Light

The accumulation of certain phenylpropanoid compounds such as flavonoids and anthocyanins, in the vacuoles of the epidermal and subepidermal cell layers, plays a significant role in mitigating UVB-induced damage (Bharti and Khurana 1997). Significant increase in UV-absorbing compounds in several rice cultivars exposed to UVB radiation was found in greenhouse (Teramura et al. 1991) and growth chamber experiments (Dai et al. 1992). However, in the case of rice cultivars, it is questionable whether or not differences in UVB sensitivity can be related to varying foliar content of UV-absorbing compounds. Dai et al. (1992) reported no significant correlation between sensitivity to UVB radiation and the accumulation of flavonoids among four rice cultivars. Kang et al. (1998) showed that UV-absorbing compounds in rice leaves were effective in reducing susceptibility to CPD induction by challenge UVB exposure, but had no effect on steady-state CPD levels during growth under chronic exposure to supplementary UVB radiation. Further tracing the question of direct involvement of pigment accumulation, Maekawa et al. (2001) produced near isogenic lines (NILs) for three rice purple leaf genes, *Pl*, *Plw* and *Pli*, with a genetic background of Taichung 65 (T-65), a wild-type rice variety from Taiwan. The *Pl* gene controls expression of chalcone synthase, the first enzyme of the flavonoid biosynthetic pathway (Dooner 1983). Interestingly, the growth of the NILs (T-65 Pl) was reduced significantly by supplementary UVB radiation, despite an increase in the accumulation of foliar anthocyanins and other UV-absorbing compounds. This result makes the isogenic line T-65 Pl well suited for use in investigating the role of anthocyanins and other UV-absorbing compounds in the response of rice to elevated UVB radiation levels. Using these two strains (T-65 and T-65 Pl), Hada et al. (2003) examined: (1) the relationship between changes in steady-state CPD levels and accumulation of flavonoids (anthocyanins and other UV-absorbing compounds) in leaves relative to leaf age; (2) the susceptibility to CPD induction by UVB radiation and (3) the ability to photo-repair CPDs. They showed that flavonoids functioned by effectively reducing susceptibility to CPD induction by a challenge UVB exposure, but not for the steady-state CPD levels during growth under chronic exposure to elevated levels of UVB radiation. In the work of Murchie et al. (2002), acclimation of rice to high irradiance has been shown to involve modular alteration in lutein to beta-carotene ratio and also hike in carotenoid to chlorophyll ratio in the thylakoid. Elucidative finding of the study demonstrated that in IR72 variety, there were increases in the carotenoid (Car) to chlorophyll (Chl) ratio in high light–treated (HL) plants compared with low light–treated (LL) ones, which were because of the decreases in Chl content. In the other chosen NPT variety, the changes in Car to Chl were smaller, indicating that the carotenoid content also fell in HL plants. The composition of carotenoids differed notably in both of these varieties. For IR72, the content of xanthophyll cycle carotenoids increased from below 18% in LL to nearly 30% in HL plants. The changes were smaller in the case of NPT leaves, and hence, the xanthophyll cycle pool size was higher in LL and lower in HL compared with IR72. Thus IR72 could be identified as the better high light–acclimated rice variety.

5.4 Free Radical Scavenging to Diminish ROS Stress Generated by UV or High Irradiance

UV and high light induce the expression of genes encoding various enzymes such as phenylalanine ammonia-lyase (PAL) and chalcone synthase (CHS) that are involved in the phenylpropanoid pathway that generates antioxidant compounds. Plants also have several categories of ROS-scavenging enzymatic systems as well. ROS are rapidly dissipated by antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) ascorbate peroxidase(APX) and glutathione S-transferase (GST), as well as by low molecular weight antioxidants such as ascorbate, glutathione and carotenoids (Mackerness 2000). Flavonoids like anthocyanins act not only as UV filters but also as ROS scavengers (Gould et al. 2002). SOD catalyses O_2- radicals into H_2O_2 and O_2 and CAT converts H_2O_2 into water and O_2, whereas POD dismutates H_2O_2 by the oxidation of co-substrates such as phenolic compounds and/or antioxidants (ascorbate and glutathione). APX is a plant-specific H_2O_2-scavenging enzyme, which is active in chloroplasts in the ascorbate glutathione cycle (Mittler 2002). The non-enzymatic and non-pigment antioxidants are smaller molecules which also play certain roles. Ascorbate and glutathione are localized in the aqueous phase (intracellular fluid), while tocopherol and carotenoids are active in cell membranes (Noctor and Foyer 1998). In all these ways, plausible enhancement of the ROS-scavenging system may help to build up UVB resistance in rice.

5.5 UV Stress and ROS Co-ordinated Signal Transduction

Prolonged UV exposure and ROS generation trigger the expression of UV-protective genes, and ROS delimiting cascades are escalated. UVR8 (UV Response LOCUS8) is identified as an UVB-specific signalling component that stimulates UV-protective genes involved in DNA repair and ROS scavenging pathway under low influence of UVB. Furthermore, it is demonstrated that transcription factors Elongated Hypocotyl5 (HY5) and HY5 Homolog (HYH) mediate the responses of the UVR8-dependent pathway (Brown and Jenkins 2008). Multifunctional E3 ubiquitin ligase, Constitutively Photo-morphogenic 1 (COP1), is a negative regulator of HY5/HYH and causes the repression of photo-morphogenesis like light inhibition of hypocotyl growth. Under supplementary UVB, UVR8-COP1 interaction changes COP1 substrate specificity and activates the expression of UVR8-mediated genes (*HY5*/*HYH*), which incidentally confers UV acclimation of higher plants (Favory et al. 2009). The pathway also has a switch-off mechanism that is essential for optimal plant growth and development. REPRESSOR OF UV-B PHOTOMORPHOGENESIS (RUP)1 and RUP2 regenerate UVR8 homodimers by disrupting the UVR8–COP1 interaction, which halts UVB signalling (Heijde and Ulm 2013). The Radical-induced Cell Death1 (RCD1) protein is a regulator of several ROS and abiotic stress-related responses, interacting with several transcription factors which are involved in the UVB signalling pathway (Jiang et al. 2009). However, the precise UV-induced signal transduction pathway leading to hike of ROS scavenging enzymes is still poorly understood. Interestingly, some plant hormones such as salicylic acid, jasmonic acid and ethylene have been shown to be key regulators of gene expression in response to UVB exposure (Holley et al. 2003). The relevant studies till date have indicated that ROS plays a pivotal role as secondary messengers in a number of UVB signal transduction pathways among key regulators. Therefore, identification of ROS-associated cascades, and early events in the signal transduction pathway following UVB stimulus perception, might provide a useful bioengineering resource for enhancement of the activity of ROS-scavenging enzymes and accumulation of UV-shielding compounds. Figure 2 shows the intricate UV responsive signalling cascades functioning in plants.

6 Various Approaches of Rice Quality Improvement Against UV and High Light Exposure Stress

Understanding of physiological responses of rice under UV or high irradiance stress gives multitude of clues that can potently guide us in course of crop improvement. Such disciplines include selection of UV stress markers, quantitative trait loci (QTL) or resistance conferring cassettes that might help in selective breeding approach. The

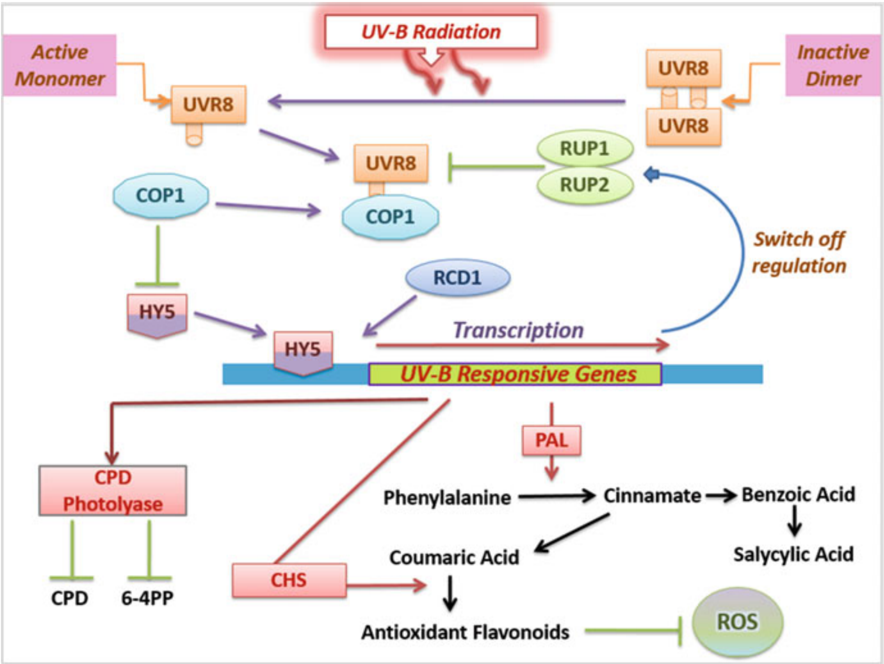

Fig. 2 UVB radiation responsive pathway in plants; UV leads to the formation of active UVR8 monomers which occupy COP1, liberating HY5 transcription factor. RCD1 and HY5 regulate the transcription of UV responsive genes. PAL and CHS synthesize flavonoids that reduce ROS accumulation. Salicylic acid continues hormonal signalling cascade. CPD photolyases cure CPD and 6-4PP lesions on DNA. There is also a switch off mode governed by RUP1/2 which release COP1 from UVR8, leading to HY5 blockage and inactive UVR8 dimer formation

thorough understanding of the transcriptomic and proteomic signature of rice under UV or high light gives us better idea about the functions of genes involved in effective signalling cascades. Identification and overexpression of resistance genes through transgenic approach has already started paving the way for modified super-resistant crop production. All these methods of crop improvement against UV and high irradiance stress are keys to secure the yield of rice in future if ozone depletion scenario gets worse.

6.1 Selection of QTLs in Rice Cultivars Comparing UV Resistance

Quantitative trait loci (QTLs) analysis is a powerful tool to analyse traits controlled by multiple genes. The detection of QTLs associated with UVB resistance in rice can allow its practical application in breeding for a multifaceted complex trait and may lead to the identification of genes and their functions. Considerable variation in UVB resistance exists within cultivated rice, but its detailed genetic control mechanism has not been well elucidated. In rice, molecular genetic maps and markers have facilitated the identification of QTLs for the trait of interest (Lin et al. 1998). Furthermore, DNA marker-assisted selection has made it possible to develop near-isogenic lines and chromosome segment substitution lines for QTL regions affecting agronomic and physiological traits including resistance to various environmental stress (Ma et al. 2002). In addition, molecular cloning of genes at QTLs has been achieved by a map-based strategy. Thus, understanding natural variation has become a useful resource for knowing the genetic control mechanism of traits such as UVB resistance. On this note of relevance, detailed study by Sato et al. (2003) can be discussed. Their group detected putative QTLs associated with the resistance to enhanced UVB radiation in rice, using 98 BC1F5 (backcross inbred lines; BILs) derived from a cross between Nipponbare (a resistant japonica rice variety) and Kasalath (a sensitive indica rice variety). They further used 245 RFLP markers to construct a framework linkage map. BILs and both parents were grown under visible light with or without supplemental UVB radiation in a growth chamber. In order to evaluate UVB resistance, the relative fresh weight of aerial parts (RFW) and the relative chlorophyll content of leaf blades (RCC) were considered. The RFW of Nipponbare was 66%, while that of Kasalath was 19%. The RCC of Nipponbare and Kasalath was 112% and 32%, respectively. The RFW and RCC of BILs observed continuous distribution with one peak ranging from 12% to 85% and from 8% to 122%, respectively. The normal distribution of RFW, and the continuous distribution of RCC showed in BILs, suggested that multiple genes may regulate UVB resistance in this population and that the alleles, which increased UVB resistance, were from Nipponbare. Three putative QTLs for RFW were detected near the marker C1370 on chromosome 1, C746 on chromosome 3 and R2174 on chromosome 10. Furthermore, three putative QTLs for RCC, another indicator of UVB tolerance, were also detected on chromosomes 1, 3 and 10. These putative QTLs were located near the marker C122 on chromosome 1, C746 on chromosome 3 and R2174 on chromosome 10. Chromosomal locations of two QTLs for RCC on chromosomes 1 and 10 were coincident with those of the QTLs for RFW. In these cases, the alleles from Nipponbare contributed to the increase in RFW and RCC. On the contrary, Kasalath alleles at QTLs on chromosome 3 contributed to the increase in RCC. This suggested that these putative QTLs located in three chromosomal regions were largely responsible for the difference in UVB tolerance between Nipponbare and Kasalath. Figure 3a, b is a simplified diagram showing the putative QTLs in rice chromosomes and location map of qUVR-10 region which is being discussed next.

6.1.1 Major QTL with Assigned CPD Photolyase Activity in Rice

Rice qUVR-10, a quantitative trait locus (QTL) for ultraviolet-B (UVB) resistance on chromosome 10, has been identified by genomic map-based strategy. Plants homozygous for the Nipponbare allele at the qUVR-10 locus were found to be more resistant to UVB compared to the Kasalath allele. High-resolution genome-wide mapping using 1850 F2 plants enabled to precisely locate qUVR-10 to a 27-kb genomic region. This particular region was identified as a gene encoding the cyclobutane pyrimidine dimer (CPD) photolyase. Activity of CPD photo-repair in Nipponbare has been found to be higher than that of Kasalath and nearly isogenic

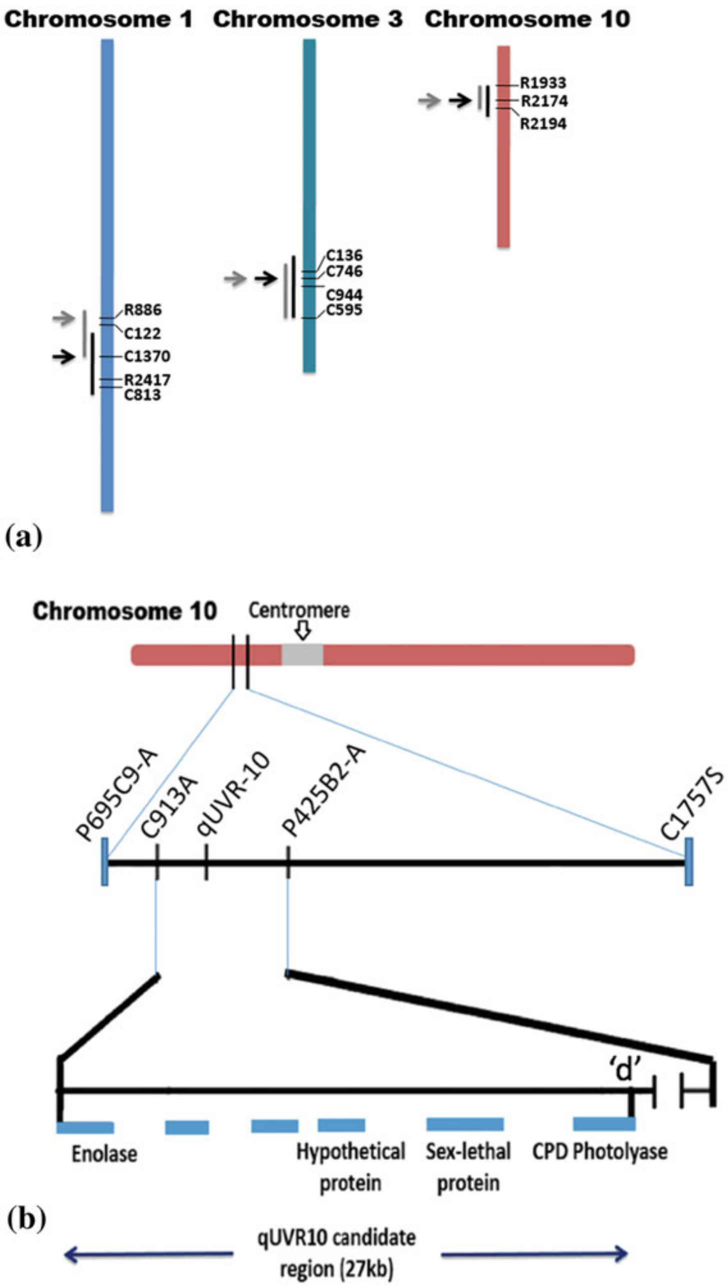

Fig. 3 (**a**) Location of QTLs in chromosome 1, 3 and 10 assigned for UV resistance in rice cultivar Nipponbare. The grey bar marks putative regions for RCC and black bar marks that of RFW. Assigned numbers are indicating nearest marker loci for respective QTLs. (Modified from Sato et al. 2003). (**b**) Genetic linkage map and detailed physical map of 27 kb stretched qUVR10 candidate region (spanned between SCAR markers : C913A and 'd') with CPD photolyase, on chromosome 10 of Nipponbare rice cultivar. (Adapted from Ueda et al. 2005)

with qUVR-10, which suggests that the CPD photolyase of Kasalath is defective (Ueda et al. 2005). Fundamental findings of the novel work by Ueda et al. (2005) can be categorized as follows:

6.1.2 qUVR-10 Is a Precise Genomic Region

To delimit the candidate genomic region of qUVR-10, a high-resolution linkage map was constructed after subjecting 1850 F2 plants. Out of these, 231 plants were further sorted in which recombination occurred in the vicinity of qUVR-10 locus, and the genotype class was determined by progeny testing. Some particular sequence-characterized amplified region (SCAR) markers were developed by using the nucleotide sequence of Nipponbaire PAC accession P0025D06 to define the region of qUVR-10 more precisely. The candidate region of qUVR-10 could be finally delimited to a 27-kb region between SCAR markers C913A and ‘d’. Bioinformatics analysis predicted six open reading frames (ORFs) in the candidate regions among which a CPD photolyase gene was found that had already been cloned by Hirouchi et al. (2003).

6.1.3 qUVR-10 Shows Changes in CPD Photo-Repair Efficacy

The measured in vitro CPD photo-repair activity in Nipponbare, Kasalath and NIL (qUVR-10) provided a comparative picture. As a result of an affirmatory experiment, CPDs were removed in Nipponbare, but half remained in Kasalath and NIL (qUVR-10) after a 60-min illumination with blue (photo-reactivating) light. This result clearly indicated that the photo-repair activity in Nipponbare was higher than that in Kasalath and NIL(qUVR-10). The research group also analysed CPD photo-repair activity in the plants in which recombination occurred in the regions flanking qUVR-10. Therefore, the CPD photolyase gene was the major likely candidate for qUVR-10.

6.1.4 Molecular Explanation of Allelic Difference in qUVR-10

The genomic sequence of the CPD photolyase in Kasalath was compared with the corresponding Nipponbare sequence. Many sequence variations were found in the promoter region, along with six single-base substitutions, a 12-base insert, a 2-base insert in the Kasalath introns, a 3-base insert in the Kasalath 5′-untranslated region (UTR), and three single-base substitutions in the Kasalath exons. Of the three single-base substitutions in the coding regions, two were synonymous and one caused an amino acid change: Glu-296 (CAG) in Nipponbare to His-296 (CAC) in Kasalath. Most importantly, the allelic difference between Nipponbare and Kasalath qUVR-10 was found to be caused by a single-base substitution in exon #4. This glutamine residue in exon #4 was verified to be conserved among other higher eukaryotic CPD photolyases.

6.2 Transcriptomic/Proteomic Signature of Rice Under UV Stress

Transcriptomic and proteomic analysis is an effective method to investigate the integral change in gene expression of plants during environmental stress at the protein level. The identification of UV-responsive proteins provides a better understanding of the molecular mechanism of plant responses to UV stress. To study the molecular mechanisms underlying the morphological and physiological UV-induced responses of rice, two-dimensional electrophoresis (2-DGE) study of proteins from time-gradient-wise UV-treated plants and untreated control plants is the foremost laboratory approach. Protein spots showing consistent upregulation or downregulation under UV radiation are further subjected to MALDI-TOF (matrix-assisted laser desorption ionization time of flight) mass spectrometry analysis which clarifies the proteomic picture and helps to discover stress-responsive protein elements. A thoroughly descriptive study by Du et al. (2011) has documented the proteomic signature of UV stress responses in rice. Proteomic and qPCR analysis identified one upregulated and two induced proteins with important functions: tryptophan synthase a chain (production of radical oxygen species), glyoxalase I (detoxification/anti-oxidant activity) and a Bet v I family protein (defence regulative function). Conclusive categorical discussions of the mentioned work include the following.

6.2.1 Plant Hormone Synthesis-Mediated Regulative Pattern Under UV Stress

The phyto-hormones like ethylene and auxin orchestrates plant physiological responses to plethora of environmental stresses (Chen et al. 2005). The natural auxin IAA controls many crucial and stress-induced damage-prone processes like embryo development (Ljung et al. 2002). SAM synthase is known to catalyse the formation of S-adenosylmethionine as the precursor for ethylene (Yan et al. 2006). Tryptophan synthase alpha chain catalyses the last step of the synthesis of tryptophan as the predecessor of IAA. In addition, tryptophan is reported to be easily degraded by UVB, accompanied by ROS generation (Ljung et al. 2002). In the study by Du et al. (2011), upregulation of tryptophan synthase alpha chain which is pivotal in relation to IAA synthesis and ROS induction and SAM synthase which has role in generation of ethylene precursor, presumably elevated phyto-hormone synthesis and signal transduction in rice seedlings during UV exposure, which were perceived to be key components for UV-induced injurious responses.

6.2.2 Increment of UV Protective Compound Synthesizing Protein Machinery

Accumulation of UV-absorbing compounds is considered as the most effective protection strategy in response to UV radiation (Frohnmeyer and Staiger 2003). SAMS catalyses the formation of S-adenosylmethionine, which is not only ethylene precursor but also the precursor of various polyamines (Yan et al. 2006). Polyamines may mitigate UV stress, possibly through scavenging the free radicals as a potent antioxidant (Jordan 1996). Experimental results observed by Du et al. (2011)

suggested that upregulation of SAMS related to polyamine synthesis and significant accumulation of UV-absorbing compounds has to be a basic protective response in rice seedlings during prolonged UV exposure. In the same study, the well-known detoxification/antioxidation of protein glyoxalase I was induced at the protein level and significantly upregulated at the mRNA level at 12 and 24 h in our study. This indicated that glyoxalase I might confer detoxification of methylglyoxal and defence against oxidative damage at the assimilation and damage stages in rice plants. The other induced proteins at 12 or 24 h in the study were defence response proteins, chitinase and Bet v 1 allergen family protein, which might have played a key role in constructing a self-defence mechanism together with glyoxalase I. Chitinase (PR-3 family) and Bet v I allergen family protein (PR-10) belong to the pathogenesis-related (PR) group of defence proteins (Breiteneder and Ebner 2000). Relevant previous studies also indicate that Hsp-related proteins play a role in response to UV in some species including rice (Murakami et al. 2004). Hsp oligomers can recognize and bind to stress-induced proteins with non-native conformations, minimize and remove the aggregated non-native proteins from the cell (Feder and Hofmann 1999). Currently discussed study by Du et al. (2011), the upregulation of four Hsp-related proteins, namely Hsp70-related protein, Hsp70 mitochondrial precursor, putative Hsp70 mitochondrial precursor and HspST1 was documented and perceived to be linked with UV stress resistance.

6.3 Candidate Genes for Producing UV and High Irradiance Resistant Rice Through Transgenic/Overexpression Approach

Schematic studies adhering to QTLs, proteomic signature analysis and metabolic pathways associated with UV or high irradiance stress have paved the identification and characterization of potent candidate genes that can be employed in the development of UV or high light–tolerant transgenic rice. In this segment, the different transgenic approaches along with the respective backgrounds of candidate gene conceptualization and the improved outcomes are being categorically elucidated.

6.3.1 Transformed Rice Expressing qUVR-10 Encoded CPD Photolyase

qUVR-10 locus of Nipponbare rice was identified to code for a CPD photolyase and transgenic studies revealed enhanced UV resistance in qUVR-10 null background, as per the observations of Ueda et al. (2005). In the mentioned experiment, a 9-kb PstI genomic fragment containing the Nipponbare CPD photolyase gene (N-PstI) and an 8-kb Nipponbare genomic fragment (60B04) derived from a shotgun clone was inserted in binary vector for *Agrobacterium*-mediated transformation. Plants regenerated from transformed calli (T0 plants) were grown in an isolated greenhouse. Self-pollinated plants of each T0 plant (T1) were grown, and T2 lines were examined for CPD photo-repair activity under UVB irradiation. To evaluate the UVB resistance of each T1 plant carrying multiple copies of the transgene, T1 plants were grown under 1.3 W m^{-2} UVB radiation for 3 weeks, and then their phenotypes were observed. After that, these T1 plants were maintained without UVB radiation for 45 days to recover UV induced damage so that the accumulation of CPD photolyase mRNA could be analysed. After the completion of southern blot and complementation analysis, they could assort two T0 plants carrying multiple copies of the Nipponbare CPD photolyase gene. One of them had NIL(qUVR-10) genetic background and the other RS24-13 genetic background. T1 plants derived from these two plants were grown under the fixed UVB radiation for 3 weeks. The transgenic plants which carried six or seven tandemly transformed copies of the Nipponbare CPD photolyase gene (60B04 fragment) were observed with increased levels of CPD photolyase transcripts. After 3 weeks of 1.3 W m^{-2} UVB radiation, leaves of transgenic rice showed no necrotic lesions, and negligible retardation of growth rate. In contrast, both RS24-13 and NIL(qUVR-10) non-transformed plants with respective empty vector controls showed large streaks of necrotic lesions, correlated with prominent retardation in growth. These result clearly suggested that UV resistance can be achieved by increasing the production level of CPD photolyase through transgenic approach in rice.

6.3.2 Overexpression of Silicon Transporter Lsi 1 Regulates UV Tolerance of Rice

Silicon is taken up through plant roots in the form of silicic acid and later becomes polymerized and accumulated on the leaf tissues in the form of silica acting as a protective shield. Ma et al. (2007) reported that the uptake of Si in rice is mediated by two transporters, low silicon rice 1 (Lsi1) and low silicon rice 2 (Lsi2) which belong to Nod26-like major intrinsic protein (NIP) subfamily. Lsi1 is an influx transporter, while Lsi2 is an efflux transporter of Si. Therefore, it is clear that Lsi1 functions in controlling Si accumulation in rice, meaning that inhibiting the gene expression would reduce the uptake of Si in rice, which might result in decreased tolerance to radiation-related stresses. Study by Wu et al. (2007) pointed out that the amount of silica on the adaxial surface in UVB-tolerant rice Lemont was higher than that in the UVB-sensitive-type Dular, suggesting that the characteristics of silica accumulation might be one of the mechanisms for rice to defend enhanced UVB radiation. So, it was previously hypothesized that regulating the transcript level of Lsi1, combatting UV stress might get aided in a good way. Chasing this hypothesis, Fang et al. (2011) investigated whether or not Lsi1 plays a role in regulating rice tolerance to UVB stress by employing gene suppression and overexpression approaches in Lemont and Dular background. Molecular responses to enhanced UVB radiation was detected by adapting suppression subtractive hybridization (SSH) approach (Diatchenko et al. 1996). Results obtained from transgenic rice showed that Si concentrations in the rice leaves were 10.17 mg g^{-1} leaf dry weight in case of Lsi1-RNAi transgenic Lemont, 21.08 mg g^{-1} in the WT and 38.32 mg g^{-1} in Lsi1-overexpressed transgenic of Lemont. For Dular rice accession, Si concentration in Lsi1-overexpressed plants was 22.34 mg g^{-1}, which was significantly higher than that (12.15 mg g^{-1}) in its WT. After this affirmation that Lsi1 indeed played a role in higher levels of silica accumulation, attempts were made to decode the regulatory network of Lsi1 in the defence response to UVB irradiation. Downregulated genes in Lsi1-RNAi transgenic line of Lemont, as well as upregulated genes in Lsi1-overexpressed transgenic lines of Lemont and Dular were taken into the comparison with WT under enhanced UVB radiation treatment using SSH. Eventually, the research group found that a resistance-related non-coding RNA (nc1) was upregulated in Lsi1-overexpressed transgenic lines of Lemont and Dular, but downregulated in Lsi1-RNAi transgenic line of Lemont compared to their corresponsive WT plants. It was ascertained that resistance-related non-coding RNA (ncRNA1) was an inducible RNA previously detected in plant hopper-infested *O. sativa* indica cultivars. Hence, this result suggested that the regulation of rice UV-B tolerance by Lsi1 might be orchestrated by differential expression modes of ncRNA, which switches off the activation of plant defence and initiates tolerance response to UV-B radiation. Also, there was noteworthy increase of mRNA expression of the antioxidant enzymes glutathione S-transferase and metallothionein-like protein type 1, which was precisely upregulated in Lsi1-overexpressed transgenic lines of Lemont and Dular, in the comparison with WT. This study also showed that the overexpression of Lsi1 was able to significantly promote the upregulation of phenylalanine-ammonia lyase (PAL), CPD photolyase (PL) and 4-coumarate Co-A ligase (4CL) like protein which are associated with UV shielding phenolics and flavonoid synthesis in rice. Lastly, it was further speculated that the overexpression of Lsi1 in rice could activate amino acid selective channel proteins, in cell and zinc-finger proteins, kinases, which are associated with signal transduction in the pathway to increase UVB tolerance in rice through the feedback regulation of Lsi1 function. Figure 4 provides a simple model to notice the upregulation targets of Lsi 1 under UV stress.

6.3.3 Fibrillin 5 (FBN5) of Rice Acting in Plastoquinone Biosynthesis Can Alleviate High Irradiance Stress

Plastoquinone-9 is known as a photosynthetic electron carrier with a role in the regulation of gene expression and enzyme activities via its redox state. Moreover, it acts as an antioxidant in plant leaves, accomplishing a central photo-protective. These photo-acclimation processes aided by Plastoquinone 9 include phosphorylation of thylakoid membrane proteins (Depège et al. 2003), expression of photosystem I and II genes in the chloroplast (Pfannschmidt et al. 2001) and expression of genes such as ascorbate peroxidase (Karpinski et al. 1997), plastocyanin transcription factors (Adamiec et al. 2008), etc. PQ-9 also participates in the biosynthesis of carotenoids as a cofactor for the desaturation of phytoene and z-carotene (Norris et al. 1995), and reduced PQ-9 shows antioxidant activity under abiotic and biotic stresses (Kruk and Szymańska 2012). Recently, Kim et al. (2017) found that fibrillin5 (FBN5), a plastid lipid binding protein with n-terminal lipocalin ‘motif 1’, is an essential structural component of the PQ-9 biosynthetic pathway, showing functional conservation in monocots as well. Moreover, OsFBN5 was found as the functionally equivalent AtFBN5 ortholog in rice (*Oryza sativa*), and the solanesyl diphosphate synthase SPS–FBN5 complex needed for synthesis of the solanesyl diphosphate tail in PQ-9 was well understood in Arabidopsis and rice. To focus on the relevance of FBN5, the overall pathway of plastoquinone generation and the proteins involved in this task within chloroplast needs to be thoroughly elaborated. In the biosynthetic cascade of plastoquinone-9, the solanesyl moiety is generated by

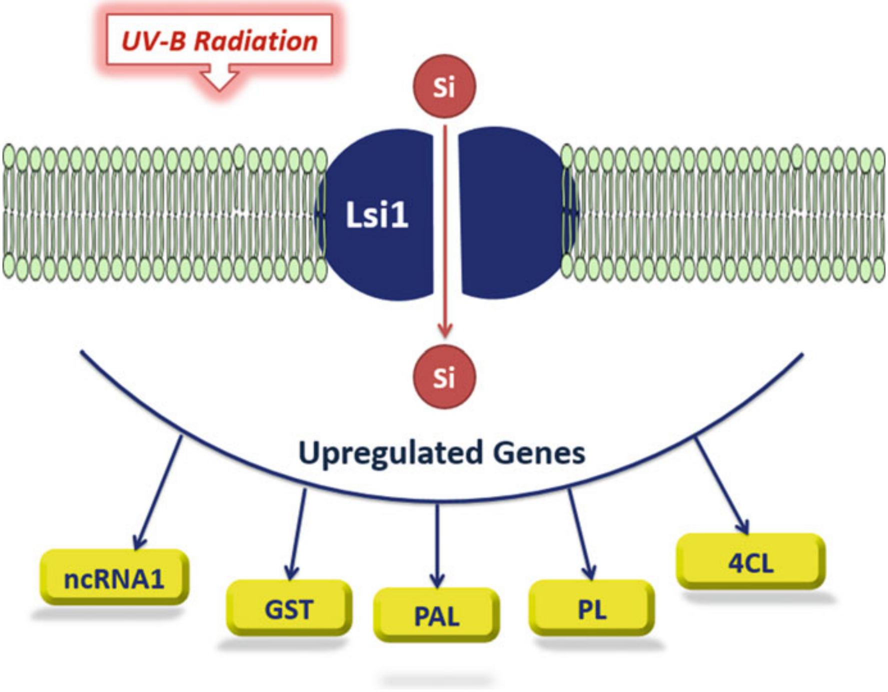

Fig. 4 Lsi 1 and its upregulation targets; Lsi 1 is an NIP subfamily silicon influx transporter activated under UV stress, which upregulates stress-responsive genes like GST, PAL, PL and 4CL, also hiking a non-coding RNA (ncRNA1) similar to that of grass hopper–infested indica rice cultivar

trans-type consecutive condensation of isopentenyl diphosphate (IPP; C5) and its isomer dimethylallyl diphosphate (DMAPP; C5), which are synthesized through the mevalonic acid pathways with geranylgeranyl diphosphate (GGPP; C20). This process is catalysed by solanesyl diphosphate synthases (SPSs) (Hirooka et al. 2003). Only recently it has been deduced that FBN5 interacts with SPS1 and SPS2 in chloroplasts. It is hypothesized that FBN5 enhances the enzymatic activity of SPS1 and SPS2 by binding to the hydrophobic solanesyl moiety required for plastoquinone-9 formation and helping to release this moiety from the enzymes, as investigated in Arabidopsis background. Thus, it is quite understandable that, characterization of FBN function would increase our knowledge about increasing photosynthetic efficiency and stress tolerance in rice. On this note of relevance, details of the study by Kim et al. (2017) are being categorically highlighted. Firstly, Tos17 (an LTR retrotransposon active in rice) insertion at exon 6 of OsFBN5 generated two mutants in Nipponbare rice, namely Osfbn5-1 and Osfbn5-2. Unlike the Atfbn5-1 plants, the Osfbn5 homozygous mutant plants were not seedling lethal, but however, phenotypically they were smaller than the WT rice plants. Expectedly, the amount of PQ-9 was reduced about sevenfold in the leaves of Osfbn5-1 and

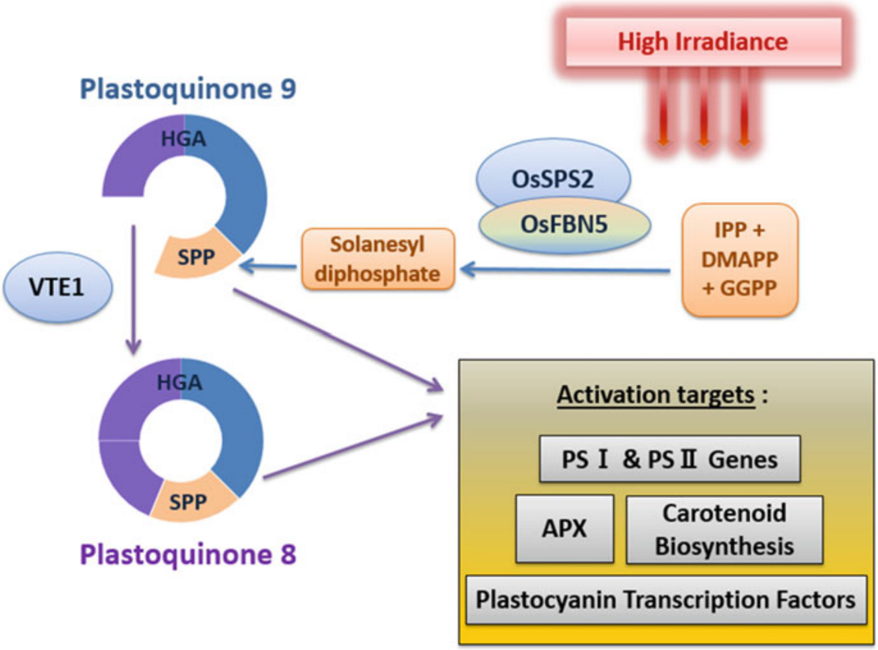

Fig. 5 Fibrillin 5 and its relevance in plastoquinone synthesis under increased light; OsFBN5 interacts with OsSPS2 and generates the SPP moiety of PQ-9. By the action of tocopherol cyclase (VTE1), PQ-9 is cyclized into PQ-8, and both of these molecules activate photo-protective genes like *APX*, *PSI* and *PSII* genes, carotenoid biosynthesis genes and plastocyanin transcription factors

Osfbn5-2 mutants, and PC-8, the product of PC-9 cyclization, was not detectable in either of the mutants, in contrast to WT plants. The level of beta carotene in the Osfbn5-1 plants was reduced by about 30% in comparison to the WT rice, and the level of antheraxanthin was increased in the Osfbn5-1 plants, indicating that the mutant plants were clearly more photo-stressed than the WT plants. This way, it was affirmed that FBN5 has a definitive role in plastoquinone synthesis in rice, and the absence of it leads to photo-stressed condition. Yeast two hybrid studies further showed that OsFBN5 interacted with AtSPS1 and AtSPS2, as well as with OsSPS2. The OsFBN5–OsSPS2 interaction in the chloroplast was cross checked by fluorescence complementation experiments in maize mesophyll protoplast. Most importantly, when Atfbn5-1 mutants were complemented with OsFBN5 under overexpression promoter, the lethal phenotype was successfully rescued and Atfbn5-1 C 35S:OsFBN5 lines displayed an overall WT-like phenotype as well as elevated levels of PQ-9, PQ-8 (formed as tocopherol cyclase VTE1 cyclizes PQ-9) and beta carotene. These results demonstrate that rice OsFBN5 is a promising candidate gene which might be used to generate transgenic lines capable of photo-stress acclimation. Primary relevance of OsFBN5 in PQ-9 biosynthesis and activation targets of PQ-9 and PQ-8 has been picturized in Fig. 5.

6.3.4 OsGGR2 Is a Geranylgeranyl Reductase in Rice Synthesizing Antioxidant Tocopherol

Tocopherol (Toc) and tocotrienol (T3) are both forms of vitamin E which is biosynthesized in the photosynthetic organs of plants and cyanobacteria and plays essential roles in both plant physiology (Munné-Bosch 2007). T3 is characteristically abundant in rice bran and is known to have greater antioxidant activity compared to Toc (Minhajuddin et al. 2005). Geranylgeranyl reductase (GGR) plays crucial roles in both vitamin E and chlorophyll biosynthesis. GGR has two functions: reduction of geranylgeranyl pyrophosphate (GGPP) to phytyl pyrophosphate (PPP) and reduction of geranylgeranyl-chlorophyll to chlorophyll. T3 is biosynthesized from GGPP and homogentisic acid (HGA), whereas Toc is biosynthesized from PPP and HGA. PPP is generated from GGPP via the chlorophyll degradation pathway or direct reduction of GGPP by GGR catalytic activity. Recent publication of Kimura et al. (2018) reveals that rice has at least two GGRs. OsGGR1 is the ortholog of AtGGR, and OsGGR2 is a novel enzyme. The mentioned research group had developed retrotransposon Tos17 inserted mutant genotypes for GGRs, and the selected mutant line was NE1041. Preliminary quantitative analysis showed much lower amount of T3 in mutant calli and transgenic leaves as compared to the wild-type, but the presence of substantial amounts of Toc could be detected in the GGR1. Expression profile analysis revealed that *OsGGR2* was expressed at the mRNA level in the callus and leaves of OsGGR1 mutants. Further *OsGGR2* expression pattern analysis in the grain-filling stage showed that *OsGGR2* was expressed in bran, the flag leaf, the third leaf from the flag leaf, and the flag leaf sheath. Evidently, OsGGR2 seemed to possess OsGGR1-independent expression, and presumably both GGRs had specialized functional areas. Consequently, the OsGGR1/OsGGR2 double mutant showed drastic physiological stand, and the Toc content of the double mutant callus was drastically reduced compared with WT and the OsGGR1 mutant callus. These results indicate that the *OsGGR2* gene product has pivotal GGR activity and synthesizes Toc in rice plant cells. The detailed information on enzymatic activity of OsGGR2, thorough comparison of overexpression level of *OsGGR1* and *OsGGR2* and differential amount of Toc and T3 generation schemes were not further elaborated in the mentioned work. However, future projects for overexpressing GGR2 to enhance antioxidant tocopherol level in rice, to decimate high irradiance or UV-mediated ROS stress can be fairly planned.

7 Discussions

7.1 Status of Ozone Layer Protection Policies

Sustenance of tropospheric ozone is a far-running process without any clear proximate work flow in the current times. In the year 1985, 20 nations signed the Vienna Convention, which established a framework for negotiating the international regulative structure for ozone depleting substances. On 16 September 1987, the Montreal Protocol on ‘Substances that deplete the Ozone layer’ was signed and applied from

1 January 1989 onwards (Velders et al. 2007). The genesis of Scientific Assessment of Ozone Depletion*:* 2014 was the 23rd Meeting of the Parties to the Montreal Protocol held in Bali, Indonesia, and the major points of discussion included: (1) assessment of the state of the ozone layer and its future evolution, including in respect of atmospheric changes from, for example, sudden stratospheric warming or accelerated Brewer–Dobson circulation, (2) description and interpretation of observed ozone changes and ultraviolet radiation, along with future projections and scenarios for those variables, taking into account among other things the expected impacts to the atmosphere and (3) assessment of the effects of ozone-depleting substances and other ozone-relevant substances, if any, with stratospheric influences, and their degradation products, the identification of such substances, their ozone-depletion potential and other properties. Since then, recurrent studies on ozone layer depletion and application of regulatory policies are moving on a steady pace (WMO Report: 55, Scientific Assessment of Ozone Depletion 2014). Obviously, sealing off the 'ozone hole' and atmospheric shielding of the enhanced exposure of UV and high white light on a global scale are not going to be accomplished very soon in future. Instead, the focus has been practically concentrated on crop improvement against the deleterious effects of UV/high irradiance.

7.2 Summary on Phenotypic Effects of UV/High Light

Over the years, thorough field observations and experimental setup–based UV treatment results have led to the identification of certain morphological signs exhibited by various susceptible rice varieties under UV/high light intensity stress. Table 1 shows the summary of the phenotypes.

Under UV-exposed, early stage crop hampering phenotypes like foliar area reduction, stunting of height was noted in indica cultivars like IR45, IR74 (Dai et al. 1992) and japonica cultivars like Norin 1 (Hidema et al. 1996). Worth a mention, japonica cultivar Fujisaka 5 showed elongation of internodes leading to increase in height (Barnes et al. 1993). Statistically significant reduction in panicle and grain count is relatively rare in the context of UV exposure and was observed in japonica rice variety Sasanishiki (Hidema et al. 2005). High light exposure phenotypes discussed earlier in this chapter include increased leaf thickness, which is attributed by adaptive addition of multiple mesophyll layers and observed in indica cultivars pgl and Z802 (Gu et al. 2017). To be analytically correct, pgl's leaf paleness response was not induced but rather constitutive, which explains that pale green leaf condition is genoptypic.

7.3 Physiological Response Summary of UV/Irradiance Stress

Looking at the cellular/physiological (Table 2) response of UV/high light in rice, it needs to be clearly categorized that physiological manifestations are of two kinds:

injurious responses and defensive responses. Injurious responses mean the deleterious outcomes like stomatal closure, decimation of RubisCO subunits, degradation of PSII proteins and loss of activity, lowering of chlorophyll content, etc. Showing most of these mentioned physiological handicaps, japonica cultivar Norin 1 could be identified as the most vulnerable one (Fedina et al. 2010). Moreover, Norin 1's CPD photolyase sequence revealed its allelic incompetence in the course of effective photo-repair of cyclobutane pyrimidine lesions formed in DNA by UB radiation (Teranishi et al. 2004). As a mode of protective defence, it is seen that UV triggers production of shielding or photo-protective compounds. Purple rice has showed elevated accumulation of anthocyanins under UV exposure (Hada et al. 2003). Under high irradiance, an indica cultivar IR72 was found to elevate carotenoid levels, fortifying antioxidant activity (Murchie et al. 2002). On the same context of high irradiance, indica cultivar pgl was found to have reduced ROS accumulation and photo-inhibition in comparison to the other variety Z802. Combining the knowledge of morphological and physiological responses, selective breeding methods can be conceptualized to grow UV/high light–resistant rice cultivars, and susceptible cultivars can be avoided during the long summers or in case of global regions with high degrees of UV penetrance.

7.4 Crucial Importance of CPD Photolyases Greater Than UV Screening Molecules

The most lethal effect on biological organisms exerted by UV radiation is DNA damage with formation of cyclobutane pyrimidine lesions that hamper further replication of DNA. Very evidently, CPD photolyases involved in photo-repair of damaged DNA bear a magnanimous task. On the other hand, UV-absorbing compounds accumulating in the epidermal and subepidermal and mesophyll cell layers have traditionally been considered to function as UV filters, which plays another important role in countering the damaging effects of UVB radiation. Although it is true that excess accumulation of flavonoids (such as anthocyanins) reduces the amount of radiation reaching cells, but it may also sometimes lower the ability to photo-repair damaged DNA. For example, purple rice is highly UVB sensitive, despite having huge levels of anthocyanins accumulated in leaves (Hada et al. 2003). These results imply that the systems for repairing DNA damage are essential for plants to grow in light conditions containing UVB, and protective role of UV filtering molecules is a secondary support. Most importantly, CPD photolyase activity is a crucial factor determining the differences in UVB sensitivity between rice cultivars. The substitution of one or two bases in the CPD photolyase gene can alter the activity of the enzyme, and the associated resistance of the plant to UVB radiation. These findings suggest that the resistance of rice to UVB radiation can be elevated, by selective breeding or bioengineering of the genes encoding CPD photolyase.

7.5 Development of UV/High-Irradiance-Resistant Rice

Several approaches have been methodically discussed earlier in this chapter which revolve around the idea of crop improvement in case of rice. Genomic DNA sequencing–based screening of QTLs have been turned out to be fruitful and include many positive examples like discovery of qUVR-10 (Ueda et al. 2005). Other approaches concerned are proteomic pattern identification under UV stress and selection of suitable genes that can be overexpressed in transgenic lines to confer resistance. For UV stress resistance, overexpression of CPD photolyase and Lsi 1 has been discussed. In case of high irradiance stress, OsFBN5 has been tested in overexpressing plants while OsGGR2 is yet to be subjected for the overexpression study. Table 3 synoptically shows the candidate genes with their respective mechanisms of action providing the UV/high irradiance resistance.

7.6 Beneficial Perspective of UV Stress

Limited amount of reports from various works give a hint of probable benefits that can be acquired from rice plants treated with UV exposure. Hidema et al. (2005) reported that supplementary UV radiation increased storage protein and total nitrogen content in japonica rice cultivar Sasanishiki. The total grain nitrogen content significantly hiked up to 12.5% and 14.7% in the survey years 2001 and 2002, respectively. During ripening of grains, nitrogen accumulates in the grain mainly as protein, and the protein is stored in protein bodies in the endosperm. Total protein content increased by 12.5% and 13.2%, respectively, in the same consecutive years of study. Notably among all proteins, glutelin content alone hiked by 10.9% in 2001

Table 3 Candidate genes for overexpression approach and their mechanisms of action

Nature of stress resistance	Candidate genes	Tested varieties	Mechanism of action	References
UVB resistance	CPD photolyase; encoded by qUVR-10 region of Nipponbare Chr. 10	RS24-13, NIL (qUVR10)	Photo-repair of CPD lesions in DNA	Ueda et al. (2005)
	Lsi 1; NIP subfamily silicon influx protein.	Lemont, Dular	Upregulation of PAL, 4-CL to produce UV-protective compounds, photolyase activation	Fang et al. (2011)
High irradiance resistance	$OsFBN_5$; Fibrillin 5, interacting with $OsSPS_2$	Nipponbare	Produces solanesyl diphosphate (SPP) moiety after binding with $OsSPS_2$, producing PQ-9	Kim et al. (2017)
	$OsGGR_2$; a geranyl-geranyl reductase	NE1041	Generates increased level of tocopherol antioxidant	Kimura et al. (2018)

and 13.1% in 2002. UV radiation decreases the grain size but increased protein content develops a different taste of the rice grains.

8 Future Prospects

In this chapter, a summative documentation has been undertaken regarding global ozone depletion scenario and emergent problems of increasing UV/high light intensity–mediated hazards in rice. Limitations as well as promising prospects in this situation should be discussed in order to incept newer ideas leading to crop improvement. Thoughts to intrigue future research directions are being crisply represented as follows.

8.1 Fine-Tuned Differentiation of Molecular Responses Driven by UV and High Irradiance

On a comparative scale, high-incident white light intensity–mediated effects on rice have been studied lesser in comparison with UV stress. Moreover, UV and high irradiance often exert redundant molecular responses especially as far as physiological manifestations are concerned. For example, photo-oxidative stresses, disruption of PS-II activity, generation of ROS and peroxidation stress, all are commonly shared responses of UV and high light exposure. Delicate differences and fine-tuned idea of differences between these two types of exposures are not clear till date. Practically under ambient light of open fields, UV rays are always accompanied by incident white light. So, particularly which stream of light incites which cellular damage when projected together is unknown and will remain so till precisions are conceptualized in future.

8.2 Transcriptomic/Proteomic Approaches Have a Huge Scope

Till date, the known candidate genes that could be used for hyper-resistant rice development are very limited in number. For this endeavour, transcriptomic and proteomic studies and gene or protein characterization experiments play a huge role. Exploration of newer genes involved in UV resistance or high light resistance needs to be done which will unravel novel proteins capable of mitigating radiation exposure stresses in rice. This will finally lead to overexpression experiments and transgenic development in order to achieve a futuristic super resistant rice.

8.3 Search for Positive Effects of UV on Biotic Stresses

Under UV/high irradiance stress, phenyl–propanoid pathway is triggered in plants including rice. Similarly, many phyto-chemicals involved in biotic defence responses are also derived from the same pathway. Phyto-alexins, phyto-anticipins and pathogen-related proteins constitute the defensive network against pathogen attack. Discovery of coupling proteins that might bridge UV resistance and biotic resistance pathway might help to check pathogenic invasion in rice under limited UV treatment.

8.4 UV Might Increase the Amount of Antioxidants in Large Scale

It has been already discussed that UV stress induces the formation of active flavonoids like anthocyanins in rice. Certain rice varieties like purple rice are quite rich in anthocyanin content, and carotenoids are also seen to have increased in some varieties. The future might potentiate the demand of pharmaceutically active compound accumulating rice grains, and concomitant research might move forward towards large-scale productions. On daily consumption chart, if we accommodate medicinally active antioxidant producing rice, supplementary needs for synthetic drugs might decrease leading to a side effect–free healthier diet.

8.5 Food Quality and Protein Value Improvement

Increase of glutelin content has been already reported in rice (Hidema et al. 2005). If studies continue in this direction involving different cultivars worldwide especially in developing or poor countries to improve protein value in rice under UV treatment, nutritional benefit and food value will be greatly escalated. Supplementary UV treatment will be a cheaper approach than overexpression line development to hike protein content in rice.

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Improving Rice Tolerance to Low- and High-Temperature Stress Through Biotechnological Approaches

Chanda Bano, Nimisha Amist, and N. B. Singh

Abstract

Abiotic stresses are severe problem to the crop survival which someway influences plant growth and yield. Among abiotic stress, low- and high-temperature stress is important, which impacts adverse effect on rice (*Oryza Sativa* L.). Rice is one of the chief and staple cereal crops of world population. Rice in response to low- and high-temperature stress changes its gene expression pattern and protein products. Several studies concerning genome profiling mutational and transgenic plant reveal complex transcriptional mechanism that activates in response to low- and high-temperature stress. By using different strategies, biotechnology improves crop yield and quality by enhancing their capacity to defend against different stresses. This chapter focuses on the conventional and improved biotechnological approaches for the betterment of low- and high-temperature stress tolerance in rice.

Keywords

Abiotic stress · Genes · Heat shock protein · High temperature · Low temperature · Rice · Transgenic plant

1 Introduction

Biotechnology is the excellent technique to produce tolerant or resistant crop varieties which is more productive under different stress conditions. Different strategies are used in biotechnology for the upgrading of crop productivity. In this chapter, we will focus on the effect of low and high temperature on rice crop and genes which are activated during these stress and biotechnological approaches that

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Table 1 Different genes and their role in rice that provides tolerance against low and high temperatures (Adapted from Kumar et al. 2018)

Gene	Functions
Glycogen synthase kinase 3-like gene 1 (OsGSK1)	Resistance against low and high temperatures (Koh et al. 2007)
Oswrky11 (OsWRKY11)	Low- and high-temperature tolerance (Wu et al. 2009)
Sedoheptulose-1,7-bisphosphatase (SBPase)	High-temperature tolerance (Feng et al. 2007)
Mitochondrial heat-shock protein 70 (mtHsp70)	High-temperature tolerance (Qi et al. 2011)
Small heat-shock protein17.7 (sHSP17.7)	Induced tolerance against high temperature (Murakami et al. 2004)
Heat-shock factor binding protein 2 (OsHSBP2)	High-temperature tolerance (Rana et al. 2012)
Heat-shock factor binding protein 1 (OsHSBP1)	High-temperature tolerance (Rana et al. 2012)
Osmyb55 (OsMYB55)	High-temperature tolerance (El-kereamy et al. 2012)
Rice carbon catabolite repressor 4(ccr4)-associated factor 1 g (OsCAF1G)	High-temperature tolerance (Chou et al. 2014)
Rice carbon catabolite repressor 4(ccr4)-associated factor 1 h (OsCAF1H)	High-temperature tolerance (Chou et al. 2014)
Small heat-shock proteins (shsps) oshsp16.9 (HSP 16.9)	High-temperature tolerance (Jung et al. 2014)
A 26s proteasome with two subunits (TT1 (Os03g0387100))	High-temperature tolerance (Li et al. 2015b)
Manganese superoxide dismutase (SOD 1 (MSD1))	High-temperature tolerance (Shiraya et al. 2015)
Spl7 (HSFA4d)	High-temperature tolerance (Yamanouchi et al. 2002)
Receptor-like kinase ERECTA (ERECTA)	High-temperature tolerance (Shen et al. 2015)
Stress-responsive nac gene (SNAC3)	High-temperature tolerance (Fang et al. 2015)

enhance tolerance against low and high temperature. Different genes and their role in rice in providing tolerance against low and high temperature are mentioned in Table 1. Different processes in a crop such as physiology, biochemical and development are affected due to low- and high-temperature stress. Stresses directly or indirectly result in free radical production, oxidative stress and reactive oxygen species (ROS). Development of stress-resistant plant is the main aim of agriculture-related biotechnological studies. Rice (*Oryza sativa* L.) is the chief staple crop for almost half of the world's population. Three billion people of the world lives depending on the rice crop. It has been grown under a wide range of climatic conditions. At least 114 countries planted rice in about 159 million hectares yearly (Tonini and Cabrera 2011). Of the total grown rice, almost 90% of rice crop are consumed in Asia (Pareek et al. 2010). Rice crop is normally grown in tropical and temperate climate zones. It is susceptible to high temperatures during flowering and grain-filling stages (Wassmann et al. 2009a, b). Low temperature also affects rice crop at the reproductive and seedling stages (Mukhopadhyay et al. 2004). Poor germination, seedlings stunted growth, yellowing or withering and decreased tillering are the consequences of low-temperature stress and almost all the growth stages of the plant are affected by high temperature. In India, Pakistan, Bangladesh, China, Thailand, Sudan and African countries, high temperature affects rice crops (Li et al. 2004; Xia and Qi 2004; Yang et al. 2005; Tian et al. 2009). Crop tolerance against low and high temperature is defined as the plant's ability to grow and produce an economic yield during low and high temperature. Low-temperature stress also affects the reproductive stage delay, heading consequently pollen sterility and ultimately decreasing rice grain yield (Kaneda 1974; Mackill 1997; Andaya and Tai 2006; Suzuki et al. 2008). High temperatures have damaging effect on different stages such as microsporogenesis, anthesis and fertilization of rice seedlings. High temperature influences growth of plant, cell division, dehiscence of anther, pollen dispersal and germination results in sterility of spikelet and ultimately yield of rice crop (Yoshida 1981; Wassmann et al. 2009a, b; Shah et al. 2011; Prasanth et al. 2005; Tenorio et al. 2013; Sanchez-Reinoso et al. 2014). Biotechnology strategies are used to improve rice tolerance exposed to low and high temperatures. Development of rice varieties which is tolerant to low- and high-temperature stress is one of the best approaches to maintain its production efficiency.

2 Tolerance to Low-Temperature Stress

Different plants have different capability to tolerate stress. Tropical plants typically have no ability to cope low-temperature stress. Additionally, plants can endure low temperatures ranging from −5 to −30 °C varying from species to species. Plants already present in colder region normally withstand temperatures even lesser than this. The plant seedling pretreated with low temperature is better to acclimatize low-temperature treatment in later stage of their life. Chilling temperature (at −5 °C) is lethal for wheat plants to grow under normal condition; however, when plants grow beneath 10 °C, they can be acclimatized to cold and can stay alive under chilling temperature down to −20 °C. For centuries, plant breeders frequently select cold tolerance traits, as different plants have different surviving capacities under freezing condition. However, from the past two decades, conventional breeding was widely used to improve the tolerance capability of major crops, rapidly instigating researches in the direction of probing molecular solutions to this problem. The time taken for acclimatization is termed as 'amid', many chilling-activated proteins synthesized by plants that probably take part in the succeeding cold (low temperature) tolerance. Around 50 chilling-activated proteins have been reportedly synthesized in a range of plant species. Each of them has a similar high degree of hydrophilic property and furthermore is composed of moderately basic amino acid, with recent motifs. Late embryogenesis abundant (LEA) proteins are such proteins that have been already identified and appear to play a protective role during seed desiccation. Several genes have been identified that encode protein in response of low temperature. Low-temperature-driven genes are still not known, but it is assumed that they may contribute exclusively to low-temperature tolerance by reducing the damaging effect of dehydration associated with freezing. In this way, low-temperature-induced protein overexpression could be used to confer low-temperature-stress resistance. In transgenic plants, low-temperature-induced protein expression is found. In *Arabidopsis*, chloroplast-targeted COR protein COR 15a increased the low-temperature tolerance of in situ frozen chloroplast or in vitro frozen protoplast. In a recent study, expression of COR 15 has no distinguishable effect on plant under frozen condition. One of the possible explanations for this study is that the cold-induced proteins might be associated with several weak segments of the cell, and altogether, they are essential for protecting the cell. It can be concluded that *COR* genes have to be altered in transgenic crop to obtain a substantial change in cold resistance. Studies are now focused on engineering a multigene trait, after the identification of few distinct chill resistance-related genes. The genes have been found to include relative regulatory elements in their promoter, such as the C-repeat (CRT) element/low-temperature-response element (LTRE)/lack of hydration- or dehydration-responsive element (DRE). It has been observed that a transcription factor CBF1 binds with the core element CRT/DRE/LTRE and triggers the expression of this group of genes, which contain the COR. The end result is that the overexpression of *CBF1* gene stimulates the acceptance of this whole group of COR cold-resistance gene. In transgenic *Arabidopsis* plants passing on a 35S promoter, *CBF1* gene construct has been developed. It was observed that these transgenic *Arabidopsis* plants express various COR genes and have resistance towards cold even without prior chill acclimation. However, in certain cases, it was reported that the level of tolerance towards cold was lower in plants where overexpression of individual COR protein, COR15a, occurred when compared to that of plants showing CBF1 overexpression. Several similar investigation provided evidences about the interconnected nature of various stress reactions. In transgenic *Arabidopsis* CBFF1 homolog expression and DREB1A, a DRE restricting protein regulated by stress-activated promoter provides enhanced tolerance against drought, salt and cold. Several genes that are activated by stresses have been identified by genetic engineering and molecular studies. Stress-activated genes encode proteins which are useful in improving stress tolerance. Transcriptional activators DREB1/CBF (Kasuga et al. 1999; Liu et al. 1998; Kreps et al. 2002) stimulate the stress-response gene that confers low-temperature tolerance (Zhang 2003). The DREB/CBF traits have been efficiently used to produce low-temperature stress tolerance in rice (Dubouzet et al. 2003; Ito et al. 2006), pepper (Hwang et al. 2005), chickpea (Mantra et al. 2007) and potato (Rensink et al. 2005). Low-temperature-tolerance gene screening is a complex procedure. A number of experiments have been performed for low-temperature stress tolerance in plants. Almost all low-temperature tolerance–associated genes are composed of regulatory elements in their promoters, the C-repeat component and low-temperature-responsive component.

3 Rice Genes That Confer Tolerance to Low-Temperature Stress

3.1 MYBS3-Dependent Pathway in Rice Provides Low Temperature Tolerance

Plants exposed to low temperature activate many genes (Hughes and Dunn 1996; Thomashow 1999). These genes encode proteins that help in tolerance against stress and are also involved in osmotica biosynthesis (Chen and Murata 2002; Taji et al. 2002), antioxidants production (Prasad et al. 1994) and enhanced membrane fluidity (Murata and Los 1997; Orvar et al. 2000). In *Arabidopsis* and rice, cold-response CBF/DREB1-dependent pathway plays a principal function and provides low-temperature tolerance (Thomashow 1999; Yamaguchi-Shinozaki and Shinozaki 2006; Chinnusamy et al. 2007). Several cold-responsive (COR) gene promoters have cis-acting elements DRE (dehydration-responsive element) (Yamaguchi-Shinozaki and Shinozaki 1994; Stockinger et al. 1997) and CRT (Baker et al. 1994; Gilmour et al. 1998; Jaglo-Ottosen et al. 1998; Liu et al. 1998; Medina et al. 1999). DRE and CRT cis-acting elements bind and are activated by DREB1/CBF family which contains DREB1A/CBF3, DREB1B/CBF1 and DREB1C/CBF2 (Roychoudhury et al. 2013; Basu and Roychoudhury 2014). In response to low-temperature stress in rice, DREB1A and DREB1B are activated. Their constitutive overexpression leads to the stimulation of the expression of stress-response genes and enhanced tolerance towards high salt and low temperature, along with retardation of growth in transgenic *Arabidopsis* and rice under control conditions (Dubouzet et al. 2003; Ito et al. 2006). Rice is more susceptible to low-temperature exposures because it does not go through the acclimation process. In rice, 22 cold-regulated genes are found by microarray analysis; in *Arabidopsis*, these genes have not been reported (Rabbani et al. 2003). The above-mentioned studies have demonstrated that plant species vary in their potential to acclimatize under low-temperature stress. Some other proteins involved in low-temperature tolerance in rice have also been identified like a zinc-finger protein, iSAP1, and provide cold, dehydration and salt resistance in transgenic tobacco (Mukhopadhyay et al. 2004). MYB4 transcription factor enhances *COR* gene expression in rice and proline accretion in *Arabidopsis* and thus helps in elevating tolerances in plants exposed to chilling and freezing stress (Vannini et al. 2004). MYB4 also improves transgenic apple tolerances towards cold and drought by osmolyte accumulation (Pasquali et al. 2008). In rice, MYB3R-2 (an R1R2R3 MYB gene) overexpression in response to cold, drought and salt confers tolerance against these stress by regulating some genes involved in the CBF-dependent or CBF-independent pathways in *Arabidopsis* in stress response (Dai et al. 2007; Ma et al. 2009). DREB1expression is subject to regulation by numerous factors. *Arabidopsis* cbf2 mutant with disrupted CBF2/DREB1C is tolerant over wild-type plant in response to higher freezing, dehydration and salt, which demonstrate that CBF1/DREB1B and CBF3/DREB1A expression is inhibited by DREB1C/CBF2 (Novillo et al. 2004). In *Arabidopsis*, DREB1/CBF expression was induced during cold acclimation with the help of certain inducers like a transcription factor CBF

Expression1, a MYC-like basic helix-loop-helix-type (Chinnusamy et al. 2003), a $Ca^{2+/}H+$ transporter like CAX1 (Catala et al. 2003), a Ca^{2+} sensor like CBL1 (Albrecht et al. 2003), and a DEAD box RNA helicase called as LOS4 (Gong et al. 2002). However inhibitors like FRY2 (a transcription factor) (Xiong et al. 2002), HOS1 (a putative RING finger E3 ligase) (Lee et al. 2000), and ZAT12 (a C_2H_2 zinc finger transcription factor) (Vogel et al. 2005) inhibited DREB1/CBF expression but the mechanism behind the inhibition is still not clear. In *Arabidopsis* MYBS3 homolog of rice is induced by abscisic acid (ABA), metal treatment ($CdCl_2$), and salinity (Yanhui et al. 2006). In previous studies it has been observed that MYBS3 expression induced in response to low temperature. The enhanced or reduced expression of MYBS3 helped in the identification of MYBS3-induced genes through transcription profiling of transgenic rice and also facilitated in recognizing the different functions of genes. In rice signalling pathway for cold-induced response is DREB1-dependent and inhibited by MYBS3. Previously, studies have observed that studies in rice DREB1- and MYBS3-dependent pathways may balance each other and act successively to adapt to instant and long-term low-temperature stress.

3.2 Cold-Induced Transcription Factor, CBF1/DREB1b Confers Tolerance Against Cold Stress in Transgenic Rice (*Oryza sativa* L.)

Plants exposed to low temperatures behave differently, and in response, variation is observed in the method of gene expression and protein products (Cattivelli and Bartels 1992; Guy 1990). Plant distribution and survival and their yields are decided by their adaptation ability to low temperature. Tropical or subtropical plants are injured or killed by low temperatures. In response to low temperature, plants exhibit a variety of chilling injury symptoms like chlorosis, necrosis or growth reduction (Wang 1990). Low-temperature-resistant plants have the capability to grow under low temperature (Larcher et al. 1973; Rédei 1969). From plants, several low-temperature-activated genes have been isolated. Generally, all factors concerned with low-temperature stress-tolerance response (Crosatti et al. 1996; Guy 1990; Houde et al. 1992; Hughes and Dunn 1996; Mohapatra et al. 1989) along with C-repeat/dehydration-response element binding (CBF/DREB1) transcription factors are implicated in regulating the expression of the number of genes. In *Arabidopsis* DNA-binding proteins, AP2/EREBP family-related three *CBF/DREB1* genes such as *CBF3/DREB1a*, *CBF1/DREB1b*, and *CBF2/DREB1c* have been recognized (Riechmann and Meyerowitz 1998; Gilmour et al. 1998; Kasuga et al. 1999; Liu et al. 1998; Stockinger et al. 1997). *CBF1/DREB1b* and *CBF3/DREB1a* overexpression induces *COR* (cold-regulated) genes that increases low-temperature tolerance (Gilmour et al. 2000; Jaglo-Ottosen et al. 1998; Kasuga et al. 1999; Liu et al. 1998), and their overexpression also alters biochemical processes like sugar and proline accumulation (Gilmour et al. 2000). *CBF/DREB1* genes are considered to be activators that combine several cold acclimation components that consequently

Table 2 List of genes and their functions that provide tolerance against low temperature (Adapted from Kumar et al. 2018)

Gene	Function
SICZFP1	Cold-responsive gene regulation (Zhang 2010)
OrbHLH001	Participated in the regulation of metabolic or homeostasis of ionic (Li et al. 2010)
Osmyb4	Transcription factor (Laura et al. 2010)
OsLTP	Total soluble sugar accretion enhanced, increased proline content and enhanced antioxidant and superoxide dismutase activity (Qin et al. 2011)
OsSPX1	Proline and sugar accretion (Zhao et al. 2009)
OsDREB1D	Transcription factor (Zhang et al. 2009)
OsiSAP8	Cytoplasmic zinc finger protein participated in signal transduction (Kanneganti and Gupta 2008)
OsDREB1F	Transcription factor (Wang et al. 2008a, b)
GS2	Glutamine synthase (Hoshida et al. 2000)
P35S-ZFP245	Proline accretion, pyrroline-5-carboxylate synthetase and proline transporter genes and ROS-scavenging enzyme activation (Huang et al. 2009)
OsP5CS2	Proline accretion (Hur et al. 2004)

enhance low temperatures (Gilmour et al. 2000; Guy 1990). Toenniessen (1991) reported that rice is negatively affected by temperatures ranging from 10 to 20 °C as compared to other crops that show cold tolerance at 0–4 °C. The main responses related to low-temperature stress are poor seedling vigour and poor fertility which ultimately decreased crop yields. Transgenic strategies have been widely adopted to improve low-temperature tolerance. In transgenic rice, improved salt and low-temperature tolerance was obtained by overexpression of either *E. coli* choline oxidase or trehalose-6-phosphate synthase fused to trehalose-6-phosphate phosphatase which led to the accumulation of two compatible solutes, i.e., glycinebetaine and trehalose (Jang et al. 2003; Sakamoto et al. 1998). Introduction of chloroplast glutamine synthetase enhances photorespiration capacity to both salt and low-temperature tolerance (Hoshida et al. 2000). In transgenic rice plants, calcium-dependent protein kinase, CDPK7 overexpression enhanced cold and salt/drought tolerance (Saijo et al. 2000). In transgenic canola and tomato plants, *CBF/DREB1* gene expression also improved low-temperature tolerance (Hsieh et al. 2002; Jaglo et al. 2001) (Table 2).

4 Tolerance to High Temperature

In the current scenario throughout the world climate changes and temperature increases, and this fluctuation in temperature increases the average temperatures. When temperatures increase further than normal levels of tolerance, it affects the plant growth and development. High temperature causes severe impact on the yield of many crop plants, rice (*Oryza sativa*) crop as well. Biotechnological strategies make crop plants more tolerant of heat for this knowledge of precise molecular information is very important for exploitation and exploration of appropriate genes for use in the development of tolerant or resistant crop. Development of tolerant crop is of immediate necessity which is genetically upgraded and survives during heat stress. Rice (*Oryza sativa* L.) is a globally extensively consumed food crop, and its productivity is threatened by high temperature, intensified by climate fluctuation. Here, we are talking about molecular and biotechnological approaches which are helpful in the development of high-temperature-tolerance plants and rice cultivars as well.

5 Molecular and Biotechnological Strategies for the Development of Heat Stress Tolerance in Plants

Molecular strategies are escalating to recognize the heat stress tolerance concept in plants very evidently other than different physiological and biochemical mechanisms. By modulating numerous genes and by regulating the gene expression in diverse pathways, plants develop tolerance during stress conditions (Vinocur and Altman 2005).

5.1 Heat-Shock Proteins (HSPs)

HSPs are dynamic products and are extremely important for plant's survival during detrimental high-temperature stress. Mostly HSPs are produced by the overexpression of several heat-inducible genes, commonly referred as "heat-shock genes" (HSGs) (Chang et al. 2007). Constitutive expression of most of HSPs at high temperature protects intracellular proteins from degradation and conserves their integrity and function by protein folding; hence, they function as chaperones (Baniwal et al. 2004). Recently a number of researches on this active protein family have increased, and this protein family is very heterogeneous in nature. HSP expression is limited to certain developmental stages of plants such as seed germination, embryogenesis, microsporogenesis and maturation of fruit (Prasinos et al. 2005). HSPs can be grouped into five diverse families: HSP100 (or ClpB), HSP90, HSP70 (or DnaK), HSP60 (or GroE) and HSP 20 (or small HSP, sHSP) in plants (Swindell et al. 2007; Banerjee and Roychoudhury 2018). Among HSPs, HSP70 and HSP60 proteins are highly conserved proteins in nature and play significant role in response to high-temperature stress (Kulz 2003). There are mutants that show normal productions of HSPs which are defective in thermotolerance (Hong et al. 2003). Plants possess maximum diversity of low molecular mass (12–40 kDa) HSPs (Morrow and Tanguay 2012). HSP expression is induced due to the temperature-tolerant nature by high-temperature exposure. HSP expression is induced by the activation of HSGs which occurs due to binding of heat-shock elements (HSEs) in the promoter region of HSGs. Palindromic nucleotide sequence (5-AGAANNTTCT-3) in HSE provides recognition as well as binding site for heat-shock transcription factors or simply heat-shock factors (HSFs) (Nover et al. 2001). In plants thermotolerance in response to high-temperature stress has been accomplished by transferring heat-shock regulatory proteins. In normal situation, most of the plant species constitutively expressed HSFs, and these proteins (HSPs) occur in monomeric forms attached to one of the HSP70. However, when a plant is exposed to high temperature, HSP70 dissociates from cytoplasmic monomeric HSFs, it enters into the nucleus, and trimer formation occur that can attach with the HSEs (Lee et al. 1995). Binding of heat-shock factors employs other transcriptional component which leads to instant gene expression under enhanced temperature (Fig. 1). HSGs have HSE-conserved sequence. HSF gene overexpression on nearly all HSGs provides protection during high-temperature stress. Plants have numerous sets of these genes in contrast to animals, and yeasts may have four or fewer HSFs. In tomato more or less 17 and in *Arabidopsis* 21 different *HSF* genes has been reported. On the basis of their flexible linkers and oligomerization domains, these *HSF* genes have been placed under three groups (classes A, B and C) (Nover et al. 2001). Various HSFs activate in response to high temperature and demonstrate that the particular HSF is concerned with particular gene transcription, and their activation may alter depending on the stress timing and intensity. Generally, HSF overexpression in plant can improve thermo-tolerance. However, individual HSF gene knockouts had slight impact on plant survival exposed to enhanced temperature. In response to enhanced temperature, numerous non-HSP transcripts are unregulated (Morrow and Tanguay 2012). Cytosolic ascorbate peroxidase gene (*APX1*) activity is upregulated by enhanced temperature in *Arabidopsis*, and in its 5′-promoter region, functional heat-shock element (HSE) is present. It was observed that in *Arabidopsi*s the early response of many genes on exposure to high temperature is controlled by *HSFA1a* and *HSFA1b*, whereas other HSFs accountable for the genes induction are expressed later. One of the HSFs, i.e. *HSFA1*, has been anticipated to be the ‘master regulator’ of the high-temperature response in tomato. Normal HSP formation does not take place if activity of this gene is censored consequently, and the plant is susceptible to high temperature (Mishra et al. 2002). It was found that other HSFs act synergistically. Therefore, the HSF system in plants appears to have capability to control the expression of high temperature induced genes. Several observations suggested that positive association among HSP level in the plant cell and particular stress tolerance (Sun et al. 2001; Wang et al. 2005). How HSPs provide tolerance against high temperature is still not known. Recent studies paying attention on the in vivo thermoprotection function, controlled by sHSPs (Miroshnichenko et al. 2005).

Under high-temperature stress, heat-shock protein, individually or in chaperone form, helps in cell protection mechanisms. Heat-shock proteins are also accountable for the synthesis of protein and their targeting, maturation and degradation, and in protein and membrane stabilization as well, and renaturation of protein exposed to high temperature stress (Torok et al. 2001). During high-temperature stress, protein denaturation takes place because reduced cellular volume increases the degradative molecular interaction probability. HSPs play important role in maintenance and repairing the structure of the accompanying protein along with targeting incorrectly accumulated and non-native proteins for the degradation and elimination from plant cells (Reis et al. 2012). HSPs play very crucial role in folding and conformation of

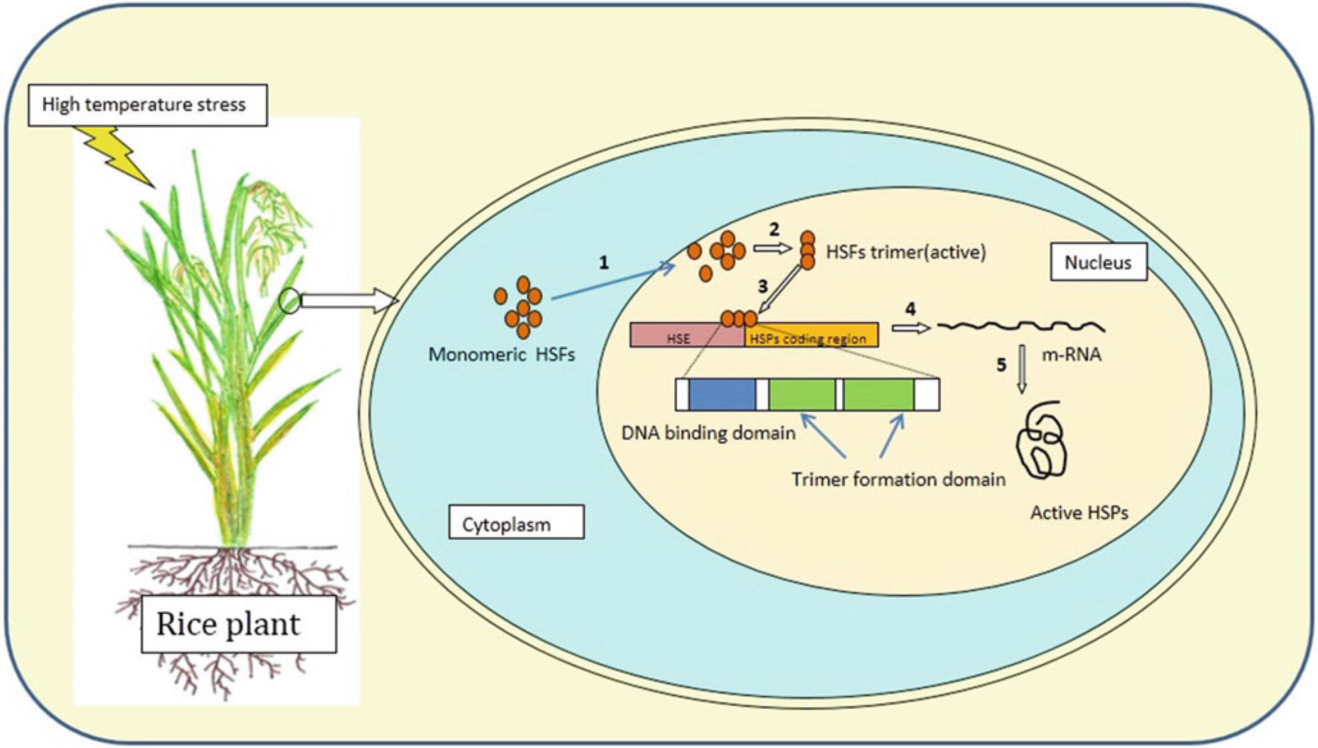

Fig. 1 Diagrammatic representation of molecular regulatory mechanism of heat-shock proteins. (1) Monomeric heat-shock factors (HSFs) enter in nucleus. (2) HSF monomers form active trimer. (3) HSF binds to heat-shock element (HSE) of the respective heat-shock gene (HSG). (4) Transcription. (5) Translation produces functional HSP to save plant cell and provide high-temperature stress tolerance

both structural and functional proteins and also control the functions of many cellular proteins under high temperature. Among these, NtHSP70-1 in tobacco constitutively overexpressed and participated in plant stress response and tolerance (Cho and Hong 2006). Transforming plants with HSP24 from *Trichederma harzianum* were found to improve resistance to high temperature stress when in *Saccharomyces cerevisiae* constitutively expressed (Liming et al. 2008). In the present time genetic analyses, the main aim is to improve the tolerance level in major crop plants exposed to high temperature. In plants tolerance against high temperature is controlled by number of genes (polygenic trait) and in plants at different stages of development or in different tissues several other tolerance components are also important. Therefore, it shows spatiotemporal mechanism and regulation (Bohnert et al. 2006). Hence, genetic stocks having diverse degrees of high-temperature tolerance, connection and co-segregation analyses and molecular biology techniques are potential strategies to dissect the genetic basis of plant's temperature tolerance (Maestri et al. 2002).

5.2 Genetic Engineering and Transgenic Approaches in Conferring Heat Stress Tolerance in Plants

High-temperature-induced unfavourable effects are mitigated by creating crop plants with better tolerance by means of different genetic engineering and transgenic strategies (Rodríguez et al. 2005). Constitutive expression of specific proteins enhances tolerance against high temperature. Transgenic plants with varying degrees of high-temperature stress tolerance have been formed by means of the researches relating to sHSPs/chaperone expression and HSF gene expression manipulation. Interestingly, in *Arabidopsis* plants by altering the transcription factor (*AtHSF1*) which is responsible for HSPs, Lee et al. (1995) effectively manipulate the heat-shock protein (HSP) expression level and generate transgenic high-temperature stress-tolerant *Arabidopsis*. *AtHSF1* is constitutively expressed in this plant. Its actions for binding of DNA, formation of trimer and transcriptional activation of HSP genes are inhibited. Transcription factor was inactive for high-temperature tolerance while *AtHSF1* gene was overexpressed. Fusion protein is formed when this gene attaches with the *N* or *C* terminus of *gusA* reporter gene (for β-glucuronidase synthesis), and in the absence of high-temperature stress this fusion protein is able to trimerize itself and/or with the additional HSFs. Fusion protein transformation into *A. thaliana* produced transgenic plants that constitutively expressed HSPs and showed increased thermotolerance. Transgenic carrot cell lines and other plants showed increased thermotolerance by carrot *Hsp17.7* gene-driven *CaMV35S* promoter constitutive expression (Malik et al. 1999). Previously, it is found that in mitochondria of tomato, small HSP (*MT-sHSP*) has a molecular chaperone function in vitro (Liu and Shono 1999). This gene is used for the thermotolerance transformed tobacco production (Sanmiya et al. 2004). In transgenic rice, it was observed that the incorporation of *HSP* genes increased the tolerance level against heat. To enhance thermotolerance in transgenic rice, Katiyar-Agarwal et al. (2003) introduced *Arabidopsis Hsp101* overexpression gene. Furthermore, in *E. coli* overexpression of chloroplast *HSPs* (*Oshsp26*) gene of rice improved tolerance to high-temperature stress and different oxidative stress as well (Lee et al. 2000). In rice plants, overexpression of *sHSP17.7* develops high-temperature stress tolerance (Murakami et al. 2004). *Dnak1* gene from the salt-tolerant cyanobacterium *Aphanothece halophytica* introduced in tobacco improves high-temperature stress tolerance (Ono et al. 2001). In *Arabidopsis* insertion of elevated levels of the compatible solute glycinebetaine (GB) through alteration with a bacterial choline oxidase gene engineered to target the protein of chloroplast was observed to be important for increase in germination rates and seedling growth of seeds exposed to elevated temperatures (Alia et al. 1998). *BADH* gene involves in the production of GB, and their transformation in plants for the over GB osmolyte production will improve tolerance against high temperature (Yang et al. 2005). Rubisco activase involves in rubisco activity. Rubisco activase stability towards high temperature is desirable for the preservation of its activity (Salvucci and Crafts-Brandner 2004). Altering tobacco with Rubisco activase gene for the reversible Rubisco decarboxylation helped in achieving thermotolerance. In the protection of photosynthetic apparatus, this tolerance mechanism helps (Sharkey et al. 2001). Lipids composed of fatty acids and modification in fatty acid enhance stability of the photosynthetic membrane against high temperature. It also enhances tolerance against high temperature and limits photo-oxidation because of free radical formation as well. Alterations in membrane fluidity may also change the stress perception via lipid signalling, consequently altering the protective mechanisms response. Transgenic tobacco with altered chloroplast membrane has been produced through silencing the gene encoding chloroplast omega-3 fatty acid desaturase (Murakami et al. 2000). In tobacco (*ANP1*/*NPK1*), H_2O_2 responsive MAPK kinase (MAPKKK) constitutive expression protects plants against the damaging effect of high temperature (48 °C, 45 min) (Kovtun et al. 2000). Rizhsky et al. (2004) observed *NPK1*-related transcript was considerably increased by high temperature. *Arabidopsis* plants constitutively expressing the barley *APX1* gene show modest augment in high-temperature tolerance (Shi et al. 2001). At high temperature, by β-carotene hydroxylase, overexpression of xanthophyll cycle intermediates double, resulting in protection against high temperature (Davison et al. 2002). By *HSP* gene overexpression or by changing HSF level that control heat-shock and non-heat-shock gene expression and also through trans-acting factors like DREB2A, bZIP28 and WRKY proteins, transgenic plants are developed having potential to tolerate high temperature (Grover et al. 2013). Development of transgenic plants with high-temperature tolerance is achieved by genetically modifying proteins concerned with osmotic adjustment, ROS removal, photosynthetic reactions, polyamines production and biosynthesis of protein. Table 3 summarizes the representation of gene and its functions that provide tolerance against heat.

Table 3 List of genes and their function that provide tolerance against heat (Adapted from Kumar et al. 2018)

Gene	Function
HSP 101	Heat-shock protein synthesis for resistance against temperature (Queitsch et al. 2000)
APX 1	Detoxification of H_2O_2 and provide tolerance against heat (Shi et al. 2001)
HSP 70	Heat-shock protein synthesis against temperature (Montero-Barrientos et al. 2010)
Cod A	Synthesis of glycinebetaine for tolerance against temperature (Alia et al. 1998)
HSP 17.7	HSP synthesis (Malik et al. 1999)
FAD 7	Responsible for increased unsaturated fatty acid level and temperature tolerance (Sohn and Back 2007)
AtPARP2	Enhanced heat stress tolerance (Vanderauwera et al. 2007)
TLHS1	Class I HSP synthesis (Park and Hong 2002)
AtHSF 1	HSF combined with β-glucuronidase and this manipulation enhances the production of HSP (Lee et al. 1995)
MT-sHSP	Molecular chaperone (Sanmiya et al. 2004)
Dnak 1	Temperature tolerance (Ono et al. 2001)
BADH	GB osmolyte excessive synthesis increases tolerance against heat (Salvucci and Crafts-Brandner 2004)

6 Recently Known Genes of Rice Provide Tolerance to High Temperature-Stress

Several genes have been recognized in rice by different ways such as microarrays and RNA-Seq, proteomics data, protein–protein interactions and genome-wide gene-indexed mutant populations (Chandran and Jung 2014). Gene functions are identified by the help of these data. Genetic analyses, gene-indexed mutants or genetically modified plant help us to know the functions of number of genes in rice (Yamamoto et al. 2012). Table 3 represents the genes that are involved in high-temperature stress and that provide tolerance against high-temperature stress. Several studies recognize genes through expression profiling that are produced in response of stresses (Rabbani et al. 2003; Bray 2004; Yamaguchi-Shinozaki and Shinozaki 2006; Zhang et al. 2012). In heat-sensitive 'Nipponbare' rice and the heat-tolerant '996' rice, expression of gene in reproductive and ripening tissues at the transcriptome level has been observed through microarrays (Endo et al. 2009; Zhang et al. 2012). In addition, with the help of RNA-Seq, the genes of reproductive tissues during anthesis of heat-tolerant 'N22' rice and the sensitive cultivars 'IR64' and '6264' were also evaluated (Gonzalez-Schain et al. 2016). In response to high-temperature stress, more than 1400 gene expressions were manipulated in the anthers from 'Nipponbare' plants (Endo et al. 2009). Likewise in young florets, 2500 heat-responsive genes were observed during meiosis (Zhang et al. 2012). Among 630 heat-responsive genes, few are core heat-responsive (Gonzalez-Schain et al. 2016). The combined effect of drought and heat stress upregulated 20 gene activities in pollinated rice pistils (Li et al. 2015a). Table 3 shows genes expressed in plants under high-temperature stress and that provide tolerance against this stress. In rice reproductive and ripening tissues, heat-responsive genes are expressed that are used to improve high-temperature stress tolerance. Expression profiling provides a large number of differentially expressed genes (DEGs) that will be helpful in future studies and also increases our knowledge about molecular responses and their probable pathways involved under stress condition. In rice reproductive stage, TFs are overrepresented among the DEGs in response to high-temperature stress (Endo et al. 2009; Zhang et al. 2012; Gonzalez-Schain et al. 2016). In rice, the genes that control pathways involved in sugar and amino acid metabolism play significant role in high-temperature stress tolerance (Li et al. 2015a). Recently, studies suggested that several DEGs in rice can aid us to comprehend the connections and probable role of these key reproduction-related genes in stimulating high-temperature stress tolerance. During the caryopsis maturation stage, chalkiness and smaller grains are distinctive of rice under high temperature stress. It seems that decreased or limited expression of starch synthesis-related genes such as sucrose transporter (*SUT1*), invertase (*CIN2*), UDP-glucose pyrophosphorylase (*UGPase*), ADP-glucose pyrophosphorylase (*AGPS*), soluble starch synthase (*SSIIIa*) and branching enzyme (*BEIIb*) (Nishi et al. 2001; Kawagoe et al. 2005; Fujita et al. 2007; Wang et al. 2008a, b; Woo et al. 2008; Ito et al. 2009) also induce variation like chalkiness and small grains at the time of maturation in transgenic and mutant rice. The expression of genes involved in sucrose import/degradation and starch biosynthesis and as well as others are downregulated by high-temperature stress while starch degradation genes are upregulated proved by transcriptomic profiling (Yamakawa et al. 2007; Yamakawa and Hakata 2010). Rice grain proteome study under high-temperature stress has exposed differential expression for 61 genes concerned with diverse physiological and metabolic functions, like synthesis of protein, redox homeostasis, metabolism of lipid and biosynthesis/degradation of starch (Kaneko et al. 2016). High-temperature stress affects proteins involved in starch metabolism, storage proteins accretion and also impact the physicochemical characteristics of rice flour and brewing quality. In various conventional cultivars and transgenic lines of rice under high temperatures, enhanced expression of small heat shock proteins or redox homeostasis-related genes is involved in chalky grains formation (Lin et al. 2005, 2014; Liu et al. 2010, 2011). Under high temperatures in developing caryopsis, metabolomic analysis demonstrated decreased levels of sugars (glucose and fructose), sugar phosphates and organic acids that participate in glycolysis/gluconeogenesis and the TCA cycle while enhancing the quantity of sucrose, UDP glucose and ADP glucose (Yamakawa and Hakata 2010). High-temperature-induced reduction in grain weight and quality is an extremely intricate event proven by genetic and transcriptome profiling, and this event requires the interactions of different metabolic pathways, including those for sugar import/degradation, synthesis/degradation of starch, storage protein synthesis and homeostasis maintenance.

7 Conclusion

In this chapter, the effect of environmental stress, both low and high temperatures, on cultivated plants was evaluated, and it also emphasizes on the role of abiotic stress in manipulating the large annual fluctuations in crop yield. Molecular genetics is a very significant tool for understanding the fundamental biology of plants under abiotic stress condition. Molecular mapping helps in building a fine-scale map to isolate the position of genes involved in shaping vital agronomic traits. Development of genetically engineered plants with improved tolerance towards high and low temperatures is a crucial challenge in rice biotechnology research. Rice being the world's most important food crop is also used as a model for cereal genomic studies. Hence, research for rice transformation hold a major place in cereal biotechnology. The most prominent tool for improving rice tolerance against abiotic stress is genetic engineering. Several genes have been recognized in rice by different methods like RNA-Seq and proteomics data. Genes that are involved in high-temperature stress and provide tolerance against high-temperature stress have been recognized and successfully introduced into rice create transgenic crops with enhanced stress tolerance. The development of transgenic plants with high tolerance against temperature is undoubtedly beneficial but is also important to assess the risk imposed by transgenic plants/crops to the environment. There is a need for a well-developed machinery to assess the risk through a scientifically sound and transparent mechanism. The adoption of transgenic plants depends entirely on the evaluation of the risks or benefits, regulatory approval, cost input and time required along with the economic status, requirements and values of different countries. We can conclude that biotechnological approach is helpful in increasing rice tolerance towards high and low temperatures, but implementation of gathered information should be done with keeping the risk involved in considerations.

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Biotechnological Approaches to Develop Rice Tolerance to Low and High Temperature Stress

Nita Lakra, Aarti Soni, and Renu Munjal

Abstract

Temperature above or below optimum has a negative impact on plant growth and performance, which leads to a great loss in economic yield. Rice (*Oryza sativa* L.) is one of the major staple food crops around the world and highly sensitive to temperature stress during the reproductive stage. Rice productivity in the tropics, subtropics, and temperate areas is in danger due to low (cold stress) and high (heat stress) temperatures. Both low and high temperatures equally affect both vegetative and generative organs of rice and sometimes lead to complete sterility. In response to temperature stress, a significant variability exists among rice germplasms. Stresses evoke cascades of physiological and molecular events and to increase the global production under such environment, we need to understand the mechanism which will help in the mitigation of stress. In response to temperature stress, protection of structural proteins, enzymes, and membranes, and expression of heat shock proteins (HSPs) are one of the major biochemical processes that impart thermotolerance. All these traits should be exploited for the development of thermo-tolerant varieties and finally the replacement of the sensitive varieties with tolerant ones. Various biotechnological tools, along with integrated management and conventional breeding, can help us to develop novel rice genotypes with better grain yield under stress. In this chapter, we will discuss the effects of low and high temperature on rice crop and various biotechnological approaches and tools including genetic engineering and omics for improving the thermal stress tolerance of rice.

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Keywords

Cold stress · Heat stress · HSPs · MAS · QTL mapping · Molecular breeding

1 Introduction

Due to ever-increasing world population, we have been forced to take necessary steps to increase crop production by 50% before 2030 (Tomlinson 2013). However, the global climate change, which has negative impacts on agricultural productivity, is adding to the challenge (Jagadish et al. 2012). Nowadays, the increasing temperature is one of the major threats worldwide. Over the next 30–50 years, the average temperature is expected to rise by 2–3 °C (Hatfield and Prueger 2015).

About one-tenth of the agricultural land is utilized for the cultivation of rice (Wang and Peng 2017), and sustains the lives of three billion people (Krishnan et al. 2011 Asia (growing on ~90% acreage) contributes a major share of rice consumption and production (Suh 2015). Rice is a source of protein (~14%) and fat (2%) (Kennedy and Burlingame 2003). In India, about 44.5 Mha land is used for the production of rice and more than 70% of the population consumes it as a staple food. It is the main energy food source for most of the people. The optimal temperature for the rice cultivation is 25–35 °C, and temperature below or higher than optimal negatively affects the growth, physiology, and yield of crop. Temperature stresses disrupt membrane integrity, severely affect the photosynthesis, and increase photorespiration, thereby altering the normal homeostasis of plant cells. High temperature (>35 °C) negatively affects the growth of roots and shoots, hampers pollination, causes poor anther dehiscence, and leads to spikelet sterility. However, the responses of rice to high temperature stress vary with the quantum of temperature increase and its duration. Likewise, low temperature (<20 °C) delays rice germination and seedling establishment, hampers tiller formation, affects flowering, causes panicle sterility, and finally leads to lower grain yield (Hussain et al. 2019). Previously increases in crop production have been achieved through the use of fertilizers and chemicals, although these depend on limited resources and are generally unsustainable. The advancement in biotechnology and their application in crop plants have helped us to increase rice yields compared to that achieved by conventional breeding. Genomics has laid the foundations for sustainable intensification of agriculture and development of climate resilience crops. Molecular breeding (MB) approaches rely on various biotechnology tools to improve the stress management potential of crop plants. Marker-assisted selection (MAS) is one of the approaches employed to improve breeding efficiency for decades (Xu and Crouch 2008; Leng et al. 2017). With the advancement in high-throughput phenotyping platforms, genotyping technology, and sequencing methods has transformed molecular breeding to genomics-assisted breeding (GAB) and the focus of plant breeders has gradually switched from phenotype-based to genotype-based selection.

Various biotechnology tools have been reported, which helps in enhancing breeding efficiency in rice through the following strategies:

- DNA marker technology for enhancing precision and accuracy in breeding program.
- Genetic engineering for transferring agronomically useful traits across species barrier.
- Genomic tools for identifying new and useful genes/alleles.
- Manipulation of target trait alone, without affecting the nontarget regions.
- Shortening the breeding cycle of crops.

The major focus of this chapter is on the traditional as well as new biotechnological strategies for the abiotic stress tolerance improvement in plants. It covers the molecular breeding, genomic-assisted breeding methods, Omics, double haploid technique, and transgenic approach that helps in the unwrapping of complicated regulatory pathways controlling stress tolerance mechanisms by high-throughput expression profiling and gene inactivation techniques. Further, an account of stress-inducible regulatory genes is discussed. Here we also discuss about the sensitivity of rice at various developmental stages against the low and high temperature and propose methods for enhancing the tolerance of rice to thermal stress where the reproductive and grain-filling stages of rice is the major concern.

2 Effect of Low and High Temperature Stress and Mechanism of Tolerance

Nowadays, temperature stress is becoming the main area of concern for the scientists around the world due to global climate change, and has devastating effects on plant growth and metabolism (Suzuki and Mittler 2006; Suzuki et al. 2011; Bita and Gerats 2013). Every plant species needs an optimum temperature for its growth and development, and any alteration in this temperature has drastic effects on plant growth and metabolism (Hasanuzzaman et al. 2012, 2013). Temperature stress may significantly affect the various vital processes such as seed germination, photosynthesis, membrane stability, respiration, metabolites level, hormone level, and loss of function of tapetal cells (Wahid et al. 2007; Endo et al. 2009; Hemantaranjan et al. 2014; Ahmad et al. 2016; Waqas et al. 2017). High temperature also affects pollen germination, spikelet fertility, and grain yields (Jagadish et al. 2010; Hasanuzzaman et al. 2013; Zafar et al. 2018). High temperature affects grain formation and development through (1) poor anther dehiscence due to restricted closure of the locules, leading to reduced pollen dispersal and low number of pollen on the stigma, (2) changes in pollen proteins lead to a significant reduction in pollen viability and pollen tube growth and finally cause spikelet sterility, (3) delay in heading, (4) reduced starch biosynthesis in developing grain, (5) increased chalkiness of grain with irregular shaped starch granules, and (6) a shortened grain-filling period, resulting in low grain weight. It is reported that high temperature may hamper the vegetative as well as reproductive stages (Katiyar-Agarwal et al. 2003); however, booting and flowering are the most critical stages, which may cause complete sterility in rice (Shah et al. 2011).

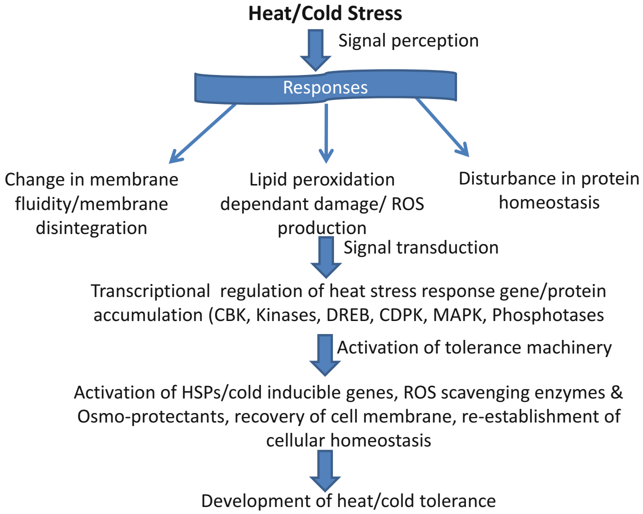

Fig. 1 Effect of thermal stress and responses of crop plants including rice

Low temperature is another major environmental factor that affects plant growth and crop productivity and leads to significant crop losses. Cold stress influences reproductive development through (1) delayed in heading, because of effect on anther respiration, which increases sucrose accumulation, protein denaturation and asparagine levels (2) pollen sterility as a result of tapetal hypertrophy and associated nutrient imbalances, (3) reduced activity of invertase in the tapetum, (4) impaired fertilization due to inhibited anther dehiscence, stigma receptivity, and ability of the pollen tube to germinate via the style toward the ovary, and (5) floret sterility, which restricts grain size, increases grain abortion, and leads to reduce grain yield. Cold stress is one of the major constraints on rice (*Oryza sativa* L.) growth at the early-seedling stage and because of low temperature stress, in south and southeast Asia, modern rice varieties cannot be planted in an estimated seven million hectares of land (Sthapit et al. 1998). During early vegetative stages, cold stress severely inhibits seedling growth, plant height, and cause tiller number reduction (Tian et al. 2011; Iba 2002). There are substantial genetic variations in rice germplasm exist, which can thrive better under the prevailing temperature environments (Shah et al. 2011) and the tolerance responses include adjustments to various morphological, physiological, and biochemical traits. Hasanuzzaman et al. (2013) suggested that temperature stress triggers the expression of certain genes and metabolites production, which provides tolerance to plant. Plants have evolved more than one mechanism, which includes escape, avoidance, or survival under temperature stress (Fig. 1). These mechanisms impart brief time avoidance or long time resistance. At the cellular level, tolerance processes including LEA proteins, factors involving in signaling cascades, ion transporters, antioxidant defense, osmolytes, and transcriptional control are required to balance the stress effects (Rodríguez et al. 2005). In a world where population growth is outstripping food supply, only plant biotechnology has surpassed all previous expectations, and the future is even more promising by improving the productivity of crops by enhancing their ability to resist or tolerate the abiotic stresses. In biotechnology, different strategies are involved for the improvement of yield and quality and it should be integrated with physiology and breeding as an aid to classical breeding, and for generation of engineered plants for abiotic stress tolerance into agricultural production systems.

3 Biotechnological Approaches for Improving Rice Tolerance

3.1 Molecular Breeding

The main objective of plant breeding is to accumulate favorable alleles that contribute to stress tolerance in a plant genome. Genes that confer stress tolerance can be sourced from germplasm collections, including wild relatives of crops that are held in gene banks or organisms that currently live in the habitats of extreme temperature that have evolved to cope with those conditions (Nevo and Chen 2010). Although some progress has been made through conventional breeding (Blum 1985), traditional approaches are limited by the complexity of stress tolerance traits and/or filled with difficulties because of the multigenic nature of trait and low genetic variance of yield components under stress conditions and the lack of efficient selection techniques. The application of quantitative trait loci (QTLs) mapping is one approach to dissect the complexity of plant stress tolerance. Furthermore, quantitative trait loci (QTLs) that are linked to tolerance at one stage in development can differ from those linked to tolerance at other stages. In brief, breeding for abiotic stress tolerance is constrained by the following factors: (1) by the complex nature of abiotic stress tolerance because of timing, duration, intensity, frequency, and thereby its quantification and repeatability; (2) because undesirable genes are also transferred along with desirable traits; and (3) because reproductive barriers limit the transfer of favorable alleles from the diverse genetic resources. Now, biotechnology is a viable option for developing genotypes that can perform better under harsh environment. Advances in genomics coupled with bioinformatics and stress biology can provide useful genes or alleles for conferring stress tolerance. Superior genes or alleles where they have been identified in the same species can be transferred into elite genotypes through molecular breeding (MB). Molecular breeding is the application of molecular biology tools in plant breeding, which generally includes marker-assisted selection (MAS) and genetic engineering (genetic transformation) in addition to QTL mapping or gene discovery. Moreover, by using genetic engineering (GE), there is no barrier to transfer useful genes or alleles across different species. The integration of genomic tools with high-throughput phenotyping to improve breeding practices through molecular markers facilitates the prediction of phenotype from genotype.

Now genome sequences are available for many crop species such as rice, (Goff et al. 2002; Yu et al. 2002; IRGSP 2005) poplar (*Populus trichocarpa*) (Tuskan et al. 2006), sorghum (*Sorghum bicolor*) (Paterson et al. 2009), maize (Schnable et al.

2009), and soybean (*Glycine max*) (Schmutz et al. 2010). Furthermore, next-generation sequencing (NGS) technologies have made it possible to sequence the genomes/transcriptome of any species relatively quickly and cheaply (Varshney et al. 2009). As a result, genome sequences are now available for less studied crops such as cucumber (*Cucumis sativus*) (Huang et al. 2009a, b), pigeon pea (*Cajanus cajan*) (http://www.icrisat.org/gt-bt/IIPG/home.html) and complex genome species such as wheat (http://www.genomeweb.com/sequencing/wheat-genome-sequenced-roches-454) and barley (*Hordeum vulgare*) (http://barleygenome.org/). These genome/transcriptome sequences coupled with genetic approaches can be used for identifying suitable genes conferring stress tolerance.

Marker-assisted selection (MAS) is a method used to select desirable individuals based on DNA molecular marker patterns instead of, or in addition to, their trait values. It is a tool that can help plant breeders select more efficiently for desirable crop traits. MAS is the process of using DNA testing results for the selection of individuals to become parents for the next generations. The submergence-tolerant rice cultivars was developed through marker-assisted back cross (MABC), which has improved yields in >15 million hectares of rain-fed lowland rice in south and southeast Asia (Septiningsih et al. 2008).

Collard and Mackill (2008) outlined three fundamental merits of marker-assisted selection (MAS) compared with conventional breeding.

1. MAS is simple, time saving, cost effective, and facilitates a nondestructive assay.
2. Selection can be carried out at any growth stage.
3. It facilitates differentiation of homozygous plants from heterozygous ones in backcross, bulk and pedigree breeding methods, and thus helps in early-generation selection of superior recombinants, particularly for those traits controlled by recessively inherited genes.
4. Screening can be done even without exposure to stress.

The advantage to conduct MAS is that a large number of molecular markers are available for rice and many traits have been tagged with molecular markers. However, MAS has a disadvantage: MAS is only effective when the target traits exist in rice germplasm. In this case, transgenic engineering is useful, which could introduce the new traits into rice by transferring the target gene from other species and its expression could produce the target trait. Moreover, by using genetic engineering, there is no barrier to transferring useful genes or alleles across different species.

Genetic engineering approach (GE) has advantages over other techniques due to the following reasons: (1) The identification of genes encoding, TFs, signaling proteins and effector proteins, and stress-responsive promoters controlling stress tolerance. (2) The identification of genes regulating opening and closing of stomata and induced expression for the enhancement of water use efficiency in crops. (3) The genetic transformation and development of elite crop genotypes with tolerance to temperature stress and other environmental stresses. (4) The assessment of promising transgenic lines for multiple stress tolerance under field conditions.

(5) The deregulation of transgenic lines to enable the release of a superior line or variety.

3.2 Genomics-Assisted Breeding for Developing Climate-Resilient Cultivars

Genome assisted breeding (GAB) enables breeders to start out with a large population of only genotypically characterized offspring, and then only use a selected subset for phenotypic evaluation, which is more expensive (Cooper et al. 2014; Peng-fei et al. 2017). Genomics-assisted breeding allows rapid identification of genes for breeding of crops adapted to a changing climate. The increasing volumes of genomic data facilitate the move toward plant genomics, providing deeper insights into the diversity available for crop improvement and developing of new cultivars. Reference crop genome sequences are the basis for crop genetic and genomic studies, as they provide insights into genomic variation, gene content, and genetic basis for agronomic traits. Since the completion of the first human draft genome in 2001, the study of genome sequencing in other species has been growing rapidly. Sanger sequencing, the first generation of sequencing technology, has been used to assemble several plant genomes. Despite the long read length and high assembly accuracy, the low throughput and high cost have limited the widespread adoption of Sanger sequencing for genome assembly (Metzker 2005). Second-generation sequencing (SGS) technologies such as Illumina are faster, with higher throughput and lower cost, and have become dominant (Goodwin et al. 2016). According to the National Center for Biotechnology Information (NCBI), there are over 100 publicly available plant reference genome sequences and majority of them were assembled using SGS data. Genomics-assisted breeding can aid in the transfer of only the desired genes, improve gene combinations, fast recovery of elite lines with targeted traits, and a clear targeted strategy, allowing supplementation of available genetic variation with precisely tailored genes and accelerating gene pyramiding efforts (Varshney et al. 2006). Steps for developing candidate lines using MAS and GAB have been shown in Fig. 2. In brief, initially breeding generations resulted from intercrosses (F1 to Fn) and from backcrossing to a parental cultivar (BC1F1 to BCnFn). The selection of parents, from the germplasm, exhibiting contrasting traits or characters, is the fundamental step for breeding. For MAS, parents should be homozygous. After the parents are selected, they are crossed to obtain the F1 generation (PA x PB) or genome-edited elite cultivar (PGE) developed and then genomic methods assisted in the conventional backcrossing (BC1F1 to BCnFn) and intercrossing (F2 to Fn) population. The phenotypic and genotypic data generated from F2 generation is used for the construction of QTLs maps, analysis, and validation. This is followed by screening to detect the presence of the marker alleles. The hybrids exhibiting the presence of markers are selected. In a final step, genome edited cultivars and candidate lines selected from the succeeding generations of backcrosses or intercrosses are tested for broad viability in advanced multi-environment field trials for evaluation of the yield,

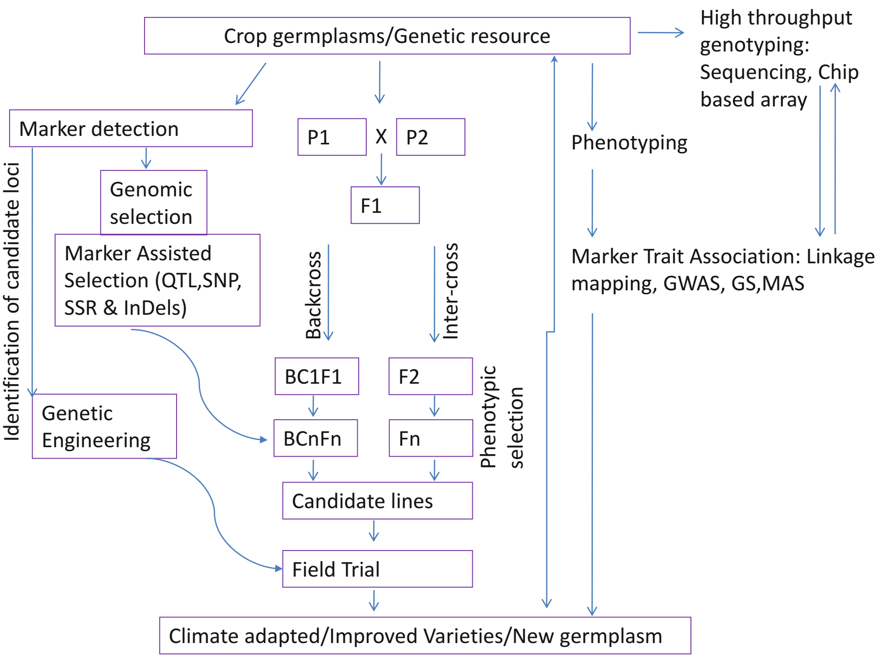

Fig. 2 Genomics-assisted breeding for the development of climate-resilient cultivar

resistance, quality, and many more traits of interest and lead to select novel climate resilient cultivars.

3.3 Integrated Biotechnology Approach

Although the biotechnology community has remained focused on either MB or GE approaches (Narayanan et al. 2004), it is evident that there is a need to target complexity of temperature stress by using integrated biotechnology approaches. In addition to MB and GE, some new approaches are currently available that should be integrated with MB and GE to tackle such complex stresses (Fig. 3). These approaches include (1) next-generation sequencing (NGS), transcriptomics, and proteomics approaches for isolating novel genes and promoters for abiotic stress tolerance (Varshney et al. 2006); (2) gene targeting for the genetic modification of crops (Zhang et al. 2010); (3) development of marker-free transgenic crop (Parkhi et al. 2005); (4) the development of cis-genics (Jacobsen and Schouten 2007); (5) allele mining for the candidate. The integration of genomic tools with high throughout phenotyping to assist breeding practices through molecular markers to facilitate the prediction of phenotype from genotype.

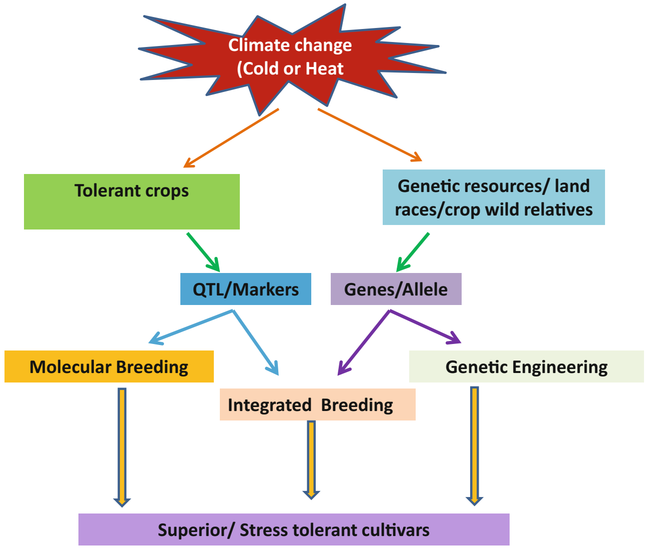

Fig. 3 Integrated approach to develop better adapted crops for climate change

3.4 Molecular Markers

Molecular markers are the small part of the DNA sequence, which is associated with a specific portion of the genome and acts as a "flag" for that specific position of a distinct gene or the inheritance of a distinct characteristic. Markers that are closer together have fewer recombination events between them during meiosis than markers that are far apart; thus, the alleles at closely linked genes tend to be inherited together. In a given cross, the attribute of interest will usually stay linked with the molecular markers. Thus, individuals can be selected in which the molecular marker is present, since the marker indicates the presence of the desired traits. DNA marker technology refers to the application of DNA-based markers in breeding programs to improve the selection efficiency (Sarma and Sundaram 2005). Breeders can use these markers to supplement the classical breeding and can select segregating plants based on the DNA marker rather than waiting long to observe the phenotype. Molecular markers are used to develop tightly separated genetic maps of a specific genome utilizing an desired mapping population of plants (Sehgal et al. 2008).The larger part of these maps has been made using restriction fragment length polymorphism (RFLP) markers, and different PCR-based markers for the development of genetic map (Sehgal et al. 2008): for example, amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs), random amplified polymorphic DNA (RAPD), and sequence tagged sites (STSs) permit loci controlling quality related to stress tolerance to be identified and mapped in the genome. Various markers have been developed and are applied to a wide range of crop species. These are the restriction fragment length polymorphisms (RFLPs), amplified

Table 1 Comparison of most commonly used marker systems (adopted from Korzun 2003)

Feature	RFLPs	RAPDs	AFLPs	SSRs	SNPs
DNA required	10	0.02	0.5–1.0	0.05	0.05
DNA quality	High	High	Moderate	Moderate	High
PCR based	No	Yes	Yes	Yes	Yes
Number of polymorphic loci analyzed	1.0–3.0	1.5–50	20–100	1.0–3.0	1.0
Ease of use	Not easy	Easy	Easy	Easy	Easy
Amenable to automation	Low	Moderate	Moderate	High	High
Reproducibility	High	Unreliable	High	High	High
Development cost	Low	Low	Moderate	High	High
Cost per analysis	High	Low	Moderate	Low	Low

fragment length polymorphisms (AFLPs), random amplification of polymorphic DNAs (RAPDs), sequence tagged sites (STS), simple sequence repeats (SSRs) or microsatellites, and single nucleotide polymorphism (SNPs). The advantages and disadvantages of these markers are given in Table 1.

A plenty of markers are now available for rice improvement. Depending on the specific purpose, the marker system can be different, as each marker system has specific advantages over others. The suitability of a marker is determined by several considerations such as ability to discriminate between individuals, ease of assay, the frequency of occurrence of the marker, and more importantly the type of marker: codominant or dominant. In rice, many PCR-based markers like random amplified polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and intersimple sequence repeats (ISSRs) are being used. Among the PCR-based DNA markers, microsatellites or simple sequence repeats (SSRs) are highly preferred for gene tagging and mapping efforts due to the high level of polymorphism and versatility and their reproducibility and amenability for automation (McCouch et al. 2002). In rice, around 20,000 SSR markers have been developed so far and their chromosomal location and polymorphism levels have been determined (IRGSP 2005; http://www.gramene.org). Because of the availability of such information, for MAS applications, SSRs are one of the best options in the case of rice. The availability of the complete sequence of the rice genome has expedited the process of cloning of many genes. If the functional nucleotide polymorphism, specific for the functional allele of the gene, is identified, it can lead to a more powerful gene-based or functional marker (Andersen and Lübberstedt 2003), which is called as a "perfect marker." Functional markers are now available for the fragrance traits (Sakthivel et al. 2009), wide-compatibility gene locus S5 (Sundaram et al. 2010), QTL controlling grain size/shape, qtl GS3 (Ramkumar et al. 2010), and blast resistance gene Pi54 (Ramkumar et al. 2010). In rice, a special class of markers called single nucleotide polymorphisms (SNPs) is gaining wide-spread recognition. Many SNPs have been identified between *indica* and *japonica* subspecies of rice, and information regarding these is freely available (http://www.plantgenome.uga.edu/snp;http://www.ricesnp.org). McNally et al. (2009) reported genome-wide SNP variation among 20 diverse elite rice varieties/ landraces and reported 160,000 nonredundant SNPs (http://www.oryzasnp.org). Even though SNP markers are not widely used for marker-assisted breeding (MAB) in rice, it is expected in future they will replace the currently used SSRs as markers of choice now.

3.4.1 Application of Molecular Markers in Rice Improvement

1. To find the allelic status of genes [i.e., determination of homozygous or heterozygous] conferring identical phenotypes.
2. Marker-assisted introgression of agronomically important genes/QTLs into the genetic background of elite cultivars/parental lines through backcross breeding
   - Marker-assisted gene pyramiding for both abiotic and biotic stresses.
   - Marker-based early generation selection.
   - Marker-based whole genome selection.
   - Combination of MAS with phenotypic selection.
   - Estimation of genetic variability, identification, and characterization of germplasm.
   - Fingerprinting of genotypes.
   - Estimation of genetic distances among population, inbreeds, and breeding materials.
   - Detection of quantitative trait loci (QTLs).
   - Identification of sequences of useful candidate genes.

To pyramid genes conferring resistance to various stresses, many molecular markers have been utilized in MAS (Septiningsih et al. 2008; Das and Rao 2015; Shamsudin et al. 2016, Kumar et al. 2018). For example, submergence tolerance is improved by deploying MAS strategy (Septiningsih et al. 2008; Luo and Yin 2013; Das and Rao 2015; Ma et al. 2016). Similarly, tolerance to insects (Das and Rao 2015) and diseases (Luo and Yin 2013; Das and Rao 2015), salinity, and drought (Das and Rao 2015; (Shamsudin et al. 2016) is improved using MAS in breeding. Similarly, various SNPs associated with tolerance to heat have been identified. Because of the complex nature of the trait, each marker makes a relatively small contribution toward variance. Hence it is important to introgress several QTLs-associated markers into a cultivar to improve tolerance. Multiple QTLs conferring heat tolerance have been mapped along with their associated markers especially at flowering or grain-filling stages in rice (Cheng et al. 2012). QTL mapping studies involving seed set rate or grain quality traits as an evaluation index revealed that HT is controlled by polygenes, which were distributed on 12 chromosomes. Cold conditions during the reproductive stage cause an average annual yield reduction of 5–10% (Darvey and Zhao 2007): one of the major factor responsible for this is pollen sterility. Cheng et al. (2012) reported four QTLs (*qSRS1*, *qSRS7*, *qSRS11a*, and *qSRS11b*) associated with survival rate of seedlings (SRSs) under cold treatment at the seedling stage on chromosomes 1, 7, and 11 in rice. Agrawal et al. (2018) showed improvement of Safri-17 and Dubraj varieties of rice for providing

Table 2 Genes/QTL related to rice thermal tolerance

Gene/QTL	Function	References
qPSST-3, qPSST-7, qPSST-9, qSCT1a, qSCT2, RM231, RM1377, RM24545, RM3602 RM1211	Cold tolerance	Jena et al. (2012), Kim et al. (2014)
qHTSF4.1 4	Heat tolerance	Ye et al. (2015b), Nogoy et al. (2016)
qHTSF1.2 1	Heat tolerance	Ye et al. (2015a)
qHTSF6.1 6	Heat tolerance	Ye et al. (2015a)
codA choline oxidase A	Cold tolerance	Sakamoto and Murata (1998)
CBF1/DREB1b DRE-binding protein	Cold tolerance	Lee et al. (2004)
HOS10 encodes an R2R3-type protein	Cold tolerance	Zhu et al. (2010)
MYBS3 DNA-binding repeat MYB	Cold tolerance	Su et al. (2010)
OsMYB3R-2 DNA-binding domain	Cold tolerance	Ma et al. (2009)

resistance against bacterial leaf blight through MAS. In an another study, Kumar et al. (2018) also used MAS strategy to pyramid two or more QTLs for quantitative trait grain yield under drought in rice. In their study, they closely followed the introgression of two or more QTLs for drought stress in the background of rice varieties; Swarna-Sub1, IR64-Sub1, TDK1-Sub1, Samba Mahsuri, and MR219 from F3 to F6/F7/F8 generations. The pyramided lines with two or more QTLs had high grain yield advantage in Swarna-Sub1, IR64-Sub1, TDK1-Sub1, Samba Mahsuri, and MR219 backgrounds.

Tolerance to drought and salinity (Das and Rao 2015; Shamsudin et al. 2016), insect (Das and Rao 2015), and diseases (Luo and Yin 2013; Das and Rao 2015; Ma et al. 2016) was improved using MAS in breeding. However, reports for improving thermotolerance are scanty in rice (Lang et al. 2015). In the current scenario, SNPs have gained substantial popularity than that of SSRs due to their high abundance in rice genome. Multiple QTLs conferring heat tolerance especially at flowering stage in rice have been mapped along with their associated markers (Cheng et al. 2012). These markers can be deployed in initiating MAS for pyramiding genes to breed for high heat tolerance (Cheng et al. 2012). For an actual application of SSR markers in breeding programs for heat tolerance in rice, a group from Vietnam used RM3735 as one of their six markers to select heat-tolerant lines in backcross population at flowering stage (Lang et al. 2015). Rice breeders can employ these QTLs in pyramiding genes to increase heat tolerance in rice. Cold damage of rice occurs at the budding, seedling, booting, flowering, and grain filling stages. QTLs related to cold stress have been identified with different methods. Backcross generation was analyzed in 213 lines of Xieqingzao B and Dongxiang wild rice with the seedling mortality rate at low temperature as an indicator; it was found that the seedling mortality rate was continuously distributed in the population: that is, cold tolerance is a quantitative trait controlled by multiple genes; further, the major effect QTL was found on chromosome 8 in rice (Rao et al. 2013). Wang et al. (2009) investigated a set of Recombinant inbred lines (RILs) using the germination rate under low-temperature stress as an indicator and detected seven QTLs for cold tolerance on chromosomes 4, 6, and 9 in rice. The majority of known QTLs for cold tolerance are mainly located on chromosomes 4 and 8, accounting for 16.22% and 13.51% of the total QTLs, respectively (Rao et al. 2013). At present, few studies have attempted to enhance cold tolerance of rice by molecular breeding. This is probably because cold tolerance in rice is a cumulative trait regulated by multiple genes, and single-gene transformation techniques have limited efficiency in improving rice cold tolerance. A QTL for cold tolerance at the booting stage was also reported on chromosome 7 and named qCTB7. Twelve putative cold tolerance genes from this QTL region were identified by fine mapping and candidate gene cloning (Zhou et al. 2010). A single QTL for booting stage cold tolerance was reported on the long arm of chromosome 3. This QTL was named qLTB3 and this explained 24.4% of the phenotypic variance (Shirasawa et al. 2012a, b).

A genome-wide association study was carried out to identify the genetic loci associated with cold tolerance (CT) by using a 1033-accession diversity panel. They identified five CT-related genetic loci at the booting stage. Accessions carrying multiple cold-tolerant alleles displayed a higher seed-setting rate than did accessions that had no cold-tolerant alleles or carried a single allele. At the seedling stage, eight genetic loci related to CT have been identified. Among these, LOC_Os10g34840 was identified as the candidate gene for the qPSR10 genetic locus that is associated with CT in rice seedlings. A single-nucleotide polymorphism (SNP), SNP2G, at position 343 in LOC_Os10g34840 is responsible for conferring CT at the seedling stage in rice. Further analysis of the haplotype network revealed that SNP2G was present in 80.08% of the temperate japonica accessions but only 3.8% of the indica ones. Author used marker-assisted selection to construct a series of BC4 F3 near-isogenic lines possessing the cold-tolerant allele SNP2G. When subjected to cold stress, plants carrying SNP2G survived better as seedlings and showed higher grain weight than plants carrying the SNP2A allele. The CT-related loci identified here and the functional verification of LOC_Os10g34840 will provide genetic resources for breeding cold-tolerant varieties and for studying the molecular basis of CT in rice (Xiao et al. 2018). Some genetic loci involved in CT at the booting stage, such as Ctb1 and Ctb2, have been identified by quantitative trait locus (QTL) mapping (Saito et al. 2004, 2010). Dai et al. (2004) detected nine QTLs distributed on chromosomes 1, 3, 4, 6, 7, 10, and 12, and Kuroki et al. (2007) detected a major QTL, qCTB8, on chromosome 8S, which explained 26.6% of the phenotypic variance. Shirasawa et al. (2012a, b) also mapped a major QTL, qLTB3, on chromosome 3L in the rice cvLijiangxintuanheigu. Using the cold-tolerant cvKuchum, Endo et al. (2016) also identified a major QTL, qCT-4, at the booting stage on chromosome 4. By resequencing introgression lines, Zhu et al. (2015) identified six QTLs for CT at the booting stage on chromosomes 3, 4, and 12. Ctb1 was the first cloned QTL associated with cold resistance at the booting stage in rice (Saito et al. 2004).A genome-wide association study (GWAS) has been applied to investigate the genetic architecture of important agronomic traits in crops (Han and Huang 2013; Ogbonnaya et al. 2017). Several genes have been fine-mapped, including Ctb1 (Saito et al. 2004), qCT8 (Kuroki et al. 2007), qCTB7 (Zhou et al. 2010), qCTB3 (Shirasawa et al. 2012a, b), and qCT-3-2 (Zhu et al. 2015) for cold tolerance at the booting stage; qCTS4 (Andaya and Tai 2007), qCtss11 (Koseki et al. 2010), qSCT1 and qSCT11 (Kim et al. 2014), qLOP2 and qPSR2-1 (Xiao et al. 2015) for CTS; qLTG3-1 for germination cold tolerance (Fujino et al. 2008). Two QTLs for rice cold tolerance, Ctb1 and COLD1, have been cloned and functionally characterized (Saito et al. 2010; Ma et al. 2015). Ctb1 encodes an F-box protein and physically associates with Skp1, a subunit of the E3 ubiquitin ligase, suggesting the potential involvement of the ubiquitin–proteasome pathway in rice cold resistance (Saito et al. 2010). The newly identified COLD1 gene confers cold tolerance in japonica rice at the seedling stage. Molecular characterization revealed that COLD1 functions as a GTPase-accelerating factor and regulates G-protein signaling by sensing cold in order to trigger Ca^{2+} signaling for cold tolerance (Ma et al. 2015). Using GWAS, Pan et al. (2015) recently mapped 51 QTLs for cold tolerance at the germination and booting stages with 174 Chinese rice accessions that were genotyped with 273 SSR markers. Fujino et al. (2015) also identified 17 QTLs responsible for rice low temperature germinability in 63 Japanese varieties genotyped with 115 SSR markers and two other markers. In addition, Lv et al. (2016) used 527 rice cultivars to identify 132 QTLs for both rice natural chilling and cold shock stresses. Unlike traditional QTL mapping, GWAS enables the identification of rare alleles in natural populations and of high-resolution SNP markers (Gupta et al. 2014). Although Pan et al. (2015) and Fujino et al. (2015) recently reported the association mapping of cold tolerance QTLs in rice, the low resolution of SSR marker genotyping data affects the interval distance of the mapped QTLs. In this study, we mapped 67 QTLs associated with CTS in 295 rice accessions by comparing the current and previously published results (Yang et al. 2015; Lv et al. 2016).

Zhang et al. (2014) summarized various QTLs related to chilling tolerance at different growth stages. Bonnecarrère et al. (2014) has summarized various molecular markers associated with phenotypes related to chilling response, such as membrane integrity, visual assessment of damage, and photoinhibition. SSR marker RM22034 is associated with membrane integrity, RM144 with visual assessment of damage and RM6547 and RM14978 with photoinhibition. Three more QTLs, qPSST-3, qPSST-7, and qPSST-9, were verified from ten chilling-tolerant lines with spikelet fertility (SF) of 51–81% compared to 7% (chilling-sensitive parent) and 73% (chilling-tolerant donor) by Jena et al. (2012) (Table 2). In brief we can say the integration of genomic tools with high throughout phenotyping to assist breeding practices through molecular markers to facilitate the prediction of phenotype from genotype.

3.5 Mutation Breeding for the Development of Stress Tolerance

Mutation breeding has been used since the 1930s to accelerate the process of developing different traits for selection, such as disease resistance, tolerance to harsh environment, and valuable agronomic traits. Mutation breeding uses a plant's own genetic resources mimicking the process of spontaneous mutations and it generates random genetic variations, with new and useful traits. Mutation breeding has many advantages: it is cost-effective, quick, robust, transferrable, ubiquitously applicable, nonhazardous, and environmentally friendly (http://www-naweb.iaea.org). Physical and chemical mutagens are used for inducing mutagenesis in plants. Chemical mutagens includes ethyl methane sulphonate (EMS), *N*-methyl-*N*-nitrosourea (MNU), and sodium azide (SA). Physical mutagens include gamma rays, X-rays, UV-rays, fast neutrons, and ion beams (https://mvd.iaea.org/). A successful mutation breeding program starts with well-defined objectives and common targets are: (a) to improve one or a few specific traits of a variety or an elite line; (b) to induce a morphological marker (color, awns, bracts, hairiness, etc.) and/or to induce male sterility or fertility restoration, making a variety/line useful for hybrid variety production.

In case of mutation breeding, the chosen variety should have sufficient uniformity in most of the important agronomic traits. Once the decision on the genotype to be mutated is made and a homogenous seed stock is available, the next step is mutagenesis. The seeds before treatment are known as the M0 generation. A untreated (control) population should grow to compare the effect of treatment various parameters such as germination, growth, survival, M1 injury, and sterility, also provide a "repurified" parent genotype as a backup for initiating a new M1 generation to be grown during the same season with the M2 generated from the first M1 if needed.

Selection of mutagen and dose of treatment should be done carefully and preferably use three doses of the selected mutagens. It is recommended that large quantities of seeds be treated to ensure that an adequate population will be available in M1 population for screening (IAEA). M1 is the generation produced directly from the mutagen-treated plant parts without a recourse to sexual or asexual reproduction. M2, M3, M4, etc. are the subsequent generations derived from M1, M2, M3, etc. plants through selfing or clonal propagation (http://ecoursesonline.iasri.res.in). Mutation breeding is widely used for the improvement of rice grain and nutritional qualities. Till now, more than 200 new mutant rice varieties have been developed with improved grain and nutritional qualities such as MDU-4 (ACM-15) and Pusa-NR-162(SOURCE: https://mvd.iaea.org/) under thermal stress. Recently, Bangladesh has developed a new mutant rice variety, Binadhan-19, through carbon ion beams (https://mvd.iaea.org/). In India, a number of rice mutant variant have been registered for different characters and 48 stable lines (17 KMR3/*Oryza rufipogon* introgression lines [KMR3 ILs], 15 Swarna/Oryzanivara ILs [Swarna ILs] along with their parents, Nagina 22 [N22], and its 4 ethyl methanesulfonate–induced mutants and seven varieties) were examined for heat tolerance (Prasanth et al. 2017). Koh et al. (2007) reported that knockout (KO) mutants of OsGSK1 showed enhanced tolerance to cold, heat, salt, and drought stresses when compared with non-transgenic segregants (NT). Overexpression of the full-length OsGSK1 led to a stunted growth phenotype similar to the one observed with the gain-of-function BIN/AtSK21 mutant. Furthermore, a heat-tolerant mutant with higher photosystem II efficiency was identified (Poli et al. 2013). The gain-of-function mutant osmads57-1 and the OsMADS57-OE line had improved chilling tolerance, whereas the loss-of-function mutant osmads57-2 and the OsMADS57-AS line were sensitive to chilling (Chen et al. 2018). Creating variability through mutations has therefore grown to be one of most important tools to improve rice. In summary, we can say mutation breeding offers an effective means of adapting agricultural crops to changing weather conditions, while also increasing yields to feed a growing population.

3.6 Double Haploid Technique for Breeding

Doubled haploid (DH) technique has twin advance of speed and efficiency and due to this, it has become most effective tool for the plant breeders to attain homozygosity of recombinants in shortest possible time. This technique has been used to produce water-logging tolerance under sodic soils and has yielded double haploids tolerant than both of the parents. Doubled haploid (DH) plants are true-breeding plants. Production of DH plants from the hybrids of crosses takes only one generation to fix genetic segregation as compared to conventional plant breeding techniques that would take six to eight generations. It will enable the breeder to reduce the time and cost for variety development as compared to conventional breeding. Microspore embryogenesis has proven to be a very efficient system to produce homozygous doubled haploid plants, whereas production of DH plants through anther culture is very simple and intensively used in many laboratories. However, microspore culture has advantages over the anther culture, it is highly efficient in callus production, giving a single genotype per callus, and there is no risk of incorporating calli from other anther-derived tissues. Darvey and Zhao (2007) reported strategies on how to improve cold tolerance of Australian rice through microspore culture, incorporating cold tolerance from Chinese germplasm.

3.7 Tissue Culture/Genetic Engineering for Rice Improvement

Tissue culture is an in vitro culture of cells, tissues, organs, or whole plant or plant parts (leaf, stem, root, flower, anther, ovule, etc.) under controlled and aseptic conditions include optimum temperature, pH, nutrients, and proper light without any microbial infection. In plant tissue culture, artificial nutrient media is used to grow plants in vitro under aseptic and controlled environment. The technique is based on the totipotency of cells. Totipotency is the ability of any cells to express its full genome by cell division, growth, and metabolism; it needs an artificial nutrient medium, which is generally composed of micronutrients, macronutrients, vitamins, carbon source (sucrose), plant hormones, gelling agent (for solid medium), and other organic components required for normal growth and development of plant (Murashige and Skoog 2006). Tissue culture approach is a cost-effective and convenient technique for the development of stress tolerance in plants. This technique operating under controlled environment with less time and space has a high potential for the development of stress-tolerant plants. It is used to understand the physiology and biochemistry of plants cultured under various stress condition. Plant tissue culture includes various techniques such as micropropagation, anther culture, protoplast culture, embryo culture, and somatic hybridization (Basavaraju 2011).

Plant tissue culture provides immense opportunities for plant propagation, production, and engineering of plants with desirable agronomic traits including greater yield, salt resistance, drought tolerance, frost tolerance, disease resistance, and herbicide resistance.

Stroud et al. (2013) showed that rice plants regenerated from tissue culture contain stable epigenome changes. They generated whole-genome, single-nucleotide resolution maps of DNA methylation patterns in a number of regenerated rice lines and found that all tested regenerated plants exhibited significant losses of methylation compared to non-regenerated plants.

3.7.1 Genetic Engineering for Rice Improvement

Since the appearance of the first report on successful production of transgenic rice plants of Japonica in 1988 (Zhang et al. 1988), a large number of rice varieties have been developed with agronomically important genes. The fusion of protoplast (Datta et al. 1990), gene-gun approach (Christou et al. 1991), and *Agrobacterium*-mediated method (Hiei et al. 1994) are the direct DNA transfer methods, commonly used for rice transformation. Though rice is the most important cereal crop of the world, transgenic rice development and the field release of GM rice is still lagging behind other GM crops. In 2005, Iran was the first country to approve commercial growing of transgenic rice and contribute less than 0.1 million hectare to Bt rice. Though China has completed the field trials of Bt rice, it is yet to start commercial cultivation. The main bottlenecks are (1) several regulatory and ethical issues associated with GM rice, (2) majority of rice is produced and consumed locally in poor countries. Although GM rice has huge potential in alleviating the problems faced by farmers such as reducing attack of pests and diseases, reducing losses due to various stresses and poor rice consumers through nutritional enhancement and above all protecting the environment by reducing the consumption of toxic pesticides. Genetic engineering allows scientists to transfer very specific genes into plants, resulting in the introduction of one or more defined traits into a particular genetic background. This process is called transformation and the genes involved are expressed to form a protein responsible for the particular trait. High temperature tolerance has been genetically engineered in plants mainly by overexpressing the heat shock protein genes or indirectly by altering levels of heat shock transcription factor proteins. Apart from heat shock proteins, thermotolerance has also been altered by elevating levels of osmolytes, increasing levels of cell detoxification enzymes, and through altering membrane fluidity. Kumar et al. (2018) has summarized number of genes identified for heat and cold tolerance by number of scientists around the world.

Genetic engineering of heat shock factors (HSFs) and antisense strategies are instrumental to the understanding of both the functional roles of HSPs and the regulation of HSFs. Manipulations of the HS response in transgenic plants have the potential to improve common abiotic stress tolerance like thermal tolerance (Wahid et al. 2007). Transgenic rice plants overexpressing *Oshsp17.7* gene showed increased thermotolerance as well as increased resistance to UV-B irradiation (Murakami et al. 2004). A Ca^{2+}-dependent molecular chaperone, calnexin, yielded *Arabidopsis* and tobacco plants with enhanced heat and drought tolerance, respectively.

Scafaro et al. (2018) overexpressed the genes encoding thermostable variants of the photosynthesis heat-labile Rca (Rubisco activase) protein from a wild relative *Oryza australiensis* in domesticated rice (*Oryza sativa*). They found that rubisco activase variant show improved growth and seed yield under heat stress in rice. Kumar et al. (2017) have carried out genome-wide identification and analysis of genes, conserved between *japonica* and *indica* rice cultivars, that respond to low-temperature stress at the vegetative growth stage. Overexpression of OsWRKY71 enhanced cold tolerance in 'Dongjin,' (*japonica* cultivar). Gene Ontology enrichment analysis indicates that the "L-phenylalanine catabolic process" was highly overrepresented under cold-stress, implying its significance under cold stress in rice to regulate multiple aspects of plant growth and development. Ma et al. 2017 reported that rice OsOFP6 (OVATE family proteins; OFPs) regulates growth and development, and alters responses to drought and cold stresses. OsOFP6 is expressed in various tissues and developmental stages. Knock-down of OsOFP6 by RNA interference (RNAi) resulted in semidwarf phenotype, altered grain shape, and shorter lateral roots. Under cold treatment overexpressed and RNAi plants exhibited lower and higher relative electrical conductivity (REC), respectively, suggesting OsOFP6's role in response to cold stress.

Overexpression of the tomato C_2H_2 zinc finger protein transcription factor, SlCZFP1, confers enhanced cold tolerance in transgenic Arabidopsis and rice (Zhang et al. 2011). GsZFP1, another C_2H_2-type zinc finger protein and GsZFP1-overexpressing Arabidopsis, resulted in a greater tolerance to cold. Rice zinc finger proteins ZFP245 (Huang et al. 2009a, b) and ZFP182 (Huang et al. 2012a, b) were induced by various abiotic stresses, and overexpression of these proteins in rice significantly enhanced stress tolerances, including salt, cold, and drought stress. Overexpression of rice zinc finger protein OsCOIN increased tolerance to chilling, salt, and drought stress and also enhanced proline level in rice (Liu et al. 2007). Rice plants are more sensitive to cold stress at the booting stages than at the seedling stages (Xu et al. 2008a, b). Leucine-rich repeat receptor-like kinase CTB4a, which conferred cold tolerance at the booting stage, was mapped and cloned (Zhang et al. 2017). Saito et al. (2010) mapped and cloned the QTL Ctb1 encoding an F-box protein, which is responsible for normal anther development under cold stress. Zhou et al. (2010) fine-mapped qCTB7 for cold tolerance at the booting stage on rice chromosome 7 using a near-isogenic rice. Kuroki et al. (2007) mapped qCTB8 for cold tolerance at the booting stage on rice chromosome 8. In Arabidopsis, C-repeat binding factor (CBF)/dehydration responsive element binding protein (DREB) is the major transcription factor responsible for induction of cold tolerance (Thomashow 2010) and overexpression of CBF in monocots showed enhanced tolerance to diverse abiotic stresses including cold and freezing. Overexpression of TaDREB3, TaCBF14, and TaCBF15 from wheat and HvCBF2a from barley resulted in enhanced frost tolerance by increasing the transcript levels of downstream target genes such as COR14b and DHN5 (Kovalchuk et al. 2013; Jekníc et al. 2014). Moreover, overexpression of TaCBF3 enhanced tolerance to frost and cold in wheat and barley (Morran et al. 2011) and cotton-GhDREB in barley also resulted in enhanced cold tolerance (Gao et al. 2009). Indeed, overexpression of the CBF genes from rice (OsDREB1), maize (ZmCBF3), and barley (HvCBF4) resulted in enhanced cold tolerance in rice (Ito et al. 2006; Oh et al. 2007; Xu et al. 2011). Fujino et al. (2008) identified a major QTL, qLTG3-1, which controlled low temperature germinability in rice. Transgenic rice plants overexpressing OsDREB1 or AtDREB1 genes showed improved tolerance to low temperature, drought, and high-salt stresses. Another DREB1 gene (OsDREB1D) from rice was overexpressed in Arabidopsis plants, resulting in cold tolerance where the degree of cold tolerance showed a good correlation with the level of OsDREB1D expression (Zhang et al. 2009). Xu et al. (2011) overexpressed a maize CBF gene (ZmCBF3) in rice plants, and the resulting transgenics showed growth retardation only at the seedling stage, with no reduction in yield under field conditions and transgenic plants were cold tolerant. Overexpression of OsCOIN (*O. sativa*, cold inducible transcription factor) enhanced tolerance to cold, drought, and salt treatment in transgenic rice plants (Liu et al. 2007). C_2H_2 zinc finger protein transcription factor OsCTZFP8 was produced in rice. OsCTZFP8 encodes a C_2H_2 zinc finger protein, which contains a typical zinc finger motif, as well as a potential nuclear localization signal (NLS) and a leucine-rich region (L-box). Expression of OsCTZFP8 was differentially induced by several abiotic stresses and was strongly induced by cold stress. OsZFP245, a C_2H_2-type zinc finger protein, also led to rice plants with enhanced tolerance to cold and drought stresses. Overexpression of OsZFP182 (TFIIIA-type zinc finger protein) resulted in cold tolerance and accumulation of compatible osmolytes, such as free proline and soluble sugars. The authors also suggested that OsZFP182 functions as the upstream regulator of OsDREB1A and OsDREB1B, under both normal and drought conditions (Huang et al. 2012a, b). Another zinc finger protein (A20/AN1-type) from the halophytic grass, *Aeluropus littoralis* (AlSAP-Stress-Associated Protein), was previously characterized as stress associated and its overexpression in rice shows enhanced tolerance to various stress conditions (Ben Saad et al. 2010). Yang et al. (2012) generated a transgenic rice overexpressing a R2R3-type MYB gene, OsMYB2, and found increased tolerance to various stresses, adding one more transcription factor to the list of genes with potential usefulness for improving stress tolerance in rice. One member of the AP2/ERF superfamily identified in tomato (TERF2/LeERF2), and its overexpression in tomato, tobacco, and rice plants, leads to enhanced tolerance to freezing without affecting growth or agronomic traits (Zhang and Huang 2010). One member of the plant-specific OsAsr1 (Asr gene family) plays an important role during low temperature stress (Kim et al. 2009). Cold-tolerant phenotype in rice has already been achieved by overexpression of a cell wall protein (OsPRP3), which maintains the structure of mesophyll cells and retains cell wall integrity under low temperature (Gothandam et al. 2009). Heterologous expression of Antarctic hair grass DaCBF4 increased the tolerance to low temperature stress in transgenic rice plants. A comparative transcriptome analysis identified common downstream targets of DaCBF4 and DaCBF7 that resulted in the same phenotype. The genes identified from the study will facilitate genetic engineering of cereal plants with enhanced cold tolerance (Byun et al. 2018).

3.8 OMICS Approaches

The beginning of the twenty-first century is considered as the dawn of the genomics era by virtue of huge amount of genomics research on various species including bacteria, plant, and animal, as well as speedy advancement of high-throughput equipments for gene expression, whole-genome sequencing, and genome characterization, and advanced bioinformatics tools. Such swift developments have significant impact and redefined plant breeding as “molecular plant breeding” or “genomics-assisted breeding” (Varshney et al. 2006). Genomics is the study of gene function, location, and expression (Collard et al. 2008). Genomics has two components: (1) structural genomics and (2) functional genomics. Structural genomics refers to a systematic reading of all the sequences in genome and understanding the function of each of the sequenced genes is called as functional genomics. Singh and Mohapatra (2007) have enumerated the possible advantages of the rice genome sequencing in rice genetics and breeding. Genomics has a significant impact on rice breeding and further enhanced rapid gene transfer, identification of key traits genes; enhanced examination of useful genetic variation; and knowledge between the species of *Oryza* (Roychoudhury et al. 2011).

Currently, transcriptomics and proteomics have been used to identify heat stress–responsive genes. An early heat stress response was reported to induce Ca^{2+} influx and cytoskeletal restructuring, which causes the upregulation of mitogen activated protein kinases and calcium-dependent protein kinase signaling cascades (Wahid et al. 2007; Ashraf and Harris 2013). This signaling cascade leads to production of antioxidants and compatible osmolytes (for osmotic adjustment) and the expression of heat shock proteins. The main impacts of heat stress are protein denaturation, instabilities in nucleic acids and cytoskeletal structure, increased membrane fluidity, inactivation of the synthesis and degradation of proteins, and loss of membrane integrity (Howarth 2005; Wahid et al. 2007).

Stress-related proteins in plants, divided into two groups, the first group includes signaling components, such as protein kinases and transcription factors (Roychoudhury and Banerjee 2015) and the other group includes functional genes, such as heat shock proteins (HSPs) and catalase (CAT). Heat stress factor (HSF) and HSPs play a central role in the heat stress and acquired thermotolerance in plants (Banerjee and Roychoudhury 2018). The HSPs can be classified into five unlike families in plants: HSP20 (or small HSP, sHSP), HSP60 (or GroE), HSP70 (or DnaK), HSP90, and HSP100 (or ClpB) (Swindell et al. 2007). HSF serves as the terminal component of signal transduction and mediates the expression of HSP. However, attempts to increase thermotolerance through the overexpression of single HSF or HSP genes have limited effects, suggesting that HSF and HSP confer heat stress resistance only under synergistic conditions. A total of 19 and 21 (Nover et al. 2001) HSF members have been cloned in rice (*Oryzasativa*L.) and Arabidopsis (*Arabidopsis thaliana*), respectively. However, the functions of these proteins are poorly understood (Volkov et al. 2006). Heat stress is responsible for upregulation of numerous heat shock genes (HSGs)-encode HSPs (Zafar et al. 2016), which protect cells from the injurious impact of heat stress (Chang et al. 2007). In another study by Zhou et al. (2011), proteomic analysis showed downregulation of proteins related to photosynthesis, energy, and metabolism and upregulation of the resistance-related proteins. Like many other crop species, response to heat stress in rice is very complicated. For instance, it involved the up- and downregulation of various proteins including proteins involved in protection, redox homeostasis, energy and carbohydrate metabolism, biosynthesis, and degradation of proteins (Zou et al. 2011). An HSP acts as molecular chaperons, which repair proteins that are damaged by stress. Overexpression of HSP17.7 (Murakami et al. 2004) and mitochondrial HSP70 (mthsp70) protects rice from heat-induced damage; consequently, it sustains the mitochondrial membrane potential and retards the synthesis of ROS (Qi et al. 2011). Importance of HSPs is being increasingly felt as their expression occurs in a number of species, and their expression and tolerance to stress are positively correlated (Feder and Hofmann 1999). The heat shock transcription factors (HSFs) have vital role in controlling the expression of HSP genes and regulate the heat stress response (Chen et al. 2006; Zafar et al. 2016). A number of HSF genes in rice were identified, which regulate the expression of HSPs (Guo et al. 2008). The identification of HSFs opened up new avenues for conducting future functional genomic studies. Recently, genome-wide association studies (GWASs) helped in the identification of new QTLs controlling important traits including heat tolerance in crop plants with high accuracy with improved breeding value (Ma et al. 2016; Lafarge et al. 2017). GWAS is a powerful strategy to understand the genetic basis of complex traits that has been especially productive for rice (Qiu et al. 2015). “OMICS” approaches (genomics, transcriptomics, proteomics, and metabolomics) help to bridge the gap between associated genetic variants to phenotype by helping define the functional roles of GWAS loci through genome sequences, stress-specific transcripts, protein and metabolite profiles and their dynamic changes, protein interactions, and mutant screens. Further understanding of the complexity of thermal stress signaling and adaptive processes would require the analysis of the function of numerous genes involved in thermal stress response.

4 Conclusions

To meet the demands of the growing population, there is urgent need to protect rice plants from various stresses that reduce yield and quality and need to develop the tolerant variety. Presently, the conventional breeding of rice is rapidly advancing due to the integration of molecular markers and MAS techniques. Through the application of molecular markers with the help of MAS in gene pyramiding, multiple stress tolerant genes could be incorporated into a single rice variety with high yield and with enhanced nutritional quality. In this chapter, we discussed the effect of temperature stress (both low and high temperature) on plants and also presented the strategies and mechanism to develop the crops tolerant to temperature stress. Molecular genetics is used for the understanding of the underlying biology and molecular mapping is to produce a sufficiently fine-scale map to pinpoint the location of genes that play a key role in determining important agronomic traits and abiotic stress tolerance. The information of markers for thermal tolerant traits gathered here will provide a one-stop read for practical rice breeders and the like to aid in their MAB. Further, information regarding the integration of tools from sequencing to genome assembly, marker discovery, genotyping, and genome editing, together with conventional plant breeding practices has created the foundation for molecular and "precision" breeding, which will allow molecular breeding of climate ready crops. The trinity of DNA markers, genetic engineering, and genomics will certainly accelerate rice improvement programs across the world including India. There is an urgent need to integrate the biotechnological information with plant breeding to get maximum benefits from the wonderful science of biotechnology. Through a judicious application of all these technologies, development of a designer rice plant which uses lesser nutrients from soil and provides high yield, with tolerance to various stresses, may be possible in the near future.

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Influence of Night Temperature on Rice Yield and Quality

Ankur Singh, Bodhisatwa Chaudhuri, and Aryadeep Roychoudhury

Abstract

Rice is an important food crop for a substantial proportion of the human population and due to the risks posed by global warming and climate change, there is significant interest in analysing the effects of environmental factors such as temperature on rice yield and quality. Compared to studies on the influence of day temperatures on rice yield and quality, there are fewer studies on the influence of night temperature on these parameters. Any change in ambient temperature is known to activate stress-associated signalling pathways in plants. Night temperature is a crucial environmental factor that affects metabolic processes such as respiration and translocation of nutrients. An increase in night temperature reduces rice yield by reducing the translocation of non-structural carbohydrate and nitrogen which ultimately reduces grain-filling rate, grain weight and quality. Other parameters such as the percentage of chalky rice kernels are also adversely affected by an increase in night temperature. High night temperature (HNT) increases spikelet sterility, thereby decreasing pollination. At the cellular level, high night temperature decreases membrane thermal stability and increases the rate of respiration. Treatment with exogenous effectors such as salicylic acid reduces the effects of high night temperature by increasing the total anti-oxidant capacity and membrane thermal stability. An increase in Ca signalling proteins and heat shock proteins (HSPs) also enhances tolerance to high night temperature. This chapter therefore focuses on the overall influence of night temperature on rice physiology, grain yield and the endogenous signalling events.

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Keywords

Rice · Night temperature · Grain yield · Calcium signaling

1 Introduction

Rice is one of the major cereal crops of the world and a substantial proportion of the human population across the globe, being dependent on rice for nutrition, are engaged in its cultivation, storage, trade and distribution. Rice contributes to one-fifth of the total population of world caloric intake and is therefore the most important cereal crop with respect to human nutrition. It is the main dietary energy source for humans in 34 countries around the world (8 countries in Africa, 17 countries in Asia and Pacific and nine countries in America) and provides 20% of the global human dietary energy supply. Rice provides the highest supply of human dietary energy amongst all the cereal grains, followed by wheat (19%) and maize (5%).

Rice has been cultivated for several millennia since the domestication of wild species of the genus *Oryza* in Asia and is suited today to a range of environment located between the latitudes 45°N and 40°S (Sweeney and McCouch 2007). In most areas, rice is cultivated by the traditional method that involves germination of seeds followed by planting of young seedlings in the field. The field is flooded with water during or just after setting the seedlings in the soil. Flooding of fields is not compulsory for rice cultivation, but when feasible, it is cheaper than other methods of irrigation in terms of weed and pest management, because rice seedlings can grow in waterlogged fields which are unsuitable for the growth of other plant species. Depending on the availability of water, different cultivars of rice are grown in different environments and climatic zones. On the basis of environment and method of irrigation, these cultivars can be grouped into different categories as indicated in Table 1.

Table 1 Categorisation of rice cultivars based on growth environment and water availability

Environmental condition	Plants surviving in the conditions
Lowland, rainfed	Rice plants survive in medium depth and are dependent on rainfall and consequently prone to drought and flood
Lowland, irrigated	Rice which is grown in both the wet and dry seasons with irrigation
Deep water or floating	Rice which is grown in flooded areas and has longer internodes than other varieties, thus being able to keep the leaves above water
Coastal wetland	Rice which is cultivated in coastal areas and can tolerate moderate salinity
Upland	Rice which is well known for its drought tolerance

2 Temperature and Rice Cultivation

Temperature is one of the most important environmental factors that regulate the life processes of plants. Every species of plant possesses its own particular niche of optimum temperature that is related to the climatic conditions of its natural environment. Any change in this ambient temperature is known to affect photosynthesis, respiration, translocation of nutrients and other metabolic processes and can potentially lead to adverse effects on growth and reproduction. Extremes of temperature can activate stress-associated signalling pathways in plants and can potentially lead to death. Rice cultivation requires a temperature above 20 °C (68 °F) but not more than 35–40 °C (95–104 °F). The optimum temperature for rice cultivation lies between 30 and 20 °C. An increase in average daily temperature due to global warming could affect plant metabolic processes such as photosynthesis and respiration. This is a serious issue, because an increased rate of respiration would lead to an increased consumption of the total photosynthates produced by plants (Paembonan et al. 1992). An increase in average daily temperature usually leads to an increased rate of respiration during night and increased stomatal closure in leaves during the day to prevent water loss, thereby reducing the rate of photosynthesis. In addition to decreasing the rate of accumulation of biomass, this effect also alters the rate of gaseous exchange in favour of increased release of carbon dioxide which would contribute to an increase in the atmospheric carbon dioxide content, thereby contributing to a further increase in temperature. Cellular respiration in plants drives growth and maintenance of ionic gradients across cellular membranes, and the turnover of proteins and lipids (Penning de Vries 1975) is affected by changes in the environmental factors (Ryan 1991). Increased respiration caused by high temperature can stimulate the production of reactive oxygen species (ROS) in many plant species (McDonald and Vanlerberghe 2005). Reactive oxygen species are toxic for cells and their effects are minimized by antioxidants under normal physiological conditions; however, under conditions of chronic stress, the levels of ROS produced are very high and this can overwhelm the ameliorating effects of antioxidants, thereby leading to permanent cell damage (Kreiner et al. 2002). Increased cell damage caused by ROS can decrease the thermal stability of membranes, consequently disrupting the movement of water, ions and organic solutes including photosynthates across plant membranes. The damage caused by ROS can therefore affect carbon production, transport and accumulation and decrease the rate of growth of cells and tissues (Christiansen 1978).

3 Effects of Global Warming on Rice Cultivation

The global human population has increased substantially over the past century along with a concomitant improvement in quality of life across many parts of the world due to advancement of science and technology and its widespread application to many disciplines and endeavours including agriculture. However, the industrial revolution followed by urbanisation and unsustainable development has exponentially increased demand for natural resources, leading to significant disruption of ecosystems and ecological cycles globally. A persistent increase in the amount of CO_2 and other greenhouse gases in the atmosphere due to anthropogenic activities has led to an increase in average atmospheric temperature over the past century and climate change. Ecological disruption is a source of significant concern for the future of life on this planet. The value of global mean surface air temperature has increased by 0.5 °C in the twentieth century and is forecast to further increase by a minimum of 1.5 °C to a maximum of 4.5 °C in this century (Intergovernmental Panel on Climate Change 1995). In the twentieth century, the daily minimum temperature has increased at a faster rate than the daily maximum temperature in association with a steady increase in atmospheric greenhouse gas concentrations (Karl et al. 1991; Easterling et al. 1997). If these changes were to become more extreme, there is a possibility of significant adverse impacts on agricultural patterns and food security. This is especially true for a labour-intensive crop like rice, the cultivation of which is highly dependent on stable climatic patterns and seasonal rainfall in many parts of the world such as in South Asia. In the current scenario, global rice production has to increase by about 1% in order to meet the demands of a growing human population in the immediate future and most of this increase in yield has to occur in existing areas under cultivation in order to avoid further loss of natural ecosystems and environmental degradation.

A study of the impact of increased minimum temperature on the yield of cultivated rice by Peng et al. (2004) found that small but significant increases in night temperature reduced the yield of irrigated rice in the dry season and this observed effect was independent of solar radiation. They observed a robust negative linear relationship between the concentration of spikelets and minimum temperature in the dry season. There was also a significant negative relationship between minimum temperature and harvest index in the dry season. This negative relationship led to a 10% decrease in biomass production for every 1 °C increase in the minimum temperature during the dry season.

Other such field-based studies have also suggested that rice yield and growth rate have already decreased in many parts of Asia, as a result of rising temperatures and decreasing solar radiation (caused due to pollution and persistent atmospheric haze) during the later years of the twentieth century when compared to what would have been observed had the temperature and solar radiation trends not occurred (Welch et al. 2010). The study was based on records from a total of 227 farms located across Bangladesh, China, India, Nepal, Pakistan, Thailand and Vietnam. The production of crops was reduced by 10–20% at some of these locations. This fall in yield might be due to increased respiration during warm nights, which causes plants to expend energy without being able to photosynthesize adequately.

4 Effects of Elevated Night Temperature on Grain Yield and Quality

There is significant interest in analysing the effects of environmental factors such as temperature variability on rice yield and quality. Any deviation from optimum temperature during growth and reproductive cycles is known to disrupt plant processes at the molecular, cellular, physiological and behavioural levels. Even a modest amount of increase in global mean temperatures is significant in terms of the relative increase of average minimum temperature, and therefore, it becomes necessary to consider the effect of night time temperatures on rice yield and quality. One of the major physiological effects of an increased night time temperature is an increased rate of respiration in plants. A number of studies have shown that this increase in the rate of respiration in rice plants in response to an increased night time temperature is associated with a decrease in biomass production (Mohammed and Tarpley 2009). An increased rate of dark respiration increases the proportion of assimilates required for maintenance and uncoupled respiration (Beevers 1970), which consequently affects the carbon status of plants (Turnbull et al. 2002).

A number of studies have revealed that the reduction in rice yield caused by high night temperature (HNT) is due to an adverse effect on a number of anatomical and physiological characteristics of rice plants. This effect, however, is not uniform and varies depending on the rice cultivar which necessitates a screening for genetic diversity. Some of the observed adverse effects of elevated night temperatures on rice plants are a reduced number of panicles (Peng et al. 2004), a decrease of final grain weight and reduced spikelet fertility (Mohammed and Tarpley 2009).

The temperature at night has been speculated to affect flowering dynamics in the following morning (Kobayashi et al. 2010), and grain filling in rice is determined by accumulation of photo-assimilates that are either redistributed from the vegetative tissues before the onset of flowering or generated in situ during flowering. The rate and duration of grain filling determines the value of final grain weight and therefore determines the yield. The sequence of yield-influencing processes also influences grain quality.

Shi et al. (2012) undertook a genetic diversity screening of rice (36 accessions) followed by both field- and laboratory-based analyses of the impact of elevated night temperature on rice grain yield and quality between two contrasting rice genotypes, viz., tolerant N22 and sensitive Gharib. Such a preliminary analysis of genetic diversity is important in order to rule out an overestimation of temperature effects and to screen for beneficial traits of resilience. They have compared N22 and Gharib in their study in order to elucidate the plasticity of plant responses to elevated night temperature (28 °C) observed in the tolerant N22. They observed that although the grain weight of the tolerant N22 was not affected by high night temperature in the field study, the number of spikelets per panicle was reduced significantly and was accompanied by a higher seed set, thereby demonstrating a possible yield-maintaining plastic response of N22 to elevated night temperature. The plant stem is known to be a better competitor for assimilates than the spikelets and N22 exhibited a 6.5% increase in height and a 14.6% decrease in spikelet number under elevated night temperatures. A similar plastic response to elevated night temperature was not observed for the sensitive Gharib.

The duration and the rate of grain growth determine the grain filling which is regarded as the final state of cereal growth. The rate and duration of grain filling are inversely related to each other (Yang et al. 2008). Continuous supply of nutrients from 0–10 to 10–20 days is regarded as an important parameter for the expansion of endosperm and filling of grains respectively (Nagata et al. 2001): 1000-grain weight and the productivity of grain declined in the sensitive rice cultivar, Gharib when nitrogen (N) and non-structural carbohydrate (NSC) content of the surrounding environment was reduced up to the ripening stage of grains. Translocation of NSC to the panicles was found to be more active due to the contribution from stem NSC as compared with leaf NSC (Fu et al. 2011). During high temperature, the decrease in yield can be supported by the fact that at higher temperature, the nutrient supply is not sufficient enough to meet the rate of higher grain filling (Kobata and Uemuki 2004). Dias and Lidon (2009) have reported a synergistic correlation between grain weight and filling rate in grain in durum and bread wheat when influenced by high temperature. The impact of high night temperature (HNT) in rice cultivars can be reduced by inducing the translocation efficiency of the nutrients which can ultimately result in higher grain quality.

High temperature affects the grain quality such as percentage of chalky rice kernel and reduces milling quality of rice including yields of the rice grains. Counce et al. (2005) have shown that high temperature (24 °C) causes more reduction in yields and width of the grains as compared to that of low temperature (18 °C) in two rice cultivars, viz., LaGrue and Cypress. Again, higher length and thickness of the grains were noted which represent a diverse effect of HNT on the biochemical and physiological parameters of the plants.

Reduced yield of milled rice along with lower grain quality adversely affects the price of the rice in the market, which marks the negative effect of HNT on the productivity of the crops. Ishimaru et al. (2009) and Morita et al. (2005) have suggested that higher chalk formation and lower grain width in rice cultivars due to higher temperature may occur because of abnormal packing of amyloplast or reduction in the area of endosperm cells respectively. Studies demonstrating the relation between source and sink have established a close relationship between the supply of assimilate and formation of chalk (Tsukaguchi and Iida 2008). Higher supply of assimilates could help overcome the formation of chalk under high temperature in rice cultivars (Kobata and Uemuki 2004). Mohammed and Tarpley (2010) reported that higher respiration rate along with higher temperature could decrease the supply of assimilates. N22 maintains a higher rate of grain filling which ultimately reduces the chalkiness of grains even under HNT (Shi et al. 2012). Ziska and Manalo (1996) conducted an experiment where they showed that increase in day/night temperature reduces the plant biomass as well as the grain yield and quality of the rice cultivar, viz., IR-72.

Li et al. (2011) investigated the different effects of elevated night temperature and elevated day temperature on rice quality and seed protein accumulation profiles during grain filling in indica rice “9311.” The result of rice quality analysis indicated that compared with elevated day temperature, elevated night temperature exerted less effect on head rice rate and chalkiness, whereas greater effect on grain weight (Li et al. 2011). It is clear from a number of both laboratory- and field-based studies that there is a decrease in rice grain yield in response to elevated night temperature, irrespective of the cultivar; however, the effect of elevated night temperature on rice grain quality parameters and plant physiology and biochemistry displays plasticity based on the specific characteristics of the cultivars.

5 Cellular and Molecular Bases of Heat Stress and Tolerance

Increased temperature can potentially alter a number of structures and processes at the cellular and molecular levels of organism and it is important to comprehend this in order to understand the mechanism of action of heat stress and devise solutions for tolerance and mitigation of adverse effects. An increase in temperature is known to affect the stability of cell membranes and proteins. The best technique to estimate the performance of the plants under high temperature is to determine the membrane thermal stability (Sullivan 1972). Thermal stability of the membrane controls the properties of the thylakoid membrane, key enzymes and the photosynthetic system (Björkman et al. 1980). Adaptivity and productivity of the plants against high temperature mainly depends on the stability and function of the cell membrane system (Raison et al. 1980); thus, damage to the cell membrane caused due to HNT may reduce the yield. High night temperature increases the respiration rate and decreases the stability of the cellular membrane which ultimately results in lower yield of the crops (Ziska and Manalo 1996; Baker 2004; Peng et al. 2004; Counce et al. 2005). High temperature also increases the respiration rate (Albrizio and Steduto 2003; Lambers 1985) and electrolyte leakage (Reynolds et al. 1994; Ismail and Hall 1999) of the cells which contributes to the lower yield of the crops (Johnson and Thornley 1985; Hemming et al. 2000). Several other studies have demonstrated a direct relationship between crop productivity and membrane stability (Reynolds et al. 1994; Ismail and Hall 1999), development of floral buds and viability of the pollens (Prasad et al. 1999; Ahmed and Hall 1993), rate of respiration (Lambers 1985), content of starch and sugar of plants tissues (Turnbull et al. 2002), size of seeds, time of appearance of flower (Gibson and Mullen 1996) and rate of growth of different crop species (Morita et al. 2005; Seddigh and Jolliff 1984). The yield of the grain in rice is positively affected by the stability of the membrane, whereas it is negatively regulated by the respiration rate of the leaves (Ismail and Hall 1999; Lambers 1985; Reynolds et al. 1994).

Li et al. (2011) have shown the effect of higher day and night temperature on grain filling and quality of the rice cultivars, where they showed the changed level of proteins using proteomics approach under high temperature stress. The identified isoforms of the major defence proteins such as pyruvate orthophosphate dikinase (PPDK) and pullulanase showed different accumulation patterns when exposed to high day and night temperature.

6 Resilience and Mitigation of Adverse Effects

There is abundant information to indicate that elevated night temperature affects metabolism and therefore important life processes in rice plants. Many of these adverse effects are correlated to the activation of abiotic stress–associated signalling pathways. There have been a number of studies that not only investigated the key factors of these pathways, but also investigated avenues for ameliorating the effects of stress. Plants that are pre-treated with antioxidants such as α-tocopherol, glycine betaine and salicylic acid become tolerant against high temperature stress and less prone to damages against oxidative damages induced by high temperature (Diaz-Zorita et al. 2001; Fryer 1992; Larkindale and Knight 2002). Glycine betaine protects the action of several enzymes such as citrate synthase and RuBisCo against high temperature, whereas α-tocopherol acts as singlet oxygen quencher (Caldas et al. 1999; Mäkelä et al. 2000). Salicylic acid stabilises the trimers of heat shock factors (HSFs) and also takes part in detoxification of the superoxide radicals which lowers the injury caused by high temperature stress (Larkindale and Knight 2002). Detoxification of superoxide radicals can also occur due to enhanced level of antioxidants which ultimately protects the cellular membrane and also the functional activity of major enzymes (Diaz-Zorita et al. 2001). Protection to the cell membrane results in lower respiration rate which is required to maintain the integrity of the membrane in absence of any protective agents during temperature stress (Amthor and McCree 1990). Mohammed and Tarpley (2009) have shown the protective role on glycine betaine, salicylic acid and α-tocopherol when exogenously applied in rice plants against HNT. A close association between the concentration of the antioxidants and tolerance of the plants against environmental stress condition was earlier reported by Smirnoff (1995).

Tolerance in rice cultivars against high temperature was associated with enhanced expression of HSFs and Ca-signalling proteins along with efficient repair mechanism of the damaged proteins (Shi et al. 2012). HSPs perform several functions such as repair of protein damaged due to high temperature stress and providing protection to the cells (Sarkar et al. 2009; Wang et al. 2004; Jagadish et al. 2011).

Higher expression of peptidyl-prolyl *cis–trans* isomerase was noted in rice cultivars which help to maintain the synthesis and transportation of the proteins during filling stage of grains. This protein also works in association with HSP90 to maintain correct folding of protein in *Arabidopsis thaliana* (Hagai et al. 2007) and also induces the process of flowering in wheat during high temperature stress (Kurek et al. 1999). HSPs were further upregulated when Ca triggers a cytosolic burst of Ca^{2+} ions and the signal was perceived by several Ca-binding protein such as calmodulins and Ca^{2+}-dependent protein kinases (Yang and Poovaiah 2003; Liu et al. 2003). Acylation of histones by histone acetyl transferase and general control non-repressed protein 5 also regulates the transcription of the genes encoding HSPs which helps to maintain the proper folding of the proteins under high temperature stress (Bharti et al. 2004; Han et al. 2008).

7 Conclusion and Future Prospective

Anthropogenic activities have led to an abrupt rise in the normal temperature of the environment which ultimately affects the crop productivity and quality of the grains. Rice is the major food crop for human populations. Rice cultivation requires an optimal temperature for its better growth and yield. Higher temperature triggers the stress signalling pathways and can ultimately result in permanent cell membrane damage. HNT adversely affects the quality of kernel and forms a chalk-like appearance which decreases the market value of the grains. Plants have several protective machineries such as antioxidative and radical scavenging molecules which can reduce the signs of damage on cell membrane caused due to the production of ROS when plants are exposed to high temperature. In addition to this, several chemicals have also been applied exogenously which helps to ameliorate the effect of stress. HSPs present in the cells also play an important role by maintaining the proper structure and folding of the major protein structures.

To meet the increasing demand of food crop due to the growing population of the world, it is necessary to maintain and increase the yield of the crops. Thus, in near future, transgenic methods need to be more focused to improve the tolerance level of the plants against rising temperature of the earth due to global warming. In addition, the protective roles of other chemicals need to be studied against high temperature stress in plants to provide easy and cost-effective technique to the field workers.

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Rice Tolerance to Multiple Abiotic Stress: Genomics and Genetic Engineering

Anirban Bhar

Abstract

Changing environment has a huge impact on bio-resources and global agriculture. Abiotic stress factors are dramatically increasing along with these uncontrolled environmental changes. Rice (*Oryza sativa*) is the most important crop providing food toward more than half of the world populations, and India is one of the major rice growing country. This important crop plant experiences massive yield loss due to abiotic out-lashes, e.g., salinity, drought, heat stress, cold shock, UV damage, and mineral toxicity. The sessile nature of plants make them easy targets of several environmental odds, but long-term evolutionary interaction of plants with environment in turn shapes reprogramming of its defense signaling networks tightly. The subtle changes in the environment can be sensed by the plant very efficiently and are portrayed by their genetic orchestrations. Due to enormous development in modern genomics, technologies, and biotechnological applications, the minute changes in gene expression and modification of metabolic functions can now be precisely recorded. Besides, complex modulations in metabolic network through biotechnology are implicated to overcome the situations in a positive way. Studies focusing on specific abiotic stress and its protection have long been implicated in different plants including rice. Unfortunately, growing yield loss in rice due to multiple abiotic stress factors supersedes increasing demand of this crop. Recently, a versatile approach has been flourished to meet the yield–demand ratio against multiple abiotic stresses. The present chapter describes various important abiotic stresses in rice plants, their complex defense signaling mechanism, and recent developments to combat these multiple stress factors comprehensively.

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Keywords

Cold shock · Drought · Heat stress · Multiple stress · Salt stress · UV light

1 Introduction

Rapid population outburst and associated urbanization constantly threaten global agriculture and crop production. Uninterrupted human interference in nature not only reduces agricultural lands but also changes environmental homoeostasis. Moreover, different abiotic stresses, i.e., drought, salinity, high temperature, chilling or cold stress, heavy metal toxicity, and UV-induced damage, are shoddily affecting plant populations. Environmental imbalance due to uncontrolled and improper usage of them worsens the situation further. Plants due to their non-motile nature face these environmental odds more than other animals. Crop plants are affected by any environmental stress more intensely as they have limited genetic variability due to constant selection on the basis of yield potentiality and quality. It has been also noticed that during domestication, many abiotic tolerances were reduced significantly (Koziol et al. 2012).

Rice (*Oryza sativa*) is the most important crop plant that feeds more than half of the global population. Asian population ranks first in both production and consumption of different rice varieties (Khush 2005). India is one of the major rice producing country in the world (Kakar et al. 2019). The production of rice is seriously hampered by different abiotic stress factors and xenobiotic interference (Hoang et al. 2015). Among them, drought-, salinity-, heat stress-, and cold-induced injuries are the major yield limiting abiotic stress in rice. Although drought and salinity are considered most deleterious for rice production, salt stress alone reduces the yield 30–50% annually (Eynard et al. 2005). The most inevitable effect of all the abiotic stress is alteration of redox state of intracellular milieu and associated signaling (Steffens 2014). Besides, plant stress hormone abscisic acid (ABA) played the most crucial role in abiotic stress signaling (Grennan 2006; Roychoudhury and Paul 2012). On the other hand, the production of compatible solutes is also common in salt and cold stress (Hasegawa et al. 2000). Primarily, any stress response in plants can be divided into three basic events: (1) sensing the environmental cues or signal perception by different sensor molecules, (2) signal transduction and regulation by synthesizing stress hormones, secondary signaling molecules, and activation of kinases or actuation of different metabolic pathways, etc. and finally, (3) tolerance, which is the outcome of the above-mentioned signalling mechanisms. Many wild-type plants develop tolerance toward different abiotic stress but do not have good yield or quality of grains. Different biotechnological methods and molecular breeding technologies can incorporate tolerance to agronomically important plants. In rice also different methods are introduced to develop environmental stress tolerant plants. Due to the advent of modern genomic technologies, common gene expression patterns has been observed in rice upon different abiotic stresses (Rabbani et al. 2003). Recently, approaches have been taken to impart tolerance against multiple abiotic stress factors to meet the expanding needs of agroeconomy. The present chapter focuses on the convergence of different abiotic stresses in rice, their commonalities, recent developments in developing multiple abiotic stress-tolerant rice varieties, and future prospects.

2 Stress vs. Multiple Stresses in Rice

Stress can be any condition or substance that readily hampers normal physiology, growth, and development of a plant or somehow retard those. Sometimes stresses are in such a magnitude that causes death of the entire plant (Kissoudis et al. 2014). In most of the cases due to sessile nature of plants, these stresses spread quite rapidly and hamper the entire vegetation brutally. Crop plants are affected quiet roughly than that of the wild members due to their history of domestication and loss of resistance behavior (Smýkal et al. 2018). Rice, being the most consumed grain crop in the world, has different lines as well as varieties with enriched qualities and yield. Unlike most other plants, stress factors in rice are also divided into two major groups, biotic and abiotic. Recently, another important factor surfaced in discussion is anthropogenic stress factors. Although, anthropogenic effect is intricately associated with both biotic and abiotic factors, it affects abiotic stress more in the present context (Dukhovskis et al. 2003). The tripartite interaction of plant with other living organisms as well as environment not only explains the homoeostasis of plant but also explicates dynamic nature of odds coming to the plant (Soliveres and Maestre 2014) (Fig. 1).

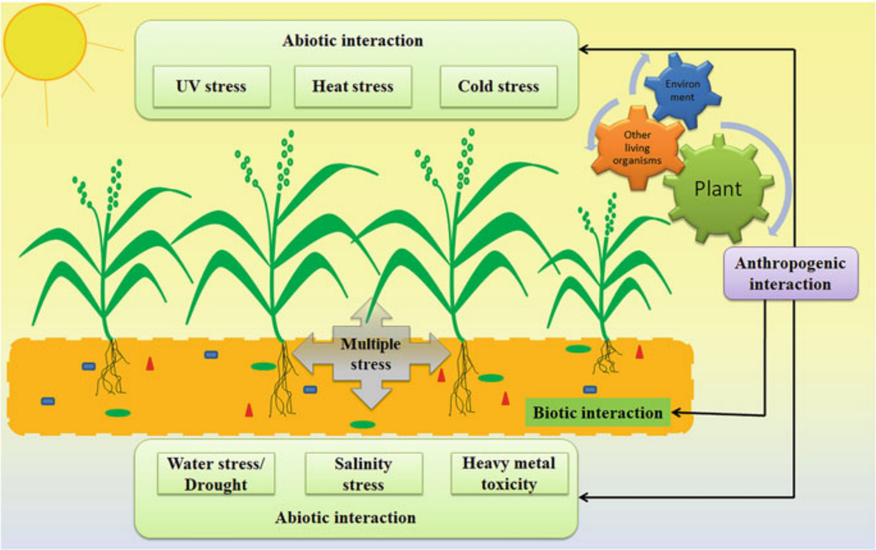

Fig. 1 Different abiotic interactions of rice in environment

Salinity and drought are considered to be major yield constrains in rice plant worldwide. Moisture requirement is very much essential for lustrous growth and also associated with crop production. India as the largest paddy growing country that produces around 125 million tons of rice in 43 million ha agricultural land (FAO, bridging the rice yield gap in India). Despite this huge agricultural production, yield remains still low around 2.85 tons/ha. The most of the agricultural production of these rice mainly relies on rain-fed and irrigated agricultural fields (Singh et al. 2017). Drought-associated loss causes more than 30% annual yield loss (Wu et al. 2019). Several attempts have been made to develop drought-tolerant variety of rice that can grow and show moderate to high level of productivity in the minimum supply of soil moisture. Crossing of natural drought-tolerant rice varieties with susceptible high yielding variety is a very common approach in developing tolerant high/moderately high yielding varieties (Venuprasad et al. 2007). In most of these cases, adequate loss of the desired traits seems to be operational and causes substantial loss of resistance phenomenon (Ceballos et al. 2015). Genetic dissections have been made to determine molecular control over drought stress in different plants (Yadav and Sharma 2016). Molecular networks in abiotic stress-tolerant pathways have broadly identified three groups of genes: stress perception genes, signal transducers, and regulators (Pandey et al. 2016). Molecular dissection also determines that different abiotic stress has common regulatory networks. For example, salinity stress has very close regulatory networks as that of drought. Broadly in both of these cases, plants experience severe water stress conditions. Plant hormones particularly abscisic acid (ABA) plays crucial role in both of these stresses (Sah et al. 2016). High-throughput microarray analyses in rice plants and *Arabidopsis* have revealed ABA biosynthesis and phospholipid metabolism have altered dramatically in response to salinity and drought stress. Besides many protein kinases, phosphatases along with several transcription factors are also playing key roles in this phenomenon (Rabbani et al. 2003; Shinozaki et al. 2003; Ahmad and Prasad, 2011, Roychoudhury and Banerjee 2017). The above study also showed that drought and salinity stress responsive gene expressions are significantly correlated with cold stress in rice plants. The comparative transcriptome analysis using rice genome database have identified 73 stress-inducible novel genes in rice, among which more than 50 genes are common with *Arabidopsis* performing similar functions (Rabbani et al. 2003). Later several studies were conducted in different plants to determine the common regulatory networks against salinity, drought, as well as cold. Recently, in cassava (*Manihot esculenta*) RNA-seq-based transcriptome analysis revealed expressional overlap in response to drought and frost. Detailed expression patterning discovered that the common gene sets were co-induced, co-repressed, as well as co-regulated (Li et al. 2017). Similar phenomenon was also observed in *Ocimum tenuiflorum* in response to multiple physical stresses (Rastogi et al. 2019). Major *cis*- and *trans*-acting elements discovered so far that act in common are ABF (ABRE-binding factor), ABRE (ABA-responsive element), AREB (ABRE-binding protein), DRE (dehydration-responsive element), DREB (DRE-binding protein), and ERF (ethylene-responsive element binding factor) (Qi et al. 2011a; Roychoudhury et al. 2013). Besides, CBF (C-repeat-binding factor) and CRT

(C-repeat) are also found to take part in these multiple stress tolerance pathway (Wang et al. 2016a; Zarattini and Forlani 2017). Despite the commonality, global gene expression demonstrated that although gene expression pattern in response to multiple physical stresses show similarities, the overall expressional matrices were poles apart in different organs in rice plants (Zhou et al. 2007; Paul and Roychoudhury 2019).

3 Signal Transduction, from Stress Sensors and Regulators to the Tolerance

Restriction in the movements, along with the absence of defined immunogenic cells and humoral immunity, makes plants rely largely on their internal signaling components to counteract diverse pathogenic and environmental cues. Harmonic orchestration and timely accumulation of a particular gene or a set of genes determine their basis of tolerance response (Basu and Roychoudhury 2014). Different abiotic stress factors may induce a group of particular genes but their activation as well as subsequent signaling pathways differ broadly based on type of stress and their amplitude (Fig. 2).

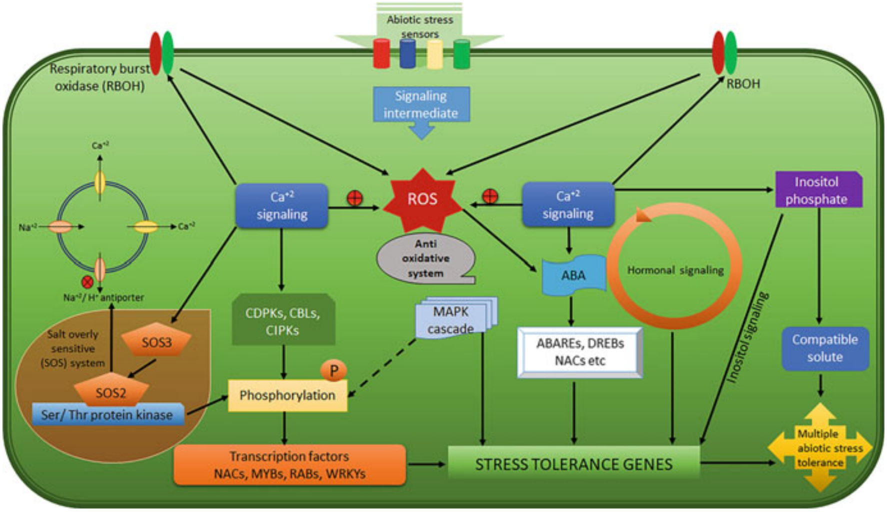

Fig. 2 Multiple abiotic stress response network. The model illustrates basic signaling events in response to different abiotic stress in rice. External stimuli have been sensed by sensor proteins, and this signal then transduced within cells by different signaling intermediates. Abiotic stress generally activates ROS pathway and calcium-dependent signaling in rice. ABA also has an inevitable role to activate stress-tolerant genes. Identification and modulation of any of these intermediate nodal genes may lead to multiple stress-tolerant rice varieties

3.1 Multiple Stress Sensors

Sensors are the group of proteins that can sense particular stimulus and transduce signal downstream. Receptors like kinase (RLK) are major group of proteins that act as sensor protein in plants against different environmental stimuli. This excessively large gene family exhibit about 610 members in rice plants (Shiu et al. 2004). Majority of RLKs are membrane bound but some are wall associated (also called wall-associated kinase, WAK), cytosolic, and apoplastic (rice root meander curling, OsRMC) (Lim et al. 2015; Zhang et al. 2015; Ye et al. 2017). Membrane-bound RLKs consist of extracellular ligand binding domain (ECLBD), transmembrane domain (TMD), and catalytic protein kinase domain (PKCD) (Walker 1994; Greeff et al. 2012). It also contains one N terminal signal peptide (SP) that specifically binds to the endoplasmic reticulum during protein synthesis (Gish and Clark 2011). Plant hormone ABA is key regulator in abiotic stress in plants and is directly related to RLK-mediated signaling event. In rice plants, two distinct pathways are operational via ABA-dependent and ABA-independent manner during drought stress. FON1 is a leucine-rich repeat family RLK (LRR RLK, also called leucine-rich repeat receptor-like kinase, LRK) which is activated during drought stress in rice and responsible for phosphorylation-dependent activation of several ABA-dependent genes (Feng et al. 2014). Whereas, another LRR RLK, *Leaf Panicle* 2 (LP2) is found to be downregulated by ABA and drought stress (Wu et al. 2015). Recently, another LRR-RLK, LRK2 has been identified in rice that also positively regulates drought tolerance in rice. Yeast two hybrid and bimolecular fluorescence complementation (BiFC) assay demonstrated that LRK2 interacts with elongation factor 1 alpha (EF1α) and increases lateral root formation (Kang et al. 2017). AtLRK 10 L1.2 (a wheat ortholog of LRK10) is also known to control drought stress tolerance in a ABA-dependent manner (Lim et al. 2015). OsSIK 1 and OsSIK2 are members of LRK family and also involved in drought and salinity response in rice by ameliorating stomatal frequency and antioxidative defense response (Ouyang et al. 2010; Chen et al. 2013).

Like drought stress, salinity stress is also sensed by some RLKs. Salt intolerance 1 (SIT1) is a common RLK activated by increased amount of NaCl concentration in rice plants. SIT1 is known to phosphorylate MPK3/MPK6 cascade as downstream regulation (Li et al. 2014). As discussed earlier, OsRMC is involved in salt sensing protein, and it regulates further signaling through OsEREBF transcription factor (Jisha et al. 2015).

Calcium plays crucial role in many physiological effects and abiotic stress responses. One calcium-regulated RLK (CRLK1) is found to take part in cold stress in plants effectively (Yang et al. 2010). A recent study identified a cold stress responsive receptor, COLD1, in rice. This sensor protein was found to be highly expressed in rice under chilling stress (Yuan et al. 2018). Low temperature induces many cold-regulated genes (COR) that are also induced under different other abiotic stresses. For example, responsive to desiccation 29 (RD29A), kinase 1 (KIN1) were highly expressed in cold stress to fix membrane freezing (Thomashow 1999). Dehydration response element-binding protein (DREB1) or

CRT/DRE-binding factors were also found to be induced in chilling stress (Yuan et al. 2018).

3.2 Abiotic Stress Regulators

3.2.1 Calcium Signaling

Calcium signaling event is pivotal in any abiotic stress response. This Ca^{+2} plays important role in transducing signal from external que to the internal specific regulators. In response to multiple abiotic stress factors, different calcium influx channels present in the plasma membrane and efflux channels located in vacuolar membrane are activated (Cao et al. 2017). Their activities in consortia increase the cytosolic calcium level ($[Ca^{+2}]_{cyt}$) significantly. Primarily, intracellular calcium concentration activated respiratory burst oxidase (RBOH) to produce ample amount of reactive oxygen species (ROS) (Kurusu et al. 2015). The *Oryza sativa* respiratory burst oxidase homolog B (OsRbohB) was found to be activated by intracellular Ca^{+2} levels in rice. This RBOHB was also activated by phosphorylation events (Takahashi et al. 2012). Genome-wide identification of phosphorylation sites in RBOHs from rice and *Arabidopsis* revealed that AtRbohD and OsRbohC contain highest phosphorylation sites, and many calcium-dependent protein kinases were found to be associated with protein–protein interaction map (Kaur and Pati 2018). A rice calcium-dependent protein kinase, OsCPK9 improves drought tolerance in rice by regulating stomatal dynamics. The expression of OsCPK9 was noticed and also be modulated through NaCl and ABA concentrations (Wei et al. 2014). Biphasic induction of Ca^{+2} concentration is very common in higher organism in response to unfavorable environmental conditions. In animal cell cultures, it is quiet easier to impose artificial Ca^{+2} induction to study the end effect. In plants, although difficult but Ca^{+2} ionophore-mediated studies suggested strong correlation of $[Ca^{+2}]_{cyt}$ with abiotic stress responses (Monroy and Dhindsa 1995; Sheen 1996). In two different studies, it has been showed that OsCDPK13, a rice calcium-dependent protein kinase was induced by both cold stress and gibberellic acid treatment (Yang et al. 2003; Abbasi et al. 2004). In a separate study, it has been observed that overexpression of OsCDPK7 leads to improved tolerance toward salt, drought as well as cold (Saijo et al. 2000). On the other hand, calcineurin B-like protein (CBL) was also involved in response to several abiotic stress response. Semi-quantitative RT-PCR-based gene expression pattern showed that NaCl, PEG, cold stress, as well as external treatment with ABA activated CBL gene families. Transgenic lines overexpressing OsCBL8 were tolerant to salt stresses than the wild-types (Gu et al. 2008). Another CBL interacting protein kinase (CIPK) family protein OsCIPK23 expressed in pistil and another found to control drought stress response in rice. RNAi-mediated silencing study suggested its dramatic dual role in rice reproduction as well as water stress conditions (Yang et al. 2008). Full-length DNA over expressor (FOX) gene hunting system conferred the involvement of OsCPK21 in salt tolerance in rice (Asano et al. 2011). Among wide varieties of rice seedlings, CDPKs (particularly, 47 kDa) were induced largely under cold stress, mainly in leaf and stem tissues (Li and Komatsu 2000). Salt overly sensitive (SOS) is a very important gene family that regulates salt stress and bridges with calcium signaling event in plants (Gong et al. 2001). Calcium sensor protein SOS3 activates ser/thr protein kinase SOS2, that in turn controls the activity of plasma membrane bound Na^+/H^+ antiporter, SOS1 and other sodium channels (NHX1) in tonoplast membrane. These SOS family proteins largely controlled by osmotic stress and ABA (Chinnusamy et al. 2005). *Oryza sativa* SOS3 and NHX1 along with some stress-induced proteins (NAC1, RAB 16A, DREBP2, CRT/DREBP, WRKY24, WRKY71) were activated dramatically against variety of abiotic stresses against rice plants (Basu and Roychoudhury 2014).

3.2.2 ROS Signaling

Generation of ROS is an inevitable event in any abiotic stress response in plants. In rice plants, oxidative outburst is also evident from salt and/or drought, cold, heat, and other abiotic cues (Ashraf et al. 2018). ROS homoeostasis in plants maintained through the balance between the production of these toxic molecules in response to some stimuli and its scavenging through subtle antioxidant system (Das and Roychoudhury 2014). The ROS includes hydrogen peroxide (H_2O_2), superoxide radical ($O_2^{\bullet -}$), hydroxyl radical (OH•), and singlet oxygen ($^1O_2$) derived from improper reduction of molecular oxygen in different metabolic processes (Apel and Hirt 2004; Miller et al. 2010; Bhar et al. 2017; Banerjee and Roychoudhury 2018). Antioxidative enzymes, catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione *S*-transferase (GST), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR), peroxiredoxin (PRX), etc. in plants effectively scavenge these reactive molecules (You and Chan 2015; Bhar et al. 2017). Comparative study with tolerant and susceptible varieties of rice revealed that oxidative burst and associated antioxidative defense phenomenon determines resistance in rice cultivars (Guo et al. 2006). The endogenous glutathione pool, glutathione (GSH)/glutathione disulfide (GSSG) were found to be dramatically altered in response to abiotic stress in rice and maize, which indicates involvement of glutathione as antioxidant mechanism in response to abiotic stress in the same (Szalai et al. 2009). NADPH oxidases are the major group of plant respiratory burst oxidases. NADPH oxidases are membrane-bound proteins with c terminal FAD and NADPH binding domains. Its cytosolic N termini have an EF hand motif that contains calcium interacting sites and also phosphorylation sites, thus controlling ROS-mediated gene regulation (You and Chan 2015; Bhar et al. 2017). *Oryza sativa* histidine kinase 3 (OsHK3) was found to be interacted with NADPH oxidase during ABA signaling (Wen et al. 2015). An ERF family transcription factor, OsLG3 was positively controlled over drought stress in rice by regulating oxidative burst (Xiong et al. 2018). In another study, it has been implicated that silencing of *Oryza sativa* glutaredoxin 17 (OsGRXS17) increases intracellular ROS level and enhances tolerance to drought stress by stomatal closure (Hu et al. 2017). Salt stress dramatically induces polyamine (PA) concentration and that exerts effect on SOD to counteract associated oxidative burst in rice plants, but the exact mechanism of controlling SOD by PA is unknown (Alcázar and Tiburcio 2018). Trehalose a non-reducing sugar is also accumulated in plants in a ROS-dependent fashion (Kosar et al. 2018). In a different study, it has been showed that external melatonin treatment ameliorated with anti-oxidative system and thus relieved stress in cold-stressed rice seedlings (Han et al. 2017). The effect of different chemical substances on the antioxidative responses in plants and negative effect of this on different abiotic stress have led the idea of priming the plants with different chemicals to combat abiotic stress. Pretreatment of seeds by soaking with different chemicals before germination showed greater tolerance than that of non-soaked seeds (Ashraf et al. 2018). Cold and heat priming of the plants also gained popularities to induce cross-tolerance (Hossain et al. 2018). ABA was also found to be important priming agent in rice against alkaline stress by inducing oxidative cycle. ABA-induced priming has increased the expression of ABA-responsive genes (SalT and OsWsi18) along with stress-regulated genes (OsJRL, OsPEX11, OsNAC9, OsAKT1, and OsHKT1) significantly under drought condition to impose resistance (Liu et al. 2019).

3.3 Transcriptional Control

3.3.1 ABA, Master Regulator of Abiotic Transcriptional Reprogramming

Abscisic acid is considered to be universal plant stress hormone with varied role on transcriptional reprogramming. ABA-regulated genes have unique and conserved *cis*-regulatory elements that can bind to their upstream region in an ABA dependent fashion; these are called ABA-responsive elements (ABREs). AREB/ABFs are bZIP family transcription factors known to upregulate during water stress conditions in plants (Roychoudhury et al. 2008; Banerjee and Roychoudhury 2017). AREB1, AREB2, and ABF3 control ABA-dependent gene expression in relation to drought stress by activating SRK2D/SnRK2.2 transcription factor. Triple mutant (*areb1 areb2 abf3*) showed reduced drought tolerance in *Arabidopsis* as compared to wild-types (Yoshida et al. 2010). AREB/ABFs are also found to be pivotal *cis*-regulatory element in rice genome in relation to water stress and ABA gene signaling (Fujita et al. 2013). In a separate study, it has been observed that OsABF1 is necessary for the ABA-dependent drought stress in rice, and this protein targeted toward nuclei to control further downstream gene regulation (Hossain et al. 2010). Dehydration responsive element binding (DREB) proteins, another class of transcription factor that are activated in response to drought, salt, and cold stress in plants by interacting with *cis*-acting element DRE/CRT present near upstream region of stress-responsive genes (Zhang et al. 2009). The integral effect of stress leads to transcriptional network among major regulatory proteins. After sensing external drought/salinity or any water stress situation, ABREs are actuated by AREB/ABF factors in an ABA-dependent way, whereas another ABA-independent pathway is activated by DREBs (DREB1 and DREB2), which in turn instigate DRE/CRT elements in stress-responsive genes. This DRE/CRT can also be activated by cold stress via CBF/DREB1 directly (Shinozaki et al. 2003). NAC domain containing transcription factors plays critical role in stress tolerance. Many of these NACs are known to control in ABA-dependent manner. Recently, OsNAC14 is found to control drought, salinity, as well as temperature stress by interacting with $RAD_{51}A_1$ in ABA-dependent manner (Shim et al. 2018). In rice, OsNAC5 and OsNAC6 are very well known in ABA-dependent abiotic stress tolerance (Nakashima et al. 2012). OsNAC5 controls proline and soluble sugar synthesis in rice to combat salt and drought stress. RNAi-mediated silencing study have elaborated the lower proline and sugar content-associated decreased tolerance (Song et al. 2011). In another study, it has been noticed that OsNAC022 also controls the similar function by activating ABA-responsive genes (OsNCEDs and OsPSY) (Hong et al. 2016). These sugars and proline act as compatible sugar or osmolytes in plants. Osmolytes are wide range of chemicals which have high solubility potential, uncharged, polar, and significantly low reactivity with other cellular components and hence can change the osmotic properties of cytosol (Roychoudhury et al. 2015). They mainly include sugars, polyols, and amino acids, i.e., proline and glycine (Gupta and Huang 2014). Sugars (e.g., glucose, fructose, fructans, and trehalose) mainly function as carbon storage and energy production, but some also help in mitigation of ROS as electron donor (Gupta and Huang 2014). Accumulation of the sugars is observed under salt stress condition in different plants (Parida et al. 2004). A non-reducing disaccharide of glucose, trehalose was highly accumulated in salt-stressed plants, and overexpression in rice plants showed enhanced resistance toward drought and temperature stress (Garg et al. 2002). Transcription factor with APETALA 2 (AP2) domain is gaining importance in plant abiotic tolerance day by day. AP37 and AP59 were found to control abiotic stress particularly drought stress in rice by ameliorating trehalose concentration. The overexpression lines were constructed under the control of constitutive promoter OsCc1 and OsCc1: AP37 rice plants were more drought tolerant in field conditions (Oh et al. 2009). Ethylene-responsive transcription factors (ERF) control drought, salt, and chilling stress by activating DRE/CRT and GCC box *cis*-acting elements in a ABA-dependent manner. One tomato ERF gene, *JERF1* when overexpressed in rice plants induced drought tolerance and proline concentration by co-activating proline biosynthetic gene *OsP5CS* (deltal-pyrroline-5-carboxylate synthetase) (Zhang et al. 2010).

3.3.2 MAP Kinase Cascade

MAP kinases are the central player in many abiotic and biotic stress responses in plants. These proteins transmit signals from external receptors to the internal effectors and mainly rely on sequential phosphorylation in tandem (MAPKKK-MAPKK-MAPK) for activation. MAPKKK generally interacts directly with the receptor kinases for their activation and transfers phosphate group to ser/threonine residues of S/T–X3–5–S/T motif of MAPKK. MAPKK activates MAPK by phosphorylating serine and tyrosine residue, in turn MAPK can activate a variety of target proteins mainly by phosphorylating serine/threonine residue (Nakagami et al. 2005). In rice, many MAPKs were reported so far to control abiotic stress tolerance. Among many others, MAPK4 and MAPK6 are essentially involved in salt, drought, cold stress response (Ichimura et al. 2000). Recently, OsMKKK63 was found to function upstream of OsMKK1 and OsMKK6 during drought, salinity, as well as chilling stress (Na et al. 2019). A novel rice MAPK, OsMSRMK2 was also upregulated in response to salt, drought, UV exposure, as well as heavy metal stress (Agrawal et al. 2002). OsMPK3, OsMPK7, and OsMPK14 were also found to work upstream of WRKY30 to control drought tolerance in rice (Shen et al. 2012). In a separate study, five MAPKK genes (*MAPKK1*, *MAPKK3*, *MAPKK4*, *MAPKK6*, and *MAPKK10-2*) from pusa basmati rice variety have been studied in response to drought, salt, heat, and cold stress. MAPKK4 and MAPKK6 were strongly regulated by salt and cold stress, whereas MAPKK was responded against salt and drought stress. MAPKK10-2 was only regulated by cold stress (Kumar et al. 2008). In *japonica* rice cultivars, OsMAPK44 was prominently induced under drought stress and ABA (Jeong et al. 2006). Interestingly, map kinase HOG1 from *Piriformospora indica* (PiHOG1), one root colonizing endophytic bacteria, showed salinity tolerance dramatically in rice (Jogawat et al. 2016).

4 Tolerance

4.1 Role of Genomics in Multiple Abiotic Stress Tolerance

Development of effective tolerant variety of plant requires proper identification of gene or gene loci meant for resistance phenomenon and introgression of those into the plant in a sustainable way. Identification of tolerant gene against a single or multiple abiotic stress can lead to the application of those in different biotechnological approaches to confer tolerance. Genomics and functional genomics are two powerful tools in this century which revolutionize the study to determine the genetic structure of a species. Quantitative trait loci (QTL) are the loci present in the genome of an organism that positively or negatively correlates with some quantitative characters. By incorporation, integration, or deletion of this genome segment, the desirable character of a plant can be modulated (Tétard-Jones et al. 2011). Rice, unlike other plants, has several QTLs associated with different abiotic stress factors. It has long been utilized in breeding procedure in rice to develop different superior varieties. About 126 SSR (simple sequence repeats) markers have studied to identify one QTL (qtl12.1) that is present on chromosome 12 and found to be associated with abiotic stress associated yield in rice (Bernier et al. 2007). Another loci containing some putative transcription factors associated with submergence tolerance in rice is transferred to Swarna variety to develop tolerant cultivars (Mackill et al. 2007). Most of the QTLs for abiotic stress tolerance are identified from wild low yielding varieties of rice that limits their utilization in crop improvement. Whole-genome scan and bulked segregate analysis of some high yielding mega rice varieties (Swarna, IR64, MTU1010 crossed with drought tolerant *aus* cultivar N22) have identified QTL for rice grain yield under reproductive phase drought stress (Vikram et al. 2011). Mostly cDNA AFLP (amplified fragment length polymorphism) and

SSR marker-associated identification have been employed for the identification of the QTL markers. Although these techniques have identified hundreds of QTLs for a varied functions, the process is slow and laborious. At the same time, simultaneous sequencing and scanning of whole genome of a particular species are necessary for the rapid identification of genetic loci imparting multiple stress tolerance. Recently, speedy development in genomic era and brisk employment of next-generation sequencing (NGS) platforms in crop improvement widely open the door for genetic dissection of plants.

4.2 DNA-Seq and Genome-Wide Association Study (GWAS)

GWAS is recently emerged as a popular broad spectrum genetic association analysis platform to detect a particular genetic loci of interest. The variation of genetic organization in a population clearly entails the inclination of loci toward a particular trait. The draft whole-genome sequence of *indica* and *japonica* cultivars were announced long back using short gun sequencing method (Yu et al. 2002; Goff et al. 2002). Due to the advent of NGS-based DNA-seq platforms, genome of different rice varieties has been decoded (McNally et al. 2006; Bindusree et al. 2017). DNA-seq-based whole-genome analysis of Godawee, a salt-tolerant variety from Sri Lanka has identified 28 salt-tolerant genes among which OsHKT2 was found to have the highest single-nucleotide polymorphisms (SNPs). Moreover, upstream analysis showed the association of WRKY transcription factor with *OsAPx8*, *OsMSR2*, *OsTIR1*, *OsHKT2;3*, *OsHKT14*, and *OsSOS1* genes (Singhabahu et al. 2017). Besides, *OsHKT1* is also known for salt tolerance in rice. Allelic variation indicated that T67K mutation destabilizes transmembrane domain of OsHKT1, whereas P140A alteration increases its phosphorylation, thus affecting salt tolerance efficacy (Negrão et al. 2013). Single-nucleotide and insertion–deletion (InDel) polymorphisms were studied extensively in different Chinese rice varieties to develop PCR-based markers (Markkandan et al. 2018). GWAS of *indica* rice varieties in response to salt stress revealed Saltol, and the salt stress QTL is associated with high Na^+/K^+ concentrations and located in chromosome 1. Besides, other salt-tolerant QTLs were also observed in chromosomes 4, 6, and 7 (Kumar et al. 2015). These loci are also known for the drought tolerance in rice. In another GWAS in rice with controlled population by eigenvectors and combined kinship matrix approach eighteen, five and six associated loci were identified respectively for plant height, grain yield, and drought resistance. OsPYL2, OsGA2ox9, and OsRLK5 were found to be critical regulators for water stress in rice (Ma et al. 2016). Same study with 202 *O. sativa* mini core accessions for chilling tolerance suggested that *indica* rice varieties have more chilling tolerance potential. Around 48 chilling-tolerant QTLs had been identified, and those are distributed among all 12 chromosomes. Low-temperature seedling survivability (LTSS) QTLs, qLTSS3-4 and qLTSS4-1 were novel in their class to control chilling stress in rice (Schläppi et al. 2017). On the other hand, OsMYB2 a very common transcription factor was found to be associated with cold-tolerant loci in *indica-japonica* rice varieties

(Lv et al. 2016). MYB particularly R2–R3 types are also well known for other abiotic stress tolerance mechanisms in rice, i.e., heat stress, salt, and drought (Dai et al. 2007; El-Kereamy et al. 2012; Xiong et al. 2014). Manipulation of such regions can impart resistance against wide ranges of abiotic factors. Moreover, SNP-based association mapping of rice diversity panel 1 (RDP1) entailed that *japonica* variety is more cold tolerant than that of *indica* and *aus* varieties (Wang et al. 2016b). The enhanced cold tolerance of *japonica* varieties is due to the presence of cold-tolerant loci COLD1 within its genome. This encodes a G-protein signaling intermediate that interacts with α subunit of membrane-bound G protein and perks of calcium signaling (Ma et al. 2015). Multi-parent advanced generation inter-cross (MAGIC) is the recently emerging genetic resource tool that combines different useful agronomical traits with different abiotic and biotic tolerance factors in consortia (Huynh et al. 2018). This method not only combines good characters in a parent to develop mega parental type but also dissect the complex genetic architecture of an organism for the crop advancement. MAGIC has been employed in different plants for crop improvements, as well as development of multiple stress-tolerant super crops (Sannemann et al. 2015; Huynh et al. 2018; Stadlmeier et al. 2018). Unlike other plants, in rice also different MAGIC populations have been developed. In a study, four MAGIC populations, namely *indica* MAGIC (comprising eight *indica* rice varieties), MAGIC plus (containing eight *indica* parents with F1 intercrossing lines), *japonica* MAGIC (composing eight *japonica* parents), and Global MAGIC (16 parents, 8 from *indica* and 8 from *japonica*) were introduced with higher yield potential, improved grain quality, and combined biotic /abiotic stress tolerance factors (Bandillo et al. 2013). Recently, MAGIC lines with abiotic stress tolerance and other agronomic traits were screened for cooking and eating quality (CEQ) of rice (Ponce et al. 2018). Association mapping of *indica* MAGIC populations for heavy metal tolerance was also analyzed (Meng et al. 2017). Multi-Environment Trials (MET) data were combined in crop modeling, and this method identified rice varieties with multi-environment adaptability (Li et al. 2016). Hence, these advanced methods have remarkable potentiality to develop multiple stress-tolerant rice cultivars with other advanced desired agronomic traits. Although many works has been done so far in this field, more integrated and systematic approach in rice genomics is necessitated to develop sustainable multiple stress-tolerant varieties.

4.3 Functional Genomics

Functional genomics is a powerful tool to identify the function of a gene for the utilization of the same in genetic manipulation and crop improvement. The advent of NGS technology and advanced RNA-seq platforms boons this gene mining method further. Holistic crop improvement is directly proportional to the number of functional annotations present within the genome of the same crop (Roychoudhury et al. 2011). With this mission "rice 2020" project has initiated to decode the functions of all genes present within genetic architecture of rice plants. Along with the full-length genes, micro-RNA libraries and insertion mutant development are also envisioned

(Zhang et al. 2008). Comparative transcriptome analysis between drought-tolerant Nagina 22 (N22), salinity-tolerant Pokkali with susceptibility to both salt and drought IR64, revealed that NAC and DBD transcription factors are mainly involved in salt tolerance. On the other hand, bHLH- and C2H2-type zinc finger transcriptional control was observed over drought tolerance in rice. Moreover, secondary metabolism was found to play a pivotal role in both the cases (Shankar et al. 2016). In another study, it has been shown that aerobic adaptation to water scarcity required MADS box transcription factors (MADS5, MADS6, MADS7, and MADS15) along with sugar transporters (SWEET3A) (Phule et al. 2019). In silico approach to find out commonly regulatory genes in rice against combined abiotic stresses (CAbS) including salinity, drought, submergence, and metal toxicity unveiled the co-expression network in rice (Muthuramalingam et al. 2017). Transcriptome analysis of high-temperature germinated (HTG) rice showed overexpression of DREB, LEA, and RAB domain proteins, which were also known for their inevitable contribution in drought and salinity control in rice (Mangrauthia et al. 2016). Heterodimeric G proteins are popular molecular regulator for several physiological events in plants. Transcriptome analysis of G alpha subunit null mutants (RGA1) with wild-type rice cultivars explained multiple role of G protein in varied abiotic stresses, i.e., heat, cold, salt, and drought (Jangam et al. 2016).

4.4 Biotechnological Approach

The final step of conferring tolerance to plants relies on the introduction of a resistance gene within the plant genome or altering metabolic/enzymatic regulations conveniently. Transgenic approach has long been utilized as a basic method to develop abiotic stress-tolerant rice plants. Between two distinguished rice varieties, *indica* is quiet easy to genetically transform than that of *japonica* varieties (Latha et al. 2017).

4.4.1 Classical Transgenic Approach

As discussed earlier, several candidate genes, transcription factors, as well as enzymes or regulatory proteins are discovered so far for the development of abiotic stress-tolerant rice lines. Transgenic approach has been considered mainly to introduce transcription factor genes and osmolyte regulatory genes to confer resistance against salinity and drought tolerance in rice (Paul and Roychoudhury 2018). Among all transcription factors, heat shock proteins are common players in salinity, drought, as well as temperature stress in rice. Several approaches have been taken to introduce different HSPs in the rice plants to make rice plants multiple stress-tolerant ones. Most of the abiotic stresses significantly modify cellular proteome by inactivation of its function and eventual disintegration. Different HSPs act as molecular chaperones. Hence, HSP/chaperone network can modulate several protein structure function relation upon multiple stress-indicated protein destabilization (Jacob et al. 2017). HSP90 alone can restructure 1–2% of total cellular proteins from destabilization (Krukenberg et al. 2011). HSP70 and HSP90 together with their chaperone network can control all cellular degradation (Huang et al. 2014). Cytosolic class I HSPs were analyzed from rice and found to be distributed in chromosome 1 and 3. These HSPs were induced in different heavy metal treatment (Guan et al. 2004). Global expression profiling of rice HSPs and heat shock factors (HSFs) confirmed overlapping function upon salt, drought, cold, and heat stress (Hu et al. 2009). Overexpression of one mitochondrial OsHSP70 reduces programmed cell death in rice plants significantly (Qi et al. 2011b). OsHSP90 transcript accumulation has been observed in response to multiple abiotic stress, i.e., salinity, high pH, high temperature, and transformed plants with OsHSP90 showed enhanced resistance against all these abiotic factors (Liu et al. 2006). Small heat shock protein, OsHSP18.6 significantly restored transformed rice plants from multiple abiotic stresses (Wang et al. 2015). Besides HSPs, different other transcription factors were also introduced within rice to confer tolerance. The ABA-dependent AREB/ABF and MYC/MYB domain containing transcription factors were introduced frequently to impart tolerance. On the other hand, bZIP transcription factor (OsbZIP46CA1) showed enhanced drought tolerance in overexpression rice plants. In *Arabidopsis*, bZIP transcription factor ABF3 also showed increased drought tolerance in transformed plants (Kang et al. 2002).

Different ABA-independent transcription regulators, e.g., DREB/CBF and NAC domain, containing transcription factors were also introduced within rice plants to build up abiotic stress resilience. Overexpression of rice plants with OsDREB2A and OsDREB2B showed increased tolerance toward drought (Chen et al. 2008; Cui et al. 2011), whereas OsDREB2A isoform, OsDREB2B2 transcripts were accumulated by cold, heat, drought, and salinity stress (Matsukura et al. 2010). NAC domain containing transcription factors as described earlier are emerging as multiple stress regulators. In rice genome, wide NAC domain transcription factors were identified (Nuruzzaman et al. 2010), and this resource have been utilized in the development of overexpression lines (Shao et al. 2015). OsNAC045, overexpression lines significantly enhance tolerance against drought, high salt, and low temperature stresses (Zheng et al. 2009). Another NAC TF, OsNAC52 isolated from *O. sativa* and constitutive expression under 35 s promoter leads to enhanced drought tolerance (Gao et al. 2010). The potentiality of NAC TFs in multiple abiotic stress tolerance is emerging and can be employed more in future (Tran et al. 2010).

4.4.2 Cisgenics

Transgenic approach has long been used from functional study of a gene to the improvements of crops. The modification of a genetic background of a species and recurrent use of that argues with ultimate loss of gene pool. Hence, genetically modified organisms (GMOs) have always been in controversy in terms of public acceptance and strictly controlled by GM legislations. In this situation, Schouten et al. (2006) proposed “cisgenics,” where genes are introduced using recombinant technology from crossable donor plants. A detailed survey has been made to study the acceptance of cisgenic crops over GM crops in India (Shew et al. 2016). As during cisgenesis, genes are incorporated from its crossable partner; hence, it does not alter the species gene pool and is rather a variant of classical breeding techniques

(Telem et al. 2013). *DREB 2A* gene was introduced in GR11 rice varieties in cisgenic approach to confer drought resistance (Raj et al. 2015). Multiple pathway regulatory genes controlling abiotic stress can be identified from reproductive members and introduced within crop plants to develop sturdy multiple stress tolerance in cisgenic way (Jacobsen and Nataraja 2008; Parvathi and Nataraja 2017).

4.4.3 CRISPR-Cas9-Based Genome Editing

CRISPR (clustered regularly interspaced short palindromic repeats) are a class of small DNA sequences present in the prokaryotic DNA such as bacteria and archaea, which are derived from previously infected viral genomes. These sequences function to determine new viral infections from the sequence complementarities and induce resistance. Cas9 (CRISPR-associated protein 9) is an enzyme that recognizes CRISPR complementary sequences and cleaves them. This technology is widely used now in biotechnological application to edit genome according to the guide RNA molecules. Cas9 nuclease-mediated cleavage of targeted DNA is governed by single guide RNA (sgRNA), which recognizes target DNA by normal base pairing in 20–22 nucleotide stretch (Jain 2015). The custom-designed zinc finger nuclease under the control of heat shock protein promoter was used to modify the expression of ABA INSENSITIVE 4 in *Arabidopsis* (Osakabe et al. 2010). Although several CRISPR-Cas9-mediated genome editing has been done in rice, the instances conferring abiotic stress are rare (Zhang et al. 2014). Micro-RNAs (miRNAs) have diverse target sites within genome that controls several abiotic stresses accordingly. The modification of miRNA target sites is a complex procedure and is difficult to achieve due to its versatility. The advent of CRISPR technology makes that easy to target diverse sites in the genome in a single stance. CRISPR-Cas9-mediated altered miRNA mutagenesis particularly OsMIR408, OsMIR528, OsMIR815, and OsMIR820 was achieved in rice and demonstrated different abiotic stress response (Zhou et al. 2017). Genome editing involving *OsPDS*, *OsMPK2*, and *OsBADH2* enhances abiotic stress tolerance in rice (Shan et al. 2013). Recently, targeted mutation in $OsRR_{22}$ gene by Cas9-RR_{22}-sgRNA was generated and demonstrated enhanced salinity tolerance at seedling stage (Zhang et al. 2019). This method has wide possibilities to achieve multiple abiotic stress-tolerant rice in future.

5 Conclusion and Future Questions

As a staple crop of majority of world population, rice has no alternative, and popularity of this important crop is getting in pace due to huge increase in population size. Climatic change and abiotic stress deleteriously affect the production of rice globally. To maintain the balance between human need and rice production, modern agriculture should rethink the long-drawn tolerance strategies in light of cutting-edge genomics and biotechnological applications. Although different abiotic stress factors have some obvious specific mechanisms, genomic intricacies also overlap at some point in striking ways. These overlapping nodes in complex interacting pathways are readily detectable due to recent advancement in technological developments.

Besides, the advent of next-generation sequencing technologies revolutionize genomics and functional genomics applications. Although many studies have been advocated till now to develop multiple stress-tolerant rice varieties, all those trials are discrete. More systematic approach is necessitated to create new possibilities in this field. On the other hand, scanning of entire metabolic pathways under different stress is also required to choose the exact nodal point of interest that can be targeted by multiple stress factors. Technological advancement will only become thriving when it will successfully improvise in field. Detection of the most efficient multiple stress-tolerant pathways and their booming "lab to land" transition are next challenges to the scientific community to impart multi-stress tolerance in rice.

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Targeting Metabolic Pathways for Abiotic Stress Tolerance Through Genetic Engineering in Rice

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Abstract

Rice is one of the leading staple foods and widely consumed worldwide. The production of rice is mostly affected by major abiotic stresses like drought, salinity, cold and high temperature by disturbing the metabolic processes involved in its growth and development. Engineering those sensitive metabolic pathways by targeting the metabolites involved could make the plant tolerant to these abiotic stresses. Therefore, targeting metabolic pathways through genetic engineering in rice is one of the major strategies for crop improvement. Metabolic engineering is a powerful tool for optimizing a targeted metabolite regulatory process to increase the cellular production of the specific metabolite. Engineered metabolic pathways can be achieved by modification in a single stress-responsive gene or multiple associated genes. Therefore, primary step to metabolite engineering is the identification of target genes involved in abiotic stress-responsive metabolic pathways. Metabolic pathways such as osmolyte pathways, reactive oxygen species pathways, hormonal pathways and other signal transduction pathways are the potential targets of genetic engineering for abiotic stress tolerance in rice. On the other hand, emerging techniques such as CRISPR Cas9/Cpf1 targeting negative regulatory metabolic pathway genes add more possibility to

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develop sustainable abiotic stress tolerance in rice. Overall, this chapter summarizes the earlier major findings to the most recent discoveries in the field of metabolic engineering by targeting important pathways involved in abiotic stress tolerance in rice. The novel genetic engineering tools and proposed potential targets of metabolic engineering for abiotic stress tolerance would have huge impact on sustainable productivity and rice crop improvement.

Keywords

Metabolic engineering · Abiotic stress · Rice · ROS · CRISPR · TALEN · ZFN · Meganuclease

1 Introduction to Plant Metabolic Pathways Engineering for Abiotic Stress

Metabolic engineering is the modification of endogenous metabolic pathways either by increasing the concentration of endogenous desired molecule or a new molecule (Capell and Christou 2004) or degradation of the compound. The increase of the targeted metabolite can be achieved by overexpression and/or suppression of a single or multiple genes. The products of the genes are either direct desired molecule/ metabolites or enzymes involved in the production of the targeted molecule. The metabolites are the results of upregulation of genes responsible for the synthesis of osmolytes. Subsequently, the accumulated osmolytes result in the easiness from osmolytic stresses. Metabolic engineering for abiotic stress tolerance can also be achieved by targeting the key enzymes involved in the stress tolerance pathways. Sometimes, the targeted molecules are the regulatory genes such as transcription factors that control the expression of the targeted molecule. Understanding of such relationships between the targeted genes and the associated factors offer more upgraded possibilities for targeted metabolic engineering.

2 Fundamental Requisite for Plant Metabolic Engineering

The foremost development in the areas of plant metabolic engineering is due to the advancement in the basic tools to drive metabolic engineering. The basic tools are required for understanding the relationship between the substrate and the desire product, identification of the target gene, methods to clone the target gene, transformation of the targeted gene followed by its downstream analysis of the subsequent product. Furthermore, efficient molecular biological technologies such as gene cloning, promoter analysis, protein targeting, plant transformation, biochemical genetics and other areas of plant biology are requisite. Identification of novel genes due to the advancement of different platform for transcript profiling under non-stress and stress conditions employing the naturally available abiotic stress tolerant genotypes is possible. Those novel genes are the potential “candidate genes” for plant metabolic engineering for abiotic stress tolerance.

3 Metabolic Pathways Engineering in Rice

Rice is popularly consumed worldwide, and its demand is increasing undoubtedly with the growing population. The production of rice is limited or even reduced due to the decrease in available land races for agriculture. Another important factor for decline in crop productivity is the frequent occurrence of unfavourable environmental conditions, viz. drought, salinity, cold and high temperature (Fig. 1). Therefore, the challenge lies in increasing the rice crop production during unfavourable conditions at the same time preserving the environmental quality. To meet the challenge, various approaches have been employed to confer tolerance against those major abiotic stresses. Among them, plant genetic transformation through metabolic engineering is one of the most promising approaches. Although rice

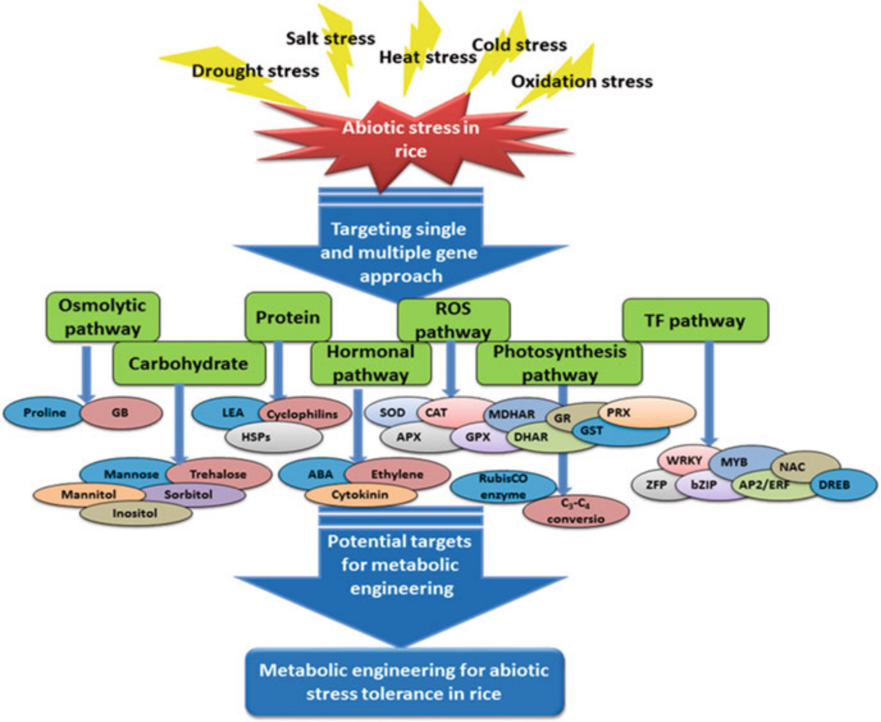

Fig. 1 Metabolic pathway engineering in rice for abiotic stress tolerance. Rice plant responses to the major abiotic stresses, viz. drought, salt, cold, heat and oxidation by upregulating the genes responsible for abiotic stress tolerance. Those stress-responsive genes were the potential targets for rice metabolic engineering for abiotic stress tolerance

full-genome sequence is already available, increasing data on functional genomics resources specifically in abiotic stress conditions enrich more detailed information in rice. Moreover, due to the availability of most of the tolerant germplasm in rice, major abiotic stresses offer more possibilities for selecting novel candidate genes. Those identified or characterized genes are the potential targets for metabolic engineering in rice to produce transgenic rice with promising traits.

4 Targeting Osmolyte Pathways for Abiotic Stress Tolerance in Rice

4.1 Proline

Proline is a conditionally essential amino acid, which plays important function during plant growth and development. During abiotic stress, proline is considered to be a key player for conferring tolerance to plant abiotic stress. Drastic increase in proline accumulation is widely reported when plants are exposed to abiotic stresses. The increased accumulation is the result of accelerated synthesis, decreased degradation, lower utilization or lower hydrolysis of proline. Proline confers abiotic stress tolerance by three mechanisms, namely stabilizing the sub-cellular structures as osmoprotectants, scavenging free radicals generated from the stressed organelles and adjusting the cellular redox potential. Alternatively, proline triggers the expression of intracellular stress-responsive genes (Roychoudhury et al. 2015). Therefore, elevating the accumulation of proline by metabolic engineering, the genes for proline biosynthesis could confer more abiotic stress-tolerant plants. In rice, expression of D1-pyrroline-5-carboxylate synthetase (P5CS) transgene from mothbean under the control of a stress-inducible promoter led to stress-induced overproduction of P5CS enzyme and subsequent proline accumulation (Zhu et al. 1998). Generally, proline in higher plants is synthesized from glutamate and ornithine. In glutamate-based proline biosynthesis, glutamate-γ-semialdehyde (GSA) is formed from glutamate by the action of the enzyme $^{1}\Delta$-pyrroline-5-carboxylate (P5C) synthetase (P5CS) followed by cyclization of GSA to form P5C. P5C is then reduced to proline in a NADPH-dependent reaction catalysed by P5C reductase (P5CR). P5CS is the rate-limiting enzyme in glutamate-based proline biosynthesis in higher plants (Kishor et al. 1995). Plants have at least two genes encoding the P5CS. In rice, there are two forms of P5CS, *OsP5CS1* is constitutively expressed, while *OsP5CS2* is primarily involved in response to hyperosmotic stress (Hur et al. 2004). During ornithine-based proline biosynthesis, the enzyme ornithine-δ-aminotransferase (OAT) converts ornithine into GSA. OAT is the key enzyme of the ornithine biosynthetic pathway. The list for engineering rice for abiotic stress tolerance with the proline biosynthesis genes is presented in Table 1. Both the pathways share the last reaction where P5C is reduced to proline in the presence of functional P5CR. On the other hand, two mitochondrial enzymes, flavin-dependent proline dehydrogenase (PRODH) and NAD^+-dependent P5C dehydrogenase (P5CDH), involve in the

Table 1 List of genes engineered for abiotic stress tolerance in rice

Targeted pathway	Targeted metabolite/pathway	Name of the gene	Source of the gene	Abiotic stress	Remark	References
Osmolyte	Proline	*δ-OAT*	Arabidopsis	Salt and drought	Accumulates higher proline	Liangqi et al. (2003)
		P5CS	Mothbean	Salt and water stress	Expression under the control of stress-inducible AIPC-ABA promoter Complex shows increase yield	Zhu et al. (1998)
			Mothbean	Salt and water stress	Stress inducible proline synthesis confers faster growth under stress conditions than that with constitutive synthesis	Su and Wu (2004)
	GB	*codA*	*Arthrobacter globiformis*	Salt and cold	Enhances the production of GB with increased survival after normal rehydration	Sakamoto and Murata (1998)
			Arthrobacter globiformis	Water stress	GB-induced water stress tolerance is associated with the upregulation of several stress-responsive genes	Kathuria et al. (2009)
		COX	*Arthrobacter pascens*	Salt	Expressed under the control of SIP (stress-inducible promoter) or a ubiquitin (UBI) gene promoter resulted enhanced production of GB	Su et al. (2006)
		CMO	Spinach	Salt and heat	Tolerance to salt stress and temperature stress with lower accumulation of GB	Shirasawa et al. (2006)
		BADH	Barley	Salt, cold and heat	Converts high levels of exogenously applied betaine aldehyde to GB more efficiently	Kishitani et al. (2010)
Carbohydrate	Trehalose	*AGPase*	Rice		Overexpression in leaf and seed tissue increases biomass and seed number in rice	Oiestad et al. (2016)
Protein	LEA	*OsEm1*	Rice	Drought	Increases ABA sensitivity and enhances osmotic tolerance in rice	Yu et al. (2016)
		LsEm1	*Lactuca sativa*	Drought and salt	Shows high germination performances, higher survival rates with less yield loss	Xiang et al. (2018a, b)
		OsLEA4	Rice	Drought, salt and heavy metal	MDA, proline and sugar content are increased	Hu et al. (2016)

(continued)

Table 1 (continued)

Targeted pathway	Targeted metabolite/ pathway	Name of the gene	Source of the gene	Abiotic stress	Remark	References
		OsLEA3-1	Rice	Drought	Driven by drought-inducible promoter of *OsLEA3-1*, the *CaMV* 35S promoter, and the rice *Actin1* promoter results in increase in yield	Xiao et al. (2007)
		OsLea14-A	Rice	Dehydration, salt, $CuSO_4$ and $HgCl_2$	Multiple stress tolerance and hg accumulation	Hu et al. (2019)
		OsLEA3-2	Rice	Salt	Transgenic lines are able to recover after stress	Duan and Cai (2012)
	Cyclophilin	*CcHyPRP*	*Cajanus cajan*	Drought, salinity and heat	Increases biomass and survival rate	Mellacheruvu et al. (2016)
		OsCYP21-4	Rice	Salt and oxidative stress	Increases tolerance to hydrogen peroxide treatment and peroxidase activity	Lee et al. (2015)
	HSPs	*OsHsp17.0 and OsHsp23.7*	Rice	Drought and salt	Shows higher germination	Zou et al. (2012)
		HsfA2a, HsfA2c and HsfA2d	Rice	Cold	Co-expression of Hsfs induces heat shock response under cool conditions	Yasuda et al. (2013)
		Oshsp16.9	Rice	Salt and drought	*Oshsp16.9* is localized specifically in the cytoplasm	Jung et al. (2014)
		OsHSP18.6	Rice	Heat, drought, salt and cold	Lower levels of malondialdehyde (MDA) and greater activities of catalase (CAT) and superoxide dismutase (SOD)	Wang et al. (2015)
		OsHSP50.2	Rice	Drought	Modulates ROS homeostasis and osmotic adjustment	Xiang et al. (2018a, b)

Hormonal	ABA	*OsbZIP42*	Rice	Drought	Elevated expression of the ABA-responsive *LEA3* and *Rab16* genes and was hypersensitive to ABA	Joo et al. (2019)
		OsPYL/RCAR5	Rice	Drought	Induces the expression of many stress-responsive gene	Kim et al. (2014)
		MAIF1	Rice	Osmotic stress	Involves in multiple signalling pathways in regulating root growth	Yan et al. (2011)
		OsJAZ1	Rice	Drought	More sensitive to drought stress treatment than the WT	Fu et al. (2017)
	Ethylene	*TERF2*	Tomato	Cold	Increases accumulation of osmotic substances and chlorophyll	Tian et al. (2011)
		OsERF71	Rice	Drought	Alters rice root structure and drought resistance	Lee et al. (2016)
		OsEREBP1	Rice	Drought and submergence tolerance	Along with abiotic stress tolerance, it confers disease resistance too	Jisha et al. (2015)
		TSRF1	Tomato	Drought	Improves the osmotic and drought tolerance of rice seedlings without growth retardation	Quan et al. (2010)
ROS	SOD	*Sod1*	Mangrove	Salt	More tolerant to methyl Viologen-mediated oxidative stress	Prashanth et al. (2008)
		OsCu/Zn-SOD	Rice	Salt	Germination rate is higher	Guan et al. (2017)
		MSD1	Rice	Heat	The grain quality of rice with constitutive High expression is significantly better than that of the wild type	Shiraya et al. (2015)
	GR	*gr3*	Rice	Salt	Gene knockout of *gr3* results in increased sensitivity to salt stress	Wu et al. (2015)
	GST	*OsGSTU4*	Rice	Salinity and oxidative stress	Results in better growth and higher GST	Sharma et al. (2014)
TF	ZFP	*OsCTZFP8*	Rice	Cold	Higher pollen fertilities and seed setting rates than WT	Jin et al. (2018)

(continued)

Table 1 (continued)

Targeted pathway	Targeted metabolite/ pathway	Name of the gene	Source of the gene	Abiotic stress	Remark	References
		ZFP179	Rice	Salt	Hypersensitivity to exogenous ABA	Sun et al. (2010)
	NAC	*OsNAC14*	Rice	Drought	Exhibits higher number of panicle and filling rate compared to WT	Shim et al. (2018)
		ONAC022	Rice	Drought and salt	Higher survival ratios and better growth than WT	Hong et al. (2016)
		EcNAC67	Finger millet	Salt and drought	Higher root and shoot biomass during stress and shows better revival ability	Rahman et al. (2016)
		OsNAC2	Rice	Salt and drought	Regulates both abiotic stress responses and ABA-mediated responses	Shen et al. (2017)
	MYB	*OsMYB6*	Rice	Drought and salt	Higher proline content, higher CAT and SOD activities, lower REL and MDA content	Tang et al. (2019)
		OsMYB2	Rice	Salt, cold and dehydration	Enhances upregulation of genes encoding proline synthase and transporters	Yang et al. (2012)
		OsMYB48-1	Rice	Drought and salinity	Hypersensitive to ABA at both germination and post germination stages and accumulates more endogenous ABA	Xiong et al. (2014)
	bZIP	*OsbZIP71*	Rice	Salt and drought	ABA-mediated drought and salt tolerance	Liu et al. (2014a, b)
		OsbZIP46	Rice	Drought	Many stress-responsive genes are upregulated	Tang et al. (2012)
	DREB	*OsDREB1F*	Rice	Salt, drought and cold	Participates in ABA-dependent pathway	Wang et al. (2008)
		OsDREB6	Rice	Cold and osmotic stress	The overexpressing lines are tolerant to abiotic stress, whereas the RNAi lines are sensitive to the stresses	Ke et al. (2013)

oxidation of proline. P5CDH plays a key role in controlling proline level in plant cells (Kiyosue et al. 1996).

Reports also indicated the alternative way for increasing the proline accumulation by metabolic engineering of other stress-responsive genes in rice. Heterologous expression of helicase gene also confers abiotic stress tolerance by increasing the proline accumulation. In rice, overexpression of helicase genes such as Pea DNA helicase 45 (*PDH45*) (Gill et al. 2013), Suppressor of Var 3 (*SUV3*) (Tuteja et al. 2013) and *PDH47* (Singha et al. 2017) confers salinity and drought tolerance. Glutamine synthetase (GS), *TERF1* (encoding a tomato ERF protein), *ICE1*, an *Arabidopsis thaliana* transcription factor gene, *SIDP361*, which encodes a DUF1644 family protein, Rice NAC Gene *SNAC1*, and calcineurin B-like protein (CBL)-CBL interacting protein kinase (*CIPK*) gene in transgenic rice accumulated higher levels of proline (James et al. 2018; Gao et al. 2008; Xiang et al. 2008; Li et al. 2016; Liu et al. 2014a, b).

4.2 Amines: Glycine Betaine (GB)

The metabolites that act as compatible solutes like glycine betaine (GB) or *N*,*N*,*N*-trimethylglycine perform an important function in plant abiotic stress tolerance. They are neutral molecules that are non-toxic to the cell. During stress, the major role of GB is to protect the transcriptional and translational machineries of the sub-cellular structures as a molecular chaperone from ROS through osmotic adjustment (Roychoudhury and Banerjee 2016; Hasanuzzaman et al. 2019). Such mechanism of GB accumulation during abiotic stress is not present in all the crops, and rice is one of them. Therefore, metabolic engineering of GB pathways in rice could increase the GB accumulation during stress. Metabolic engineering is now easily possible with the development of various gene engineering strategies. Also, the availability of wide knowledge on genomics and proteomics made it even easier. The specific genes of the GB metabolic pathways are identified and overexpressed that confer tolerance to various abiotic stresses. The possible target enzymes for GB pathways for metabolic engineering are the key enzymes involved in GB biosynthesis pathways. In higher plants, GB is synthesized from chlorine via betaine aldehyde (BA). The GB biosynthesis reactions are catalysed by choline monooxygenase (CMO) and NAD$^+$-dependent betaine aldehyde dehydrogenase (BADH). On the other hand, GB is synthesized by a single chlorine oxidase enzyme, COD or COX by soil bacteria of the genus *Arthrobacter*. The GB is also synthesized in other organisms from chlorine and glycine. From choline via a membrane-bound chlorine dehydrogenase (CDH) and BADH, GB is synthesized in *E. coli*. From glycine, GB is synthesized via three successive *N*-methylations, *S*-adenosylmethionine-dependent methyl transferases, glycinesarcosine methyltransferase (GSMT) and sarcosine dimethylglycine methyltransferase (SDMT) in halophilic microorganisms. In rice, the GB pathway is engineered by overexpressing the *codA* gene from *Arthrobacter globiformis* and *Arthrobacter pascens*. The transgenic rice showed better performance than its wild type with upregulation of several stress-responsive genes during salt, cold and water stress (Sakamoto and Murata 1998; Su et al. 2006; Kathuria et al. 2009). Generally, rice plants do not accumulate glycine betaine but possess a gene encoding BADH with a very low level of activity. The BADH encoding gene from barley is metabolically engineered in rice for abiotic stress tolerance. The transgenic rice plants can convert the exogenously applied betaine aldehyde to GB more efficiently as compared to the WT during salt, cold and heat stress (Kishitani et al. 2010).

4.3 Carbohydrates

Occurrence of unpredictable abiotic stress delays plant growth and development by disturbing the plant photosynthesis and production of reactive oxygen species (ROS). The generation of ROS superoxide radical ($\bullet O^{2-}$), hydrogen peroxide, singlet oxygen and hydroxyl radicals ($\bullet OH$) disrupts the cell equilibrium by disintegrating the biological membranes, disrupting the protein structure or by disruption of osmotic homeostasis and metabolic functions. Generally, response to such stresses is the production of osmotically compatible solutes or osmoprotectants that can scavenge the ROS. Major category of osmotically active compounds like carbohydrates is synthesized by plant during photosynthesis. Accumulation of such osmotically active compounds maintains the disturbed osmotic equilibrium during abiotic stresses. Furthermore, the accumulated sugar triggers the activation of stress-responsive genes. Nevertheless, not all the types of plants can significantly produce the osmolytes under stress. In those cases, increasing such metabolite accumulation through metabolic engineering, with transgenic genes responsible for osmolyte synthesis, could be an alternative for improved rice production. The osmotically active soluble sugars are glucose, sucrose, fructose, trehalose and mannose. Metabolic engineering of mannose-6-phosphate reductase enzyme in non-mannitol-producing plants resulted in the accumulation of the osmolyte mannitol (Khalil et al. 2017). Apart from soluble sugars, the insoluble form of sugar in cereals is the storage starch. Storage starch is the major determinant of yield. The higher content of amylose: amylopectin in rice starches absorbs more water and is generally more desirable for cooking purposes. Unlike other cereal crops, rice is facing most of the unfavourable environmental stresses resulting in reduction in its production. The unfavourable environmental condition could hinder the amylase content and thus hampers the quality of storage starch. The unfavourable environmental conditions like lower temperature hamper the carbohydrate metabolism by disrupting the enzymatic mechanisms of starch synthesis in rice and are damaged by temperature below 15 °C (Howarth and Ougham 1993). The important enzymes involved in the synthesis of starch from sucrose in the endosperm are sucrose synthase (SuSy), ADP-glucose pyrophosphorylase (AGPase), starch phosphorylase (SPase), starch branching enzyme (SBE), soluble starch synthase (SS) and granule-bound starch synthase. The rate of starch synthesis during higher temperature is reduced in the developing storage organs in cereals. The reduction in the starch storage is not because of the reduced supply of photosynthate but due to the heat inactivation of

SS, an enzyme in the pathway of starch synthesis (Denyer et al. 1994). Metabolic engineering of those key enzymes involved in starch synthesis with high- or low-temperature stress-tolerant enzymes could increase the starch accumulation. The metabolic engineering of transgenic rice overexpressing AGPase in leaves or in seeds increased the leaf and seed starch content (Oiestad et al. 2016). On the other hand, AGPase is heat sensitive, and their activity is severely affected by heat stress during seed maturation. In such cases, development of heat stable AGPase enzyme could be a solution. Hwang et al. (2019) developed heat stable AGPase enzyme by engineering the two dominant AGPase small and large subunits expressed in developing endosperm. Therefore, such rice AGPase mutants could be applied to improve starch production in cereal plants especially under heat stress conditions. Although wide information on carbohydrate metabolism is available in rice, the area of carbohydrate metabolic pathway engineering for abiotic stress tolerance is mostly untouched.

5 Targeting Protein Pathways for Abiotic Stress Tolerance in Rice

5.1 Late Embryogenesis-Abundant (LEA) Proteins

Late embryogenesis-abundant (LEA) proteins are a type of highly hydrophilic glycine-rich protein in plants and animal and protects from abiotic stresses. LEA has antioxidant, metal ion binding, membrane and protein stabilization, hydration buffering and DNA and RNA interaction properties (Roychoudhury and Nayek 2014). LEA was first reported during late embryogenesis, and later, LEA is reported to be present in other organs at any phase of growth. LEA proteins in higher plants including rice are classified into subgroups such as LEA1, LEA2, LEA3, LEA4, LEA5, LEA6, LEA7, dehydrin (DHN) and seed maturation protein (SMP). LEA plays an important role during diverse abiotic stresses (Banerjee and Roychoudhury 2016). On the other hand, metabolic engineering of LEA biosynthesis pathways by overexpressing the LEA encoding genes confers tolerance to abiotic stresses such as drought, salt and heavy metal in plants. Wide array of research has been done for abiotic stress tolerance in rice by manipulating the expression of the LEA encoding genes (Table 1).

5.2 Heat-Shock Proteins (Hsps)/Molecular Chaperones

Proper folding and maintaining the functional conformation of protein are necessary for its function. Heat-shock proteins (Hsps)/molecular chaperons are ubiquitous proteins that function for the proper folding of the proteins by interacting, stabilizing and remodelling the polypeptides. The unfavourable environmental stress conditions cause misfolding of most of the proteins. However, many chaperons are upregulated during heat-shock protein misfolding. Based on the molecular weight, HSPs are classified into Hsp100s, Hsp90s, Hsp70s, Hsp60s and a group of sHsps (small heat shock proteins) (Banerjee and Roychoudhury 2018a). Depending on the mode of action, the chaperons are divided into three subclasses. The first subclass is the ATP-dependent folding chaperones including DnaK and GroELG. They rely on ATP-driven conformational changes to mediate the net refolding/unfolding of their substrates. The second subclass is the holding chaperones (e.g., Hsp33, Hsp31 and IbpB) and maintains partially folded proteins on their surface upon stress abatement. The third class of chaperone is the solubilizing chaperons including ClpB that promotes the solubilization of aggregated proteins during stress. The DnaK, GroEL and ClpB belong to the HSP70, Hsp60/Hsp10 and Hsp100 family of heat-shock protein, respectively. The productivity of rice is mostly affected by change in unfavourable temperature stress. To overcome such problems, metabolic engineering of rice by overexpressing the HSP is one of the effective ways for engineering abiotic stress tolerance. Higher plants code higher number of Hsfs, and they are reported to play role during high-temperature tolerance. Targeting the overexpression of those stress-responsive Hsfs confers diverse abiotic stress tolerance in rice (Table 1).

5.3 Cyclophilins

Cyclophilins, a subfamily of peptidyl-prolyl isomerases (PPIase), are a group of stress induced ubiquitous proteins. Cyclophilins catalyse slow conformational change from cis to trans isoforms of Xaa-proline peptide bonds in case of short peptide sequence, which is one of the rate-determining steps in protein folding. Cyclophilins confer cellular protection during stress and help the organism to adapt to the changing environment. Cyclophilins are reported to be responsive to abiotic stresses in plants including bean, maize, sorghum, pigeon pea, Arabidopsis, tomato, wheat, rice and even the algae. Therefore, attempts were made to genetically transform the stress-responsive cyclophilin gene to confer stress tolerance in *E. coli, S. cerevisiae*, tobacco and yeast (listed in Table 1).

6 Targeting Hormonal Pathways for Abiotic Stress Tolerance in Rice

In nature, plants are often exposed to multiple abiotic stresses. The major abiotic stresses are drought, heat, cold and salinity. Phytohormones play important role in perceiving such stress signals and the response to the stress throughout the life cycle of plant. Moreover, their ability to cross talk among them is ideal for defence response. The major plant hormones in regulating plant response to abiotic stress are the ABA, ethylene, cytokinin, auxins, gibberellins and brassinosteroids. Identifying and understanding the mechanism they followed to counteract the abiotic stress conditions is important to employ them for more plant stress tolerance.

Therefore, recognizing such pathways could be the metabolic engineering targets for producing abiotic stress-tolerant crop plants.

6.1 Ethylene Pathway

Ethylene is commonly regarded as plant stress hormone besides its important role in normal plant growth and development (Abeles et al. 1992). Ethylene is synthesized from methionine by de novo as well as salvage pathway in plants. In de novo pathway, 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase (ACS) is the rate-limiting enzyme that converts activated methionine, *S*-adenosyl-methionine (SAM) to ACC and 5′-methylthioadenosine (MTA), where MTA is a by-product. ACC oxidase (ACO) catalyses the second committed step of ethylene biosynthesis. It metabolizes ACC to ethylene. During abiotic stresses, the level of ethylene is induced to a significant amount. Application of ethylene or its precursor ACC could improve plant tolerance to high salinity (Cao et al. 2007). The transcription factor, ethylene response factor (ERF) play a crucial role during abiotic stresses by involving in the ethylene response. The ERFs are reported to regulate the expression of ethylene-responsive genes. Metabolic engineering of ERF genes in rice conferred cold, drought and salt stress tolerance by increasing the expression genes associated with ABA signalling and proline biosynthesis (Tian et al. 2011; Lee et al. 2016; Kumar et al. 2017; Tang et al. 2017; Li et al. 2018). The pathways for abiotic stress tolerance involved cell wall modification to not only enable root morphological adaptations, increase accumulation of osmotic substances and chlorophyll content but also reduce reactive oxygen species and malondialdehyde content in rice without affecting growth or agronomic traits.

6.2 ABA During Abiotic Stress

During abiotic stresses, plants can induce the expression of stress-responsive proteins that protect them during abiotic stresses. Among them abscisic acid (ABA) plays important roles in gene expression regulation for abiotic stress adaptation. Therefore, ABA is also known as stress hormone (Zhang 2014; Roychoudhury and Banerjee 2017). Apart from stress response, ABA regulates seed dormancy, seed germination and stomatal closure. ABA is an isoprenoids and synthesize from a five carbon precursor isopentenyl (IDP) under stress conditions. ABA is also synthesized from β-carotene via a number of enzyme catalysed steps. The important enzymes involved are zeaxanthin epoxidase (ZEP), 9-cisepoxycarotenoid dioxygenase (NCED), ABA-aldehyde oxidase (AAO) and molybdenum cofactor sulphurase (MCSU) (Roychoudhury and Paul 2012). Genetic engineering of these genes coding the enzymes has improved plant abiotic stress tolerance. One of the important gene, the ZEP gene is reported to increase the accumulation of ABA when identified, cloned, and overexpressed in other organisms (Park et al. 2008; Cao et al. 2018). Apart from the other enzymes, in rice, short-chain dehydrogenase/reductase-like

(SDR) and cytochrome P450 are also related to ABA biosynthesis. The ABA accumulation during stress is the balancing result of ABA biosynthesis and its catabolism. In rice, silencing and overexpression of a key ABA catabolic gene, *OsABA8ox3* regulates the ABA accumulation and its consequent sensitivity to abiotic stress. *OsABA8ox3* RNAi lines showed significant improvement in drought stress tolerance while the overexpression seedlings were hypersensitive to drought stress when compared with wild-type (Cai et al. 2015). During stress, ABA interacts with the stress-responsive signalling molecules and modifies the expression level of genes (Paul and Roychoudhury 2019). On the other hand, transcription factors are involved in regulating the expression of ABA-responsive genes (Banerjee and Roychoudhury 2017). In rice, the ABA signalling network in response to stress is well characterized. The ABA receptors regulate the ABA-dependent gene expression in rice.

7 Targeting Reactive Oxygen Species Pathways for Abiotic Stress Tolerance in Rice

Reactive oxygen species (ROS) including hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet -}$), hydroxyl radical (OH•) and singlet oxygen ($^1O_2$) plays significant role during abiotic stress adaptation processes by involving in signal transduction pathways. They are the result of excitation or incomplete reduction of molecular oxygen by the aerobic organisms (Banerjee and Roychoudhury 2018b). ROS is naturally produced in chloroplast, mitochondria and peroxisomes in low amount during photosynthesis (Fig. 2). At low level, ROS plays significant role as a signalling molecule during abiotic stress response. However, at higher accumulation, ROS creates oxidative damage to the cellular compartments by reacting with proteins, lipids and nucleic acid which ultimately results in cell death. Furthermore, they cross talk with other signalling molecules. The ROS accumulation to a tolerable level is maintained by the balance between its production and ROS scavenging through antioxidant system. Targeting the genes that have been characterized to involve in ROS production and ROS scavenging could regulate the ROS homeostasis. The genes coding for the enzymes involved in apoplastic ROS production is NADPH oxidases, amine oxidases, polyamine oxidases, oxalate oxidases and a large family of class III peroxidases that are localized at the cell surface or apoplast are contributed to production of apoplast ROS (Apel and Hirt 2004; Cosio and Dunand 2009). ROS scavenging follows both enzyme based and non-enzymatic based detoxification system. ROS scavenging through antioxidant system involves the enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT), glutathione peroxidase (GPX), monodhydroascorbate reductase (MDHAR), dehydroascorbatereductase (DHAR), glutathione reductase (GR), glutathione *S*-transferase (GST), peroxiredoxin (PRX) and non-enzymatic antioxidants, including ascorbic acid and glutathione (GSH) (Scandalios 2002; Das and Roychoudhury 2014; You and Chan 2015). Among the enzymes involved in the production of apoplastic ROS, NADPH oxidases are the most studied enzyme as they are the key

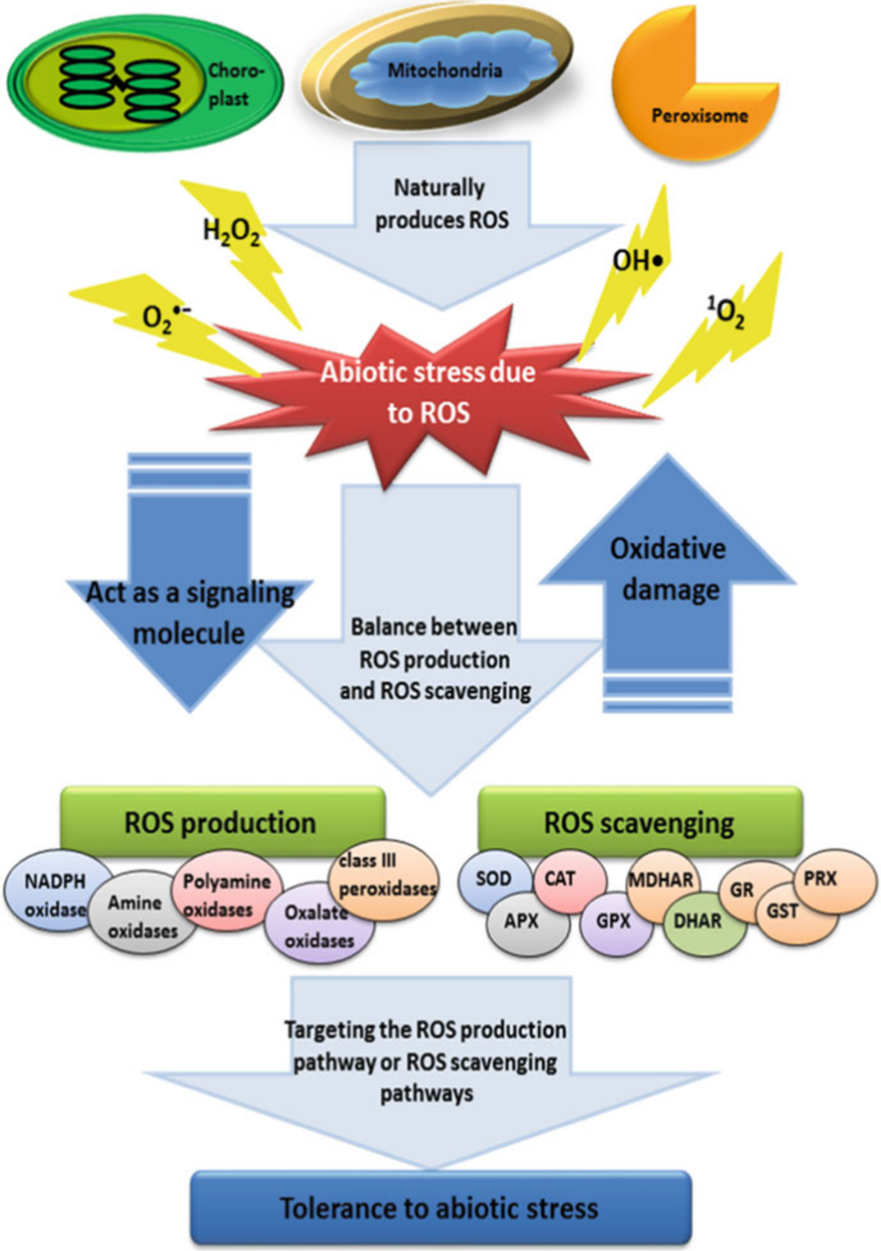

Fig. 2 ROS is naturally produced by the chloroplast, mitochondria and peroxisome. ROS at lower concentration acts as a signalling molecule whereas at higher concentration causes oxidative damage. Maintaining the balance between the ROS production and ROS scavenging results in plant tolerance to abiotic stress

signalling molecule in ROS production. NADPH oxidases are also known as respiratory burst oxidase homologues (RBOHs) and catalyse the production of superoxide. The gene coding for this enzyme is not recommended as a target for metabolic engineering as the overexpression of this will lead to the increased production of ROS which is not desirable. Moreover, the expressions of those genes are naturally increased during abiotic stresses in rice (Wang et al. 2013). Along with the ROS-generating pathways, the ROS-scavenging pathways also works together. Among the ROS-scavenging enzymes, SOD, GR, GST and CAT are mostly studied in rice. SOD acts as first-line of defence against superoxide anions and catalyses the conversion of superoxide anions into H_2O_2 and O_2. The transgenic rice overexpressing chloroplast copper/zinc SOD showed increased detoxification capacity of ROS with higher activity of SOD and reduced oxidative damage during salt, drought and oxidative (Prashanth et al. 2008; Guan et al. 2017). Similarly, targeting of other potential stress-responsive genes in rice is listed in Table 1.

8 Targeting Photosynthetic Pathways for Abiotic Stress Tolerance in Rice

Photosynthetic organisms fix atmospheric CO_2 into sugars by converting the light energy into chemical energy. Ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is the first key enzyme of photosynthesis by catalysing the fixation of both CO_2 and O_2. The large (LS) and small (SS) subunits of Rubisco enzyme was overexpressed with the Rubisco assembly chaperone RUBISCO ASSEMBLY FACTOR 1 (RAF1) resulted in increased content of Rubisco in maize (Salesse-Smith et al. 2018). The increased Rubisco content enhances the photosynthesis and growth in C_4 plants (Mueller-Cajar 2018). Similarly, such approach for targeting the photosynthetic pathways can be utilized for increasing the Rubisco content in other important crop plants such as rice. Another important area in metabolic engineering is the inter conversion of C_3 and C_4 photosynthetic pathways. It is observed that the C_4 photosynthetic pathways are much more efficient than the C_3 pathways. The C_4 plants can inhibit the carboxylation reaction significantly and eliminate photorespiration by increasing the concentration of CO_2 around the Rubisco enzyme in chloroplasts of bundle sheath cells, so that the carboxylation reaction work at a much more efficient rate. Furthermore, the C_4 pathways are more efficient during abiotic stresses as compared to the C_3 pathways. For photosynthetic pathways engineering in rice, researches are now focused mainly for the metabolic engineering of the two-celled C_4 characteristics into rice with increased photosynthetic efficiency and yield. Dr. William Paul Quick and his associates are trying to engineer the two-celled C_4 shuttle in rice by metabolic engineering the C_4 enzymes NADP-malic enzyme (NADP-ME). Similar to the C_4 mechanism, the RuBisCO and glycine decarboxylase expression will be restricted to bundle sheath in the engineered rice (Kajala et al. 2011). The outcome of such major research for the conversion of metabolic pathway of C_3 to C_4 photosynthesis is being considered as one of the possible routes to increase productivity of rice crop to a significant extent.

9 Targeting Transcription Factors for Abiotic Stress Tolerance in Rice

Transcription factors (TFs) are proteins which act as one of the important key player in abiotic stress response regulation by interacting with *cis*-elements present in the promoter region of various abiotic stress-responsive genes (Mukherjee et al. 2006). A complex adaptive mechanism is activated by a single TF that regulates the expression of other multiple stress-responsive genes. The identification of such abiotic stress-responsive TFs has the potential to overcome the limitations of crop losses by developing abiotic stress tolerant crops. Therefore, the area of research on TFs has huge role in plant stress response. TFs are potential targets of plant metabolic engineering for abiotic stress tolerance (Roychoudhury and Banerjee 2015). Furthermore, discoveries of novel TFs and its elucidated functions open up more approaches for developing plant stress tolerance through genetic engineering. Some potential TFs, engineered in rice, are zinc finger protein, WRKY, bZIP, MYB, TFs of AP2/ERF, NAC family TFs, Homeobox TFs and DREB TFs. Plant zinc finger proteins (ZFPs) mainly involves in plant abiotic stress response. The ZFPs are classified into six different groups based on the conserved domain architecture as C_2H_2, C_3H, C_2C_2, A_{20}/AN_1, $C_3H_2C_3$ and C_3HC_4. The C_2H_2 group of proteins are the largest member among them. They play critical role during plant response to abiotic stress by regulating the abiotic stress-responsive genes. The soybean C_2H_2-type zinc finger protein conferred cold tolerance by positively regulating cold-regulated (COR) gene expression (Huang et al. 2009). Overexpression of such proteins belonging to these groups confers cold, drought and oxidative stress tolerance (Table 1). The WRKY TF is reported to play an important role during salt and drought in other organisms (Table 1). They contain one or two DNA-binding domains with the highly conserved sequence WRKYGQK at its N-terminus and a zinc-finger motif at C-terminus (Banerjee and Roychoudhury 2015). The first WRKY TF was identified in sweet potato. Another important TF that plays crucial role in abiotic stress tolerance is the basic leucine zipper (bZIP) transcription factor. bZIP TFs contain two conserved regions such as a basic region and a leucine zipper linked by a hinge region (Roychoudhury et al. 2008; Wei et al. 2012). The proteins of the MYB TF are present in all the eukaryotes and contain conserved MYB DNA-binding domain. MYB TFs regulate genes under abiotic stress either by involving in ABA response or by interacting with other stress-responsive TFs. On the other hand, NAC family of TFs regulates the abiotic stress adaptation processes as a negative or positive regulator. In rice, a single NAC gene can respond to several abiotic stresses, viz. drought and salt stress. NAC can also regulate the ABA-dependent genes for abiotic stress tolerance. Most of the important TFs are considered as potential targets for genetic engineering for abiotic stress tolerance in rice. The TFs engineered in rice for abiotic stress tolerance are listed in Table 1.

10 Tools for Engineering Metabolic Pathways in Rice

The development of abiotic stress tolerance in plants through genetic engineering is a challenging task because of its diverse nature. A variety of crop plants are being engineered by abiotic stress-tolerant genes and have shown the stress tolerance. These have been made possible due to the progress in diverse techniques for plant genetic engineering. One such tool like genome editing technology is capable in precisely targeting the genome and allows changes as whole gene insertion or deletion, gene pyramiding and precise modification in genomic element of interest. In the recent years, several classes of genome editing technologies have been discovered which are employed for genome modification in various crop model plants. Even though the appearance of genome editing technology is quite new-fangled in recent years, it has contributed significantly to the rapid development of biological research and has broad prospects in application (Baltes and Voytas 2015; Ishii 2015). The use of sequence-specific nucleases for efficient targeted mutagenesis has provided a powerful new approach for understanding gene function and developing new traits. These nucleases create DNA double-strand breaks at chromosomal targeted sites that are primarily repaired by the homologous recombination (HR) or non-homologous end joining (NHEJ) pathways. The NEHJ can introduce mutation at the target site as it is often imprecise, which may lead to the loss of gene function. On the other hand, HR uses a homologous DNA template for repair and can be employed to create site-specific sequence modifications or targeted insertion (Moynahan and Jasin 2010).

Since 2009, various breakthroughs have been made in the genome editing technologies and over the use of these sequence-specific nuclease like zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated Cas9 (CRISPR/Cas9) tolerance for different abiotic stress in various model and crop species have been achieved. (Ishii 2015; Weeks et al. 2016). From the past decades, the remarkable progress have been witnessed in the genome analysis and gene discovery of agronomically important traits in rice (Jiang et al. 2012; Lo et al. 2016). The complex genetic makeup of rice allows continuous progress in the fending off biotic and abiotic stress, increasing yields and other important aspects. The great genetic diversity of rice also allows novel and improved genetic traits fuelled by new discoveries and synthetic biology. Thus, other cereal crops could also be benefitted from the knowledge obtained from rice. To develop tolerance in rice for abiotic factors, several sincere efforts have been made using these genome editing methods. Different metabolic pathway engineering approaches were employed for the genetic modification and to achieve the desired traits in rice. These methods of metabolic pathways engineering can be summarized as following:

11 Meganucleases

The first class of sequence-specific nuclease used in plants were meganucleases (Puchta et al. 1996). The nucleases are also referred as homing endonucleases and initially found to be encoded by mobile introns. Meganucleases continue to be deployed to achieve complex genome modifications (D'Halluin et al. 2013) because of the advantage of their size. The relatively small size of 165 aa and large DNA recognition sequence (18 bp) of meganucleases make them favourable option for genome engineering. On the other hand, meganucleases are challenging to redesign for new target specificity in comparison to the other sequence-specific nucleases. The redesigning of meganucleases is hindered by the non-modular nature of the protein. For example, the meganucleases LAGIDADG family, the amino acid responsible for binding DNA and the amino acid responsible for DNA cleavage overlap (Prieto et al. 2007). So the attempts to alter the DNA-binding domain can affect the enzyme's catalytic activity. Consequently, in the plant system, the use of meganucleases has been limited to either naturally occurring nucleases (e.g., I-SceI, I-CreI) or the redesigned nuclease made by the experts.

12 Zinc Finger Nucleases (ZFNs)

The zinc finger nucleases are the chimeric fusion proteins, comprising a DNA binding domain and a DNA-cleavage domain. Usually the DNA binding domain is comprised of a set of Cys2His2 zinc fingers. The targeting of DNA by using zinc-finger nucleases is achieved by arrays, in which each array typically binds to a nucleotide triplet. The zinc finger associates typically 3 bp of DNA and arrays of 3 or 6 fingers recognize 9 or 18 bp, respectively. The FokI restriction enzyme generates the DNA cleavage domain of zinc finger. According to the report of Kim et al. (1996), the FokI activity requires the dimerization, therefore to cleave DNA on specific site, two zinc finger nucleases were designed to bind to DNA in a tail to tail orientation. Due to the relatively small size of zinc finger nucleases (300 aa per zinc finger nuclease monomer) and further progression in the redirecting targets, these nucleases should continue to be an effective technology for genome editing in plants (Sander et al. 2011).

The redesigning of zinc finger DNA binding domain is much easier than the meganucleases, but there are still challenges achieving new target specificity, mostly due to the influence of context on zinc finger function. For instance, the GGG nucleotide sequences recognized by zinc finger in one array may not be recognize this sequence when positioned next to different zinc fingers. Therefore, limited success has been achieved in the modular assembly zinc finger (Ramirez et al. 2008). Maeder et al. (2008) reported that one of the successful methods for redirecting targeting involves screening libraries of three zinc finger variants to identify, recognize and bind to their intended target sequences. In another report of Sander et al. (2011), the modular methods for constructing zinc finger arrays have been found successful in which two finger units were used to minimize the context effects. As a result, the functional zinc finger nuclease is now attainable by most of the genomic editing research labs.

13 Transcription Activator-Like Effector Nucleases (TALENs)

The TALENs are recently added to another class of sequence-specific nucleases, and they quickly became adopted for plant genome engineering. Similarly, like the zinc finger, they are also composed of a DNA binding domain and a FokI cleavage domain. The DNA binding domain of TALENs is derived from TALE proteins found in *Xanthamonas sp*. In each TALE DNA binding domain, repeat sequences of 33–35 amino acids are found, and within each repeat are two variable amino acids (RVDs) that facilitate recognizing a target nucleotide (e.g., the most often used RVDs and their nucleotide targets are HD, cytosine; NG, thymine; NI, adenine; and NN, guanine and adenine). The DNA double-strand breaks (DSBs) can be achieved by TALENs technology into predetermined chromosomal loci. These DSB triggers DNA repair mechanisms and results in the loss of gene function either by non-homologous end joining (NHEJ) or by precise homologous recombination (HR) (Baltes et al. 2015). The modular DNA binding domain of TALENs offers an advantage over the meganucleases and zinc finger nucleases. On the other hand, the drawback of using TALENs is their repetitive nature and large size (950 aa; 1900 aa per pair), which makes delivery to the plant cell difficult. The TALENs are usually supplied to the plant cellular system via direct delivery of DNA to protoplasts or by stable integration of TALEN-encoding constructs (Baltes et al. 2015).

14 CRISPR Cas9/Cpf1

In the family of sequence-specific nucleases, CRISPR (clustered, regularly interspaced, short palindromic repeats)/Cas (CRISPR-associated) system is the most recent addition. The cas9 endonucleases and a guide RNA (gRNA) are the constituent of CRISPR/Cas system. For the cleavage of DNA, a protospacer adjacent motif (PAM) is required, and approximately 20 nucleotides within the gRNA are responsible for directing Cas9 cleavage. The double-stranded breaks (DSBs) can be generated using dCas9 (nuclease-inactive Cas9) fusions to FokI, or using Cas9 nickases to increase the target specificity. The mechanism of CRISPR/Cas9 is well known and were employed for genome editing in several crops. A summary of the type of genome modification, targeted crop, specific gene/TF along with references are presented in Table 2. The types of genome modifications can be summarized in different categories like trait stacking, gene knockout, large deletion and gene replacement (Baltes et al. 2015).

Table 2 List of Genome Modifications Achieved in Plants Using Sequence-Specific Nuclease

Type of DNA modification	Method used/nuclease	Plant	Target(s)	References
Stacking of traits	Meganuclease	Cotton	Transgenic sequence	D'Halluin et al. (2013)
	Zinc finger nuclease	*Zea mays*	Transgene	Ainley et al. (2013)
Gene knockout	Meganuclease	*Zea mays*	Intergenic sequence	Gao et al. (2010)
	Meganuclease	*Zea mays*	MS26	Djukanovic et al. (2013)
	Zinc finger nuclease	*Arabidopsis thaliana*	ADH1, TT4	Zhang et al. (2010)
	Zinc finger nuclease	*Glycine max*	DCL1a/b, DCL4a/b, RDR6a, HEN1a, transgene	Curtin et al. (2011)
	Zinc finger nuclease	*Arabidopsis thaliana*	ABl4	Osakabe et al. (2010)
	Zinc finger nuclease	*Arabidopsis thaliana*	ADH1, TT4, MPK	Qi et al. (2013a, b)
	Zinc finger nuclease	Tobacco, *Petunia hybrida*	Transgene	Marton et al. (2010)
	Zinc finger nuclease	*Arabidopsis thaliana*, tobacco	Transgene	Tovkach et al. (2009)
	Zinc finger nuclease	*Arabidopsis thaliana*	Transgene	Even-Faitelson et al. (2011)
	TALEN	*Arabidopsis thaliana*	ADH1, TT4, MAPKKK1, DSK2B, NATA2, GLL22	Christian et al. (2013)
	TALEN	*Glycine max*	FAD2-1A/B	Haun et al. (2014)
	TALEN	*Hordeum vulgare*	PAPhy_a	Wendt et al. (2013)
	TALEN	*Oryza sativa*	SWEET14	Li et al. (2012)
	TALEN	*Arabidopsis thaliana*, Tobacco	AtTT4, AtADH, NbSurB	Cermak et al. (2011)
	TALEN	*Oryza sativa*, Brachypodium	OsDEP1, OsBADH2, SPL, SBP	Shan et al. (2013a, b)
	TALEN	*Triticum aestivum*	MLO	Wang et al. (2014)

(continued)

Table 2 (continued)

Type of DNA modification	Method used/nuclease	Plant	Target(s)	References
	TALEN	*Zea mays*	PDS, IPK1A, IPK	Liang et al. (2014)
	TALEN	*Hordeum vulgare*	Transgene	Gurushidze et al. (2014)
	Cas9 nikases	*Arabidopsis thaliana*	RTEL1	Schiml et al. (2014)
	CRISPR/Cas	*Arabidopsis thaliana*	TT4, GAI, BRl, JAZ1, CHL1, AP1, transgene	Feng et al. (2014)
	CRISPR/Cas	*Arabidopsis thaliana*	ADH1, TT4	Fauser et al. (2014)
	CRISPR/Cas	*Arabidopsis thaliana*, *Nicotinana benthamiana*	AtPDS3, AtRACK1c, NbPDS3	Li et al. (2013)
	CRISPR/Cas	Tobacco, *Arabidopsis thaliana*, Sorgum, *Oryza sativa*	OsSWEET14, transgene	Jiang et al. (2013)
	CRISPR/Cas	*Oryza sativa*, *Triticum aestivum*	OsPDS, OSBADH2, Os02g23823, OsMPK2, TaMLO	Shan et al. (2013a, b)
	CRISPR/Cas	*Arabidopsis thaliana*	Transgene	Jiang et al. (2014)
	CRISPR/Cas	*Arabidopsis thaliana*, *Oryza sativa*	AtBRI1, AtJAZ1, AtGAI, OsROC5, OsSPP, OsYSA,	Feng et al. (2013)
	CRISPR/Cas	Sweet orange	PDS	Jia and Wang et al. (2014)
	CRISPR/Cas	*Zea mays*	IPK	Li et al. (2012)
	CRISPR/Cas	*Oryza sativa*	SWEET11/13/1a/1b/	Zhou et al. (2014)
	CRISPR/Cas	*Oryza sativa*	PDS. PMS3, EPSPS, DERF1, MSH1, MyB5, MYB1, ROC5, SPP, YSA	Zhang et al. (2014)
Large deletion	Zinc finger nuclease	Tobacco	Transgene	Petolino et al. (2010)
	Zinc finger nuclease	*Arabidopsis thaliana*	RPP4 gene cluster	Qi et al. (2013a, b)
	CRISPR/Cas	*Arabidopsis thaliana*	PDS3	Li et al. (2013)

(continued)

Table 2 (continued)

Type of DNA modification	Method used/nuclease	Plant	Target(s)	References
	CRISPR/Cas	*Oryza sativa*	Labdane-related diterpenoid gene cluster on chr 2, 4 and 6	Zhou et al. (2014)
Gene replacement	Meganuclease	*Arabidopsis thaliana*	Transgene	Puchta et al. (1996)
	Meganuclease	Tobacco	Transgene	Reiss et al. (2000)
	Zinc finger nuclease	*Arabidopsis thaliana*	Transgene	De Pater et al. (2009)
	Zinc finger nuclease	*Arabidopsis thaliana*	PPO	De Pater et al. (2013)
	Zinc finger nuclease	Tobacco	Transgene	Baltes et al. (2014)
	Zinc finger nuclease	Tobacco (suspension culture cell)	CHN50, transgene	Cai et al. (2009)
	Zinc finger nuclease	*Arabidopsis thaliana*	ADH1	Qi et al. (2013a, b)
	Zinc finger nuclease	*Zea mays*	IPK1	Shukla et al. (2009)
	Zinc finger nuclease	Tobacco	Transgene	Wright et al. (2005)
	Zinc finger nuclease	Tobacco	SurA/B	Townsend et al. (2009)
	TALEN	Tobacco	SurA/B	Zhang et al. (2013)
	CRISPR/Cas9	*Oryza sativa*	PDS	Shan et al. (2013a, b)
	CRISPR/Cas9	*Nicotiana benthamiana*	PDS	Li et al. (2013)
	CRISPR/Cas9	*Arabidopsis thaliana*	ADH1	Schiml et al. (2014)
Controlling gene expression	TALE repressor (SRDX)	*Arabidopsis thaliana*	RD29A, transgene	Mahfouz et al. (2012)
	TALE activator (native TALE activation domain, VP16, GAL4)	*Nicotiana benthamiana*	Transgene	Geißler et al. (2011)
	Zinc finger activator (VP16)	*Brassica napa*	KasII	Gupta et al. (2012)
	dCas9 activator (EDLL and TAL effector domain), dCas9 repressor (SRDX)	*Nicotiana benthamiana*	PDS, transgene	Piatek et al. (2014)

15 Concluding Remarks and Future Outlooks

Targeting metabolic engineering pathways for abiotic stress tolerance in rice provides huge impact in increasing the plant tolerance to those stresses leading to enhanced productivity. Various comprehensive studies have been done in this context, involving genes from different pathways including antioxidant pathways, ion homeostasis, osmolyte synthesis and regulatory genes. From the last decade, to verify the role as single function gene(s) as well as transcription factors, numerous attempts have been made. Meanwhile the nature of abiotic stress tolerance is multigenic so a shifting trend towards multigene or transcription factor has been observed. Many important pathways for metabolic engineering are discussed along with its progress so far. Here, we also presented the untouched probable potential areas of research for targeting metabolite engineering in rice including targeting the carbohydrate, ROS, Proline and photosynthetic pathways. Availability of varieties of high throughput platform along with the efficient gene engineering strategies contribute immensely for plant metabolic engineering in rice.

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Targeting Phytohormone Levels for Genetic Engineering Abiotic Stress Tolerance in Rice

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Abstract

More than 50% of the population in the world consumed rice as basic food, particularly in Asian countries. However, as a tropical cereal and also due to the changing climate, major abiotic stresses and also deficit nutrients in the soils have limited the rice production globally. These threats are not solely liable for a drastic reduction in the yield of rice but also interrupt the human beings who consume rice as a basic cereal. Usually, plants can survive against the modest level of environmental stresses. But once stresses exceed the threshold level,

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survival mechanisms including physio-biochemical and molecular mechanisms break down causing death of the plants and drastically reducing the final yield. It is recognized that plants follow several mechanisms to survive against stress, including stress escaping, avoidance, physio-biochemical mechanisms, and genes that confer tolerance against stress. In the case of physio-biochemical mechanisms, plants have numerous internal and external adaptation strategies including phytohormones (PH), osmoprotectants, signaling molecules, and trace elements to survive against abiotic stresses. Among these natural adaptation strategies, target PH such as gibberellins (PHGAs), cytokinins (PHCKs), auxin or indole-3-acetic acid (PHIAA), salicylic acid (PHSA), abscisic acid (PHAA), ethylene (PHET), brassinosteroids (PHBS), strigolactone (PHSL), and jasmonates (PHJAs) have a great role against environmental stresses. Since PH of plants have already proved their importance against abiotic stresses, therefore PH engineering could be a perfect platform to improve environmental stress-tolerant crops under the changing climate. This book chapter outlines the importance of PH, particularly against abiotic stresses, in plants including rice and also highlights the metabolic engineering of PH for enhancing the adaptability of rice against the harsh environment.

Keywords

PH engineering · Abiotic stresses · Rice · Changing climate

Abbreviations

MDA	Malondialdehyde
PAL	Phenylalanine ammonia-lyase
PHAA	Abscisic acid
PHBS	Brassinosteroids
PHCKs	Cytokinins
PHET	Ethylene
PHGAs	Gibberellins
PHIAA	Auxin or indole-3-acetic acid
PHIC	Isochorismate
PHJAs	Jasmonates
PHSA	Salicylic acid
PHSL	Strigolactone

1 Introduction

Rice is the topmost cereal next to wheat and maize, since >50% of people consumed rice as basic food in the world (Lou et al. 2011). The cultivated land occupies 156 million hectares agricultural land globally (FAO 2008; Arif et al. 2019); where

Asian countries including China, India, and Pakistan are occupied 30%, 21%, and 18% cultivated land and the leftover 30% land in Thailand, Indonesia, Japan, and Myanmar (Calpe 2006). However, the demand of rice across the globe is increasing day by day due to the increasing food demand as a result of globally increasing rice-based inhabitants. Since rice is a tropical cereal, major abiotic stresses including deficit nutrients in the soils are a common limitation for hindering the rice production globally, ultimately deteriorating the food security situation (Pareek et al. 2010; Manju Latha et al. 2017; Arif et al. 2019). Therefore, rice production areas may be shifted and would create complications in terms of access and distribution, ultimately threatening the food security of rice-consuming nations. Similarly, these threats are not solely accountable for drastically lessening the yield of rice but also interrupt the nourishment of human beings who consume the cereal as a basic food (Pareek et al. 2010; Lou et al. 2011; Arif et al. 2019).

In general, stages of specific plants can survive with modest levels of stress. However, once stresses exceed the threshold level, the morphological, physio-biochemical, and molecular mechanisms break down causing death of the plants and drastically reducing the yield of plants (Holmstrorm et al. 2000; Manju Latha et al. 2017). Normally, tolerant plants follow several mechanisms to survive against stress, including stress escaping, avoidance, physio-biochemical mechanisms, and genes that confer tolerance against stress (Fig. 1; Manju Latha et al. 2017; Arif et al. 2019).

Generally, genes that involved physio-biochemical mechanisms are linked with numerous PH, osmoprotectants, signaling molecules, and trace elements (Wolters and Jurgens 2009; Manju Latha et al. 2017; Arif et al. 2019). Among these natural adaptation strategies, target phytohormones (PH; Fig. 2) such as gibberellins (PHGAs), cytokinins (PHCKs), auxin or indole-3-acetic acid (PHIAA), salicylic acid (PHSA), abscisic acid (PHAA), ethylene (PHET), brassinosteroids (PHBS), strigolactone (PHSL), and jasmonates (PHJAs) have a great role against environmental stresses. General phytohormones work as runners to interconnect the cellular activities and harmonize various signal transduction plants' paths in stress conditions (Voß et al. 2014; Kazan 2015; Fahad et al. 2015a, b, c; Wani et al. 2016). PH act equally at their site of biosynthesis or somewhere else in plant cells subsequent to their transport (Peleg and Blumwald 2011), response to stress tolerance in plants via signaling pathways (Fig. 2).

Therefore, PH engineering might be an appropriate stage to develop abiotic stress tolerance in crop plants. This book chapter outlines the importance of PH, particularly against abiotic stresses in plants including rice, and also highlights the metabolic engineering of PH for enhancing the adaptability of rice against the harsh environment.

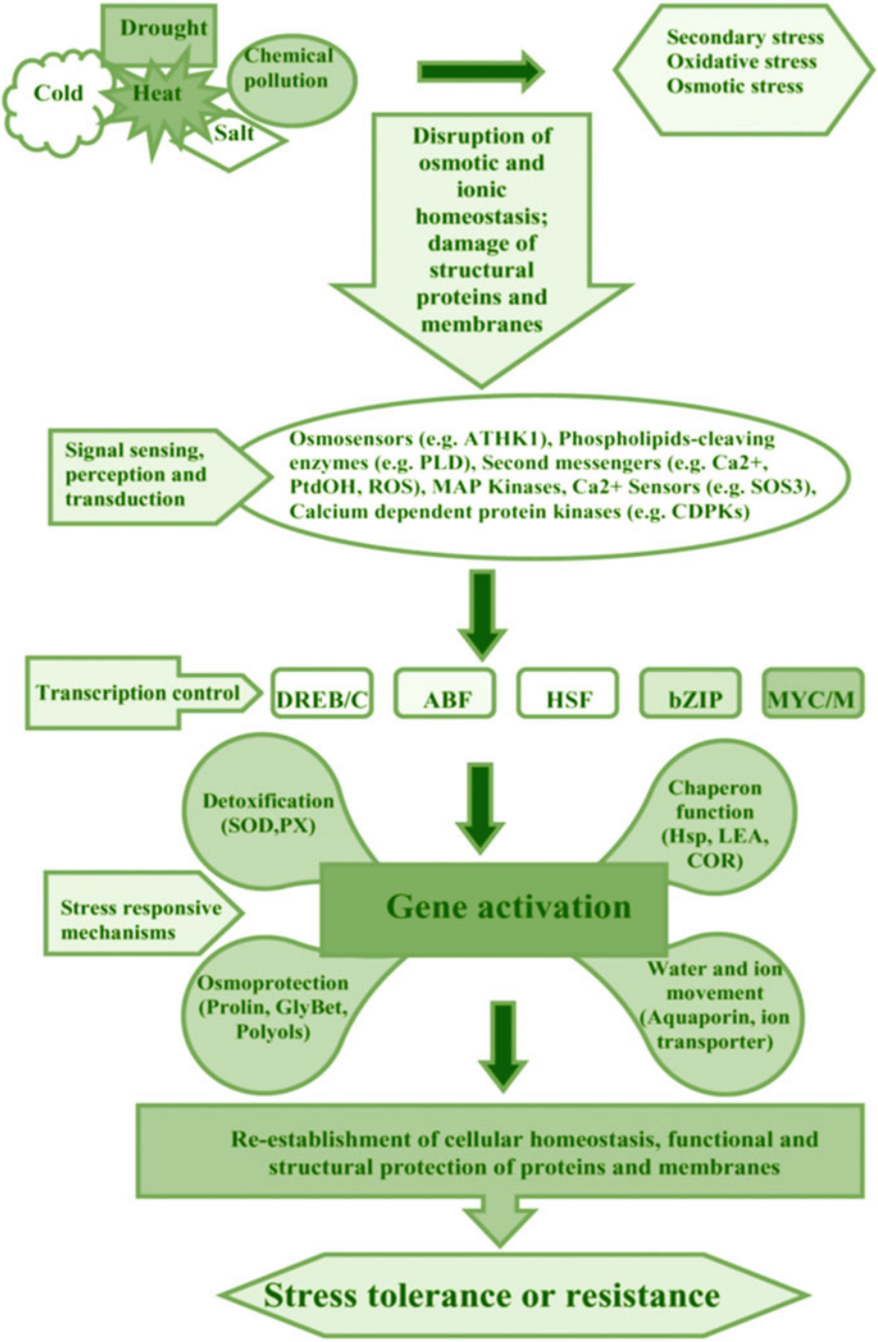

Fig. 1 Mechanisms of abiotic stress tolerance in plants. (Modified from Manju Latha et al. 2017 with authorization)

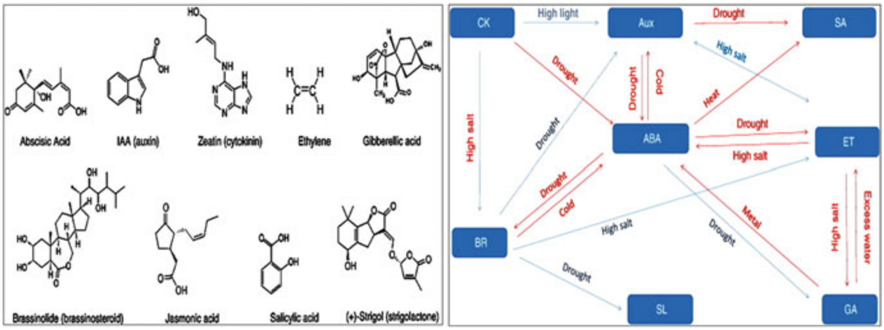

Fig. 2 The chemical formula of major phytohormones, which are linked to enhance the survival ability of plants against abiotic stresses. (Modified from Wani et al. 2016 with authorization)

2 Plant Hormones Are Key Mediators to Cope with Stresses Through Genetic Engineering

2.1 Auxin or Indole-3-Acetic Acid (PHIAA)

Auxin or indole-3-acetic acid (Aux/PHIAA) is multiple functional PH that control various plant physical and biological activities including embryogenesis, fertilization, tropisms, fruit/grain development, apical dominance, and the formation of lateral and adventitious roots (Friml 2003; Kazan 2013). Zolla et al. (2009) revealed that under salinity, the regulation of PHIAA concentration restructured the growth, direction, and several roots in plants. PHIAA also acts as a chemical messenger to communicate cell activities when crops face different environmental stresses (Hasanuzzaman et al. 2013; Sharma et al. 2014; Vardhini and Anjum 2015). PHIAA is also an effective component, which works as guard responsive equipment during environmental stresses for the regulation of several genes (Jain and Khurana 2009) through PHIAA-related pathways (Song et al. 2009; Van Ha et al. 2013).

Repressor proteins of Aux-regulated transcriptional activation are encoded by the PHIAA genes (Woodward and Bartel 2005), which control the activity of two important protein families (Aux/PHIAA and PHIAA-response factor; ARFs) (Song et al. 2009). Some Aux-responsive elements (Aux-REs) and Aux-responsive genes (Aux-RGs) have been found as promoters in the systems (Jain et al. 2006). However, other researchers (Tiwari et al. 2003) observed that the domains of ARFs in combination with Aux-REs or Aux-RGs facilitated their appearances, where Aux-RGs are stimulated by PHIAA for initiating defense mechanisms that may be categorized into three classes such as PHIAA, *GH3* genes, and minor Aux-up-RNA (SAUR) genes (Guilfoyle 1999; Kumar et al. 2016). Among them, the *GH3* genes elaborated the regulation of Aux homeostasis through altering active PHIAA to inactive PHIA (Staswick et al. 2005; Chapman and Estelle 2009). Researchers presumed that PHIAA motivates the transcript of a huge quantity of prime Aux-RGs such as *GH3* family genes in *Arabidopsis* and rice plants (Hagen and Guilfoyle 2002), which help to survive under environmental stress condition. Since the *GH3* genes such as *WES1* generally stimulate the expressions of abiotic stress-related genes such as *CBF* and *PR1*, generally the genes influence to accumulate the transcriptional level quickly by conjunction with free PH such as amino acids and PHIAA; as a result, the activity and availability of PH levels in plant cells are decreased (Woo et al. 2007).

Song et al. (2009) have recognized 25 *OsARF* and 31 *OsIAA* genes in rice, which are mostly responsible for salt- and drought-induced oxidative stress. The genes *OsIAA24* and *OsIAA20* were upregulated in rice when soil salinity level is high (Song et al. 2009). The survival ability of rice against drought stress was increased through the motivation of an Aux transporter *OsPIN3t*; the absence of this gene's consequence is abnormal crown-root initiation at the initial stage of rice seedling (Zhang et al. 2012). Other researchers reported that *TLD1* as a *GH3* gene was intensely induced by PHIAA-amido synthetase in rice under drought stress (Zhang et al. 2009), while their over-countenance reduced the PHIAA concentration, ultimately altering the plant architecture for inspiring to survive against drought stress.

Similar to other environmental consequences, cold stress also induces the modifications of growth and development in the plant, since Aux-RGs genes play a significant role in the plant for fine-tuning against abiotic stresses (Shibasaki et al. 2009). Jain and Khurana (2009) explained that cold stress induces many Aux-RGs genes in rice for adaptation against abiotic stress, while the over-appearance of *OsGH3-2* gene could increase the survival ability of rice against cold stress (Du et al. 2012).

2.2 Cytokinins (PHCK$_S$)

Plants generally sense and respond to adverse situations through signaling molecules/pathways, which are mediated by a large number of genes. Cytokinin (PHCK) is one of them which functions solely and/or with other hormones to mediate different mechanisms within plants in response to environmental fluctuations. PHCK is a significant signaling component that shows a major role under different environmental changes such as temperature, nutrient levels, osmotic conditions, etc. (Hwang et al. 2002; Wolanin et al. 2002; Li et al. 2016a, b). Since it was identified in 1950 (Miller et al. 1955), to date, different functions of the PHCKs in the plant process have been confirmed by several researchers related to germination, root and shoot development to the reproduction, cell division, senescence, and many others (Kieber and Schaller 2018).

Excessive light energy creates heat stress, and also cyclic electron transport in leaves and chloroplasts (Takahashi and Badger 2011) ultimately reduces the photosynthesis efficiency of plants, while plants having lower PHCK content show a decrease in photoprotection and an increase in photoinhibition (Cortleven et al. 2014; Yamamoto 2016). Procházková et al. (2008) observed that the chloroplast's life span is increased with antioxidant-based protection mediated by PHCKs.

Besides high-temperature stress, PHCK has a contradictory function in the cold stress reaction in plants, while the role of PHCKs under heat stress is poorly known. Cold stress leads to membrane disintegrity, ion imbalance, and also impaired water and nutrient balance. Reduction in PHCK content and signaling was reported due to cold temperatures (Maruyama et al. 2014) which may result in growth hindrance in plants. Foliar application of PHCKs in *Arabidopsis* seedlings increased cold tolerance (Jeon et al. 2010), as a result of the PHCKs signaling pathway by the stimulation of several genes including *ARR5, ARR7,* and *ARR15* (Kang et al. 2013). Jeon et al. (2016) revealed that genes such as *CRF2* and *CRF3* are the key elements of the signaling pathway of PHCKs for cold stress feedback in *Arabidopsis*, by promoting the formation of lateral roots.

During heat stress, protein denaturation and metabolic imbalance occurred due to the excessive production of ROS. To survive against heat stress, plants stimulate heat-shock proteins as a protective measure to prevent protein denaturation (Mittler et al. 2011). The upregulation of heat-shock proteins in *Nicotiana tabacum* L. and *Agrostis stolonifera* L. was recorded due to the enhancement of the antioxidant activity as an outcome of higher PHCKs in plant cells reported by Xu et al. (2009, 2010). Besides this, external application of PHCKs inhibits the damage in photosynthesis under heat stress in *Zea mays*, *Passiflora edulis*, and *Oryza sativa* (e.g., Sobol et al. 2014; Wu et al. 2016, 2017).

Similar to other abiotic stresses, drought stress also hinders the plants' process due to water unavailability. Plants have self-defense mechanisms under drought stress which includes root growth redirection (Feng et al. 2016) and transpiration regulation through stomata and cell membrane (Zhu 2016). Werner et al. (2003) and Riefler et al. (2006) revealed that shortened shoot growth and improved root growth are the survival mechanisms of plants under drought stress, while PHCK was found to control the drought stress resistance negatively in *Arabidopsis* (Nishiyama et al. 2011, 2013; Nguyen et al. 2016). However, similar to other harsh environmental conditions, drought stress also reduces PHCK signaling gene expression in both *Arabidopsis* and soybean (Nishiyama et al. 2011, 2013; Ramireddy et al. 2014; Li et al. 2016a, b; Nguyen et al. 2016). Nishiyama et al. (2011) and Nguyen et al. (2016) also observed that PHCK receptor transgenic plants with genes *AHK2, AHK3, HPTs,* and *RRBs* increased in drought stress resistance.

2.3 Abscisic Acid (PHAA)

Similar to PHSA, abscisic acid (PHAA) plays a significant role to adapt plants against abiotic stresses (Liu et al. 2015), through maintaining seed dormancy, germination process, regulation of growth, opening and closing of stomatal guard cells, fruit abscission, morphogenesis of embryo, storage proteins, and lipid synthesis (Zhu 2002; Roychoudhury et al. 2009; Sreenivasulu et al. 2010; Roychoudhury and Paul 2012). PHAA enhancement in plant cells increased the seedling growth due to the reduction of oxidative damage to plants under heat stress (Kumar et al. 2012). Under drought conditions, roots' PHAA signals to shoots as they are suffering stressful environments for starting an antitranspirant activity or water-saving activity, through closing stomatal and decrease leaf enlargement (Giuliani et al. 2005; Wilkinson et al. 2012). PHAA also enhances the signaling ability of plants to survive against hostile environments (Keskin et al. 2010). PHAA works as a key representative to increase the survival ability of plants against hostile environmental stress (O'Brien and Benkova 2013). They also found that, under stress condition, modifying protein-encoding gene expression activates specific signaling pathways for increasing PHAA levels in plant cells. Similarly, Hasanuzzaman et al. (2013) observed that PHAA is also acting as a signaling envoi and also enhances the number of other signaling molecules such as nitric oxide for thermos-tolerance.

2.4 Ethylene (PHET)

Ethylene (PHET) is a PH that involves numerous growth processes in plants, particularly ripening of fruit, flower senescence, and abscission of leaf and petiole that have a regulatory function under abiotic stress (Gamalero and Glick 2012). PHET in combination with PHJAs and PHSA often act cooperatively to enhance the adaptive ability of plants against abiotic stresses, particularly in unfavorable temperature, drought, and salinity stress (Gamalero and Glick 2012; Shi et al. 2012). Researchers observed that both PHET and PHAA work some time synergistically or antagonistically for controlling plant processes to adapt plants against stress (Yin et al. 2015). Since PHET works through controlling signals of the respective gene expression (Klay et al. 2014), however, Matilla-Vazquez and Matilla (2014) and Kazan (2015) reported that PHET has been well-thought-out as the main performers which regulate the defensive mechanism of plants against abiotic and biotic stresses.

2.5 Gibberellins (PHGAs)

Gibberellins (PHGAs) are naturally occurring tetracyclic diterpenoid carboxylic acids, since their structure is constructed on the ent-gibberellane carbon skeleton. However, only a rare form of PHGAs such as GA1 and GA4 worked as growth-regulating PH in plants (Sponsel and Hedden 2004; Munteanu et al. 2014). The PHGAs have obligatory functions for plants throughout their life cycle (Colebrook et al. 2014). The major functions of growth-regulating PHGAs are enhancing seed germination, expansion of leaf, elongation of the stem, and flower and fruit development under harsh environment (Colebrook et al. 2014; Banerjee and Roychoudhury 2019). For example, PHGAs enhanced the survival ability of *Arabidopsis* (Ogawa et al. 2003; Claeys et al. 2012; Binenbaum et al. 2018) and rice seedlings (Yamaguchi 2008) against abiotic stress-induced oxidative stress. Similar to other hormones, PHGAs also interrelate with other PH in many developing and motivational processes in plants (Munteanu et al. 2014). For example, both PHGAs and PHET phytohormones work sometimes antagonistically or synergistically to regulate plant developmental processes under adverse environmental conditions (Munteanu et al. 2014). Iqbal and Shazia (2004) observed that under salt stress, PHGA concentration was decreased; however, the foliar application of PHGAs has been proved for coping salt stress.

2.6 Salicylic Acid (PHSA)

Similar to other PH, salicylic acid (PHSA) also occurs in plant cells naturally (Miura and Tada 2014). PHSA plays a crucial part to regulate the plant's developmental process and also performances as a defensive mechanism for plants to survive under harsh environments (Rivas-San and Plasencia 2011; Hara et al. 2012; Roychoudhury et al. 2016). The biosynthesis of PHSA is responsible for enhancing the survival ability of plants to several environmental stresses including heat (Fayez and Bazaid 2014), drought (Miura et al. 2013), salinity (Khodary 2004), and chilling (Yang et al. 2012). Generally, biosynthesis of PHSA happened by two biological processes in plant cells including the phenylalanine ammonia-lyase (PAL) and isochorismate (PHIC). Among them, the PHIC pathway is the topmost for most of the plants. For example, *Nicotiana benthamiana* (Uppalapati et al. 2007) and tomato (Catinot et al. 2008) follow the PHIC pathway during the biosynthesis of PHSA under adverse conditions. Exceptional information for the synthesis of PHSA was observed by Jumali et al. (2011), who found that low concentration of PHSA improved the osmoprotectants' production ability in plants that help plants to survive against abiotic stresses, while high concentrations of PHSA enhanced the stress susceptibility. They also observed that PHSA-influenced the survival mechanism of plants against abiotic stresses consists of antioxidants, heat-shock proteins, gene-coding chaperones, and synthesis of essential subordinate molecules in plant cells (Jumali et al. 2011). However, Miura and Tada (2014) found that the combined effect of PHSA and PHAA helps plants to survive against drought stress. In another study, Miura et al. (2013) found that PHSA pathogenesis-related genes *PR1* and *PR2* (also called inducible genes) encouraged drought stress.

For example, several research findings revealed that PHSA decreases the heat-induced oxidative stress in rice seedling (Lu et al. 2009) and heavy metal-induced toxicity of rice (Mishra and Choudhuri 1997). Similarly, Kang and Saltveit (2002) observed that the chilling tolerance ability of rice shoots was increased by the application of hydroponic PHSA treatment. The biosynthesis of several chemical compounds (such as enzyme benzoic acid 2-hydroxylase and glutathione) under stress conditions is linked with the availability of PHSA (Sawada et al., 2006). For example, Wang et al. (2016, 2017) found that foliar usage of PHSA enhances the survival ability of rice against herbicide (quinclorac). Similarly, Kusumi et al. (2006) revealed that PHSA is linked with the biosynthesis of glutathione in rice, which led to enhancing the tolerance of rice against herbicide (paraquat).

2.7 Brassinosteroids (PHBRs)

Brassinosteroids (PHBRs) are a new group of PH (signaling molecules) with strong promoting potential for growth and development of plants under adverse conditions (Vardhini et al. 2006; Xia et al., 2009; Choudhary et al. 2012). PHBRs exist practically in each part of plants including leaves, shoots, roots, flower buds, pollen, fruits, and also seeds (Bajguz and Hayat 2009; Banerjee and Roychoudhury 2018). Many studies concluded that the major role of PHBRs is the regulation of different developmental processes such as elongation of stem, root, and leaf stomata, reproductive part development, male sterility, and stress resistance (Bajguz and Hayat 2009; Oh et al. 2012; Tao et al. 2015).

Similar to other PH, PHBRs have shown tremendous potential under abiotic stress-induced oxidative stress (Fahad et al. 2015a, b, c; Vardhini and Anjum 2015), including high temperature (Janeczko et al. 2011), drought (Zhang et al. 2007; Mahesh et al. 2013), salinity (Abbas et al. 2013), chilling (Wang et al. 2014), excessive production of high light-induced ROS (Janeczko et al. 2011), flooding (Liang and Liang 2009), heavy metal stress (Bajguz 2010), and organic pollutants (Ahammed et al. 2013).

Similar to other PH, many genetic factors (genes) and signaling pathways are involved in the function of hormones including PHBRs to confer the tolerance to stresses (Zhang et al. 2007). A current observation revealed that plant defense systems under abiotic stresses are linked with PHBR regulatory genes and confirmed that approximately 40,000 PHBR regulatory genes are involved to stress tolerance mechanism in plants (Anwar et al. 2018). Besides the function of genes, there are many other transcription factors, antioxidant, and signaling proteins which might interact with PHBRs under abiotic stress.

2.8 Jasmonates (PHJAs)

Jasmonates (PHJAs) comprise of methyl jasmonate (MeJa) that advances plant for blossoming at early morning (Kobayasi and Atsuta 2010), including grain development (To et al. 2019). Generally, PHJA enhances the activity of the antioxidant enzymes in plants for protective responses to low- and high-temperature stress, water deficit, and salinity stress (Achard et al. 2006; Tani et al. 2008; Clarke et al. 2009; Chen et al. 2011; Anjum et al. 2011; Farhangi-Abrizet al. 2019).

Rice is an important crop that has been studied more due to its consumption. All the homologs of PHJAs were already identified in rice (Hazman et al. 2019; To et al. 2019), but their specific role in rice growth and developmental processes is still yet to be confirmed. In rice, PHJA also plays key function during the photosynthesis process (Dhakarey et al. 2016), in interaction with another hormone signaling (Lyons et al. 2013; Liu et al. 2015), and in spikelet development and asexual progression (Hazman et al. 2019; To et al. 2019). The OsJAZs or OsNINJA1 are PHJA repressors overexpressed in transgenic rice that were reported for better root development under favorable situations (Hakata et al. 2017; Kashihara et al. 2019).

Transcription factor OsMYC2 had a controlling role in the appearance of MADS-box factors (such as OsMADS1, OsMADS7, OsMADS14, etc.) involved in the flowering process in rice (Cai et al. 2014; You et al. 2019). Mutant gene OSJAR1 or AtJ MT was found to be involved in spikelet structure and in reduced fertility of rice plants (Kim et al. 2009; Xiao et al. 2014). In terms of growth and development role, PHJAs are important signaling molecules generally induced during stress, which activates plant defense response under the harsh environments such as salt, water deficit, low- and high-temperature, and UV irradiation stresses (Seo et al. 2011; Du et al. 2013a, b). Activation of antioxidants in plants by external application of PHJAs lessens the toxic-metal stress in plants (Yan et al. 2013). PHJAs could potentially reduce the effect of environmental stresses (Dar et al. 2015). Exogenous MeJA was reported to reduce the salinity-induced osmotic stress in the seedling stage of soybean (Yoon et al. 2009), whereas in rice an enhancement of PHJA concentrations in roots reduced the salt stress effect (Wang et al. 2001). In rice, jasmonate involvement under abiotic stress has been well studied. Exogenous supply of PHJAs or transgenic plants showed changes in PHJA signaling. Both positive and negative responses of PHJAs were reviewed by BengoaLuoni et al. (2019). So, in general, PHJAs can maintain cell integrity under abiotic stresses and protect through regulating physiological and biochemical responses (Farhangi-Abriz and Ghassemi-Golezani 2019). Several components were involved in PHJA signaling in rice and many yet to characterized lefts more challenge to research. The involvement of PHJAs in stress response was reported, but the actual role in defense response mechanism is still not clear (Dhakarey et al. 2017). More attempts to dissect the role of PHJAs in rice plants need to formulate to identify new regulators and their regulation mechanisms. PHJAs and other PH interactions with abiotic stress signal conveyance can permit to dissecting important components linked in PH cross-talk. Signaling component modification through genetic engineering could improve the plants' performance under stress. However, interactions among different signaling pathways are still yet to be dissected. The application of modern tools and strategies, forward and reverse genetics, genomics, and proteomics will help in dissecting complex signaling mechanisms under abiotic stress in rice.

3 Engineering Phytohormone for Stress Tolerance of Crop Plants

To encounter the food security of growing people and to prevent the crop losses from environmental stress, identifying the mechanism developed and deployed by the plants under stress condition is a major concern to researchers nowadays. For sustainable crop production under changing environmental conditions, PH engineering could be a platform for abiotic stress tolerance, and more research should be performed towards plant abiotic stress response. The upcoming investigation must be concentrated on the progress of stable PH-engineered crops and the identification of a combination of stress response pathway for maintaining growth and survival of crops in the stressed environment since harsh environments are expected to happen concurrently in the field conditions. A specific description of PH engineering is discussed in the following subheadings:

3.1 Phytohormone Engineering Techniques

Current achievement in plant science has been made on transgenic plants through genetic engineering for improvement of crop plant tolerance to abiotic stresses. The success of the investigation mainly depends on appropriate gene-transformation approaches for stable and useful desirable genes in the plant's genome sequence (Paszkowski et al. 1984; Narusaka et al. 2012).

Two appropriate gene-transformation approaches are generally available and popular in the genetic engineering process: the first one is vector-facilitated genetic engineering, which is done by an organic vector such as *Agrobacterium*. Bacterial genes (*Agrobacterium*) may be effectively exchanged with target gene(s), and this addition does not affect the renovation consistency (Gelvin 2003; Lorence and Verpoorte 2004; Broothaerts et al. 2005), although *Agrobacterium*-mediated approaches are reliant on numerous aspects including explant, medium hormonal composition, the supplement of nutrition, culture situations earlier and at the time of inoculation, time of cocultivation, bacterium strain's virulence status, and the concentration of the usable agent composition and also bacteriostatic agent (Yookongkaew et al. 2007). The second approach is well known as the shortest/nonbiological gene transfer method, where DNA introduces in target plant cells (protoplasts) with the help of biolistics or bombardment particles. In the process, DNA absorption occurs in the cell protoplasts of target plants in the existence of polyvalent cations, protoplast fusion with bacterial spheroplasts and with liposomes comprising of foreign DNA, electrotransfection, polymer-based transfection, silicon carbide fiber-mediated DNA absorption, injection-based approaches, wave, beam-mediated alteration, desiccation-based conversion, and foliar DNA presentation and imbibition (Darbani et al. 2008; Narusaka et al. 2012).

3.2 Phytohormones' Metabolic Engineering

PH are considered as important plant growth regulators that are linked with several plant processes such as metabolism, signaling, etc., to acclimate abiotic stresses (Cabello et al. 2014). Among these PH, PHAA is well-thought-out and the most desirable hormone and may be used in PH-metabolic engineering to develop abiotic stress tolerance in crop plant. Researchers have already investigated transgenically the biosynthetic pathway of PHAA for enhancing plants' tolerance to harsh environments (Jewell et al. 2010). Park et al. (2008) reported the overexpression of PHAA-responsive stress-related genes such as C-repeat binding factor (CBF) and/or dehydration-responsive element binding (DREB) deployed from *Arabidopsis* for enhancing survival ability of water deficit tolerance in tomato (Lee et al. 2003). Similarly, expression was recorded in transgenic (CBF/DREB genes) maize plants

Table 1 Phytohormone engineering for enlightening the ability of crop plants to survive under environmental stresses

Plant hormones	References	Gene	The major function of the respective gene	Phenotypic characters of transgenic plants
Auxin	Jung et al. (2015)	OsIAA6	Rice auxin/ gene family	Transgenic rice plants showed drought tolerance through higher auxin biosynthesis
	127	OsPIN3t	Significant in polar auxin transport	Enhanced drought tolerance in rice
	Kim et al. (2013)	YUCCA6	Auxin biosynthesis	Higher auxin content in potato showed drought tolerance
ABA	Lu et al. (2013)	LOS5	PHAA biosynthesis	Transgenic maize enhanced drought tolerance
	Estrada-Melo et al. (2015)	NCED	PHAA biosynthesis	Decreased stomatal conductance and increased drought tolerance
	Zhang et al. (2016)	AtLOS5	PHAA biosynthesis	Increased salinity tolerance
	Mao et al. (2010)	SnRK2.4	Important serine/ threonine-protein kinase in PHAA signaling network	Transgenic *Arabidopsis* showed better tolerance abiotic stresses
	Zhang et al. (2015)	MsZEP	PHAA biosynthesis	Improved tolerance against salt and drought
	Li et al. (2013)	MoCosulfurase	PHAA biosynthesis	Transgenic soybean showed drought tolerance
Brassinosteroids (BR)	Li et al. (2007)	tHSD1	PHBR biosynthesis	Increased salinity tolerance through overproduction of BR
	Feng et al. (2015)	BdBRI1	PHBR-receptor gene	Enhanced drought tolerance with dwarf phenotypes of purple false brome through overproduction of BR
	Koh et al. (2007)	OsGSK1	PHBR negative regulator	Increased tolerance of heat, drought, salt, and cold stresses

(continued)

Table 1 (continued)

Plant hormones	References	Gene	The major function of the respective gene	Phenotypic characters of transgenic plants
Cytokinins	Ghanem et al. (2011)	IPT	Cytokinin synthesis	Transgenic tomato showed salt tolerance
	Pospíšilová et al. (2016)	AtCKX1	Cytokinin in dehydrogenase	Transgenic barley plants showed better drought tolerance via better dehydration avoidance
	Werner et al. (2010)	CKX	Cytokinin in dehydrogenase	Transgenic *Arabidopsis* plants showed drought tolerance
Ethylene	Habben et al. (2014)	ACC-synthase	Catalyzes the rate restrictive step in the biosynthesis of PHET	Showed better drought tolerance in transgenic maize plants through reducing ethylene
	Du et al. (2014)	ETOL1		Showed drought and submergence tolerance of rice
	Zhang et al. (2010)	ERF-1(JERF1)	Biosynthesis of ethylene as well as jasmonates	Rice plants showed increased drought tolerance through biosynthesis of ethylene as well as jasmonates
	Shi et al. (2015)	ZmARGOS	Undesirable controllers of PHET signal transduction	Undesirable controllers of PHET signal transduction improved drought tolerance of transgenic *Arabidopsis* and maize plants

(Al-Abed et al. 2007) and in transgenic (MoCo-sulfurase gene) soybean (Li et al. 2013) under stress conditions. However, in some times, over-response of the gene (s) is linked with the biosynthesis of PHAA, which enhances the survival ability against drought stress (Hwang et al. 2010). Drought tolerance in a 120-day-old potato plant by over-appearance of AtYUC6 was also detected by Kim et al. (2013).

Woo et al. (2007) observed that rice mutant “Constitutively Wilted1” was scarce in the homolog of YUCCA; however, a stimulation tagline of YUCCA7 transgenic from *Arabidopsis* boosted the water deficit tolerance (Lee et al. 2011). Sakamoto et al. (2003) amended the level of PHGA via overstimulation of gene OsGA2ox1 and found that all transgenic-rice plants exhibited severe dwarfess and also increased sterility, which indicated that PHGA is linked with both stem elongation and grain fertility.

Efforts have been made by many scientists across the globe for the engineering of phytohormone to improve stress tolerance in plants; some of these are described in Table 1.

4 Conclusion and Future Outlook

From the above discussion of the chapter, it may be concluded that due to the changing climate, the environmental stresses such as high-temperature stress, drought, salt stress, the toxicity of heavy metals, and nutrient insufficiency have great threats for rice production globally. Since major percentages of rice-cultivating regions have already been affected due to harsh environments. These threats are not only liable for a severe decline in the yield of rice but also interrupt the nutrition of humans who consume rice as a basic food. Since there are three ways plants could survive under stress such as through stress escaping, avoidance, and genes that involved tolerance mechanisms including production of PH, osmoprotectants, genes-involved signaling molecules, and trace elements to survive against abiotic stresses, where several PH such as PHIAA, PHCKs, PHAA, PHET, PHGAs, PHSA, PHBRs, and PHJAs have already shown the potentiality under adverse environmental conditions. Thus, PH engineering may be an actual platform for the sustainability of rice production under abiotic stress. In the chapter, we highlight a summary of earlier findings of PH and their key roles against abiotic stress tolerance in rice for improving food production and its quality under the changing climate.

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The Effect of Exposure to a Combination of Stressors on Rice Productivity and Grain Yields

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Abstract

Rice (*Oryza sativa*) is one of the principal cereal crops and is a major source of calories for billions of people around the globe. It has been predicted that rice production will decrease in near future due to the negative effects of global

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climate change. The increase in temperature and scarcity of irrigation water poses a serious threat to rice cultivation, as rice is very sensitive to heat and drought stress, particularly during the reproduction and grain-filling stages. Individually, the above stressors negatively affect almost all of a rice plants growth and developmental processes, leading to significant impacts on photosynthesis, floral abnormalities, pollen/spikelet sterility, grain yields and quality. Moreover, the combination of drought and heat stress, which commonly occurs under field conditions, can result in deleterious additive effects on the growth, physiology and productivity of rice cultivars. This threat to food security, and the associated economic losses, has increased the need for the development of abiotic stress-resilient rice varieties, with better yield potentials. A comprehensive understanding of the various morphological, biochemical, physiological, and molecular components governing rice yields under a combination of stressors is vital for the development of climate- resilient rice varieties. In this chapter, we provide an overview of the most common combinations of stressors faced by rice plants under field conditions, with special emphasis given to drought and heat stress, and the influence of these stressors, alone and in combination, on the growth, morphology and yields of rice. We also highlighted the potential strategies that can be used to develop rice plants adapted to the multiple abiotic stressors faced by plants under field conditions.

Keywords

Combined stresses · Drought · Heat · Rice yields · Molecular breeding · Stress management

1 Introduction

In recent years food security has become a major concern for global policymakers, as climate change is expected to threaten food production and certain aspects of food quality. According to a global climate change model, the temperature of the world is predicted to increase by 4.8 °C by the end of the twenty-first century (Stocker et al. 2013). As a result, agriculture in the near future will face multiple challenges to produce more food to meet the demand of an increasing global population. Despite the changing climate, global food production needs to be increased by about 70% by 2050, to feed the ever rising human population (Bruinsma 2009; Parry and Hawkesford 2010; Zhu et al. 2010; Cramer et al. 2011; Alexandratos and Bruinsma 2012). This goal of increasing food production will be difficult to overcome due to the ongoing changes in climate and the degradation of the agricultural land both of which are mostly because of anthropogenic activities (Kissoudis et al. 2014). Increased global temperatures leads to higher frequencies of heat stress events (Battisti and Naylor 2009) that can aggravate other plant associated stresses such as drought and increased soil salinity (Munns and Tester 2008; Zhao and Running 2010).

Plant stressors may be biotic or abiotic and can be encountered by plants either individually or in combination. How plants respond to a single stress can be different to when plants are exposed to the same stress in combination with other stressors. When exposured to multiple stressors simultaneously plants can exhibit shared and tailored responses, which are not merely the additive effects of both stresses (Rizhsky et al. 2002, 2004) and hence combined stress is considered a new state and not just a sum of the independent stressors (Mittler 2006). This variation in responses might be due to multiple interactions and cross-talk between molecular and metabolic processes in plants (Mittler 2006; Atkinson et al. 2013; Rasmussen et al. 2013; Prasch and Sonnewald 2013; Suzuki et al. 2014; Ramegowda and Senthil-Kumar 2015). This complex, but intensive interaction, depends on the nature, severity, time of occurrence, duration of exposure to the stressors and growth stages of the plant (Suzuki et al. 2014; Ramegowda and Senthil-Kumar 2015; Pandey et al. 2015).

Rice (*Oryza sativa* L.) is the staple food and the principal source of income for a vast majority of the global population (Khush 2005; Muthayya et al. 2014). It is the second most widely grown cereal crop that is cultivated in tropical and temperate regions. Due to a high water requirement for cultivation, rice is considered to be one of the most vulnerable cereal crops, which yields predicted to be greatly affected by global warming (Jagadish et al. 2011a, b, 2012). Heat stress ($\geq$38 °C) during the reproductive stages especially at flowering and grain-filling stages has already caused yield losses in the major rice-producing countries of Asia, namely China (Tian et al. 2010), Japan (Hasegawa et al. 2011), Laos and Southern India (Ishimaru et al. 2016). For example, every 1 °C rise in temperature can reduce rice yields by up to 10%, depending on cropping season and varietal use (Peng et al. 2004; Tenorio et al. 2013). Even an hour or 1 day of heat stress may cause a significant adverse effect on rice productivity by inducing spikelet sterility (Jagadish et al. 2007, 2013; Rang et al. 2011) and drought stress at the reproductive stage may cause yield reductions of up to 60–90% in rice (Ghimire et al. 2012; Sandhu et al. 2014, Vikram et al. 2011). In summary, heat or drought stress affects almost all of the growth and developmental stages of rice leading to reductions in photosynthesis, floral abnormalities, spikelet sterility, grain yield, and quality deterioration. Combined drought and heat stress at flowering and grain filling causes even more pronounced detrimental effects on rice yields and quality attributes (Jagadish et al. 2011b; Rang et al. 2011; Lawas et al. 2018). The spatial analysis by Wassmann et al. (2009) showed the concomitant occurrence of heat and drought stress in the mega-deltas of Bangladesh, the eastern part of India, southern Myanmar and the north of Thailand makes future rice production in these areas vulnerable. However, in recent years research has been carried out on the combination of heat and drought stress rice and their interactions, and this has demonstrated the potential for combined stress tolerance in rice exists (Wu et al. 2009; Jagadish et al. 2011b, 2014; Straussberger 2015; Lawas et al. 2018; Rabara et al. 2018).

It is assumed that future rice production will take place in warmer and drier environments due to global warming and therefore, rice yields are predicted to be much lower than required to feed an increasing global population (Kadam et al.

2014; Stocker et al. 2013; Jagadish et al. 2012). In this context, it is crucial to develop climate-resilient and climate-ready novel rice varieties with greater yield potentials using molecular breeding and genetic engineering approaches that are able to withstand multiple environmental stressors. This challenging task requires a thorough understanding on the impacts of combined stressors, stress intensity, timing and stress frequency on rice growth and yields, as well as in depth knowledge of the different morphological, physiological, biochemical, and molecular elements that regulate yield and the yield components of rice under stressful conditions.

This chapter covers our current understanding of the effects of combined stressors (especially heat and drought stress) on the growth, morphology and yield of rice. We also highlight potential strategies for developing single or combined stress tolerance rice cultivars, to maintain sustainable production and also to mitigate the negative effects of global climate change on rice cultivation worldwide.

2 The Various Stress Combinations Experienced by Rice Plants Under Field Conditions

Being sessile, plants are constantly subjected to a plethora of stressors. Stress often defined as an “overpowering pressure or some adverse force of influence” (Jones and Jones 1989; Sharma et al. 2013). In the broader sense, stressors can be classified into two groups: (a) biotic and (b) abiotic stress. Living organisms that include pathogens (bacteria, fungi, viruses etc.), herbivores, and nematodes as well as other plants such as weeds generate biotic stress. Extreme environmental conditions such as toxic chemicals, heavy metals, pollutants, mechanical injury, temperature, water, salinity, light, and nutrient availability lead to the generation of abiotic stress (Sharma et al. 2013; Suzuki et al. 2014). Considering the number of biotic and abiotic stressors that exist in the environments in which plants grow, stress exposure can also be grouped into following three categories; (a) single stress (b) multiple stress and (c) combined stress. Plants generate unique response patterns against these stress conditions, which can be either positive or negative.

2.1 Single Stresses

A single stress represents only one stress factor that affects plant growth and development in most of the cases (Pandey et al. 2017). For example, when rice plants are subjected to only drought or chilling stress, increased ROS generation, altered antioxidant enzymes activities, and increased lipid peroxidation and rice yield loss can occur (Sharma and Dubey 2005; Afrin et al. 2019). Similarly, salt stress on rice and other crops create disrupt germination, cause ion toxicity and ROS accumulation, breakdown membrane lipids, cause nutrient imbalances, disrupt photosynthesis and cause imbalances in hormonal homeostasis etc. (Roychoudhury et al. 2008; Banerjee and Roychoudhury 2018; Hussain et al. 2017; Mostofa et al. 2015;

Tahjib-Ul-Arif et al. 2018a, b, c, 2019). In most of the cases, a single stress with long duration and at a high level poses detrimental effect on plants.

2.2 Exposure to Multiple Stressors or Sequential Stress

When two or more stressors occur sequentially one immediately after the other, or at different time periods without any overlap, then the termed is "multiple stress" can be used (Pandey et al. 2017). Multiple stress can confer either positive or negative effect on plants, depending on the nature of the stress and the plant species in question. Hossain et al. (2018) reviewed many studies related to two stress conditions that occur at different time periods and in general memory of first stress enhanced tolerance to the second stress. They concluded that exposure of a plant to a single stress can result in increased tolerance to a wide spectrum of abiotic stressors and, even in some cases, biotic stressors, which is known as cross-stress tolerance. One possible outcome for plants exposed to stress is to become more resistant to future stress events due to the acquisition of stress memory, a response also often referred to as priming, acclimation, conditioning or hardening. It was reported that when rice plants were exposed to primary stressors like heat-shock and then a secondary stress was imposed, the rice plants showed considerable tolerance to the second stressor (reviewed in Hossain et al. 2018). For example, when rice plants were exposed to high temperatures and subsequently exposed to cadmium (Cd) stress, the rice plants generated excess H_2O_2 and had increased the activities of ascorbate peroxidase (APX) and glutathione reductase (GR) with increased expression of the *OsAPX2* and *Oshsp17.3* genes, increased the glutathione (GSH) biosynthesis and that these factor probably contributed to increased Cd tolerance (Cao et al. 2012; Hsu and Kao 2007; Chao et al. 2009).

2.3 Exposure to a Combination of Stressors

When two or more stresses occur simultaneously with significant degree of interaction between them, the term used in combined stress. Recent evidence shows that, in the field, plants encounter different stress factors mostly often concurrently and that this generates a new state of stress, the combined stress state. The combined stress state can be categorized into: (a) abiotic and abiotic interactions (b) biotic and biotic interactions (c) abiotic and biotic interactions. Simultaneous drought and heat stress during summer is an example of an abiotic and abiotic stress combination (Lawas et al. 2018). Similarly, simultaneous attack by two different pathogens is considered a biotic-biotic stress combination (Bonnet et al. 2017). When biotic and abiotic stresses occur concurrently then it is termed an abiotic and biotic stress combination (Rejeb et al. 2014; Audebert et al. 2000). For example, when nematodes and drought occur simultaneously, it was found that the severity of the combined stress was much more pronounced than the single stressors (Audebert et al. 2000). Evidence shows that plant response to a combination of stress factors is unique compared to how they responses to individual stressors (Pandey et al. 2015, 2017). When plants are exposed to different stress combinations, a variety of interacting signal transduction pathways are induced (Mittler 2006). In most cases, plant responses to combined stresses deviate from responses to the individual stresses at the molecular, biochemical and physiological levels (Prasch and Sonnewald 2013; Rasmussen et al. 2013). An obvious difference found between the individual and combined stress responses is in the transcriptome profile. For example, Rasmussen et al. (2013) compared transcriptome changes in ten Arabidopsis (*Arabidopsis thaliana*) ecotypes using cold, heat, high-light, salt, and flagellin treatments as single stress factors as well as combinations of two on the above stressors and found that some 61% of the transcriptome changes in response to double stress exposure were not predicted from the responses to single stress treatments. Another difference found between the combined and single stresses is in stomatal opening and closing. During heat waves, plants open their stomata in order to release water to cool their leaves by evapotranspiration. When plants were incubated for 30 min at 23, 30 or 35 °C, stomatal openings were 0.66, 2.76 and 4.28 μm respectively (Feller 2007). In contrast, during combined drought and heat stress, it was found that plants opened their stomata less and as a result leaf temperatures increased due to less evapotranspiration (Rizhsky et al. 2002). It has also been shown that plants prioritize between potentially antagonistic responses for only 5–10% of the responding transcripts which suggest that plants have evolved to cope with combinations of stresses and, therefore, may be bred to improve crop yield under combined stress situations (Rasmussen et al. 2013). Moreover, under salt and/ or drought stress, physiological parameters such as H_2O_2 content, relative water content (RWC), free proline content, chlorophyll content, carotenoid content, superoxide dismutase (SOD) and catalase (CAT) activity etc. of sunflower (*Helianthus annuus*) were measured. Then it was found that changes undergone by plants as a result of combined stresses are markedly different to responses to individual stresses (Umar and Siddiqui 2018). These findings suggest that combined and individual stress exert differential response to plants, which might be correlated with the reason behind the yield gap of crop plants under field condition where myriad stressors exist. In order to increase the yields of rice and improve food security, it is important to understand the nature of combined stress responses. To date, a limited amount of study has been undertaken to understand the influence of combined stresses on rice plants, and so it is suggested that the possible stress combinations proposed by Mittler (2006) are followed. These potential interactions could have important implications for rice productivity. Studies on the effects of combined stress on rice are summarized in Table 1.

Table 1 Effects of combined stress on growth, development and yield attributing traits of rice plants

Cultivar of Rice	Stress combination and experimental conditions	Stress dose and duration	Effects of combined stress on rice plant	References
IR-36, Fujiyama-5	CO_2 + UV B Controlled condition	Temperature: 40/33/37 °C and CO_2 330 and 660 μmol mol^{-1} 16 weeks	Cultivar IR-36 • Decreased leaf area and leaf weight • Increased SWL, root weight and shoot root ratio, yield • No significant change in stem weight, biomass • Decrease number of tillers • Increase panicle weight and harvest index • No effect on number of panicle and number of panicle per tiller • Decreased stomatal limitation Fujiyama-5 • Increased leaf weight, stem weight, root weight and shoot and root ratio, biomass, yield • No effect on leaf area and SLW • Increased panicle weight • No significant effect on number of tiller, number of panicle, number of panicle per tiller and harvest index • Decreased apparent carboxylation efficiency • Increased maximum rate of O_2 evolution at saturated light	Ziska and Teramura (1992)

(continued)

Table 1 (continued)

Cultivar of Rice	Stress combination and experimental conditions	Stress dose and duration	Effects of combined stress on rice plant	References
Liangyou 287 (temperature sensitive)	Heat and CO_2 Field condition	Moderate stress: Temperature 26–31 °C+ CO_2 550 μmol mol^{-1} Intense stress: Temperature 28–33 °C+ CO_2 650 μmol mol^{-1}	• Decreased the growth at various phases of plant growth such as seedling stage, tillering stage, elongation stage, heading stage, filling stage, and maturation stage • Decreased leaf area in different growth stages • No significant variation observed on plant height in different growth stages • Malondialdehyde content increased in all the growth stages except in intense stress of heading and blooming stage • Proline content increased at moderate stress of heading and blooming stage whereas decreased in intense stress of filling stage • Soluble protein increased in heading and blooming stage. • Soluble sugar content increased in moderate stress of filling stage • SOD activity increased in moderate stress of filling stage • Head rice rate, chalky rice rate and protein content decreased in moderate stage • Amylose content showed no change	Liu et al. (2017)

N22, Dular, Anjali	Drought and heat Field condition	Soil water potential reached up to −61 and −57 kPa resulting in up to 1.75 and 1.17 °C higher canopy-air temperature	• Decrease transcriptional conductance • Lower panicle conductance during flowering and grain filling stage • Reduction of plant height and biomass • Significant reduction in yield (Dular showed highest reduction) • Increased chalky grains • Grain width was significantly reduced • Grain length was increased under stress conditions in N22, but was reduced in Dular and Anjali • Amylose content also varied between treatment	Lawas et al. (2018)
Koshihikari	O_3 and CO_2 Growth cabinet	800 $cm^3\ m^{-3}$ CO_2 + 0.1 and 0.3 $cm^3\ m^{-3}$ O_3 3 h	• Increased net photosynthetic rate • Declined stomatal conductance • Decreased L-ascorbic acid content	Imai and Kobori (2008)
KhaoDawk Mali 105	O_3 + CO_2 Indoor climate control chambers	O_3 40 ppb + CO_2 700 ppm and O_3 70 ppb + CO_2 700 ppm 28 days	• Increase photosynthesis • Decrease plant height, leaf chlorophyll, total soluble sugar in extreme stress	Phothi et al. (2016)
Pokkali CSR 1 IR 36 IR 26 IR 2153	O_3 and NaCl Controlled environment chamber	O_3: 88 nmol mol^{-1}+ NaCl: 25 and 50 mM	• Decreased plant height, shoot and root dry weights, root: Shoot ratio, and number of tillers • Decrease the length of fourth and fifth leaves • Decrease K^+ ion content • Increase Na^+ ion uptake in Pokkali and CSR 1 whereas decreased in IR 36, IR 26 and IR 2153 • Cl^- ion uptake varied among the cultivars	Welfare et al. (1996)

(continued)

Table 1 (continued)

Cultivar of Rice	Stress combination and experimental conditions	Stress dose and duration	Effects of combined stress on rice plant	References
			• Decrease net photosynthesis, transpiration and stomatal conductance	
Shanyou 63	CO_2 and O_3	604 $\mu LL^{-1}CO_2$+ 78.6 $nL\ L^{-1}\ O_3$	• Non-significant increase in chalkiness degree • No significant change found in mineral composition (K, Zn, Mg, Cu, P, Mn) under combined stress • Decrease individual grain mass • Non-significant change on nitrogen concentration, non-protein nitrogen (NPN) concentration and protein nitrogen (PN) concentration • Total starch concentration decreased and gelatinization increased	Wang et al. (2014a)
IR-30	CO_2 and temperature Controlled condition	Temperature: 28/21/25, 34/27/31 40/33/37 CO_2 concentrations: 330 and 660 μmole mole^{-1}	• Grain yield increased in mild stress but decreased under severe stress • Increased number of pinnacle per plant under mild stress but inhibited under high stress • Filled grain decreased • Decreased pinnacle biomass • Grain mass decreased • Total above ground biomass decreased • Harvest index decreased	Baker et al. (1992)

IR-8	Brome Mosaic virus (BMV) and drought Growth chamber	Withholding water application at 28 days post inoculation	• Elevates antioxidants (α-tocopherol, ascorbic acid content) • Elevates osmoprotectant and signaling molecule (trehalose, putrescine, salicylic acid, proline, and sucrose content) • Shows less change in metabolites • Drought symptoms appeared later and leaves maintained water longer than their uninfected counterpart	Xu et al. (2008)
IR 72	CO_2+ temperature	(ambient +4 °C and 200 μL L^{-1}) And (ambient +4 °C and 300 μL L^{-1})	• Green leaf area, leaf weight, stem weight and root weight increased in combined stress • Pinnacle weight increased but harvest index compared to ambient temperature and CO_2	Ziska et al. (1997)
Japonica unwaxy	Cr + Cd Controlled environment	20 μg each	• Decreased seedling height and biomass, chlorophyll content (SPAD) • Decreased activities of catalase and ascorbate peroxidase in leaves H^+-ATPase in roots/leaves • Elevated superoxide dismutase and peroxidase activities in leaves • Increased malondialdehyde accumulation both in Leaves and roots	Cao et al. (2013)
Indica cv. 9311	Drought + high light Controlled environment	PEG (5,10, 15, 20, 25, 30%) + 800 μmol m^{-2} s^{-1} at 25–28 °C (HL, high light)	• Assimilation rates and stomatal conductance decreased, especially under high light • Reduced the activities or activation states of Rubisco • No significant effect on the activities or	Zhou et al. (2007)

(continued)

Table 1 (continued)

Cultivar of Rice	Stress combination and experimental conditions	Stress dose and duration	Effects of combined stress on rice plant	References
			activation states of NADP-dependent malate dehydrogenase • Reduced the membrane stability index, but increased malondialdehyde and H_2O_2 content • Decreased relative water content drastically	
94D-22,94D54, 94D64, Gui630, YY-1, and KY1360	Copper (Cu) and cadmium (Cd) Greenhouse	100 mg/kg Cu + 1.0 mg/kg Cd	• Combined treatment increased Cd uptake more than single Cd treated plants • Combined stress treatment increased less uptake of copper than only copper treated plants	Huang et al. (2009)
CG14 IDSA6	Cyst nematodes and drought Field experiment	10 cyst nematodes + withholding irrigation	• The combination of both stresses had similar but yet more pronounced effects than the single stresses on stomatal conductance, leaf water potential and leaf dry weight in CG14 • Only the combined stresses resulted in a significant reduction in osmotic potential and root dry weight compared with the control, for either CG14 or IDSA6	Audebert et al. (2000)

KS-282, Basmati Pak, Shaheen Basmati, KSK-434 and 99417	Salinity and Iron (Fe) deficiency Wire house	50 mM NaCl + 3 μM Fe	• Reduced shoot and root growth, photosynthetic and transpiration rates, chlorophyll concentration, and stomatal conductance recorded in combined stress compared to single stresses • Showed highest Na^+ and lowest k^+ and Fe^{2+} content in combined stress condition compared to individual stresses	Abbas et al. (2015)
Oryza sativa cv. Bomba	Combined heat stress and water stress Growth chamber	38/33 °C+ 45% field capacity	• Higher growth inhibition occurred in combined stress compared to individual stresses • Decreased leaf area • Decreased instantaneous rates of leaf photosynthesis	Perdomo et al. (2015)

3 The Common and Distinctive Features of Individual and Combined Stress in Rice Plants

Plant acclimation to a particular abiotic stress condition requires a specific response that is tailored to the precise environmental conditions the plant encounters. Thus, morphological, biochemical and physiological processes set in motion by a specific stress might differ from those caused by a different composition of environmental parameters.

3.1 Growth and Biomass

Reduced plant growth is one of the initial effects of abiotic stress exposure. When rice plants are expose to a combination of stressors inhibitory effects were shown for plant growth related parameters such as shoot length, root length, shoot and root fresh weights, leaf area and leaf weight (Ziska and Teramura 1992; Welfare et al. 1996; Zhou et al. 2007; Phothi et al. 2016). During simultaneous elevated CO_2 and UV-B stress and individual stress exposure, the plant growth parameters above responded differentially. For example, while both individual and combined stress exposure of IR-36 rice plants reduced leaf area, the reduction in leaf area was greater in the combined elevated CO_2 and UV B stress treatment. Elevated CO_2 enhanced stem weight of the plants but combined exposure did not influence it. Similarly, in terms of root, elevated CO_2 increased the root weight of rice plants but only UV B stress did not influence the root weight of rice plants. Moreover both combined stress and only UV stress decreased leaf weight but elevated CO_2 did not influence leaf weight (Ziska and Teramura 1992). Similar differential responses for the growth and biomass of rice plants were observed for combined elevated CO_2 and heat stress (Liu et al. 2017), drought and heat stress (Lawas et al. 2018), CO_2 and ozone (O_3) stress (Phothi et al. 2016), O_3 and NaCl stress (Welfare et al. 1996), drought and high light (Zhou et al. 2007).

3.2 Photosynthesis

One of the physiological processes that is greatly affected by abiotic stressors in plants is photosynthesis and a decline in the photosynthetic capacity of plants due to stress is directly associated with reductions in yield (Singh and Thakur 2018). Net photosynthesis, transpiration rate and the stomatal conductance of rice plants was measured in plants exposed to O_3 and NaCl stress alone or in combination. It was found that while exposure to an individual stressor reduced net photosynthesis, transpiration rate and the stomatal conductance, compared to the control rice plants, exposure to combined stress caused a more rapid decline in these parameters (Welfare et al. 1996). So it is evident that the combined effect of ozone and salt exposure caused an additive effect on photosynthetic parameters. A similar additive result was found for combined O_3 and elevated CO_2. Only ozone exposure declined the net photosynthesis whereas only elevated CO_2 increased the net photosynthesis. Interestingly, combination of ozone and elevated CO_2 sharply decreased the net photosynthesis rate. On the other hand, stomatal conductance decreased both in individual and combined application of ozone and elevated CO_2 (Imai and Kobori 2008). Therefore, detailed information on plant responses on photosynthetic apparatus could help in developing new crop plants with more robust photosynthetic machinery capable of higher yields even under combined stressed environments.

3.3 Yield

When plants encounter with stressors, it have to adapt in a physiologically costly way against these stressors, resulting in reduced availability of resources to produce biomass, seeds. As a result, a significant yield reduction occurs. Moreover, the combination stress factors may further cause trade-off between plant responses that are appropriate for adaptation to one stress (Dresselhaus and Hückelhoven 2018). Therefore, it is urgent to gain an insight into how a combination of stressors influences the rice plants. It was found that combined drought and heat stress inhibited the grain yield, spikelet per panicle, seed set, 100-grain weight and harvest index whereas panicles were increased. Moreover degree of inhibition was dependent in their respective cultivar (Lawas et al. 2018). Similarly during elevated CO_2 and high temperature stress, rice plants yield components such as grain yield, panicles, filled grain, grain mass and harvest index were inhibited completely whereas moderate CO_2 and temperature combination decreased the yield component compared to control plants (Liu et al. 2017). Similar decrease of yield components were also observed in combined elevated CO_2 and UV stress (Ziska and Teramura 1992). This sharp decline in grain yield in combined stress treatment in mentioned experiment suggests the potential additive negative effects of the stress on rice production.

3.4 Mineral Content

Abiotic stressors can interfere with the uptake of essential mineral and promote the accumulation of toxic elements, which can negatively impact the normal growth and physiology of plants (da Silva Lobato et al. 2016). Rice plants show differential response patterns when exposed to a wide array of abiotic stresses. For example, under Cu stress, rice plants accumulated Cu both in the shoots and the roots. Compared to control plants, Cu treatments elevated the Cu concentrations in rice plants by 3.0–4.6 fold in the roots and 1.2–2.9 fold in the shoots. However, the extent of accumulation depends on the cultivar (i.e., KY1360 had the highest increment in root Cu). On the other hand, in only Cd treatment, rice plants showed negligible influence on Cu accumulation but increased Cd concentrations both in root and shoot. Among the cultivars, KY1360 has the highest Cd content accumulation. Interestingly, combined Cu and Cd treated soil triggered to accumulate more Cd in rice plants compared to the only Cu and only Cd treatment suggesting an interactive relationship where Cu can enhance Cd uptake and accumulation in rice plants (Huang et al. 2009). Similarly combined NaCl and iron deficiency increased the Na^+ content and reduced the K^+ and Fe^{2+} content of rice plants resulting stunted growth of rice plants (Abbas et al. 2015). However, differential response of nutrient uptake were found in the stress combinations such as combined O_3 and NaCl stress (Welfare et al. 1996) and combined elevated CO_2 and O_3 stress (Wang et al. 2014a).

4 The Combined Effect of Drought and Heat Stress on the Physiology, Morphology, Growth and Yield of Rice Plants

The concomitant existence of multiple abiotic stresses rather than single individual stressors is a common phenomenon in plants under field conditions (Mittler 2006). In general, plant age, the inherent stress-resistant or susceptible nature of the plant and the severity of the stresses all influence the impact of stress combination. Plant responses to stress combination are majorly determined by the more severe stress (dominant stressor) and therefore, plant physiological and molecular responses under combined stresses often resemble those observed under more severe individual stress conditions (Pandey et al. 2015).

The simultaneous occurrence of heat and drought stresses is the most prevalent combined stress that is encountered by rice plants in rainfed rice ecosystems (Mittler 2006). The individual effects of heat or drought stress on rice plants have been extensively studied, but limited information is available on the combined effects of heat and drought stress for rice. Recent studies using combined drought and heat stress show more serious deleterious effects on the morphology, growth and physiology during various chronological stages and yields of rice, in comparison to drought or heat stress alone (Craufurd and Peacock 1993; Savin and Nicolas 1996; Shah and Paulsen 2003; Altenbach et al. 2003; Porter and Semenov 2005; Prasad et al. 2011; Kadam et al. 2014). The co-occurrence of drought and high temperature stress at critical growth stages (flowering and early grain filling) of rice plants under field condition is often inevitable in the major rice producing countries of Asia (Cohen et al. 2005) and this has resulted in significant reductions in yields (Wassmann et al. 2009; Zhang and Huang 2012). The combination of drought and heat stress will in the future have further detrimental impacts on rice production in arid and semi-arid regions (Jagadish et al. 2014). Scientists have classified the life cycle of the rice plant into major three stages such as vegetative, reproductive, and grain filling. The reproductive and grain-filling stages being the most important stages for rice production are considered the most sensitive phases to heat and drought stress. Temperatures greater than critical the threshold of 33 °C can greatly reduce rice yields and grain quality (Jagadish et al. 2007). In Fig. 1, we have summarized some of the changes in physiological, morphological, biochemical, molecular processes and alterations in yield parameters of rice under combined drought and high temperature stress.

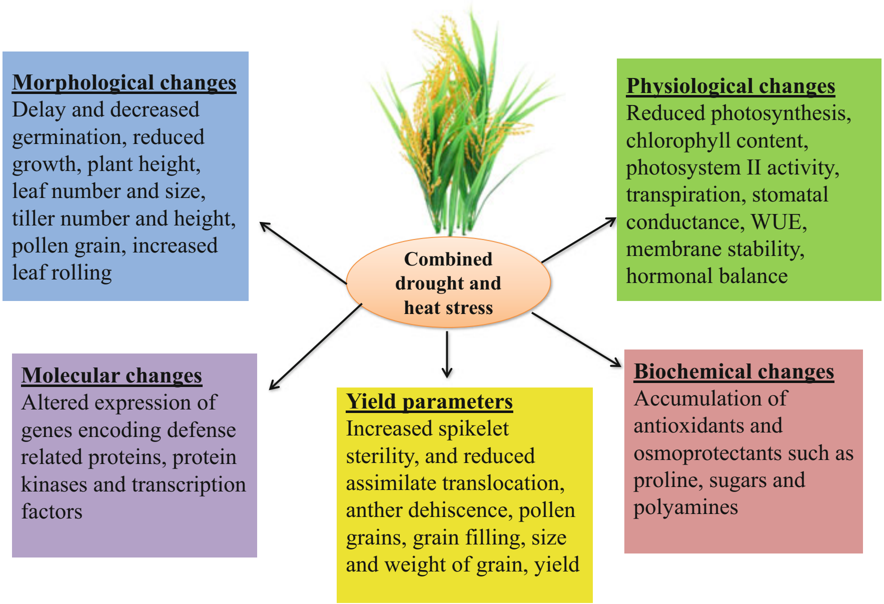

Fig. 1 Various responses of rice plant to combined drought and heat stress. Combined drought and heat stress lead to morphological, physiological, biochemical and molecular changes of plants and negatively affect yield and yield attributing traits of rice

4.1 Physiological Changes

4.1.1 Photosynthesis and Photorespiration

Both drought and heat stress at the vegetative stage of rice development can reduce photosynthesis by up to 11–36% (Rabara et al. 2018). Usually, the photosynthetic process is less affected by heat stress compared to drought stress and temperature above 35 °C is known to be detrimental for rice (Jagadish et al. 2007; Krishnan et al. 2011; Hatfield and Prueger 2015). Heat and drought stress limit photosynthesis in a different mode of action. For example, drought stress reduces stomatal conductance and thus reduces CO_2 movement into the mesophyll tissues of the leaves (Chaves et al. 2003). Besides, it also alters photosynthetic pathway components by reducing regeneration of ribulosebisphosphate (RuBP) and declining the activity of ribulose 1, 5-bisphosphate carboxylase/oxygenase (RuBisCO) (Tezara and Lawlor 1995; Parry et al. 2002 and Bota et al. 2004) and inorganic P via impairing ATP. According to Zhou et al. (2007) both of this diffusive limitations account for reduced photosynthesis under drought stress. In contrast, heat stress reduces net photosynthesis via increasing photorespiration by declining CO_2/O_2 ratio in mesophyll tissues (Lea and Leegood 1999), deactivating RuBisCO by inhibiting Rubiscoactivase (Crafts-Brandner and Salvucci 2000; Prasad et al. 2004) and destructing photosystem II (Havaux 1992). Thus, both drought and heat stress reduce CO_2 uptake by modulating stomatal conductance or internally preventing CO_2 diffusion. Lower

CO_2 levels favor oxygenase activity leading to higher photorespiration, lowering net photosynthesis. A lack of carbohydrate production due to reduced photosynthesis may cause starvation leading to plant death. Reduced photosynthesis may reduce assimilate production and transport and this may lower shoot biomass accumulation during the vegetative phase of plant growth (Wahid et al. 2007; Barnabás et al. 2008). Thylakoid membranes are highly sensitive to both drought and heat stress and thus photosynthetic disruption is one of the first indicators of this combinatorial stresses. Extended exposure to high temperatures or drought stress causes major changes in metabolic functions e.g. increased amylolytic activity and thylakoid grana disintegration and reduced chlorophyll content (Kozłowska et al. 2007; Pandey and Shukla 2015). The reduction in chlorophyll content is attributed to stress-induced pigment degradation or impairment of pigment biosynthetic pathways, loss of thylakoid membrane structure and higher lipid peroxidation (Reddy et al. 2004; Khan et al. 2017a).

4.1.2 Respiration

Under combined drought and heat stress, plants can modulate mitochondrial respiration as a shared response of stress (Prasad et al. 2008). Under field conditions, there is an intricate relation between high temperatures and root respiration, due to the co-occurrence of heat stress with drought. Mitochondrial respiration is greatly influenced by heat stress and it should be noted that heat stress can be deleterious to mitochondrial activity by disrupting respiration (Paulsen 1994). Up to 50 °C respiration increases with increasing temperature, but above 50 °C respiration decreases with increasing temperature as severe mitochondrial damage occurs. Again, temperature quotient (Q_{10}, the relative change in a process with a 10 °C rise in temperature) for root and leaf respiration reduces with rising temperature. On the other side, drought stress shortly decreases root and leaf respiration rates, while combined drought and heat stress further induce the decrease in root respiration (Byrla et al. 2001). Plant respiration as observed by Q_{10} values, responses to combined drought and heat stress depend on the crop species in question, as well as age of the plant organs (Paulsen 1994). Higher respiratory losses by rice grain can offset the higher influx of assimilate resulting in yield loss.

4.1.3 Transpiration and Water Use Efficiency (WUE)

Drought and/or heat stress alter the physiological processes of rice plants by affecting transpiration rate (Cabuslay et al. 2002), WUE (Chaum et al. 2010), relative water content (Biswas and Choudhuri 1984; Pirdashti et al. 2009; Cha-um et al. 2010) and membrane stability (Kumar et al. 2014a). Very often it is observed that during high temperature stress, plants open their stomata to cool their leaves by transpiration. However, if heat stress is combined with drought, the plants are unable to open their stomata and therefore, the leaf temperature remains higher (Rizhsky et al. 2002). Stomatal closure is thought to have a more inhibitory effect on transpiration of water than on CO_2 diffusion into the leaf tissues (Sikuku et al. 2010). Again, stomatal closure reduces leaf expansion, thereby prevents the dehydration of leaf tissues. A number of reports have shown that reduced net photosynthetic rates are associated with stomatal closure resulting in increased WUE (net CO_2 assimilation rate/transpiration) (Ruggiero et al. 2017). Recently, Akram et al. (2013) reported that photosynthetic rate, relative water content (RWC) and stomatal conductance have strong positive correlations with WUE, whereas transpiration rate has a negative correlation with WUE, in rice plants under drought stress. In plants, WUE in enhanced under moderate drought conditions (Blum 2009; Shah and Paulsen 2003; Rollins et al. 2013), while it is reduced under heat stress conditions (Shah and Paulsen 2003; Rollins et al. 2013). Interestingly, the combination of drought and high temperature stress can modulate the expression of WUE in a unique manner. Plant root responses are not similar during drought and high temperature stresses. Roots directly play a major role by maintaining a cooler canopy with efficient use of available water under drought stress (Lopes and Reynolds 2010; Pinto et al. 2010) and by increasing transpiration for cooling to maintain an ambient microclimate in response to high-temperature stress with sufficient water availability (Wassmann et al. 2009). Canopy temperature depression is also maintained by root under combined drought and high-temperature stress.

4.2 Growth and Morphological Changes

In plant growth, both cell division and cell enlargement are the major processes, where the latter is greatly affected by drought and/or heat stress. Rabara et al. (2018) showed that drought and/or heat stress of rice plants at the vegetative growth stage reduces plant height, leaf number and size, tiller number and the total dry weight of the plants, which might be due to low CO_2 assimilation and slow nutrient mobilization to the growing tissues (Barnabás et al. 2008; Lipiec et al. 2013). According to Blum (2011) reduction in rice growth is often a consequence of improper root development and poor leaf-surface traits like shape, composition of cuticular wax, leaf pubescence and leaf color, affecting the radiation load of the leaf canopy, delaying or reducing normal plant senescence rates and also inhibiting stem reserves. Reduction in leaf number, size and rate of expansion are some of the general effects of drought stress at the initial stage but at the advance stage drought stress can cease leaf growth (Prasad et al. 2011). Drought and temperature stress may also cause leaf rolling in rice plants, which reduces leaf area, light interception, and dehydration as well as transpiration (Kadioglu and Terzi 2007; Kadioglu et al. 2012), and plasticity in leaf rolling is related to leaf angle when internal water deficit occurs in plants (Chutia and Borah 2012). Leaf senescence occurs early in response to combined drought and heat stress, especially at the post-flowering stages of seed filling (Prasad et al. 2011). Root growth of rice is very sensitive to water and heat stress. Compared to other growth processes, root growth has a narrow optimum temperature range (Porter and Gawith 1999), beyond which the number, length and diameter of the roots decrease. During the reproductive stage, root growth is also reduced due to heat stress because of reduced carbon partitioning (Batts et al. 1998).

In contrast, the response of root growth to drought stress is variable depending on the severity of drought and the cultivar. Under low to medium drought stress, root growth is enhanced due to increased partitioning of carbohydrates to roots, while under extreme drought stress combined with a high temperature root development can be inhibited. Approximately a 56% reduction in root length was observed in the drought-sensitive rice variety IR64 compared to 23% in tolerant variety PSB Rc14, under drought stress (Ferrer 2015).

4.3 Yield and Yield Attributes

As a complex character yield results from the interaction of many plant characters, e.g. tiller and panicle number, spikelets per panicle, proportion of filled spikelets, 1000-grain weight etc. Drought, heat and their combination have similar effects on the reproductive development of rice plants. Severe reductions in the grain yields of rice plants due to drought or heat stress alonehas been noted by many researchers (Bouman et al. 2005; Centritto et al. 2009; Pirdashti et al. 2009; Venuprasad et al. 2011; Raman et al. 2012; Ahadiyat et al. 2014; Maisura et al. 2014; Aghamolki et al. 2014; Cao et al. 2008; Srivastava et al. 2012; Ghadirnezhad and Fallah 2014; Shi et al. 2016). However, reports highlighting the impacts of drought stress coinciding with high temperatures are few. In recent years, some studies have investigated the combined effects of drought and heat stress on rice during the reproductive and grain filling stages either under controlled (Liu et al. 2006; Jagadish et al. 2011b; Li et al. 2015; Rang et al. 2011) or field conditions (Lawas et al. 2018). These studies showed the negative relationship between these stresses and yield and quality attributes. In contrast with individual stressors, combined heat and drought stress during various phenological stages imposed additive deleterious effects on rice plants causing significant yield losses (Kadam et al. 2014; Porter and Semenov 2005). According to the study of Rang et al. (2011), about 8%, 24% and 27% shortening in peduncle length, 81%, 59% and 84% reduction in number of germinated pollen on the stigma and about 77%, 21% and 71% decline in spikelet fertility were observed by heat stress, drought stress and concomitant heat and drought stress, respectively. The enhanced damage incurred by the heat and drought stress combination as compared to individual stressors is due to the fact that heat and drought share a number of physiological traits and the overall effect of the two stresses on plants is additive and leads to aggravated stress effects (Pandey et al. 2015).

It should also be mentioned that the co-occurrence of drought and heat stress in rice plant at the flowering stage has a more significant influence on yield and its components, while combined stress during the grain filling stage had a stronger negative impact on quality parameters, mainly chalkiness, with a percent decline in rice heading (Lanning et al. 2011; Zhao and Fitzgerald 2013; Lawas et al. 2018). Both drought or water deficit (Liu et al. 2006, Wassmann et al. 2009; Raman et al. 2012; Kumar et al. 2014a) and heat stress (Jagadish et al. 2007, 2008, Matsui 2002) during flowering causes spikelet sterility in rice, by affecting anther dehiscence, pollination, pollen germination and shedding. Exposure of rice plants to drought or heat stress at the flowering and booting stages not only causes spikelet sterility, but also disrupts floret initiation, delays grain filling, reduces grain size and weight, and ultimately leads to lower productivity (Pantuwan et al. 2002; Kamoshita et al. 2004; Castillo et al. 2006; Venuprasad et al. 2007; Mostajeran and Rahimi-Eichi 2009; Ji et al. 2012; Rabara et al. 2018). According to Lawas et al. (2018) combined drought and heat stress at the flowering and early grain filling stages resulted in a significant reduction in panicles per meter square, spikelets per panicle, spikelets per meter square, percent seed-set and 1000-grain weight in three rice cultivars (N22, Dular and Anjali).

This decline in plant growth and yields may be due to stress-induced shortening of the grain filling period, disruption of leaf gas exchange and plant water relations, limitation of the size of the source and sink tissues, impaired phloem loading and assimilate translocation, reducing CO_2 assimilation rates, stomatal regulation, photosynthetic pigments, assimilate partitioning, leaf size, and stem elongation, WUE, activities of sucrose and starch synthesizing enzymes (Shahryari et al. 2008; Farooq et al. 2009a, b, 2010; Anjum et al. 2011).

5 Mechanisms of Combined Drought and Heat Stress Tolerance in Rice

Like other plants, rice has evolved physiological and molecular adaptation strategies to protect plants from heat and drought stress, both alone and in combination, with responses to combinatorial stresses being unique from individual stress responses (Pandey et al. 2015). In addition, plants exhibit shared responses that are common to individual stresses and stress combinations. The adaptation mechanisms of plants to stress combination are regulated by the stress interactions and these strategies may or may not have similarities with those to individual stresses. The probable mechanisms of rice plants to combat combinatorial drought and heat stress are shown in Fig. 2.

5.1 Accumulation of Compatible Osmolytes

Under stress, plants accumulate a variety of osmolytes such as proline (Pro), polyamines (PAs), sugars and sugar alcohols (polyols) and plant acclimation to drought and heat stress has been frequently assessed based on changes in these osmoprotectants (Jouve et al. 1993). Pro content increases in rice at temperatures ranging between 25 and 35 °C, but then decreases at 40 °C and above (Tang et al. 2008; Sánchez-Reinoso et al. 2014), which might be due to plant sensitivity to prolonged stress periods. Many studies report an increase in Pro content in rice plants under drought (Sheela and Alexallder 1995; Mostajeran and Rahimi-Eichi 2009; Bunnag and Pongthai 2013; Kumar et al. 2014b; Lum et al. 2014; Maisura et al. 2014). Pro not only serves as an osmoprotectant, but also acts as a metal chelator, and signaling and antioxidant molecule (Hayat et al. 2012; Hossain and Fujita 2014). Pro can be used as a biochemical marker for screening drought tolerant rice varieties (Fahramand et al. 2014).

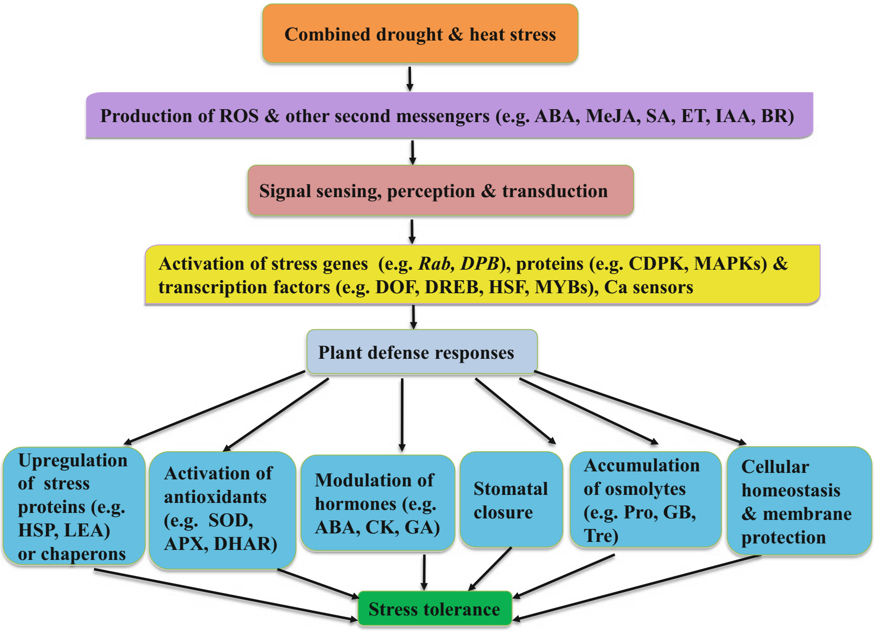

Fig. 2 A schematic diagram showing pathways involved in combined drought and heat stress tolerance in rice plant. Combined drought and heat stress stimulate production of ROS and second messengers that induce expression of signal transduction and activation of stress genes, proteins and transcription factors which subsequently modulate various metabolic processes as defense responses to develop stress tolerance

As heat or drought stress increases, rice plants enhance PAs biosynthesis, particularly spermidine (Spd) and spermine (Spm) in the free form and putrescine in the insoluble-conjugated form (Yang et al. 2007; Basu et al. 2010; Do et al. 2013; Kumar and Mallick 2019) and this is considered to be an important biochemical adaptation for drought tolerance in rice. Under drought stress higher PAs contents in plants are related to improved photosynthetic capacity, reduced water loss, as well as osmotic adjustment and detoxification. Exogenous application of PAs, especially Spm, to rice plants can alleviate drought stress by improving net photosynthesis, WUE, leaf water status, production of free Pro, anthocyanins and soluble phenolics, and can reduce oxidative damage to cellular membranes, which foliar application more effective than the seed priming (Farooq et al. 2009b; Do et al. 2013). Heat stress caused greater accumulation of free and conjugated polyamines in the callus of the heat-tolerant rice cultivar N22 than in the heat-sensitive cultivar IR8 (Roy and Ghosh 1996). Production of these metabolites can prevent stress-induced alteration in cellular components, maintain cell turgor and restore homeostasis, which is vital for stress tolerance.

5.2 Activation of Antioxidants

Drought and heat stresses can result in over-accumulation of reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) and superoxide($O_2^{\bullet -}$), singlet oxygen ($^1O_2$), hydroxyl radical ($OH^{\bullet}$) leading to membrane damage, denaturation of proteins, lipid peroxidation and cellular damage (Jaspers and Kangasjärvi 2010). The detrimental effects of ROS are limited in plants by a robust, complex antioxidant defense system where the non-enzymatic and enzymatic antioxidants are critical components whose expressions can improve tolerance to stresses (Wang et al. 2005). In rice, higher activities of the enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), peoxidase (POD), guaiacol peroxidase (GPX), ascorbate peroxidase (APX), glutathione reductase (GR), dehydroascorbate reductase (DHAR), phenylalanine ammonia-lyase, monodehydroascorbate reductase (MDHAR) and non-enzymatic antioxidants including ascorbate (AsA) and glutathione (GSH) were reported by many researchers in plants under drought (Sohag et al. 2020; Nahar et al. 2018; Kamaruddin et al. 2018; Sara et al. 2017; Lum et al. 2014; Yang et al. 2014a; Shehab et al. 2010; Sharma and Dubey 2005; Selote and Khanna-Chopra 2004) or heat stress (Khan et al. 2017b; Lee et al. 2013; Das et al. 2013; Cao et al. 2008). This increases show the mitigating role of these antioxidants to combat the oxidative damage caused by heat and drought stress. According to Li et al. (2012) mild drought pre-conditioning of rice seedlings changes antioxidant enzymes response, which helps the acclimatization of plants more efficiently to intermediate drought stress conditions. Thus, raising the levels of naturally occurring antioxidants can be an effective mitigation strategy for rice plants exposured to combined stresses.

5.3 Activation of Stress Responsive Genes and the Synthesis of Proteins and Transcription Factors

Plants response to drought and heat stress is a multigenic trait. Scientists have identified a large number of genes that respond to drought and heat stress at the transcriptional level (Shinozaki and Yamaguchi-Shinozaki 2007). In rice some of these genes function in defense against desiccation through stress perception, signal transduction and transcriptional regulatory networks (Wang et al. 2005). In rice, over-expression of genes like *DPB3-1*and *OsHTAS* enhance heat tolerance (Sato et al. 2016; Liu et al. 2016), while genes such as *OsHsfA*, *LEA*, *OsNAC14*, *OsDRAP1*, *XERICO*, *OsLG3*, and *OsHSP50.2* increases tolerance to drought (Liu et al. 2013; Xiao et al. 2007, Shim et al. 2018; Huang et al. 2018; Zeng et al. 2013; Xiong et al. 2018; Xiang et al. 2018). Furthermore, enhanced drought and heat tolerance in rice was observed by Wu et al. (2009) due to over-expression of *OsWRKY11*. Gene products can be grouped into two major classes, functional proteins and regulatory proteins, both of which are important for stress tolerance. The functional protein group includes chaperones, LEA proteins, heat shock proteins (HSPs), proteinases etc. while the regulatory protein group includes transcription factors (TFs; e.g. DREB, AREB/ABF, ERF, WRKY, MYB, MYC, bHLH, bZIP, DOF, NAC), protein kinases (e.g. MAPK, CDPK, SNF1 etc.), phospholipase C etc. (Shinozaki and Yamaguchi-Shinozaki 1997; Shinozaki et al. 2003; Seki et al. 2003). Recently El-Esawi and Alayafi (2019) reported that over-expression of the *OsRab7* gene improves grain yields and improves drought and heat tolerance in rice by modulating osmolytes, antioxidants and abiotic stress-responsive gene expression.

TFs are the major regulatory components of abiotic stress (e.g. drought, heat stress) signal transduction pathway that can interact with *cis* regulatory sequences and control the expression of a series of related genes (Nakashima et al. 2014; Pandey and Shukla 2015). For example, the activation of heat stress-responsive genes is mediated by heat stress transcription factors (HSFs), which are the terminal components of signal transduction pathways. The TFs function by balancing cross-talk in abiotic stress (drought, heat and cold) responses, affecting multiple signaling pathways (Nakashima et al. 2014), and over-expression of key TFs under the control of suitable promoters can improve drought stress tolerance in many crop species including rice (Oh et al. 2005; Ito et al. 2006; Hu et al., 2006; Takasaki et al. 2010; Jeong et al. 2010; Ishizaki et al. 2012; Datta et al. 2012; Nakashima et al. 2014). As for example, Hu et al. (2006) suggested that over-expression of an NAC-type TF, e.g. SNAC1, in rice can significantly increase drought tolerance, with better seed set compared to controls under severe drought stress at the reproductive stage under field conditions. Again, when plants are exposed to abrupt or gradual heat stress, there is a decrease in the synthesis of normal proteins, but an increase in transcription and translation of stress proteins e.g. HSPs (Nakamoto and Hiyama 1999; Schöffl et al. 1999; Bray et al. 2000; Liu et al. 2006; Sun et al. 2006; Momcilovic and Ristic 2007). The HSPs comprise the majority of the proteins synthesized during heat stress and play a pivotal role in plant survival under heat stress (Ristic and Cass 1992).

Besides, plants increase the levels of intracellular concentration of Ca^{2+} as it mitigates the adverse effects of stresses by taking care of antioxidants activity (Jiang and Haung 2001; Kleinhenz and Palta 2002; Kolupaev et al. 2005). Functioning as Ca^{2+} sensors, calcium binding proteins perceive elevated Ca^{2+} levels and activate kinases or phosphatases that phosphorylate or dephosphorylate specific TFs, thereby regulating the expression of stress responsive genes (Kudla et al. 2010; Reddy et al. 2011). Moreover, these sensors also bind with *cis*-elements of gene promoters, cross-talk with DNA-binding proteins to regulate the activation or suppression of responsive genes (Kudla et al. 2010; Reddy et al. 2011).

5.4 Hormonal Changes

The functions of phytohormones in drought and heat tolerance in plants are complex. A variety of hormones including abscisic acid (ABA), cytokinins (CK), auxins (IAA), gibberellins (GA), salicylic acid (SA), and methyl jasmonate (MeJA) play significant roles in regulating stress-induced processes at the cell, organ and whole-plant levels (Hoffmann and Parsons 1991; Maestri et al. 2002a). ABA accumulation in drought-exposed rice plants has been reported by many researchers (Yang et al. 2007; Wang et al. 2007; Ye et al. 2011; Ashok Kumar et al. 2013; Changan et al.

2018). In rice, ABA and cytokinins are involved in controlling the linkage between senescence and the remobilization of carbon reserve during drought stress, which substantially enhances ABA concentrations with a reduction in cytokinin concentrations in roots and leaves (Yang et al. 2002; Yang and Zhang 2006). Interestingly, factors associated with senescence (photosynthesis rate and chlorophyll content of flag leaf) are negatively correlated with ABA levels and positively with cytokinin levels, while remobilization of pre-stored carbon is positively liked with ABA levels, but not cytokinin levels. Recently, Reguera et al. (2013) showed that stress-induced cytokinin synthesis in rice plants promoted increase sink strength, through cytokinin-dependent coordinated regulation of carbon and nitrogen metabolism facilitating an enhanced tolerance of plants to water deficit. Plant drought responses depend on regulatory mechanisms sensing water status, turgor, hormones (e.g. ABA) and alteration in cell membranes (Chaves et al. 2003). Higher ABA concentration in roots under drought increases hydraulic conductivity leading to an increase in water uptake and a reduction in water deficit in shoots. According to Yang et al. (2001), inhibition of cell division and development of floret and seed are related to increased ABA levels in early reproductive structures caused by environmental stresses. ABA induces stomatal closure to reduce water loss and also regulates gene expression, which controls cell division and the activities of enzymes involved in carbohydrate metabolism under drought conditions (Liu et al. 2005). ABA induces drought tolerance in rice by enhancing the recovery of net photosynthetic rate, transpiration rate, controlling stomatal conductance and up-regulating the expression of various drought responsive genes (Teng et al. 2014). Dehydration signaling pathways in plants are mediated by ABA, which is implicated in osmotic stress responses (Davies and Jones 1991) and the ABA signal transduction pathway comprises a protein kinase/phosphatase cascade interacting with Ca^{2+} (Bray 2002). ABA induces the expression of many drought-responsive genes that are activated by characterized TFs thatbind specifically to promoters containing ABA responsive elements (Antoni et al. 2011; Fujita et al. 2011; Rushton et al. 2012). ABA-induced gene products are connected with drought stress tolerance, while ABA-repressed gene products are generally linked to growth (Pandey and Shukla 2015). Induction of ABA is also a major part of thermo-tolerance where drought and high-temperature stresses concurrently occur (Gong et al. 1998). GA has contrasting effects to ABA for high temperature tolerance (Maestri et al. 2002a), but recently Plaza-Wüthrich et al. (2016) showed that GA deficiency in rice confers lodging and drought tolerance. In addition, under drought stress, SA may regulate the synthesis of osmoprotectants and the activation of antioxidant systems and thus improve membrane stability, enabling rice plants to maintain tissue water status (Farook et al. 2009b). Moreover, Kim et al. (2009) suggested that rice plants produce MeJA during drought stress, which in turn stimulates ABA synthesis leading to a loss in grain yields. According to Du et al. (2013), endogenous JA levels increased in rice plants under drought stress, but decreased under heat stress. The reverse situation was observed for auxin levels i.e. the IAA content decreased under drought stress, but significantly increased during moderate heat stress, although a reduction in IAA content was observed under severe heat stress. In addition, Sharma et al. (2018)

showed that IAA synthesis and signaling play significant roles in mitigating spikelet sterility, rescuing pollen viability and stabilizing grain yields in rice plants under drought and heat stress conditions. Thus, under heat and drought stress, the ratio of plant growth regulators (PGRs) varies greatly depending upon the environmental signals (Kondamudi et al. 2011).

6 Molecular Breeding Approaches for Combined Drought and Heat Stress Tolerance in Rice

Recent developments in molecular genetics have opened new horizons for plant breeders to identify and select genes conferring drought or heat stress tolerance or combined heat and drought stress tolerance. In the following sections, we will discuss various genetics and genomics approaches that are now being practiced to enhance drought and heat stress tolerance in rice.

6.1 Qunatitative Trait Loci (QTLs) and the Marker Aided Selection (MAS) System for Heat and Drought Tolerance in Rice

Molecular techniques, such as construction of genetic maps using molecular markers, gene transfer and apomixis, have made fundamental changes in rice molecular breeding (Ashkani et al. 2015). There are two ways to create tolerant cultivars through the use of molecular markers in rice (Shabir et al. 2017). The first approach is to use grain yield as a stress tolerance index. The second approach is to identify genes associated with stress tolerance, with candidate gene sequences used as markers (Das et al. 2017). Physiological studies should be carried out before applying these strategies in order to confirm the positive effects of traits or genes related to yield under stress conditions and in different environments (Fahad et al. 2017).

The term “QTL” refers to the genetic loci controlling quantitative traits and is a region or part of a chromosome that carries a gene or genes controlling a quantitative trait (Kordrostami and Rahimi 2015). QTL mapping can be important for facilitating selection through MAS, by the use of tightly linked markers for important QTLs controlling quantitative traits, and cloning important major-effect QTLs and using them in gene transfer projects (Palanga et al. 2017). The main purpose of QTL mapping is to recognize the inheritance of markers adjacent to genes that control quantitative traits (Consortium 2003). Genetic mapping is mostly done in two ways: a) using artificial populations called QTL mapping, and b) using natural populations or germplasm collections called association mapping (Abdurakhmonov and Abdukarimov 2008).

Many studies have been conducted on drought stress tolerance in rice. In a study using SSR markers, QTLs related to initial and final germination rates were identified on chromosome 1 and at the marker interval of RM5-RM306. Interestingly, these QTLs were located near the *amy1B/A* (alpha amylase) gene (Temnykh et al. 2001). Zheng et al. (2003) related the RM160-RM215 marker interval on chromosome 9 with maximum root depth under two control conditions and drought stress. In another study on rice, 48 genes were identified on chromosome 1, between RM319-RM212 markers, hich played animportant role in controlling the 16 traits associated with drought stress tolerance (Wang et al. 2005). Yue et al. (2006) in a 2 year consecutive study on rice, mapped a major-effect QTL for drought stress on chromosome 3, between RM316-RM219 markers, which explained 14–25% of phenotypic variance. Bernier et al. (2007) in a study on 436 lines resulting from the cross between Vandan and Way Rarem cultivars under drought stress conditions, used 426 microsatellite markers to map a major-effect QTL for grain yield, which explained 54% of phenotypic variation under stress conditions. This QTL was mapped at the RM28048-RM511 marker interval on chromosome 12. Bernier et al. (2009), in another study on a rice population derived from a cross between R74371-46-1-1/2 and Sabitri cultivars, identified a major-effect QTL for rice grain yield under drought stress conditions. This QTL ($qDTY_{12.1}$) was mapped at the RM28166-RM28199 marker interval on chromosome 12. They introduced this QTL as the first major-effect QTL for grain yield under drought stress. Finally, in 2009, Bernier et al. concluded that the major-effect QTL of *qDTY12*.1 is located between the RM28166-RM28199 markers on chromosome 12. They also stated that this QTL, as the severity of drought stress increased, has an important role in increasing plant yields and adaptability to stress conditions, because it increases the water absorption by the plant and controls several traits such as plant height, biomass, harvest index and panicle number under drought stress conditions.

In a genetic diversity study, on IR62266 / CT9993 populations in rice, a major-effect QTL was reported for yield under drought stress conditions on chromosome 1, which explained 32% of the genetic variation for grain yield (Kumar et al. 2007). Salunkhe et al. (2011) reported a major-effect QTL for drought tolerance at the RM212-RM302 and RM8085-RM3825 marker intervals on chromosome 1. In another study, using three genetic backgrounds, including three populations derived from Apo/2 × Swarna, Apo/2 × IR72 and Vandana/2 × IR72 crosses, a major-effect QTL was identified for grain yield. This major-effect QTL (*qDTY*6.1) was identified by the RM510-RM19367 marker interval on chromosome 6. The effect of this QTL was generally investigated in 21 hydrological environments and five populations with three different genetic backgrounds in five seasons. This QTL in different populations had a significant effect on different aerobic conditions with drought stress, different aerobic conditions without drought stress and flood conditions. According to the results of this study, the Vandana variety was found to have an optimal allele in aerobic conditions. In total, they have proven *qDTY*6.1 as a highly effective QTL for grain yield in aerobic conditions, which can be used as an effective potential for rice molecular breeding programs in aerobic environments (Venuprasad et al., 2012). In a survey on the rice populations derived from a cross between Dhagaddeshi/Swarna cultivars, two major-effect QTLs for grain yield (*qDTY*1.1) and days to flowering (*qDTF*1.1) between the RM104-RM431 markers, as well as a major-effect QTL associated with the plant height (*qDTH*1.1) was mapped by the markers RM11943-RM431 (Ghimire et al. 2012). In another study, using 269 SSR markers to adequately cover all rice chromosomes, a population derived from the cross between IR64 and Azucena was used. Also, the QTLs were validated from other populations, including BPT5204/A67 and BPT5204/Dodiga. In this study, 22 QTLs related to drought tolerance were identified on chromosome 1–6. One of these major-effect QTLs was related to the control of germination on chromosome 1 between RM5-RM306 markers and near the alpha-amylase gene under drought stress conditions. Also, in this study, another QTL related to seed germination rate under drought stress conditions was mapped at the RM231-RM7 markers interval on chromosome 3. But, this QTL was also present on chromosomes 4 and 5 (Diwan et al. 2013). By studying grain yield under drought stress, it was found that the QTLs controlling this trait were distributed on different chromosomes (Bernier et al. 2007; Bernier et al. 2009; Mishra et al. 2013; Palanog et al. 2014; Sahebi et al. 2018; Saikumar et al. 2014; Singh et al. 2016b; Venuprasad et al. 2009; Wang et al. 2014b; Yadaw et al. 2013). According to these studies, QTLs that control this trait are distributed on chromosomes 1, 2, 3, 9 and 12. Most of these QTLs are major-effect and explain 7.5–77% of phenotypic variance. Table 1 shows the most important SSR markers tightly linked with grain yield under drought stress conditions.

In addition to grain yields under drought stress conditions, various studies have examined in rice plants QTLs related to the most important yield-related traits under drought stress conditions. For example, Lilley et al. (1996) found a major-effect QTL for osmotic adjustment trait on chromosome 8. Several QTLs also have been identified for root traits (Courtois et al. 2003; Mu et al. 2003). Interestingly, the number of QTLs is high and spread across the rice chromosomes. For example, Mu et al. (2003) identified 18 QTLs and Courtois et al. (2003) 42 QTLs for these traits spread across the rice chromosomes (Table 2).

A similar situation for the drought avoidance traits. Price et al. (2002) found 17 QTLs for this trait distributed on all the chromosome except 9. Most of these were major-effect QTLs and explained 4.4–25.6% of phenotypic variation. Babu et al. (2003) found 47 QTLs for water stress indices, phenology and production traits which were distributed on all the chromosomes except 5. Most of these QTLs were major-effect and explained 5–59% of phenotypic variation. Li-Feng et al. (2007) found two QTLs for basal root thickness and 100-grain weight under drought stress conditions, which were distributed on chromosomes 4 and 6. These were major-effect QTLs and explained 20.6–33.4% of phenotypic variation. Hu et al. (2007) also found 15 QTLs for coleoptile length and drought resistance index, which were distributed on all chromosomes except 3, 8, 11. Most of these QTLs were major-effect and explained 4.9–22.7% of phenotypic variation. Kato et al. (2008) found seven QTLs for relative growth rate and specific water use under drought stress conditions, distributed on chromosomes 2, 4, 5, 6, 7, 8. These QTLs were major-effect and explained 10.0–22.0% of phenotypic variation. Wang et al. (2014b) in a study, found 23 QTLs for filled grain number per panicle, 14 QTLs for panicle number per plant, and 21 QTLs for 1000-grain weight under drought stress conditions, distributed on all chromosomes except 1, 3, 8, 12. These QTLs were major-effect and explained 33.3–50.3% of phenotypic variation. Prince et al. (2015) found 9 and 24 QTLs for physio-morphological traits and plant production which

Table 2 Information on the microsatellite markers tightly linked with *qDTY* QTL

Marker	Forward sequence	Reverse sequence	References
RM11943	CTTGTTCGAGGACGAAGATAGGG	CCAGTTTACCAGGGTCGAAACC	Vikram et al. (2015)
RM431	TCCTGCGAACTGAAGAGTTG	AGAGCAAAACCCTGGTTCAC	Vikram et al. (2015)
RM104	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC	Yue et al. (2006)
RM12023	TGCGTACCTCTGCTCCTCTCTGC	GACGAAGCCGACCAAGTGAAGC	Vikram et al. (2015)
RM12091	TGTCCAGAGAATGACAAAGTACGC	GGATGTATATCTGCCACCAAATGC	Vikram et al. (2011)
RM12146	AGTATGCCCTGCCCACTACACTAGG	CAGCGAATGGCAAGAGCAACC	Vikram et al. (2015)
RM12233	CTTGAGTTCGAAGCGAGAAGACG	CACTTGAGCTCGAGACGTAGCC	Vikram et al. (2011)
RM416	GGGAGTTAGGGTTTTGGAGC	TCCAGTTTCACACTGCTTCG	Venuprasad et al. (2009)
RM520	AGGAGCAAGAAAAGTTCCCC	GCCAATGTGTGACGCAATAG	Vikram et al. (2015)
RM16030	GCGAACTATGAGCATGCCAACC	GGATTACCTGGTGTGTGCAGTGTC	Vikram et al. (2015)
RM236	GCGCTGGTGGAAAATGAG	GGCATCCCTCTTTGATTCCTC	Swamy et al. (2013)
RM279	GCGGGAGAGGGATCTCCT	GGCTAGGAGTTAACCTCGCG	Swamy et al. (2013)
RM423	AGCACCCATGCCTTATGTTG	CCTTTTTCAGTAGCCCTCCC	Swamy et al. (2013)
RM53	ACGTCTCGACGCATCAATGG	CACAAGAACTTCCTCGGTAC	Uday (2013)
RM555	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC	Swamy et al. (2017)
RM211	CCGATCTCATCAACCAACTG	CTTCACGAGGATCTCAAAGG	Palanog et al. (2014)
RM233A	CCAAATGAACCTACATGTTG	GCATTGCAGACAGCTATTGA	Sellamuthu et al. (2015)
RM468	CCCTTCCTTGTTGTGGCTAC	TGATTTCTGAGAGCCAACCC	Dixit et al. (2014)
RM105	GTCGTCGACCCATCGGAGCCAC	TGGTCGAGGTGGGGATCGGGTC	Swamy et al. (2013)
RM551	AGCCCAGACTAGCATGATTG	GAAGGCGAGAAGGATCACAG	Swamy et al. (2013)
RM573	CCAGCCTTTGCTCCAAGTAC	TCTTCTTCCCTGGACCACAC	Palanog et al. (2014)
RM168	TGCTGCTTGCCTGCTTCCTTT	GAAACGAATCAATCCACGGC	Dixit et al. (2014)
RM250	GGTTCAAACCAAGCTGATCA	GATGAAGGCCTTCCACGCAG	Palanog et al. (2014)
RM269	GAAAGCGATCGAACCAGC	GCAAATGCGCCTCGTGTC	Swamy et al. (2013)

were distributed on chromosomes 1, 4 and 6 respectively. These QTLs were major-effect and explained 14–36.8% of phenotypic variation.

Many studies have been conducted on rice heat stress tolerance. Although these studies are fewer than the studies on drought stress in rice. Cao et al. (2003) in a study on lines resulting from the cross between IR64 × Azucena cultivars under high-temperature stress conditions, mapped a major-effect QTL for photosynthetic rate and heat tolerance at the tillering and heading stage on chromosome 6, explaining a high percentage of phenotypic variation under stress conditions. In another study, Chang-lan et al. (2005) mapped QTLs for heat-tolerance at grain filling in a population derived from a cross between Nipponbare/Kasalath//Nipponbare and found a major-effect QTL for this trait on chromosome 3, between C1677–C361 markers. In another study on the same population, Zhu et al. (2006) found a major-effect QTL for thermo-tolerance of amylose content and gel consistency on chromosome 9 explaining a high percentage of phenotypic variation under stress conditions. Several studies have also been carried out at the seedling stage. Wei et al. (2012) and Lei et al. (2013) in their studies found QTLs controlling high-temperature tolerance at the seedling stage, on chromosomes 1 and 5 respectively. Both of these were major-effect QTLs and explained a high percentage of phenotypic variation under stress conditions. Zhang et al. (2008) in a study on a RIL population resulting from the cross between Zhongyouzao No. 8 × Fengjin cultivars under high-temperature stress conditions, mapped a major-effect QTL for heat tolerance at the tassel period on chromosome 3, explaining a high percentage of phenotypic variation under stress conditions. Spikelet fertility stage is an important stage in the rice life cycle. For this reason, various studies have taken place at this stage on different populations. Jagadish et al. (2010b), in a study on a population resulting from the cross between Bala × Azucena cultivars under high-temperature stress conditions, mapped three major-effect QTLs for heat tolerance at spikelet fertility on chromosomes 1, 2 and 8, explaining a high percentage of phenotypic variation under stress conditions. Xiao et al. (2011) and Ye et al. (2012) mapped QTLs for heat tolerance in spikelet fertility in two different populations (996 × 4628 and IR64 × N22) and found a major-effect QTL on chromosome 2 for this trait. Hirabayashi et al. (2014) in a study on the *O. officinalis* introgression lines mapped a major-effect QTL for spikelet sterility escape from heat stress on the chromosome 1. In two separate studies on the IR64 × N22 population, Ye et al. (2015a); Ye et al. (2015b) mapped a major-effect QTL for this trait on the chromosome 1 explaining a high percentage of phenotypic variation under stress conditions.

6.2 Association Mapping in Rice for Drought and Heat Tolerance

Although linkage mapping is an important tool in gene tagging, this method has some limitations. The limitations of the QTL mapping are (Mulualem and Bekeko 2016):

- High genetic distances of mostly identified QTLs with adjacent markers.

- Problems related to the use of synthetic populations resulting from the cross of two homozygous parents, including F_2, backcross, double-haploid, and recombinant inbred lines.
- High cost and time consuming of synthetic populations resulting from the cross of two homozygous parents.

In order to overcome these limitations, in recent years the method of association mapping has been introduced. Association mapping or linkage disequilibrium is a method based on linkage disequilibrium that examines the association between phenotypic diversity and genetic polymorphism (Sorkheh et al. 2008). In association mapping, the linkage disequilibrium in natural populations and germplasm sets is used. Linkage disequilibrium is a non-random relationship between two markers or two genes, or a marker and a gene. The greater the linkage disequilibrium in a population, the more successful the genetic mapping plan will be (Slatkin, 2008). Therefore, in populations that are in equilibrium, linkage disequilibrium can be due to the selection, migration, and linkage of genes, while in the artificial population, the linkage is more due to the genes linked (Xu 2010).

Although various studies have been conducted around genome-wide associations (GWAS) in different crops (Han and Huang 2013; Huang et al. 2010; Kumar et al. 2014c; Wu et al. 2015; Yang et al. 2014b), the information available is very limited for heat and drought tolerance in rice. Agrama et al. (2007) investigated the association between 123 microsatellite markers and four agronomical traits in 92 rice genotypes. They identified many traits that were previously mapped to QTL genomic loci for drought tolerance and concluded that association mapping can be an appropriate alternative to QTL mapping based on bi-parental crosses. Vasant (2012) investigated the association between microsatellite markers and drought tolerance traits (root traits and plant production) in 48 rice genotypes from different geographic locations under two different irrigation regimes. In this study, 510 microsatellite markers were used. Of these, 323 markers showed polymorphism and were used for association analysis. The results of the association analysis showed that 155 markers were associated with yield-related traits and 82 markers were associated with root traits under stress conditions. For example, markers viz., RM318 (ch. 2), RM29 (ch. 2), RM36 (ch. 3), RM3843 (ch. 4), RM170 (ch. 6), RM540 (ch. 6), RM585 (ch. 6) and RM5720 (ch. 7) linked to both root and yield-related traits under drought stress in this study. Vanniarajan et al. (2012) investigated the association between microsatellite markers and drought tolerance traits in 31 rice genotypes from Tamil Nadu, southern India, in two different irrigation regimes. The results of association analysis showed that RM302 (ch. 1), RM16 (ch. 3), RM274 (ch. 5), RM11 (ch. 7) and RM311 (ch. 10) were associated with panicle length, RM256 (ch. 8) was associated with a weight of 100 grains and RM302 (ch. 1), RM303 (ch. 4) and RM287 (ch. 11) were associated with grain yield per plant. Zhou et al. (2012) investigated the association between 125 microsatellite markers and 11 agricultural traits in 128 rice genotypes over 2 years. A total of 16 markers showed a significant relationship with the studied traits, which indicated that the use of association analysis to evaluate different rice genotypes within breeding programs is both useful and efficient. Deshmukh (2012) investigated the association between microsatellite markers and drought tolerance traits in 48 rice genotypes. Their results showed that 82 markers had a high association with root length, root dry weight, and root diameter. Also, under drought stress conditions, RM36 RM585, RM540, RM29, RM3843, RM318, RM5720, and RM170 markers associated with both yield and root-related traits. Courtois et al. (2013) investigated the association between SNP markers and drought tolerance traits in 168 traditional and improved *japonica* accessions. In this study, Diversity Arrays Technology (DArT) and a next-generation sequencing technique called DArTseq™ were used to genotype the accessions. The results of the association mapping showed that several markers belonging to the same chromosome segment in full LD were found to have the exact same level of significance (e.g., SNPs in the interval from 34,890,451 to 34,939,105 bp on chromosome 1 for a range of traits). The study found that 51 loci are associated with more than one trait. Of these, 19 loci were in the predicted genes and another 10 loci encoded proteins that had no function. 37 loci were associated with root-related traits that were located on chromosomes 1, 2, 3, 4, 5, 7, 8, 9, 10 and 11. By examining the genes that were located at the interval of ±25 kb on both sides of significant markers, 521 genes were identified, of which 261 had a specific function. Kinases were found in great abundance in the rice genome and other genes included: gibberellin dioxygenases (five on chromosomes 1, 2 and 11); multicopper oxidases (three in a cluster on chromosome 1); elongation factors (five) and glutathione-*S* transferases (two on chromosomes 1 and 11) were also found. In another study, Muthukumar et al. (2015) using 1168 SSR markers and 911,153 SNPs with 17 diverse rice lines from different geographical regions and hydrological habitats investigated the association between these markers and drought tolerance traits. In this study, a total of 130 and 118 water–trait associations were obtained with SSR and SNP markers respectively, under the stress conditions.

Association mapping of rice plants under heat stress conditions are quite limited. Lafarge et al. (2017) investigated the association between markers and for traits such as spikelet sterility, and secondary traits (fertilization process and panicle micro-climate) in 167 rice varieties for heat stress tolerance at anthesis. In their study, haplotype regression, single-marker regression, and simultaneous fitting of all markers were used to perform GWAS. They also reported that, 14 loci had a significant relationship with spikelet sterility. By examining the genes that were located at this interval, different genes with different functions, such as heat shock proteins, kinases, cell division, regulating plant response, sensing abiotic stresses, and gametophyte development, were identified in this region.

6.3 Genes Related to Heat and Drought Tolerance in Rice

Traditional methods of plant breeding were originally utilized by farmers and then reviewed and revised by plant breeders (Breseghello and Coelho 2013). Today plant breeders have developed modern breeding methods using an array of modern scientific tools. These new plant breeding methods include mutagenesis, and biotechnological methods (Lusser et al. 2011). Biotechnology uses biology tools to improve human life. The twentieth century has witnessed rapid advances in various biotechnological fields (such as microbial technology, genetic engineering, and in-vitro culture technology). In vitro culture, molecular genetics, and genetic engineering have a great deal of plant breeding (Peacock 2010). The major applications of molecular biology for rice breeding include gene cloning, molecular markers, and the use of other recently developed technologies (Singh et al. 2016a). Genetic engineering is the integration of foreign genes in the genome of living organisms. This technology is also called “recombinant DNA technology”. The production of transgenic plants has evolved as a new plant breeding method based on the potential of genetic engineering. Transformation involves the insertion of one or more desired genes into the genome of an arable crop. The advantage of this method is to cause the least disturbance in the recipient genotype (Godbey 2014). Theoretically, any gene can be transmitted through transformation. An important point in the transformation is the simultaneous transfer of the coding sequence of the gene and the gene promoter. Because promoters are the sequences determining the time and rate of expression of the coding genes. Resistance to abiotic stresses such as heat, drought, and salinity can be achieved through genetic engineering. In rice breeding, genetic engineering and transformation have helped to withstand drought stress and heat, which are discussed below.

6.3.1 Genes Related to Heat Tolerance in Rice

6.3.1.1 Heat Shock Proteins (Hsps)

Each biotic and abiotic stressor prompts a specific response. But in general, the main effect of biotic and abiotic stress is on the plant metabolism which is very essential for plant survival (Zhu 2016). Since the majority of the activity of the plant cells requires the presence of proteins and their direct or indirect effects, these stressors usually disrupt the plant’s intrinsic mechanisms by denaturing the proteins. Therefore, preserving proteins in natural conformation and preventing the accumulation of abnormal proteins, is important to protect cells, and at higher levels, plant protection against environmental stresses (Kordrostami and Rabiei 2019). One of the most important abiotic stress is temperature stress. Like other stresses, this stress can induce a group of defense mechanisms in plants. In fact, this type of response to stress (at the molecular level) is seen in all living organisms. Temporary changes in gene expression and production of proprietary proteins and the acquisition of high levels of tolerance or compatibility are the most important responses of plants to abiotic stresses (Mittler 2006). As the temperature rises, the expression of a new group of genes (heat shock genes) increases, whose role is to protect the cells by synthesizing heat shock proteins (Al-Whaibi 2011). Heat shock proteins under stress conditions are responsible for the folding of proteins, assembly, translocation and their degradation in many natural cellular processes (Park and Seo 2015). By folding proteins under stress conditions, they play a vital role in supporting plants under stressful environments and play a vital role in maintaining cell homeostasis (Efeoğlu 2009).

Katiyar-Agarwal et al. (2003) introduced an *Arabidopsis thaliana* hsp101 (*Athsp101*) cDNA into the Pusa basmati 1 cultivar of rice (*Oryza sativa* L.) by *Agrobacterium*-mediated transformation. They concluded that the integration of this gene had no adverse effect on plant growth and development. They also compared the transgenic plants with control plants under thermal stress. The results showed that transgenic plants had better growth under thermal stress conditions. They attributed this superiority to the expression of the *hsp101* gene in the transgenic plants. They also stated that the production of transgenic rice plants with heat stress tolerance would eventually increase the cultivation of rice in warmer arid areas. Qi et al. (2011) overexpressed *mtHsp70* and suppressed heat- and H_2O_2-induced programmed cell death (PCD) in rice protoplasts, as reflected by higher cell viability, reduced DNA laddering and chromatin condensation. They also observed that ROS levels in transformed cell protoplasts under thermal stress conditions were far lower than in control plants cells. They ultimately concluded that *mtHsp70* gene retains mitochondrial $\Delta\psi$ (m) and prevents the production of ROS to protect cells under thermal stress conditions. Murakami et al. (2004) in their study, isolated the *sHSP17.7* gene from the rice plants treated with the heat stress and then investigated the relationship between *sHSP17.7* gene expression and the viability of *E. coli* bacteria under thermal stress. Their results showed that *E. coli* bacteria containingthe *sHSP17.7* gene can tolerate better heat stress than the control ones. They stated that *sHSP17.7* can act as a chaperone and can protect the stressed catalase from precipitation. They also transformed the *sHSP17.7* gene to the Hoshinoyume rice cultivar using *Agrobacterium*. The comparison of growth parameters showed that the transgenic rice plants had significantly better growth compared to the control plants under heat stress conditions.

6.3.1.2 Heat Stress Transcription Factors (HSFs)

Heat stress transcription factors (HSFs) are considered to be signal transduction endpoints that mediate the activities of response genes to temperature stress and a number of other stresses (Guo et al. 2016). Compared to other eukaryotes, the HSF family of plants is more diverse (Soares-Cavalcanti et al. 2012). It is believed that these transcription factors, together with other transcription factors, constitute a complex regulatory network that is essential for the survival and development of plants under stressful environmental conditions (Guo et al. 2016). Yokotani et al. (2008) isolated the *OsHsfA2e* gene from rice and transformed *Arabidopsis* plants with the gene and exposed them to heat stress. They concluded that the integration of the gene had no adverse effect on plant growth and development. They also compared the transgenic plants with control plants under thermal stress. The results showed that the transgenic plants had better growth under thermal stress conditions. They concluded that under non-stress conditions, transgenic plants expressed a variety of *HSP*s. Also, the results of this study showed that the transgenic plants, in addition to thermal stress, had salinity stress resistance. They concluded that this gene is useful for future molecular studies of plants under changing environmental conditions.

6.3.1.3 Other Genes

Feng et al. (2007) overexpressed sedoheptulose-1,7-bisphosphatase (SBPase), the Calvin cycle enzyme in rice and concluded that the transgenic plants accumulated SBPase in their chloroplasts and that seedlings had better growth under thermal stress conditions. They also observed that the transgenic plants had better CO_2 assimilation compared with wild-type plants. Using chlorophyll fluorescence analyses, they concluded that the transgenic plants had better photosynthesis under high temperatures, which was related to the activation of SBPase rather than the functionality of photosystem II. They concluded that the transgenic plants overexpressing the SBPase, by inhibiting the activity of Rubiscoactivase, can maintain the activity of RuBisCO and enhance CO_2 assimilation under high-temperature stress. Rana et al. (2012) analyzed the expression of and functionality of the *OsHSBP1* and *OsHSBP2* genes, two important HSF binding proteins (HSBP) in rice. It should be mentioned that HSF binding proteins can affect the functionality of HSF by binding to them. They showed that under normal conditions, both genes were expressed in all tissues. Under heat stress conditions, the expression of these genes increased significantly in all the tissues. The results showed that these two genes are very important for seed development. El-Kereamy et al. (2012) analyzed the expression of and functionality of the *OsMYB55* transcription factor under heat stress conditions. They showed that the expression of the *OsMYB55* transcription factor under heat stress increased significantly and by overexpression of *OsMYB55* in the rice plants they observed better growth and development in seedlings under high-stress conditions. They also observed a significant increase in the expression of other genes specifically, the genes involved in amino acid biosynthesis in particular arginine, GABA and L-glutamic acid. They concluded that the *OsMYB55* transcription factor by linking to activators around the gene or the promoter region, activated the expression of genes including glutamine amidotransferase (GAT1), glutamine synthetase (*OsGS1*;2) and glutamate decarboxylase 3 (GAD3).

6.3.2 Genes for Drought Tolerance in Rice

There are several studies regarding the relationship between transcription factors and drought tolerance in rice. For example, Oh et al. (2009) studied the functionality of *OsAP37*, the AP2/ERF gene, under drought stress conditions. For this purpose, they made transgenic rice plants overexpressing the *OsAP37* transcription factor and evaluated the morphological traits of the studied plants at the vegetative stage. They also treated the studied plants with drought stress in the field and observed that the transgenic plants had a higher yield compared to the control plants. They concluded that the *OsAP37* transcription factor can increase the GY under drought stress conditions. Datta et al. (2012) and Ravikumar et al. (2014) analyzed DREB1A transcription factor in rice. In this study, the transgenic rice plants containing DREB1A transcription factor had a high drought tolerance compared to the controls. These transgenic plants had a higher yield and spikelet fertility and showed better vigor. Ambavaram et al. (2014) studied the functionality of HYR (HIGHER YIELD RICE), an AP2/ERF transcription factor which is related to the photosynthetic carbon metabolism under different environmental conditions. For this purpose, they made transgenic rice plants overexpressing the HYR transcription factor. They concluded that the expression of this gene enhances photosynthesis under different environmental conditions and by regulating cascades of transcription factors, photosynthesis genes, and other downstream genes enhanced grain yield under normal, high-temperature and drought stress conditions. Huang et al. (2018) characterize *OsDRAP1*, a DREB2-like gene, in rice under drought stress conditions. Their results showed that the *OsDRAP1* gene was expressed in all organs and can be induced by a variety of environmental stresses. Their results also showed that the transgenic plants overexpressing this gene were drought stress tolerant and maintained redox homeostasis, water balance, and vascular development under stress conditions. Their results also demonstrated that in the transgenic plants, *OsDRAP1* can interact with many genes/proteins and could activate many downstream DT related genes like the*OsCBSX3* transcription factor.

Saijo et al. (2000) studied the functionality of *OsCDPK7*, a calcium-dependent protein kinase (CDPK) gene, in rice under different treatments. To understand the physiological role of *OsCDPK7,* they produced transgenic rice plants with different expression levels. They observed that the level of tolerance to cold, salinity and drought stress was correlated with the level of *OsCDPK7* expression. They concluded that this gene is a positive regulator for salinity/drought tolerance. Xiang et al. (2007) studied the functionality of *OsCIPK01–OsCIPK30*, calcineurin B-like protein-interacting protein kinases (CIPKs) genes, in rice under different environmental conditions. The results of this study showed that among the studied genes, 20 OsCIPKs were induced differentially by at least one of the treatments including abscisic acid, polyethylene glycol, drought, cold and salinity. The results also showed that gene expression under salinity, drought, and abscisic acid treatments had the same pattern. But that the expression pattern differed for cold stress. For understanding the efficiency of these genes for drought and salinity stress tolerance, they made transgenic plants contained *OsCIPK03*, *OsCIPK12*, and *OsCIPK15* genes and treated them with cold, drought, and salt stress. They observed that the transgenic rice plants tolerate these stresses very well and that the transgenic plants had higher levels of soluble sugars and proline than wild-type plants. They concluded that calcineurin B-like protein-interacting protein kinases play an important role in stress tolerance in rice plants.

6.3.3 Genes for Combined Heat and Drought Tolerance in Rice

There are several studies of the relationship between transcription factors and combined heat and drought tolerance in rice. For instance, Wu et al. (2009) fused a *OsWRKY11*cDNA to the promoter of HSP101 and made transgenic rice plants overexpressing this transcription factor. They performed a heat pre-treatment and observed that the transgenic plants tolerated heat and drought stress and showed a higher survival rate of green parts of plants and slower leaf-wilting. They concluded that the *OsWRKY11* transcription factor plays an important role in combined heat and drought tolerance in rice plants.

Mutation breeding can also be used to create modified cultivars, haploid production, increase genetic diversity, overcome self-incompatibility and breed the rice plants adaptation to the environmental changes (Ahloowalia and Maluszynski, 2001). In rice breeding, mutations have helped plants to withstand drought and heat stress. For instance, Koh et al. (2007) made mutant rice plants with a mutation in *OsGSK1* gene and screened them under different environmental conditions. They observed that the mutant lines were cold, drought, salinity and heat tolerant. They concluded that *OsGSK1* gene can affect the physiology of the rice plants and make them tolerate adverse environmental conditions.

7 Conclusions and Future Perspectives

It is obvious that in near future rice plant will suffer more from the simultaneous occurrence of various abiotic and abiotic stresses under natural field conditions. Understanding the regulatory networks involved in combined stress responses may provide an option to manipulate plants conferring higher tolerance and yields. High temperatures and subsequent drought, greatly affects the production of important crops, including rice, at the global level. To deal with such stresses, plants have a variety of mechanisms altering their morphological, physiological, biochemical and molecular (gene expression) responses. Because molecular mechanisms are as important as morphological, physiological, biochemical mechanisms, identifying the genes responsive to thermal and drought stress is very important. Tolerant rice plants could be produced by increasing the expression of genes involved in drought and heat stress tolerance. Also, by transferring the genes from other tolerant plants to rice plants, rice plants that are high-yielding under severe environmental conditions could be produced. Limited research has been conducted to produce plants tolerant to combined heat and drought stress. Importantly, it is also difficult to create the combined stress conditions artificially in the field or in the lab. Therefore, application of high-throughput phenotyping and with modern genomic tools will lead to the development of combined heat and drought stress tolerance in rice. Mutation breeding can also be used to breed rice varieties adapted to combined heat and drought stress. New genomic and genome editing methods, including CRISPR-Cas9, could be used to identify new genes, their deliberate modifications, as well as the use of genes for developing heat and drought stress tolerance. Therefore, by combining conventional plant breeding and modern molecular techniques, breeders might enable to develop rice plant tolerant to combined drought and heat stress tolerance at various phases of plant growth, helping ensure food security.

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Rice Grain Quality Traits: Neglected or Less Addressed?

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Abstract

Rice (*Oryza sativa*) has been regarded as one of the oldest food crops, feeding more than half (55% approximately) of the entire world population. Seventy-five percent of the daily calorific demand is fulfilled by polished white rice in Asian population. Besides being easily digestible, rice also has a considerable amount of protein and a significant amount of minerals (magnesium, phosphorus and iron) and nutrients like niacin and thiamine. However, only a handful of rice varieties can be distinguished in terms of both yield and cooking quality traits and with significantly large areas of cultivation. Compared to the other field crops, from the economic point of view, postharvest processing, i.e. milling quality or grain quality of rice, actually determines the true market value. The manifestation of

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endless global warming and its allied climatic aberrations have caused various field crops to come across a wide range of stresses imposed by biotic and abiotic factors, either alone or in combinations. Such adverse conditions disturb the normal growth as well as yield potential of crop plants in various magnitudes. This chapter is intended to draw attention of the scientific community to look into the issues related to improving grain quality attributes.

Keywords

Rice · Grain Quality · Consumers · Stress · Yield

1 Introduction

Rice (*Oryza sativa*) has been regarded as one of the oldest field food crops. Rice has also the credit of being staple food, feeding more than half (55% approximately) of the entire world population and mainly consumed in a form known as polished white rice or PWR (Park et al. 2017). The findings of Park et al. (2017) further revealed that 75% of daily calorific demand is fulfilled by PWR in Asian people. According to an estimate (Anon 2016), 740 metric tons (MT) of rough rice (with husk) has been produced mainly in Asia and South Asian countries alone (FAOSTAT 2016). Statistical findings of FAO suggest that among 692,590,948T rice produced in Asia, India has produced 168,500,000T (24.32%), just trailing behind China. Juliano (1985), while explaining the nutritional features of rice, reported that, besides being easily digestible, rice also has a considerable amount of protein (mainly due to lysine) too. Along with these, a significant amount of minerals (magnesium, phosphorus and iron) and nutrients like niacin as well as thiamine can also be obtained from rice. Observations made by authors like Debnath et al. (2018) and Chauhan et al. (2017) indicate that approximately 100 million tons of milled rice is consumed by Indians residing in India per year. In India, the Indian Council of Agricultural Research (ICAR) has released more than 1200 rice varieties for a wide range of agroecosystems and with various quality preferences (http://icar-nrri.in/released-varieties/). However, only a handful of rice varieties can be distinguished in terms of both yield and cooking quality traits and with significantly large areas of cultivation. If we consider the "field to plate" scenario, rice milling quality traits are the most crucial factors that actually determine or modulate the economic aspect of rice, next to yield. From the economic perspective (local, international), rice cultivation generates two types of revenues: sale of unprocessed paddy rice creates producer's revenue, whereas production and sell of good-quality milled rice generates miller revenue. Compared to the other field crops, from the economic point of view, postharvest processing, i.e. milling quality or grain quality of rice, actually determines the true market value. The manifestation of endless global warming and its allied climatic aberrations has caused various field crops to come across a wide range of stresses imposed by biotic and abiotic factors either alone or in combinations. Such adverse conditions disturb the normal growth as well as yield potential of crop plants in various magnitudes (Suzuki et al. 2014; Pandey et al. 2015; Ramegowda and Senthil-Kumar 2015; Fahad et al. 2014). Here in this chapter, we would like to fetch attention of scientific community to look into the issues related to improving grain quality attributes.

1.1 Research Trends: Yield Was Prime Area of Interest

In the past half-century or so, hybrid vigour (heterosis) and semi-dwarfness are the two concepts devoted solely towards significant enhancement of harvest index (with enhanced grain production), but the qualitative aspects fetch very little or are neglected (Yuan 1997; Sasaki et al. 2002; Spielmeyer et al. 2002; Xing and Zhang 2010; Tian et al. 2009). Crop improvement schemes for developing new rice varieties were based on recognizing followed by crossing with a suitable variety(s) with desirable agronomic features. As a result of this conventional outlook few semi-dwarf varieties (with a desire trend such as better capacity to withstand a particular stress) gets adopted. Though these newly adopted varieties meets the demand of productivity but at the same time raises the production cost to some extent too. Besides conventional breeding, exploitation of genetic resources exists among different rice varieties that are also under genomic breeding programme. A considerable effort is thus directed towards identifying and characterizing various agronomical important traits. Having the smallest genome (~400 Mb, the diploid) among the cultivated cereal crops and first to be completely sequenced makes the breeders further interested in characterizing rice genome (Matsumoto et al. 2005). Climate resilience in rice production system has now become the prime focus area in rice research throughout the world. A significant amount of effort has been diverted towards understanding and characterizing the adverse effects and identifying the response of rice plant and subsequently incorporating it (Lobell and Field 2007; Schlenker and Roberts 2009; Lobell et al. 2011; Xiong et al. 2014) as well as preparing for the upcoming intensities of climate changes. In order to propose a particular mitigation plan for climate change, one has to standardize a large number of factors, besides the varietal response, agronomic practice, soil characteristics, irrigation water profile and so on. As the rice agroecosystem has been influenced by various factors, devising a particular and generalized plan is close to impossible. It is evident from the short sketch presented above that the main goal of rice researchers is to ensure better yield with minimum attention towards grain quality attributes.

1.2 Grain Quality Attributes: Why So Complex?

In the postharvest phase, rice reaches to the consumers or the buyer's hand as a milled rice either as brown rice, semi-polished rice or white polished rice. The grain quality attributes are found to be governed by various QTL's (quantitative trait loci) and are found to be modulated by environmental factors or stress (s). Speaking of rice grain quality traits from consumer's perspective, rice varieties are categorized into six different criteria or grades. Among these six criteria, the proportion of head rice to brewer's rice or intact to broken rice, chalky grains are considered as the most important. It is believed that the broken rice or the brewer's rice tampers the visual appearance of rice grain. During the course of milling process, the breakage appears through the cells; as a result, starch leaks through the broken part while cooking (Swamy and Bhattacharya 1982). The leaked starch thus forms a "glue" which surrounds the cooked rice and makes it stickier which is usually stuck at the bottom of cooking vessel and difficult to clean away. Generally, milled rice with maximum head rice and minimum chalky grain is preferred by the consumers, but they share an inverse relationship among them (Zhao and Fitzgerald 2013, USDA). Besides HRR% and chalkiness of rice grain, grain morphology is another important physical grain quality trait. Grain morphology or shape is defined by length-to-width ratio. Based on length, width rice grain can be divided into three classes: slender type (>3), medium type (2.1–3) and bold type (<2). Consumers while selecting a particular variety for consumption usually have their own preference of grain shape or size that is again a very confusing aspect and varies from region to region, socioeconomic aspect, previous experience and so on. Calingacion et al. (2014) reported that major rice-producing (producing and exporting) and rice-consuming (including importing) countries have different standards to define grain shape and own preference. In short, this variable preference regarding grain shape or morphology makes the entire business very difficult to standardize. During the course of selecting a particular variety, colour of milled rice fetches equal importance (Monsoor et al. 2004; Bergman et al. 2004; Fitzgerald and Reinke 2006). Yellowness index is used to classify milled rice based on colour of grain. It has been observed that consumers prefer white milled rice over yellow-type grain (Bergman et al. 2004). The reason behind colouration of milled rice can be attributed to inadequate storing practice (at high temperature), accumulation of 2-nonenal, fatty acid oxidation, etc. (Swamy et al. 1971; Tran et al. 2005; Lam and Proctor 2003). Authors like Champagne et al. (2009) and Matsue et al. (1994) were having view that protein content in grain has a noteworthy influence on the development of colouration, and they further added that higher protein content has an undesired trait as it influences aromatic characteristics of grain too. Amylopectin and amylose are two key components of rice starch. These two components influence many chemical parameters of rice grain quality such as pasting viscosity, gelatinization temperature (GT), amylose content (AAC), gel consistency and texture of cooked rice (Bao 2012). Among the abovementioned parameters, AAC is the most important factor that determines the eating and cooking property of rice (Tian et al. 2009). Based on AAC, rice varieties can be grouped into low (12 ± 20%; these are soft with glossy texture), intermediate (20 ± 25%; most preferable) and high (>25%; upon cooking, the cooked rice gets dry, wrinkled and hard) (Hossaina et al. 2009). Though AAC has a significant role in determining cooking and eating property of rice, other parameters such as peak viscosity, breakdown viscosity and setback viscosity also have a crucial role (Jiranuntakul et al. 2011; Han and Hamaker 2001).

Enhancement in rice grain quality attributes is considered as most complex task to execute. The grain quality attributes are found to be governed by various QTL's (quantitative trait loci) and are found to be modulated by environmental factors or stress (s). In spite of investing considerable efforts, unfortunately only few relevant genes (*OsSPL16, Chalk5*, etc.*)* were identified that influence grain quality traits. Any adverse condition or stress (biotic/abiotic or both) at any particular growth stage of the rice plant has the capability to alter its yield potential and quality traits, which later may influence the value creation associated with rice. During varietal development and subsequent field trial, evaluation and finally release of new varieties are two major criteria a breeder or an organization has to fulfil, first yield and second the quality attributes. The new variety has a strong and significant interrelationship among the farmer's need and consumer's preference. Now being a farmer, the prime concern for the adopted or selected variety is its yield potential because at the end of cropping season how much (quintal or so) he can produce. But for a consumer the main factor for choosing a particular variety is its grain quality attributes that keeps him purchasing again and again from the market (Webb 1991; Cardello 1995; Moskowitz 1995; Grunert 2005). The complexity with developing new rice variety gets intense with the factor called "consumer's preference" which cannot be ignored. Moreover, comparing to the local land races or indigenous rice varieties, the high-yielding or hybrid varieties have significantly greater yield, but they (high-yielding varieties) are found to be trailing behind in terms of quality traits. There is no doubt that besides growth and yield, grain quality attributes are also prone to agro-environmental fluctuations.

2 High Day and Night Temperature

Generally, upon encounter with various abiotic stresses, crops were found to shuffle their metabolic and molecular machinery to cope up with the odds that resulted in significant yield and quality alternation. These abiotic stresses affect the productivity of crops by triggering a series of physiological, biochemical, morphological and molecular fluctuations (Kaur et al. 2008; Fahad et al. 2015a, b). Recent evidences suggest that various abiotic stresses have a pronounced role in modulating various grain quality attributes in rice, such as elevated day and night temperature, and comparison among them was reported by Fahad et al. (2016) and Li et al. (2011). Authors like Lanning et al. (2011), Lanning et al. (2012) and Lanning and Siebenmorgen (2013) reported about the consequences of extreme night time temperature on rice quality traits, physicochemical properties and whiteness of head rice, respectively. Literature currently available indicates that various stresses in different magnitudes can alter physicochemical parameters of milling or grain quality attributes (Wassmann et al. 2009). In this regard, we can consider the findings of Counce et al. (2005), Cooper et al. (2008a, b) and Lanning et al. (2011), which suggest that elevated temperature can modulate grain quality attributes (quantitatively as well as qualitatively). Chun et al. (2009) reported about the impacts of temperature extreme on different aspects of grain quality attributes (chalkiness, cooking characteristics) along with organoleptic features.

As a result of increase in day/night temperature beyond the critical levels during flowering and post-flowering period, some grain quality parameters like amylose content (Ac), gel consistency of rice flour (GC) and test weight are reduced. The reduction in photosynthesis of flag leaf and subsequent alternation in source and sink assimilate transport can be considered as possible reason behind the modulation of grain quality traits (Lin et al. 2010). Findings of Jagadish et al. (2010) and Lyman et al. (2013) indicate that as a result of elevation in day/night temperature, not only the seed-setting rate is decreased but decrease in endosperm sink rate and inadequate grain filling are observed. According to their opinion, the modulation in expression of genes that involved starch metabolism under high temperature stress can be attributed to the deterioration in grain quality traits. Findings of Wang et al. (2008) suggest that, in the wild rice varieties, the *GIF1* or *GRAIN INCOMPLETE FILLING 1* gene has broader impact on increasing the chalkiness and reducing grain weight. During the sucrose synthase mediated sucrose cleavage (sucrose into hexoses) the greater demand of energy (as ATP) by *GIF1* gene and thus become a crucial step in sink process. This GIF1 gene become a vital factor to ensure optimum storage and maintaining grain quality traits. Due to abnormal or altered expression of GIF1 gene, it often resulted into production of smaller grain. In order to trace the reasons behind the appearance of chalky grain in rice variety under elevated temperature regime, Yamakawa et al. (2007) and Liu et al. (2010) reported that under temperature stress enhancement of starch-degrading enzymes (encoded by alpha amylase gene) and reduction of enzymes are involved in starch biosynthesis (encoded by GBSSI or granule-bound starch synthase I, BEIIb or branching enzyme IIb gene, orthophosphate dikinase gene, etc.). Singletary et al. (1997) found that elevated temperature shares a correlation between decreased activity of ADP-glucose pyrophosphorylase and starch synthase enzymes along with reduced grain weight. Further, in order to elaborate the adverse effects of elevated temperature the occurrence of chalkiness due to alternation in starch structure and reduced AC content along with variable GC content in rice grains have been observed (Chen et al. 2008; Lanning et al. 2012; Yamakawa and Hakata 2010; Patindol et al. 2014). Patindol and Wang (2003) reported that alterations in starch structure primarily due to a reduction in AC lead to variation between translucent and chalky grains. According to the view of Hakata et al. (2012), α-amylase which is involved in starch metabolism process (starch hydrolysing) is responsible for the chalkiness. The occurrence of many vacuums within the roughly filled starch granules in the chalky grain foils the transmission of light, and thus the opaque region appears in translucent grains except in glutinous rice varieties (Ashida et al. 2009; Juliano 2007). Authors like Lin et al. (2010), Li et al. (2011), Yamakawa et al. (2008) and Peng et al. (2014) from their transcriptomic analysis and QTL browsing concluded that (1) accumulation of storage proteins (globulins, prolamins) was prone to high temperature stress when compared with other similar proteins. These proteins (often located in the promoter site) somehow favour the expression of other genes like abscisic acid response element and heat stress-responsive element which influence the formation of chalkiness in grain at maturity. Larkin and Park (2003) and Larkin et al. (2003) view that the downregulation in the expression pattern of Wx protein is associated with variable starch viscosity pattern and lower AC and thus alters the grain quality traits under high temperature stress condition. Head rice recovery percentage or HRR% is a complex grain quality trait governed by multiple genic trait. This HRR% has been controlled by the triploid endosperm as well as by diploid maternal tissues (Pooni et al. 1992; Zhu and Weir 1994). Speaking of QTLs and HRR%, i.e. milling quality traits, it has been observed that HRR% is controlled by a large number of

QTLS. Moreover, grain morphology (shape and size) also shares a significant relationship with HRR% (Kepiro et al. 2008; Yuan et al. 2010; Nelson et al. 2011). Gravois et al. (1991) reported that how a particular genotype (rice variety) interacts with the environment can also determine the milling traits in postharvest phase. Agro-industries specially dealing with rice are of the opinion that the global warming has its significant adverse impact (with increase in temperature) correlated with reduction in HRR% during commercial milling process (Custodio et al. 2016; Zohoun et al. 2018). Besides these, variable consumer preference and agronomic practice favour the production of long and slender-type aromatic grains in dry season. But elevated day/night temperature causes increase in brewer's rice or lesser HRR%. Custodio et al. (2016) further added that traditional postharvest process such as parboiling of paddy rice is a widely accepted process to enhance HRR%.

2.1 Salinity Stress

Salinity is another important condition which can strongly affect grain quality (Chen et al. 2012; Paul and Roychoudhury 2019). The effect of salinity on the rice grain is determined by complex interactions of several factors including the severity of salinity, timing and the duration of salt stress (Thitisaksakul et al. 2015). Grain physical appearance and dimensions are important indicators for marketability of rice. Researchers reported that salt stress may reduce the kernel milling, cooking and sensory properties (Thitisaksakul et al. 2015). These may be due to reduction in cell numbers of the lemma and palea because the size of the outer glume greatly restricts the dimension of dehulled rice endosperms (Shomura et al. 2008). Fabre et al. (2005) also found that high salinity levels were associated with an increased fraction of unfilled spikelets and reduced grain dimensions and weight. Thus, salinity stress ultimately reduced the head rice recovery and market value of milled rice (Rao et al. 2013).

Abdullah et al. (2001) observed the negative impact of salinity stress on starch synthetase activity (α-1-4-glucanglucosyle transferases) in developing rice grains. They found that crop grown at higher salinity stress (50 mM NaCl) recorded significantly reduced starch synthetase activity in rice grains as compared to the grains obtained from the plants with no salt stress. Starch synthetase is the first enzyme in the pathway of the starch metabolism which is responsible for transfer of a glucose moiety from ADP-G to the starch primer. Salinity stress is also responsible for reduction in the number of filled spikelets due to a significant reduction in starch-condensing enzyme activity (Abdullah et al. 2001). On the other hand, Thitisaksakul et al. (2015) found no significant effect of salt stress on reducing sugar content in grain.

Interestingly, salt stress may increase some of the nutritional qualities of rice grains, i.e. total phenolic content, protein, anthocyanins, proanthocyanidins and antioxidant activities (Safitri et al. 2016; Thitisaksakul et al. 2015). Saeedpour (2014) observed that salt stress may promote the sugar accumulation in the grains of salt-tolerant rice cultivars. They also concluded that the application of exogenous abscisic acid can improve sugar accumulation under severe saline condition. Another study conducted by Siscar-Lee et al. (1990) reported that grains of both saline-tolerant and saline-susceptible cultivars grown on saline soils have higher storage protein content but less

Table 1 Summary of rice grain quality parameter as influenced by salt stress

	Effect of salt stress			
Parameters	Improved	No effect	Reduced	References
A. Physical properties				
1. Kernel size			●	Thitisaksakul et al. (2015)
2. Grain weight			●	Fabre et al. (2005)
3. Cooking and sensory properties			●	Thitisaksakul et al. (2015)
4. Alkali spreading value		●		Siscar-Lee et al. (1990)
5. Gel consistency		●		Siscar-Lee et al. (1990)
6. Head rice recovery			●	Rao et al. (2013)
B. Biochemical properties				
1.Starch synthetase activity			●	Abdullah et al. (2001)
2. Reducing sugar content		●		Thitisaksakul et al. (2015)
3. Total sugar content	●			Saeedpour (2014)
3. Total phenolic content	●			Safitri et al. (2016)
4. Grain protein	●			Thitisaksakul et al. (2015) Siscar-Lee et al. (1990)
5. Anthocyanins	●			Safitri et al. (2016)
6. Antioxidant activities	●			Safitri et al. (2016)
7. Amylose content		●	●	Juliano and El-Shirbeeny (1981)
8. Starch			●	Siscar-Lee et al. (1990)

translucent grain and lower starch and amylose content than grown on normal soil. Thus, these differences were not related to salinity tolerance (Siscar-Lee et al. 1990). In contrary to Thitisaksakul et al. (2015) and Siscar-Lee et al. (1990), Juliano and El-Shirbeeny (1981) reported that increased level of salinity may significantly reduced the protein content of cultivars but had no effect on amylose content and the alkali spreading value. These findings are also supported by Siscar-Lee et al. (1990). But Rao et al. (2013) observed the ill impact of salinity on amylose content and gel consistency of rice grain obtained from the plants grown in high-saline soil (Table 1).

2.2 Arsenic Stress

Arsenic (As) has been considered as well-known stressor in soil-water-rice agroecosystem throughout the world. Arsenic compounds can be classified into three distinct classes such as (1) inorganic (sulphides, arsenides, halides, pentoxide and trioxide salts of arsenous acid and arsenic acid), (2) organic (methylated and other such derivatives) and (3) vapour/gaseous (arsine gas) (WHO 2000; Grund et al. 2008). In rice agroecosystem, As in soil mainly exists as either As^{3+} (arsenate) or As^{5+} (arsenate) with no to negligible amount of organic As species, depending on soil and its microbial profile and acidity of soil (Moulick et al. 2019a). Besides soil, irrigation with groundwater is another important source of As in rice field. Irrigation paddy field with groundwater having As concentration beyond the permissible limit in "as-hot spot" area of the world intensifies the As accumulation-induced toxicity in rice plant as well as accumulation in grain (Santra et al. 2013; Moulick et al. 2016a; Moulick et al. 2019a, b). Adverse effects of As on rice agroecosystem have been documented regularly with phytotoxic effects on every growth phase and others. Among the phytotoxic consequences of As on rice plant in both soilless and soil-based assays, (a) inhibition of germination and seedling growth (Moulick et al. 2016b; Moulick et al. 2017) (b) reduced other essential mineral accumulation and translocation in seedlings (Moulick et al. 2018c), (c) inhibited growth and yield and facilitated greater As accumulation in grain (Moulick et al. 2018a; Moulick et al. 2018b). Scientific professionals from a wide range of backgrounds have devoted their attention to minimize the adverse effects of As on rice plants since it is recognized as toxic to humans and rice plants (Saha et al. 2019, Moulick et al. 2019a).

If we carefully noticed the research trend on phytotoxic consequences of As on soil-water-rice agroecosystem, then three different trends emerge: (1) documentation or biomonitoring of As content in components of rice agroecosystem including grain and cooked rice throughout the world (Mandal et al. 2019; Upadhyay et al. 2019; Wang et al. 2019; Oteiza et al. 2019); (2) phytotoxicity and interactive aspect with various other agents under controlled, i.e. laboratory, condition (Saha et al. 2019); and (3) field-based trials of various mitigation options. Interestingly, here also documentation(s) of As stress on grain quality attribute are almost negligible. Majority of focus fetches by the investigators looking for (a) less As accumulating rice varieties and in (b) developing suitable mitigation options along with (c) constant biomonitoring of the actual scenario. Moulick et al. (2016a) and Moulick et al. (2018d) reported that As stress can modulate selected grain quality attributes in the studied rice varieties. When the grain quality attributes of two popular rice varieties (Swarna and Minikit) cultivated in three As-contaminated districts of West Bengal (in Lower Indo-Gangetic Plain) were compared with the same varieties cultivated in As-free zone, noteworthy differences were found. Among the selected parameters, 1000 kernel weight, HRR%, AC, gel consistency value, water uptake ratio and minimum cooking time were found to have a significant correlation with As content in the milled rice. The authors further found that As content in grain or milled rice negatively influenced 1000 kernel weight, HRR%, gel consistency value and water uptake ratio, whereas it positively influenced AC (actually increases) and minimum cooking time (Moulick et al. 2016a). Moulick et al. (2018a, b, c, d) surveyed multiple As-contaminated sites and analysed the relationship between grain quality traits and grain As content. The authors found that compared to the local landraces (rice varieties), the high-yielding varieties accumulate greater amount of As and the grain As content significantly modulates the grain quality attributes.

2.3 Drought Stress

The yield is the ultimate purpose of growing crops; rice grain yield severely reduces under drought stress condition and reduction in grain size, weight (Venuprasad et al.

2007), seed-setting rate and 1000-grain weight (Ji et al. 2012). Drought stress at vegetative growth especially booting stage (Pantuwan et al. 2002), flowering and terminal periods can interrupt floret initiation, causing spikelet sterility and slow grain filling, resulting in lower grain weight and ultimately poor paddy yield (BotwrightAcuña et al. 2008). Drought stress reduces grain yield probably by shortening the grain-filling period (Shahryari et al. 2008), disrupting leaf gas exchange properties, limiting the size of the source and sink tissues, impaired phloem loading and assimilate translocation (Farooq et al. 2009). The amount of grain yield loss depends on the duration of drought, the stage of crop growth (Gana 2011) and the severity of drought stress (Kumar et al. 2014). The effect of drought stress on grain yield attributes was changes in the number of tiller plant^{-1}, number of fertile tiller plant^{-1}, spike length, and number of spikelet spike^{-1}. The grain size is an important quality trait in rice trade with different preferences among consumers (Fan et al. 2006). The study was conducted to ascertain the harmful effects of drought stress on grain quality traits in rice. The result of analysis of variance clearly indicates that genotypes significantly differ in grain quality traits under both normal and drought-prone environments.

The grain width was highly correlated with yield per plant under drought stress condition, while grain breadth was positively correlated only under normal condition. It shows that reduction in grain width has direct effect on grain yield. Drought stress is very crucial to identify how quality is affected when rice crop is under drought stress. In drought stress, grain length was positively correlated with grain width and positively correlated with grain length and width ratio. Under drought stress condition, grain width showed positive correlation with yield per plant that shows that reduction in grain width has direct effect on grain yield, while grain length and breadth were nonsignificantly correlated with yield per plant under drought stress condition. Direct effect of grain breadth on yield per plant was negative, and higher loss was observed in drought stress. Drought stress has a negative impact on gas exchange attributes, yield and yield components and grain quality. The drought stress delays the time of flowering condition leading to a reduced number of panicles and number of kernels and ultimately loss of grain yield (Kumar et al. 2009; Iseki et al. 2014). The drought-tolerant genotypes can save water and can resist a reduction in rice yield under conditions of water scarcity (Luo, 2010). Grain quality is important for the acceptance and adaptation of any new cultivar by farmers and consumers (Cooper et al. 2008a, b). In the Indian subcontinent, people prefer medium- to long-grain rice. In drought stress conditions reduction of rice grain length-to-width ratio of tolerant as well as sensitive rice cultivars (Rao et al. 2013).

Drought stress significantly reduced grain and straw yields as well as the number of panicle plant^{-1} of different rice genotypes. Under drought stress, the genotypes showed the highest and the lowest number of panicle plant^{-1}. The genotype significantly differed in panicle plant^{-1} from all the other genotypes, whereas the genotype was statistically at par with the genotype. Genotypic variation was also observed with regard to straw and grain yields. Drought stress caused a significant reduction in grain length and width of all the rice genotypes. Drought stress has a drastic effect on rice as it caused reductions in gas exchange attributes, yield and yield components and quality of rice grains. In the present study, 11 rice genotypes were exposed to drought stress which was applied at reproductive stage by withdrawing irrigation.

There was a significant reduction in photosynthetic and transpiration rates and stomatal conductance of the rice genotypes due to the drought stress in comparison to the well-irrigated control. The prolonged severe drought stresses at grain-filling stage cause grain yield loss (Pantuwan et al. 2002). Reduction in grain yield under drought stress could be due to increased spikelet sterility that reduces fertile panicles and loss of grain weight (Yue et al. 2006). Drought affects crop productivity when soil water content becomes insufficient to satisfy crop requirements and thus impede crop yield (Serraj et al. 2011). Drought stress negatively affects crop yield of any developmental stage, for example, the vegetative stage hinders plant development by limiting photosynthetic activities and unbalancing the metabolites (Okami et al. 2015). Drought stress at the reproductive stage is detrimental to rice reproductive organs and limits grain yield and yield component traits (Zu et al. 2017). It inhibits anther dehiscence, pollen shedding, pollen germination on stigma and fertilization, as well as pollen tube growth and panicle exertion (Zhang et al. 2018), causing high spikelet sterility (Serraj et al. 2011). The drought stress affects the physical, nutritional and cooking qualities of the grain, such as protein content, amylose content and starch contents, and the number of panicles per plant was significantly reduced by drought treatment at seedling stage or at combined drought treatments at seedling and tillering stages and high temperatures. This was a result of low tillering capacity and failure of panicle initiation during drought at vegetative stages, which led to a grain yield reduction. The various results confirm previous findings of panicle and spikelet development failure under high drought stress; the number of panicles per plant is an important yield attribute which was hampered through drought stress (Rajeswari and Nadarajan, 2004).

3 Conclusion

From the above discussion, we may conclude that there is an urgent shift or reshuffle required by various scientific and funding organizations (local to international) to put due emphasis on rice grain quality attributes besides ongoing priorities. If adequate steps are not encouraged or taken keeping in mind about the importance of rice grain quality attributes, one day we might achieve the greater rice yield to feed the ever-increasing population of the world, but we will come across about the details of rice grain quality attributes in scientific literatures only!

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Rice Grain Quality and Abiotic Stress: Genomics and Biotechnological Perspectives

Aditya Banerjee and Aryadeep Roychoudhury

Abstract

Rice is the staple food crop of a large number of countries across the world. Asia is the largest producer of rice grains. Due to increasing global warming, large portions of rice fields are now inflicted with abiotic stress factors like high temperature, drought, salinity and nutrient deficiency. As a result, normal reproduction is impaired and the production of rice grains is affected. The overall food security is severely threatened. The use of genomics and biotechnological markers like quantitative trait loci (QTLs) are now gradually being popularised to increase rice production during abiotic stress. This chapter concisely illustrates the recent genomic approaches used for developing stress-tolerant green super rice (GSR) varieties with higher potential of grain production under stress conditions. The development of pure lines harnessing the novel QTLs has also been found to be beneficial in grain development under suboptimal conditions.

Keywords

Rice · Grain · Genomics · Abiotic stress · Quantitative trait loci

1 Introduction

Rice is the staple food crop of several countries across the world, especially in Southeast Asia. India and China are the largest producers of rice grains (Banerjee and Roychoudhury 2019a, b). The consumption of rice and its demand in the national and international market also depends on its quality, texture, grain size, length and even flavour (Banerjee et al. 2019). The prolonged problem of global warming has increased the advent of multiple abiotic stresses in rice agriculture.

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These environmental stresses include salinity, drought, temperature, nutrient deficiency, etc. (Banerjee and Roychoudhury 2017; Banerjee et al. 2017). Abiotic stresses impose suboptimal conditions for rice seedling growth and panicle development and hence directly affect grain formation and production vigour. The stress cues negatively regulate the physiological and molecular processes in rice and significantly reduce the crop yield (Banerjee and Roychoudhury 2019c, d, e, f). Rice being a glycophyte succumbs to such molecular and biochemical fluctuations induced by extreme stress cues.

The population across the world is growing at a rapid pace. It is expected that in Asia, the human population will increase from 4.3 to 5.2 billion by 2050. Since a majority of the population in this continent largely depends on rice grains as the only staple and nutrition-deriving food, hence the production and quality of rice grains need to be steadily maintained even in the face of abiotic stress-mediated challenges (Banerjee and Roychoudhury 2018a, b, c, d, e, f). This chapter concisely discusses the genomics and biotechnological perspectives of the effects of abiotic stress in rice grain development and resulting deteriorations.

2 Effects of Environmental Stressors on Rice Grain Development

Reproduction is one of the most vulnerable stages of plant development since extreme abiotic stress conditions effectively impose sterility in the reproductive tissues. As a result of steady decrease in rice grain production, the overall yield is compromised and food economy is also affected (Welch et al. 2010). It has been observed that grain chalkiness, kernel development, fissuring, protein and amylose content are all negatively affected when rice plants are exposed to high temperature stress (Wassmann et al. 2009). Turnbull et al. (2002) reported that increased temperature inhibited the rate of photosynthesis and respiration in the leaves leading to compromised biomass generation. Dong et al. (2014) showed that elevated temperature in the post-anthesis phase reduced rice biomass and yield and also established a negative impact on the grain appearance and quality. This is possibly due to the loss in pollen viability and promotion of spikelet sterility (Rang et al. 2011). Paul and Roychoudhury (2019) presented a comparative analysis of grain development in two indica rice cultivars IR-64 (salt susceptible) and Nonabokra (salt tolerant) exposed to 250 mM NaCl-mediated salt stress. It was found that the agronomic parameters were more negatively affected in the developing grains of IR-64 compared to that of Nonabokra (Paul and Roychoudhury 2019). The authors also proposed a positive correlation between the abscisic acid (ABA)-inducible transcription factor-encoding genes and the yield-related genes associated with grain filling (Paul and Roychoudhury 2019).

Sugar metabolism and starch biosynthesis play a crucial role in the developing grains during panicle filling (Fahad et al. 2019). High temperature stress suppressed the starch biosynthetic genes like *granule-bound starch synthase* (*GBSS*), *starch-branching enzyme I* (*SBE I*) and *SBE IIb* in rice grains (Fahad et al. 2019). The seeds produced by these stressed plants contained small granules with reduced amylose and amylopectin and mimicked the related phenotype represented by the *floury-endosperm* (*flo*) mutants (Yamakawa et al. 2007; She et al. 2010). Peng et al. (2014) showed that the *Flo6* gene encoded a protein which contained a carbohydrate-binding domain in the C-terminal. The protein was found to bind with starch moieties and regulate the physicochemical properties of multifaceted synthesis of starch granules (Fahad et al. 2019). The starch biosynthesis and number of spikelets per panicle were drastically reduced in rice seedlings exposed to salt stress (Abdullah et al. 2001; Zeeman et al. 2010). The salt-tolerant cultivar, Pokkali, was found to maintain efficient starch metabolism in the grains during salt stress (Theerawitaya et al. 2012). The grains also contained a high level of soluble sugar which acted as compatible solutes in the cytoplasm during Na^+-mediated toxicity (Siringam et al. 2012).

3 Study of the Effects of Abiotic Stress on Rice Grain Development Through Genomics Approach

The use of genomics to study the effects of abiotic stress on grain development and yield in rice has not been well characterized. Some recent reports indicate at the regulation of abiotic stress responses in rice grains by specific quantitative trait loci (QTLs). Catolos et al. (2017) performed a cross between the drought-tolerant (DT) cultivar Dular and drought-susceptible variety IR-64. It was revealed that three major drought-tolerant (DT) QTLs, viz. $qDTY_{1.1}$, $qDTY_{1.3}$ and $qDTY_{8.1}$, were responsible for proper reproduction and grain yield during drought stress (Catolos et al. 2017). The $qDTY_{1.1}$ QTL was also reported to be a 'hotspot for grain yield and yield-related agronomic and root traits' (Catolos et al. 2017). Feng et al. (2018) used gene pyramiding of two DT and two low-nitrogen-tolerance (LNT) QTLs to develop green super rice (GSR) variety exhibiting tolerance to drought stress and low-nitrogen conditions. The two DT QTLs were $qDT_{3.9}$ and $qDT_{6.3}$. The two LNT QTLs were *qGY1* and *qSF8*. The authors inferred that designed QTL pyramiding (DQP) has enormous potential in molecular rice breeding of complex quantitative traits since the genetically bred rice varieties showed improved yield under suboptimal conditions (Feng et al. 2018). Dixit et al. (2014) identified QTLs responsible for generating both drought and blast tolerance in rice by crossing the high-yielding and blast- and drought-sensitive indica rice variety Swarna and the blast- and drought-tolerant japonica cultivar Moroberekan. The researchers were able to identify QTLs governing grain yield. It was concluded that the development of pure lines containing these grain yield-associated QTLs would help in increasing the crop yield even under drought stress or blast infection (Dixit et al. 2014). QTL pyramiding was also used to develop GSR varieties with improved grain yield during salinity stress (Pang et al. 2017). The researchers analysed the single-nucleotide polymorphism (SNP) data from 3K RGP and mapped three QTLs associated with increased grain production and tolerance against salinity stress (Pang et al. 2017). Kumar et al. (2018) developed a marker-assisted selection strategy for QTL pyramiding of yield-related traits in rice. The authors observed high yield during stress in '$Sub1 + qDTY_{1.1} + qDTY_{2.1} + qDTY_{3.1}$ and $Sub1 + qDTY_{2.1} + qDTY_{3.1}$ in Swarna-Sub1, $Sub1 + qDTY_{1.1} + qDTY_{1.2}$, $Sub1 + qDTY_{1.1} + qDTY_{2.2}$ and $Sub1 + qDTY_{2.2} + qDTY_{12.1}$ in IR64-Sub1, $qDTY_{2.2} + qDTY_{4.1}$ in Samba Mahsuri, $Sub1 + qDTY_{3.1} + qDTY_{6.1} + qDTY_{6.2}$ and $Sub1 + qDTY_{6.1} + qDTY_{6.2}$ in TDK1-Sub1 and $qDTY_{12.1} + qDTY_{3.1}$ and $qDTY_{2.2} + qDTY_{3.1}$ in MR219' (Kumar et al. 2018). Yadav et al. (2019) performed multi-season NIL phenotyping and QTL mapping to improve grain yield and drought tolerance.

4 Conclusion and Future Perspectives

Rice is the staple food crop of a large number of countries across the world. It is a prime source of nutrition for a large proportion of the global population. It is a water-intensive crop and is widely grown across various ecosystems. According to the International Rice Research Institute (IRRI), 690 million metric tonnes of rice grains was recovered from rice plants planted across 160 million hectares of land in Asia in 2011. It has also been surveyed that close to 50% of the fresh water in Asia is diverted for irrigation of the rice fields (Barker et al. 1998). Thus, rice production regulates a significantly large portion of the food economy in Asian countries. Abiotic stresses like temperature, drought, salinity, etc., inhibit optimum growth and physiological development of rice seedlings with steady deterioration of yield. The reproductive stages like panicle development and grain filling are inhibited and compromised. As a result, the overall production of rice grains is affected, and the food security of a large population is threatened. The use of genomics and QTL-based approaches have helped in identifying the genes and loci responsible for dictating tolerance in rice plants under abiotic stress conditions. Introgression of these newly identified molecular candidates can improve rice physiological growth under suboptimal conditions and also stimulate reproductive development and grain production. However, the field of genomics and biotechnology in assessing rice grain quality during abiotic stress is naive and requires a lot more extensive genome-wide association studies. Exhaustive experiments involving next-generation sequencing platforms can lead to the identification of more new candidate genes in grains which might be responsible for regulating grain filling under stress situations. The epigenetic regulation of stress during grain development and grain quality improvement is also unknown. Future studies might also involve the utilisation of epigenomics in identifying such epigenetic mechanism at the global scale.

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Increasing Rice Grain Yield Under Abiotic Stresses: Mutagenesis, Genomics and Transgenic Approaches

Aamir Raina, Samiullah Khan, Parmeshwar K. Sahu, and Richa Sao

Abstract

Rice is a main source of food to millions of people across the world, and hence increase in its production/yield is vital to feed the rapidly growing population. However, the yield of rice is decreased to a large extent due to the adverse effects of several kinds of abiotic stresses such as drought, salinity, heat stresses, etc. Different traditional and modern breeding approaches have been used to mitigate the damaging effects of different abiotic stresses on the rice production. Traditional breeding strategies such as hybridisation and selection have resulted in the development of few stress-tolerant varieties, but such strategies are laborious and time-consuming. The modern biotechnological approaches such as mutagenesis, transgenics and genomics have been effective in the identification, cloning and characterisation of genes that govern tolerance to different abiotic stresses. Insertion of such genes into the rice plants have decreased the yield loss caused by various abiotic stresses. Modern biotechnological tools have brought landmark achievements by developing varieties with enhanced tolerance to various abiotic stresses. The role of mutagenesis, genomics and transgenic approaches in the creation of rice varieties with improved yield under different abiotic stress has been reviewed in this chapter.

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1 Introduction

Oryza sativa L. is commonly known as rice with chromosome complement $2n = 2x = 24$, a member of Poaceae family with a huge diversity comprising of more than 40,000 cultivated varieties. Rice is an ancient staple food with the origin of centre in southern and south-western tropical Asia and origin of domestication in India and China (Vavilov 1926). *Oryza sativa* is the main species of rice cultivated across the world. To date, 23 species and ten kinds of genomes of rice including AA, BB, CC, BBCC, CCDD, EE, FF, GG, HHJJ and HHKK have been reported (gramene.org). About one in three persons on Earth consumes rice, and it is believed that more than 50% of total human population is dependent on rice for their food requirements. According to FAO forecasts, the food production must be double to feed the rapidly growing human population which is likely to rise to nine billion in 2050. In addition to sky high population; shrinking arable land, depleating natural resources, erratic rainfalls and drastic climate change further reduces the rice production. This will create a huge demand on the food production and will exceed the food supply by a greater mark. Further, the overall yield of rice is sternly reduced by several environment induced abiotic stresses (Ansari et al. 2015; Manju et al. 2017). Increasing population and economic development have been posing a growing pressure for increase in food production (Zhang 2007). All these challenges can be met by the development of rice varieties with improved yield, resistance to abiotic stresses and improvement in grain quality via the use of modern biotechnological breeding approaches (Fahad et al. 2016a, b).

1.1 Abiotic Stresses

The occurrence of global warming, depleting rice lands, exhausted water resources, erratic rainfalls, expanding urbanisation and climate-induced abiotic stresses lead to significant reduction in the production of crops including rice (Pandey et al. 2015, b). Among the abiotic stresses, drought, salinity and heat have been reported to cause substantial yield loss in various agro-economically important crops (Ahmad and Samiullah 2019; Ahmad et al. 2019). In the current scenario, it is imperative to employ breeding approaches both conventional and modern with the main aim of increasing tolerance to abiotic stresses (Leonforte et al. 2013). However, with the complex nature of inheritance of tolerance to abiotic stress and meagre understanding, it will be quite difficult to enhance tolerance in existing rice varieties (Lafitte et al. 2006). The main abiotic stresses that limit the rice production are discussed below.

1.2 Drought Stress

As compared to other cereals, rice requires a huge quantity of water to complete its life cycle (Pandey and Shukla 2015). The drought reduces the yield, and the degree of reduction depends on the variety and the timing of drought occurrence (Wassmann et al. 2009; Dixit et al. 2014a, b). In addition to yield reduction, the drought stress affects various morpho-physiological and biochemical traits in rice plants (Basu et al. 2010). The significant decline in crop yield has been attributed to the negative impact of drought on plant growth, physiology and reproduction. Nahar et al. (2016) reported that drought induced low seed germination and decreased seedling growth could be the probable reason for substantial yield drop. Drought also disrupts the regulation of stomatal opening and closing by making stomatal closure at higher rate, reduces leaf water potential and consequently leads to decrease in cell dimensions and overall plant growth. The drought stress also causes a reduction in leaf dimensions and turgor pressure within leaf cells which in turn result in leaf rolling and quick senescence. An observation of reduced cell division and root elongation on the onset of drought may be attributed to the lesser growth and yield of the plant (Singh et al. 2012). Furthermore, severe drought stress results in drop in photosynthetic and respiration rate, translocation and ion uptake and carbohydrate and nutrient metabolism and consequently leads to stunted growth (Jaleel et al. 2008; Razmjoo et al. 2008). In addition to the abovementioned effects, drought stress also causes disturbance in assimilate partitioning and photosynthetic rate which eventually lead to a considerable reduction in overall yield (Praba et al. 2009). A shrink in the photosynthetic rate also results in reduced activity of chlorophylls a and b, PSI, PSII and photosynthetic enzymes such as Rubisco and PEPcase, thus reducing the rate of carbon dioxide fixation and overall production (Asharf and Harris 2013; Banerjee and Roychoudhury 2018). In order to combat the devastating effects of drought on plant growth and development, it is imperative to have broader understanding of drought stress and its tolerance mechanism (Nahar et al. 2016).

1.3 Heat Stress

In the era of global warming due to continuous increase in the atmospheric temperatures, heat stress is considered as a potential abiotic stress that limits the overall yield of many agro-economically important crops including rice (Fahad et al. 2017). Rice is more sensitive to the heat stress occurring at grain filling stage that adversely affects many physiological and biochemical processes in crop plants, which result in reduced plant productivity (Zhang et al. 2016). Apart from various negative effects of heat stress on the grain filling stage, vegetative parts are also greatly impacted due to heat stress, and its impact depends on the variety grown and timing of occurrence. It also induces a series of morpho-physiological and biochemical variations, especially in enzyme activities with abnormal elevation or complete denaturation which consequently affects the overall growth. Heat stress also causes reduction in pollen fertility, thereby disrupting the pollination rate, and hence reduced flower and seed set. This has been attributed to substantial decrease in yield in rice (Zhang 2007; Liu et al. 2013). Heat stress at the flowering stage resulted in the increase in the number of sterile and aborted spikelet that leads to a drop in yield, while heat stress at harvesting stage did not affect overall yield (Aghamolki et al. 2014). Fahad et al. (2016c) have reported that elevated temperature at night also results in a reduction in 100-grain weight, which is considered as one of the main yield-attributing traits of rice.

1.4 Salinity Stress

Globally, salinity stress is recognised as one of the most devastating abiotic stresses that limit the overall crop production of cereals (Mondini and Pagnotta 2015). It is considered as one of the main obstacles in achieving the desired goals of rice production as the rice varieties are more sensitive to the salinity stress that incurs more than 50% yield loss. Therefore, attention needs to be paid towards the genetic improvement of rice varieties with improved tolerance to salinity (Molla et al. 2015). All the main developmental stages including germination, vegetative, flowering and seed stages are influenced by the increase in salinity stress (Fujino et al. 2004). The effectiveness of employing a proper selection of rice seedlings for the development of rice varieties with improved salt tolerance and subsequent increase in the overall yield and yield-attributing traits has been reported by Cuartero et al. (2006). However, traditional breeding approaches such as selection is cumbersome, laborious and time-consuming and would require 8–9 years on an average for the development and official release of salt-tolerant rice varieties. Therefore, to speed up the development of elite varieties, it is important to have an in-depth understanding of mechanism underlying salinity tolerance at morpho-physiological and molecular levels (Roychoudhury et al. 2008; Horie et al. 2012). Baby et al. (2010) have reported that the development of salinity-tolerant genotypes is a challenging task as the salinity tolerance is polygenic trait with complex mode of inheritance; therefore, direct selection for tolerance trait is rarely effective. Hence, to create rice varieties with improved salt tolerance, deeper research that is based on identifying key genes that govern the inheritance of salt tolerance trait is required (Bizimana et al. 2017). Several morphological and molecular markers should be employed to screen and select the rice seedlings that display high tolerance to salt stress. After screening, the rice seedlings should be subjected to multi-location trials to check the stability of tolerance trait before official release of salt-tolerant rice varieties (Roychoudhury and Chakraborty 2013).

2 Modern Breeding Approaches for Improving Abiotic Stress Tolerance in Rice

At present, efforts are made to identify key genes that play a critical role in the abiotic stress tolerance with the main objective of creation of rice varieties with improved stress tolerance. For this, several approaches have been used from time to time, and some of the main approaches include mutagenesis, genomics, transgenics, etc. (Kim et al. 2014; De Leon et al. 2017; Okazaki and Saito 2016; Manju et al. 2017). However, transgenics and genomics were more successful in developing the varieties with improved tolerance to a wide range of abiotic stresses and are discussed in detail in following subsections.

2.1 Mutagenesis

Induced mutations by chemical and/or physical agents were employed by several plant breeders to create rice varieties with high yielding potential and better tolerance to abiotic stress (Raina et al. 2016; Khursheed et al. 2019). The continuous use of traditional breeding approaches in the past several decades led to narrowing of genetic variation in several crops including rice. Among the various breeding approaches used to date, mutagenesis has proven relatively effective tool for enhancing the genetic variation and improving resistance to abiotic stresses. Mutagenesis equips the plant breeders to make the efficient selection of the genotype for the desired traits including resistance to abiotic stresses (Raina et al. 2018a; Raina and Danish 2018). Several researchers have employed different mutagens in different doses for creating varieties with desired traits in crops such as chickpea (Laskar et al. 2015; Raina et al. 2017, Raina et al. 2019), lentil (Laskar et al. 2018a, b), cowpea (Raina et al. 2018b), mung bean (Goyal et al. 2020a, b; Wani et al. 2017), faba bean (Khursheed et al. 2018a, b, Khursheed et al. 2018c), fenugreek (Hassan et al. 2018) and black cumin (Amin et al. 2016, 2019; Tantray et al. 2017). Mutagenesis has played a vital role in improving characters such as plant yield, earliness, adaptability and tolerance to a wide range of abiotic stresses (Khursheed et al. 2015, 2016; Laskar et al. 2019; Goyal et al. 2019a, b; Raina and Khan 2020; Raina et al. 2020).

At present, continuously rising global warming along with drastic climate change has led to increased occurrence of drought which is affecting the overall production of important crops such as rice (Hallajian 2016). The International Rice Functional Genomics Consortium maintains 0.2 million mutant lines of rice, with mutations in about 50% of mapped genes to date (Krishnan et al. 2009). The FAO/IAEA Mutant Variety Database (MVD) maintains information about 3322 officially released mutant varieties which include 829 rice mutant varieties (www.mvd.iaea.org accessed March 2020). Recently in April 2019, a mutant variety of rice named as ‘Trombay Chhattisgarh Dubraj Mutant-1’ has been developed and released in Chhattisgarh, India. This variety has short stature and early maturity which improved its resistance to lodging and the grain yield. Several drought-tolerant rice varieties have been created all over the world. In 2015, two drought-tolerant rice mutant varieties named as NMR 151 and NMR 152 have been developed by irradiation of popular Malaysia rice variety MR 219 (breeding line developed by MARDI) using gamma rays at dose 300 Gy (mvd.iaea.org accessed March 2020).

Up to now, nine drought-tolerant varieties of rice, viz. 202 in 1973 at China, Azmil mutant in 1976 at Philippines, RD 15 in 1978 at Thailand, CNM 6 in 1982 at India, Danau atas in 1988 at Indonesia, IACuba 23 in 1995 at Cuba, NMR 151 in 2015 at Malaysia, NMR 152 in 2015 at Malaysia and Binadhan-19 in 2017 at Bangladesh, have been developed (Table 1). Researchers at Vienna promote creation of more sustainable rice varieties with the main aim to improve the tolerance to abiotic stresses such as drought, heat and salt stress (Kaskey 2013). India made a landmark achievement by developing a mutant variety of rice, viz. CNM 6 (Lakshmi), by irradiating IR8 with 300 Gy X-rays under real field conditions at different geographical sites with improvement in tolerance to drought, reduction in maturity and stature and enhancement in aroma and yield (mvd.iaea.org accessed March 2020). Researchers at Australia were successful in developing rice varieties with improved tolerance to abiotic stresses and enhanced grain yield. Another mutant Nagina 22 (N22) was developed by treating the parent variety with ethyl methanesulfonate; the mutant variety displayed improvement in a deep-rooted system and enhanced tolerance to drought and heat stress (Panigrahy et al. 2011).

Heat stress is also one among the major abiotic stresses that restricts plant growth, metabolism and overall production of plants worldwide (IPCC 2007). Heat stress influences all growth stages of rice and incurs a huge loss in rice grain quality and yield (Nakagawa et al. 2003; Matsui et al. 1997). Mutagenesis is used to increase the overall genetic variability of rice that enables the rice breeders to make selection for the heat stress-tolerant lines. Physical mutagens such as gamma rays have broaden the scope of increasing genetic variability in various agro-economic traits. Gamma radiations were employed by rice breeders to improve genetic variations and to render them more yielding and tolerant to heat stress (Luzi-Kihupi et al. 2009). The rice mutant varieties may involve heat resistance mechanism that is linked with increased synthesis and build-up of heat shock proteins. However, the molecular mechanism making rice plant cells survive from heat stress is very complicated, and hence more information is needed. The heat-resistant mutant variety, namely, Zaoyeqing, was developed by irradiation of seeds with 200 Gy gamma rays and officially approved in 1980. Another mutant variety named as Binadhan-14 was developed in 2013 by Bangladesh. This variety had improved tolerance to high temperature, shorter height and long fine grains (mvd.iaea.org accessed March 2020). The mutant rice lines were developed using 150 and 200 Gy gamma rays with the main aim of enhancing tolerance to heat stress. Recently, Targeting Induced Local Lesions in Genome (TILLING), a reverse genetic method, has been used for the characterisation of putative heat-tolerant (HT) mutant upland rice lines from gamma ray-induced mutation and target mutation in genes linked with heat stress tolerance. Although originally TILLING was developed for use with *Arabidopsis* only, now the technique has been applied to a wide range of plants, including rice (Till et al. 2006). McCallum et al. (2000) reported its use for detection of mutations

Table 1 Role of mutagenesis in the development of rice varieties with enhanced tolerance to abiotic stresses (MVD 2019)

Name	Country	Year	Mutagen (dose)	Traits improved
202	China	1973	Gamma rays (200 Gy)	Small leaves, large panicle and tolerance to drought
CNM 6	India	1980	X-rays (300 Gy)	Early maturity, increased tillering, 10% higher yield, long grain size, resistance to drought, dwarf (85 cm), long bold grains
Danau atas	Indonesia	1988	Gamma rays (400 Gy)	Resistance to drought, high yield
RD 15	Thailand	1978	Gamma rays (150 Gy)	Early maturity and tolerance to drought
Azmil mutant	Philippines	1976	Gamma rays (200 Gy)	High yield, drought resistance
IACuba 23	Cuba	1995	Fast neutrons (20 Gy)	Resistance to drought and high amylose content
Binadhan-19	Bangladesh	2013	Physical mutagen	Shorter height, shorter duration, uniform plant growth, long and slender grains with golden colour, higher yield
NMR 151	Malaysia	2015	Gamma rays (300 Gy)	Minimal water requirement and high yield
NMR 152	Malaysia	2015	Gamma rays (300 Gy)	Minimal water requirement, high yield and longer panicle length
Zaoyeqing	China	1980	Gamma rays (200 Gy)	Large panicle and tolerance to high temperature
Fuxuan 1	China	1968	Gamma rays (300 Gy)	Early maturity, tolerance to salinity and good adaptability
Liaoyan 2	China	1992	Gamma rays	Tolerance to salinity, high yield, multiple resistance, high quality, good adaptability
Rasmi	India	1976	Gamma rays (220 Gy)	Awnless, high yield, tall plant type and tolerance to salinity
Mohan	India	1983	Gamma rays	Salt tolerance
Atomita 2	Indonesia	1983	Gamma rays (200 Gy)	Tolerance to salt, early maturity, higher protein content
Shadab	Pakistan	1987	0.5% EMS	High yield, improved grain quality, resistance to salinity
Shua 92	Pakistan	1993	Gamma rays	Resistance to salinity and high yield
NIAB-IRRI-9	Pakistan	1999	Fast neutrons (15 Gy)	Salt tolerance
A-20	Viet Nam	1990	0.015% MNH	Early maturity and high tolerance to salinity
DT17	Viet Nam	1999	NA	High yield and tolerance to salinity
VND 95-19	Viet Nam	1999	Gamma rays (200 Gy)	Strong tolerance to acid sulphate soil, sturdy stem, high yield (5–10 t/ha)

(continued)

Table 1 (continued)

Name	Country	Year	Mutagen (dose)	Traits improved
VND 95-20	Viet Nam	1999	Gamma rays (200 Gy)	Short duration (90–95 days), stiff stem, high yield, amylose content, tolerant to salinity
Lunisree	India	1992	NA	Long slender grain for coastal saline areas, high yield
GINES	Cuba	2007	The mutant variety protons (20 Gy)	Tolerance to salinity and good quality
Wonhaebyeo	Korea, republic of	2007	Gamma rays (50 Gy)	Tolerance to salinity

in rice genomes; TILLING method can be used to screen genes for specific mutations using a PCR assay and then enzymatic digestion of PCR products by CEL I (Till et al. 2006). Among the 64 putative rice lines screened for heat tolerance, 34 mutant rice lines were recognised to have mutations in heat-tolerant genes (HSP genes) and reflected enhanced tolerance to high temperatures (Yona 2015).

Mutagenesis technique also played a vital role in developing crop varieties with improvement in tolerance to salinity stress. To date, about 15 mutant varieties with enhanced tolerance to salt stress have been developed. Moreover, there are several other elite mutants that showed promising results for heat tolerance and are subjected to multi-location trials as a prerequisite for the official registration and release. The rice mutant, viz. Shu-92, developed in Pakistan showed substantial increase in yield by 40–49% margins against standard salt-tolerant checks (Balooch et al. 2003). Another rice mutant, GINES, has been developed in 2007 by Cuba which have reflected high tolerance to saline soils and higher yielding potential. Bangladesh Institute of Nuclear Agriculture (BINA) in collaboration with International Rice Research Institute (IRRI) has been successful in developing two salt-tolerant rice varieties, namely, BINA dhan 8 and BINA dhan 10, which showed substantial improvements in tolerance to salt stress (bina.gov.bd). The collaborative mutagenesis research between the IAEA and IRRI aimed at the genetic improvement of rice with the focus on improving salt tolerance in the backdrop of increased salt concentration in rice lands. A major breakthrough was the development of salt-tolerant variants from IR29, a variety which is highly sensitive to salt stress. The salt-tolerant mutant lines derived from IR29 with improved traits will play a major role in addressing the scourge of salinity in rice agriculture in Southeast Asia (Mba et al. 2007). Further attention has been paid towards the improvement for the overall plant growth and development and grain quality and quantity of wild rice species, Pokkali (Lee et al. 1996; Gregorio et al. 2002).

2.2 Transgenics

The abiotic stresses incurred a huge loss to the overall productivity of rice, and common transgenic approaches are employed to address the issues of abiotic stress (Table 2). As the understanding of mechanisms involved in tolerance to abiotic stresses increases, the identification and characterisation of multiple genes that govern the stress tolerance is becoming easier. In response to salinity, drought and heat stress plants regulate the expression of genes that control the synthesis of low molecular weight compounds, viz. GB (glycine betaine), proline, trehalose, amphoteric quaternary amines and LEA (late embryogenesis abundant) proteins to combat the effects of abiotic stresses (Roychoudhury et al. 2011; Roychoudhury et al. 2015; Roychoudhury and Banerjee 2016). Shirasawa et al. (2006) reported the increased synthesis of GB that imparted resistance to salinity and heat stress in transgenic rice overexpressing spinach choline monooxygenase. Kumar et al. (2009) have reported mutagenised gene P5CS (Δ^1-pyrroline-5-carboxylate synthase) with phenylalanine substituted for an alanine at 129-amino acid position, hence named as P5CSF129A gene in transgenic rice that leads to the increased synthesis of proline in response to exposure to salinity stress. Similarly, another gene in transgenic rice that plays a vital role in imparting tolerance to abiotic stress is OsTPS1 (trehalose-6-phosphate synthase) and results into manifold increase in the synthesis of osmoprotectant solute, proline and trehalose (Li et al. 2011). Xiao et al. (2007) and Duan and Cai (2012) have reported that overexpression of OsLEA3-2 (rice late embryogenesis abundant), OsLEA3-H and OsLEA3-S genes in genetically engineered rice imparted much better resistance to drought and salt stress. The role of polyamines in combating the abiotic stresses is not much known and is at very initial stage; however, the functional genomics have brought few significant insights into the role of polyamine synthesising genes in abiotic stress tolerance. Although no genetically engineered rice with increased polyamine synthesis have been developed, however, preliminary research has reflected that genes OsPUT1 (rice polyamine uptake transporter) and AdoMetDC (*S*-adenosylmethionine decarboxylase) are the potential candidate genes that confer the tolerance to abiotic stresses via regulating the synthesis of polyamine (Mulangi et al. 2012). To date, a huge number of transcription genes have been isolated and characterised that confer resistance to drought, heat and salinity (Kumar et al. 2013). Many genes isolated from different plants or even rice varieties that show tolerance to abiotic stresses have been introduced into widely cultivated rice varieties through genetic modification with the objective to enhance the tolerance to a wide range of abiotic stresses. Some of the noteworthy examples include the following: The transgenic rice showing overexpression of HvCBF4, ZmCBF3, OsDREB1F and OsDREB2A isolated from barley, maize and rice, respectively, introduced and overexpressed showed an increased survival under salinity and drought stress (Wang et al. 2008, b; Mallikarjuna et al. 2011). Research has shown that different transcription factors (TFs) in transgenic rice play a pivotal role in modulating the expression of genes that govern the tolerance to a wide range of abiotic stresses (Todaka et al. 2012).

Table 2 Role of transgenics in the development of rice varieties with enhanced tolerance to abiotic stresses

Gene(s)	Traits improved	References
AtHsp101, OsHsp101	Heat tolerance	Agarwal et al. (2003)
OsPIP1;3	Cold tolerance	Lian et al. (2004)
Choline monooxygenase	Salt and heat tolerance	Shirasawa et al. (2006)
SsNHX1, AVP1	Salt tolerance	Zhao et al. (2006a, b)
OsSBPase	Heat tolerance	Feng et al. (2007)
HvPIP2;1	Salt tolerance	Katsuhara (2007)
OsGSK1	Heat tolerance	Koh et al. (2007)
KatE	Drought tolerance	Nagamiya et al. (2007)
HvCBF4	Cold, drought and salt stress	Oh et al. (2007)
OsLea1-3	Drought tolerance	Xiao et al. (2007)
ZFP245	Cold tolerance	Huang et al. (2009)
RWC3	Drought tolerance	Matsumoto et al. (2009)
OsWKRY11	Heat and drought tolerance	Wu et al. (2009)
OsTPS1	Cold, drought and salt stress	Li et al. (2011)
OsNAC5	Cold, drought and salt stress	Song et al. (2011)
ZmCBF3	Cold, drought and salt stress	Xu et al. (2011)
ZmCBF3	Cold, drought and salt stress	Xu et al. (2011)
OsMYB55	Heat tolerance	El-kereamy et al. (2012)
OsCam1-1	Salt tolerance	Saeng-ngam et al. (2012)
OsMYB48-1	Drought and salt stress	Xiong et al. (2014)
GS2	Salt and cold tolerance	Hoshida et al. (2000)
OsCDPK7	Salt and drought tolerance	Saijo et al. (2000)
Adc, Samdc	Salt and drought tolerance	Capell et al. (2004)
HVA1	Salt and dehydration tolerance	Babu et al. 2004
OtsA	Salt, drought and cold tolerance	Jung et al. (2004)
pdc1, adc	Submergence tolerance	Rahman et al. (2001)
AGPAT, SGPAT	Cold-tolerant	Ariizumi et al. (2002)
Cat	Cold-tolerant	Matsumura et al. (2003)
spl7	Tolerance to heat stress	Yamanouchi et al. (2002)
OsPYL3 and OsPYL9	Drought and cold tolerance	Tian et al. (2015)
SOD2	Salt tolerance	Zhao et al. (2006a, b)
P5CS	Salt tolerance	Karthikeyan et al. (2011)
MnSOD	Drought tolerance	Wang et al. (2005)
pENA1	Salt tolerance	Jacobs et al. (2011)
miR319	Cold, salt and drought tolerance	Khraiwesh et al. (2012)

Abscisic acid (ABA) is popularly known as stress hormone due to its critical role in mitigation of a wide range of abiotic stresses. In rice varieties with improved stress tolerance, ABA-synthesising gene is unregulated and led to increased synthesis of ABA under stress conditions, and hence it is evident that ABA production is directly related to enhanced stress in rice (Roychoudhury and Paul 2012). With the increasing understanding of ABA-mediated signalling pathways, several bZIP TFs have been discovered to play a key role in drought stress tolerance, thereby improving the yield substantially (Banerjee and Roychoudhury 2017; Kumar et al. 2019). Saeng-ngam et al. (2012) have reported that MAPK (mitogen-activated protein kinase) and Ca^{2+}/CaM (calcium/calmodulin)-mediated pathways play a crucial role in stress signalling response. Several novel genes have been isolated and characterised by working on these pathways. For instance, OsCam1-1 (rice calmodulin gene) can sense the severity of salt stress and upregulate the ABA synthesising gene to build up more ABA which in turn impart increased tolerance to salinity (Saeng-ngam et al. 2012). Another gene OsMAPK44 (rice mitogen-activated protein kinase) has been shown to play a critical role in the mitigation of various abiotic stresses, particularly salinity and drought (Jeong et al. 2006). Lee et al. (2011) while studying the role of genes in stress signalling reported that downregulation of OsMAPK33 resulted in the improvement of tolerance to salinity stress in rice plants. Nagamiya et al. (2007, b) and Gu et al. (2013) have reported that several genes such as KatE (*E. coli* catalase), OsIRL (rice isoflavone reductase-like gene), PPDK (maize pyruvate orthophosphate dikinase) and PEP carboxylase (maize phosphoenolpyruvate carboxylase) introduced and overexpressed increased the survival of rice plants exposed to drought and salt stress. Several workers have postulated different underlying mechanisms that confer tolerance to salinity stress, and one such mechanism has been attributed to the upregulation of Na/H (sodium proton pump) gene that led to rapid and effective extrusion of sodium ions across the cell membranes. Another gene, viz. SOD2, (superoxide dismutase) isolated from fission yeast and introduced and overexpressed into rice imparted substantial increase in the rate of photosynthesis, increased grain yield and improved salt tolerance (Zhao et al. 2006a, b). The genetically engineered rice with overexpressed (Na^+ pumping ATPase gene) PpENA1 isolated from moss *Physcomitrella patens* revealed enhanced potential to withstand salt stress (Jacobs et al. 2011). Similarly, the OsECS (γ-glutamylcysteine synthetase) gene overexpression in genetically engineered rice showed enhanced seed germination and seedling survival in rice exposed to abiotic stress, and this has been attributed to improved redox homeostasis (Choe et al. 2013). In addition to the genes, several micro-RNAs, for instance, miR160, have been reported to play a critical role in mitigation of adverse effects of drought stress in rice (Nadarajah and Kumar 2019).

Apart from the transcription factors and signalling pathways, aquaporins, members of the major (membrane) intrinsic protein (MIP) family, have been reported to play a role in improving the ability to tolerate salt and drought stress. For instance, transgenic rice harbouring and/or overexpressing HvPIP2;1 (barley aquaporin gene), OsPIP1;1 (rice aquaporin gene) and RWC3 (rice water channel protein) plays an important role in imparting tolerance towards salinity and drought, respectively (Kapilan et al. 2018). Zhao et al. (2006a, b) while studying salt tolerance in rice reported that co-expression of SsNHX1 (vacuolar Na+/H+ antiporter gene) and AVP1 (vacuolar H+-PPase) isolated from *Suaeda salsa* and *Arabidopsis,* respectively, introduced in transgenic rice seedlings revealed improved tolerance to salinity with increased grain yield. Genetically engineered rice plants harbouring and overexpressing *ZFP245* displayed substantial increase in tolerance to drought and other abiotic stresses (Huang et al. 2009). Further, the increased stress tolerance has been attributed to augmented proline content and upregulation of P5CS and proline transporter genes. Likewise, another gene PgNHX1 (vacuolar Na+/H+ antiporter) isolated from *Pennisetum glaucum* and introduced in transgenic rice showed enhanced plant yield in severe salt stress (Verma et al. 2007). The literature is scanty on identification, isolation and characterisation of transporter genes and underlying mechanism that impart stress tolerance in rice. More attention is required to pay toward this area to develop a broader understanding of the role of transporter genes in conferring tolerance to abiotic stress (Huang et al. 2009).

Several new genes such as Athsp101 (*Arabidopsis* heat shock protein), FAD7 (*Arabidopsis thaliana* fatty acid desaturase) and OsHSP101 (rice heat shock protein) governing the heat tolerance serve as a promising approach in developing transgenic rice plants with increased yield under heat stress. Transgenic rice revealed manifold increase in heat tolerance due to downregulation of FAD gene (Sohn and Back 2007). These genes required for acclimation to heat stress and their insertion into rice resulted into the development and official release of heat-tolerant rice varieties (Agarwal et al. 2003). The knockout mutants of OsGSK1 gene showed substantial increase in the ability to tolerate high temperature (Koh et al. 2007). Likewise, the upregulation of SBPase gene imparted heat tolerance along with the simultaneous increase in grain yield due to increased rate of carbon dioxide fixation in genetically engineered rice plants (Feng et al. 2007). Additionally, the upregulation of OsMYB55 (rice myb transcription factor 55) activates stress genes associated with increased heat tolerance, amino acid metabolism and grain yield in transgenic rice (Deeba et al. 2017).

2.3 Genomics

To feed the rapidly increasing human population, creation of varieties with high yielding potential and improved resistance towards abiotic stresses is prerequisite (Leonforte et al. 2013). Chee et al. (2005) have documented that conventional approaches of breeding such as mutagenesis are cumbersome and require a longer time span; modern breeding strategies such as marker-assisted breeding (MAB) have proven effective for creating varieties with enhanced yield and stress tolerance in a short span of time with better precision. MAB has been employed in several agricultural and horticultural important crops in rice with the objective to screen and isolate varieties with abiotic stress tolerance (Mondal et al. 2013). Molecular markers, viz. RFLP, RAPD, AFLP, SSRs and SNPs have played a vital role in marker-assisted breeding (MAB) for developing crops with improved tolerance to abiotic stress (Hussain 2006; Raina et al. 2019). QTL mapping has been employed to understand the underlying mechanism of abiotic stress tolerance in rice (Shanmugavadivel et al. 2017).

Table 3 Role of genomics in the development of rice varieties with enhanced tolerance to abiotic stresses

Gene(s)	Traits improved	References
DSM1	Dehydration tolerance	Ning et al. (2010)
OsCPK12	Salt tolerance	Asano et al. (2012)
OsCPK4	Salt and drought tolerance	Campo et al. (2014)
OsSIK1	Salt and drought tolerance	Ouyang et al. (2010)
SIT1	Salt tolerance	Li et al. (2014)
OsPP18	Osmotic and oxidative stress tolerance	You et al. (2014)
OsANN1	Drought tolerance	Qiao et al. (2015)
Os SAPK2	Drought tolerance	Lou et al. (2017)
Dro1	Drought tolerance	Uga et al. (2011)
Saltol	Salt tolerance	Das and Rao (2015)
qDTY2.2, qDTY3.1 and qDTY12.1	Drought tolerance	Shamsudin et al. (2016)
SKC1	Salinity stress	Emon et al. (2015)
DST	Salinity stress	Emon et al. (2015)
qSCT-11	Chilling-tolerant	Chen and Li (2005)
qPSST-3, qPSST-7, qPSST-9, qSCT1a and *qSCT2*	Cold stress	Jena et al. (2010)
qHTSF4.1	Heat stress	Ye et al. (2015)
ABo28184	Drought tolerance	Khattab et al. (2014)
AJ578494	Drought tolerance	Khattab et al. (2014)
OsNAC2	Salt, drought, cold stress	Hu et al. (2006)
OsDREB2A	Salt, drought, cold stress	Chen et al. (2008)
OsNAC5	Salt, drought, cold stress	Hu et al. (2008)
OsHOX24	Salt, drought, cold stress	Bhattacharjee et al. (2017)
OsCMO	Salinity stress	Burnet et al. (1995)
Erf68	Salinity stress	Steffens (2014)

Increased salt concentration in the rice lands incurs a huge loss in overall yield and monetary loss worldwide. In order to improve the yield in rice, it is important to identify, isolate and characterise genes that improve acclimation to salt stress (Table 3). Therefore, global collaborative research has been initiated with the main objective of screening QTLs that govern improved salt stress tolerance in rice. Since the tolerance to salinity is polygenic trait, it is difficult to screen QTLs that are linked with salt tolerance and is at the central place in broadening the concept of stress responses in a wide range of crops. A landmark achievement was made by developing a set of introgression lines (ILs) from "Pokkali" which served as donor of genes that confer tolerance to salinity stress in a "Bengal" rice cultivar with better yield but sensitive to mild salt concentration in soil. These ILs were subjected to genotyping using SSRs and SNP markers and lead to the identification of 18 and 32 QTLs, respectively, that are linked with salt stress tolerance and can be used to transfer salinity tolerances to other crops. They concluded that salt tolerance may be attributed sodium dilution and compartmentalisation and build-up of osmoprotectant compounds (De Leon et al. 2017). Similarly, molecular dissection of SST by means of a high-density rice genetic map was constructed using a cross between varieties Bengal and Pokkali that resulted into the development of 187 recombinant inbred lines (RILs). These RILs were evaluated for several morpho-physiological and biochemical characters associated with salt tolerance and were also genotyped with 9303 SNP markers. The study leads to the identification of 85 QTLs that govern several traits, viz. shoot Na+and K+ concentration, shoot Na+/K+ content, shoot length/root length, salt injury score, chlorophyll content and dry weight of shoots. Hence, construction of genetic linkage map provides new clues in understanding the mechanism of salinity tolerance and identification of novel genes that govern salt tolerance (De Leon et al. 2016). Cheng et al. (2012) reported isolation of salt-tolerant QTLs by employing two sets of reciprocal IL derived from a cross between Xiushui09 and IR2061–520–6-9 with former being salt-resistant and latter salt-sensitive rice variety. A total of 26 QTLs linked with salt toxicity symptoms (SST) and days to seedling survival (DSS) were identified and can be used to further improve the salt tolerance. Gregorio et al. (2002) were successful in isolating *Saltol* QTL that confers salinity stress tolerance in Pokkali by improving Na^+/K^+ homeostasis. This QTL can be intergressed into salt-sensitive varieties through marker-assisted backcross breeding and will equip the plant breeders to create salinity-tolerant version of otherwise high yielding rice varieties (Singh et al. 2018; Valarmathi et al. 2019).

Drought causes substantial reduction in the overall yield of rice crops and hence is considered main obstacle in achieving the desired goals of rice production. Hence, plant breeding programmes are required to plan with the main aim of developing drought tolerance in crops (Venuprasad et al. 2007). The isolation of QTLs that govern drought tolerance plants could be useful to enhance the survival of rice seedlings exposed to drought stress (Prince et al. 2015). Hence, MAB research is being carried out to have a broader understanding of mechanisms of drought tolerance and to quicken the progress of developing varieties with improved drought tolerance (Dixit et al. 2017a, b Muthu et al. 2020). For instance, root length and density (RLD) play an important role in mitigation of adverse effects of drought stress, and hence indirect selection for drought tolerance through RLD is an emerging research area in stress studies. Due to severe drawbacks in traditional breeding, selection for RLD is cumbersome, time-consuming and laborious; hence, advanced breeding approaches such as MAB may prove effective in improving the drought tolerance (Siddique et al. 2015). With the advent of molecular marker techniques, a very minute details and a broader understanding of genetics involved in tolerance against drought in several crops including rice became available in data repositories. A global collaboration between stress-oriented research institutes such as IRRI, Philippines, initiated with the aim of mapping of drought-responsive QTLs in rice (Kumar et al. 2007; Bernier et al. 2007; Venuprasad et al. 2009; Vikram et al. 2011; Dixit et al. 2014a, b). Some of the noteworthy QTLs, viz. $qDTY_{1.1}$ and $qDTY_{2.1}$, from the drought-resistant genotype, Apo, have been identified and are suitable for improving the drought tolerance in drought-sensitive genotypes (Muthu et al. 2020). Recently, Uga et al. (2011) identified a key QTL (RDR Dro1) in rice on chromosome 9 that governs deep rooting/root growth angles, thereby playing a role in making water available under severe drought. Shamsudin et al. (2016) carried out MAS in Malaysian rice cultivar MR219 that leads to the identification of three drought yield QTLs, qDTY2.2, qDTY3.1 and qDTY12.1.

The QTL mapping strategy has been employed to unfold the complex genetic control of heat tolerance in rice which is also counted as one of the main stresses that limit the rice production to a great extent (Poli et al. 2013). In order to overcome the deleterious effects of continuous rise in atmospheric temperature, it is imperative to create rice varieties with improved heat tolerance (Chang-lan et al. 2005). Rice plants usually face the negative effects of high temperature at the reproductive stage, and hence it is very important to identify QTLs that are responsive to heat stress at this stage. The main effects include anomalous pollination, increased pollen sterility, reduced seed set and lower yield. Hence, the progress in breeding for heat resistance can be rapid by detection of QTLs and candidate genes governing heat stress tolerance at reproductive stage (Qingquan et al. 2008). A cross was made between IAPAR-9 (heat susceptible) and Liaoyan241 (heat resistant) that leads to the creation of RIL mapping population and identification of heat-stable QTLs viz., qNS1, qNS4, qNS6, qRRS1, qHTS4 and qRRS4 (Li et al. 2018). Similarly, two QTLs *qPF4* and *qPF6* were shown to affect the pollen fertility under high temperature (Xiao et al. 2011). Ye et al. (2015) identified heat tolerance QTL, viz. qHTSF1.2, qHTSF2.1, qHTSF3.1 and qHTSF4.1, at the reproductive stage in rice. Zhao et al. (2016) while investigating the key components of molecular mechanism for heat stress tolerance at flowering stage were successful in identifying 11 QTLs that are linked with spikelet fertility, flowering and pollen maturation timing. Among several QTLs, qPSLht4.1 was shown to control pollen attributes and is useful to improve the pollen maturation and pollen tube growth. Shanmugavadivel et al. (2017) investigated the mapping of heat tolerance QTLs and developed 272 recombinant inbred lines in the F8generation by crossing Nagina22 (heat resistant) and IR64 (heat susceptible) rice varieties. This experimentation has resulted in the identification of two QTLs qSTIPSS9.1 and qSTIY5.1/qSSIY5.1 associated with heat tolerance at reproductive stage.

3 Conclusion

Overall, the modern biotechnological tools such as mutagenesis, transgenics and genomics have led to the identification, cloning and characterisation of genes (from different organisms), followed by its insertion into the rice plants with the aim of decreasing the yield loss incurred by the different abiotic stresses. Such approaches have brought landmark achievements by developing varieties with improved tolerance to various abiotic stresses.

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