Shabir Hussain Wani *Editor*

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Prof. Sudhir Kumar Sopory *is an Indian academician and eminent plant molecular biologist. He is a stalwart of Indian Scientific Research with prominence in the areas of Molecular Plant Physiology, Stress Biology and Plant Biotechnology.*

Prof. Sopory had his education first in the University of Kashmir for his graduate and postgraduate degree and then completed his doctorate at the University of Delhi in the field of Plant Molecular Biology. He began his academic career in the year 1973 as a faculty at the School of Life Sciences, Jawaharlal Nehru University, and worked there till 1996. His teaching and research career spans over 45 years inclusive of that as visiting scientist at Max Planck Institute

for Plant Breeding Research, Germany; visiting Fulbright fellow at the Department of Botany, University of Texas, USA, and Plant Molecular Biology Lab of the US Department of Agriculture, USA; and visiting Humboldt professor at the University of Munich, Germany.

He has broadly worked in the area of light signal transduction and plant stress biology. His group identified the involvement of a number of novel genes in abiotic stress responses and was the first to work out in details the role of glyoxalase pathway in plant stress biology. Many groups across the globe are now working on this pathway. His researches have been documented in over 250 articles published in peer-reviewed journals. He has been the editor of 13 books and has contributed more than 50 chapters to books written by others. He has also been the president of the Indian Society of Plant Physiology; the vice president of the National Academy of Sciences, Allahabad, India; Indian Society for Plant Physiology and Biochemistry; Indian National Science Academy, New Delhi; and Society for Plant Biochemistry and Biotechnology, New Delhi, and is a former secretary of the Plant Tissue Culture Association of India.

Prof. Sopory has been recipient of many national and international honours for his pioneering contributions to scientific research and teaching, including the 1987 Shanti Swarup Bhatnagar Prize by CSIR, the highest Indian award in the Science and Technology categories. Some of the other notable awards include the Chakravorty Award, Birbal Sahni Medal of the Botanical

Society, Birbal Sahni Birth Centenary Award of Indian Science Congress, Godnev Award Lecture of Belarus Academy of Sciences, T. N. Khoshoo Memorial Award, B. M. Johri Memorial Award, NASI Prof. R. N. Tandon Memorial Award, and Padma Shri, the fourth highest civilian honour by the Government of India. He is an elected fellow of several major Indian science academies, namely, the Indian National Science Academy (New Delhi), Indian Academy of Sciences (Bangalore), National Academy of Sciences (Allahabad), National Academy of Agricultural Sciences (New Delhi), as well as The World Academy of Sciences (Trieste, Italy). He is the first Indian to receive Corresponding Membership Award of the American Society for Plant Biologists in 2010.

After he left JNU in 1996, he joined the International Centre for Genetic Engineering and Biotechnology, New Delhi, as a group leader of research in plant molecular biology and also became the interim director of the institution. He was the eleventh vice chancellor of the Jawaharlal Nehru University from Jan. 2011 to Jan. 2016. He was appointed as Arturo Falaschi emeritus scientist at ICGEB, New Delhi, since 2016, and currently, he is working as SERB distinguished fellow of the Dept. of Science and Technology, Government of India, at ICGEB, New Delhi.

Foreword

I am pleased to hear that Dr. Shabir Hussain Wani has edited this volume entitled *Recent Approaches in Omics for Plant Resilience to Climate Change* for the well-renowned publisher, Springer Nature. I personally know him since the year 2009 when he was working as research associate in the Biotechnology Laboratory at the ICAR-Central Institute for Temperate Horticulture, Srinagar, Jammu and Kashmir, India. He had a good experience to work in the area of plant biotechnology particularly the *omics* techniques for abiotic stress tolerance in plants. I was overwhelmed with his passion and dedication for science, including research, teaching, and dissemination of scientific knowledge. Hence, this is the book edited by him in the area of *omics* approaches. Therefore, a book coming from him in the said area for plant resilience to climate change is a commendable task.

Climate change has led to many aberrations in extreme temperatures and increases in other abiotic stresses which hinder plant growth and productivity. Recent *omics* approaches are the key to overcome such limitations and can help in opening vistas for novel approaches of improving plant resilience to major stresses which are otherwise very slow or impossible with the conventional plant improvement approaches like plant breeding. Climate change has resulted in the widespread occurrence of abiotic stresses, such as drought, extreme temperatures, salinity, etc. These stresses are responsible for the reduction in yields in many crop plants worldwide. While noteworthy developments have been made in unravelling the plant resilience to abiotic stresses, due to the complex and quantitative nature of these resilience traits, very less success has been achieved through the conventional plant breeding approaches. Many novel omics technologies, including genomics, proteomics, metabolomics, and ionomics, have progressed during the last few decades to scientifically investigate the changes in the genome, transcriptome, proteome, and metabolome, which are occurring as a result of various changes in plants' response to changing stress conditions. This book by Dr. Wani is an emerging area of plant science and is more demanding in both the developing and developed nations as efforts are being made to elucidate the molecular mechanisms underlying the complex traits of stress tolerance in plants.

Dr. Wani has done an excellent effort by bringing up this volume comprising of high-quality chapters from the international- and national-level experts in various research fields. The 13 chapters included in this book are well written by experts including from various developed nations, such as the USA. Diverse chapters include the overview on *omics* approaches under changing climate and application of various *omics* approaches, including genomics, proteomics, and metabolomics, in important commercial crops, like rice, maize, cotton, chickpeas, etc. This book is a suitable reference source for academicians, researchers, and graduate students working in the area of climate resilience in plants using *omics* approaches. I congratulate Dr. Wani for editing this wonderful book volume.

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Preface

Human population is growing at a startling pace and assumed to exceed 9.7 billion by 2050, whereas, at the same time, the agricultural productivity is dwindling due to the growing environmental constraints as a result of global climate change. Climate change has resulted in pervasive episodes of abiotic stresses, such as drought, extreme temperatures, salinity, flooding, etc. These stresses are liable for the decrease in yields in many crop plants at global level. While significant accomplishments have been made in extricating the plant resilience to abiotic stresses, due to the multifaceted and quantitative nature of these resilience traits, very less success has been achieved through the conventional plant breeding approaches. Many novel omics technologies, including genomics, proteomics, metabolomics, and ionomics, have progressed during the last few decades to scientifically investigate the changes in the genome, transcriptome, proteome, and metabolome which are occurring as a result of various changes in plants' response to changing stress conditions. Through this book *Recent Omics Approaches for Plant Resilience to Climate Change*, an effort has been made to include chapters describing the implication of climate change on global food security and its management using the recent novel omics tools. This book is an incredible and a comprehensive reference material for researchers, teachers, and graduate students involved in climate change-related abiotic stress tolerance studies in plants using omics tools by unraveling principles of lately developed technologies and their application in the development of abiotic stress resilience in plants. The chapters are written by reputed researchers and academicians in the field of plant stress biology. I express sincere thanks and gratefulness to my venerated authors; without their untiring efforts, this book project would not have been possible. I am also thankful to Springer Nature for providing such an opportunity to complete this book project. I am thankful to all my family members, especially my wife, for their support during the language editing process.

Finally, I bow in reverence to Almighty Allah who gave me the intellect and strength to complete this book project.

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About the Editor

Shabir Hussain Wani is Assistant Professor (Senior Scale) at Mountain Research Centre for Field Crops, Khudwani—192101, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, J&K, India. He received his Ph.D. degree in plant breeding and genetics on "transgenic rice for abiotic stress tolerance" from the Punjab Agricultural University, Ludhiana, India. After obtaining his Ph.D., he worked as a research associate in the Biotechnology Laboratory, Central Institute of Temperate Horticulture (ICAR), Srinagar, India. He then joined the Krishi Vigyan Kendra (Farm Science Centre) as programme coordinator at Senapati, Manipur, India. He teaches courses related to plant breeding, seed science and technology, and stress breeding and has published more than 100 papers/chapters in journals and books of international and national repute. He served as guest editor and reviews editor for the journal *Frontiers in Plant Science* (2015–2018). He has also edited several books on current topics in crop improvement for abiotic stress tolerance published by Springer Nature and CRC Press USA. His Ph.D. research fetched first prize in the North Zone Competition, at national level, in India. He was awarded a Young Scientist Award from the Society for Promotion of Plant Sciences, Jaipur, India, in 2009. He is a fellow of the Society for Plant Research, India. Recently he also received the Young Scientist Award (Agriculture) 2015 from the Society for Plant Research, Meerut, India. He also served as visiting scientist in the Department

of Plant Soil and Microbial Sciences, Michigan State University, USA, under the UGC Raman Post-Doctoral Fellowship programme. Currently, he is leading the wheat improvement programme at MRCFC Khudwani SKUAST Kashmir.

Omics Technologies for Abiotic Stress Tolerance in Plants: Current Status and Prospects

Sahil Mehta, Donald James, and M. K. Reddy

1 Introduction

In nature, plants are complex, sessile organisms and are hence continuously exposed to a number of environmental stresses from vegetative to the post-reproductive stage (Jakab et al. 2005; Zhao et al. 2007; Mosa et al. 2017; Parida et al. 2018). These environmental factors have a detrimental effect on the growth, development, and productivity of the plant. Due to these stresses, there is a severe decline in plant yield and productivity due to the imbalance at cellular, molecular, physiological, and developmental levels (Xiong and Zhu 2002; Singh et al. 2018). These environmental factors are generally divided into two categories, abiotic and biotic stress. The abiotic stress factors include high and low temperatures, drought, salinity, freezing, heavy metals, high irradiance and ultraviolet (UV) light, and low oxygen conditions (Reyes and Cisneros-Zevallos 2007; Singh et al. 2018). The term biotic stress encompasses mainly pathogens and pests such as bacteria, fungi, viruses, insects, nematodes, rodents, etc. In the current scenario, abiotic stresses are poised to be most detrimental as they severely reduce crop yield and productivity. This is evident from the reports of the Intergovernmental Panel on Climate Change (IPCC) (http://www.ipcc.ch). The report concludes that in the near future abiotic stresses will delimit the productivity of standing crops more adversely because of global warming, depletion of water resources, deforestation, and anthropogenic activities (Singh et al. 2018).

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In order to enhance stress tolerance and increase the plant productivity, the focus of research has already shifted toward understanding the key molecular targets, regulators, and their signaling involved in plant interactions with the environment (Mosa et al. 2017; Singh et al. 2018; Parida et al. 2018). In the past two decades, a new integrative "omics" approach has gained momentum in the plant biology research field, fueled by advancements in nucleic-acid sequencing platforms, peptide-sequencing platforms, mass spectrometry (MS) technology, advanced computational capabilities, and statistical methodologies. This is evident from the fact that the keyword "Plant omics" fetched 75,700 publications in Google Scholar website (https://scholar.google.co.in) in 2018. This integrative "omics" method gives a snapshot of the development, functioning, and interactions of a cell, tissue, or organism by characterizing and quantifying all its biomolecules in a high-throughput approach (Soda et al. 2015; Mosa et al. 2017; Parida et al. 2018).

2 Insights into Omics in Plant Abiotic Stress

In the past 20 years, research has shown that the plant's response to stress is controlled by a set of genes being upregulated and downregulated dynamically. As a result, many researchers have applied various "omics" approaches to get an integrated view of the response of plants to various abiotic stresses (Govind et al. 2009; Mochida and Shinozaki 2010, 2011; Burgos et al. 2011; Witt et al. 2012; Bowne et al. 2012; Collino et al. 2013; Chen and Thelen 2013; Dubery et al. 2013; Duque et al. 2013; Cusido et al. 2014; Kumar et al. 2016; Freund and Hegeman 2017; Zhu et al. 2017; Parida et al. 2018; Gupta et al. 2018; Zhang et al. 2018a, b). Omics approaches have emerged as essential tools to address and understand the plant molecular systems and their functions; to gain insights into biological networks; and promote the translational research (Burgos et al. 2011; Kumar et al. 2016; Parida et al. 2018). Omics approaches are aimed at characterizing the plant's biomolecule pool because these molecules play roles in maintaining homeostasis as well as signalling responses to altering environments. Although initially much work progressed in genomics, it became clear that an integrative approach involving the study of other omics levels, including transcriptional, proteomic, and metabolic profiles, and their flux distributions is essential for a more comprehensive understanding (Vidal 2009; Shen et al. 2018). Due to technical advances in the experimental protocols, data analysis, and visualization techniques, the expression, and activity of any gene, its interacting partners and regulators in the whole system can be studied at any time (Sussman et al. 2009). The advent of omics-based approaches has thus led to investigations on biologically relevant patterns shifting largely to "data and knowledge-driven" from being purely "hypothesis-driven" (Mousavi et al. 2016; Zhang et al. 2017). Furthermore, progress in computational biology has led to the application of data mining methods to reconstruct the biomolecular networks for each omic level.

Various omics-based approaches have been utilized for understanding plant abiotic stress biology (Li et al. 2006; Skirycz et al. 2010; Bowne et al. 2012; Pant et al. 2015;

Narayanan et al. 2016a; Zhu et al. 2017; Bajwa et al. 2018). The various omics-based approaches include genomics (Agarwal et al. 2014; Shen et al. 2018), transcriptomics (Iyer et al. 2013; Shen et al. 2018), proteomics (Liu et al. 2015; Kosová et al. 2018), metabolomics (Colmsee et al. 2012; Khan et al. 2018), miRNAomics (Song et al. 2017), lipidomics (Pant et al. 2015; Zhang et al. 2018a, b), ionomics (Huang and Salt 2016), interactomics (Vandereyken et al. 2017), secretomics (Krause et al. 2013), phenomics (Yang et al. 2013b), microbiomics (Lakshmanan et al. 2017), proteogenomics (Zhu et al. 2017), primeomics (Yang et al. 2018), etc (Fig. 1). All these approaches focus on the elucidation of key genes, their regulators and interactors, and the characterization of changes at various levels in plants exposed to abiotic stress. The derived knowledge is used in targeting the key regulators and/or signaling pathways prevailing under abiotic stress and enhancing the tolerance against different abiotic stresses in plants. Thus, various omics-based approaches seek to provide novel insights into the integrated mechanisms and regulation involved in plant abiotic stress response and to translate this knowledge for better utilization in crop improvement programmes.

3 Genomics: Elucidating Stress-Responsive Genes

Genomics is a branch of "omics" which deals with the study of a given genome and reveals valuable data about the biology of the organism (Gilliham et al. 2017). The researchers identify intragenic and gene sequences, structures of genes, and provide annotation (Duque et al. 2013). The advance of genomics has been exponentially boosted by rapid developments in genome sequencing technology which began in the 1970s (first generation), continued into the mid-1990s (next-generation sequencing-NGS), and currently utilizes third-generation sequencing technologies (El-Metwally et al. 2013, 2014a). The study of genomics involves a series of steps including DNA extraction, amplification, sequencing, assembly, quality assessment, and most importantly, structural and functional annotation of the genome. This whole procedure provides valuable data about the genomics structure of the organism.

Functional genomics has been successfully utilized in identifying various genes involved in abiotic stress responses in plants (Govind et al. 2009; Ramegowda et al. 2013, 2014; Zhang et al. 2017; Wang et al. 2018). Many of these genes have also been successfully utilized in developing abiotic stresses tolerant crop plants (Yao et al. 2011; Le et al. 2012; Chen et al. 2012; Shankar et al. 2013; Agarwal et al. 2014; Thiry et al. 2016; Wang et al. 2016a, b, c; Gilliham et al. 2017). Additionally, the huge online genomic data—repositories developed in the genomics—era serve as a foundation for transcriptomics, proteomics, and genome engineering studies (Mochida and Shinozaki 2010, 2011; Jung and Main 2014; Alter et al. 2015; Mousavi et al. 2016; Shen et al. 2018; Zhang et al. 2018a, b). The advances in genomics of wild germplasm and weedy relatives of crop plants have led to the identification of several novel gene candidates and/alleles for abiotic stress tolerance. For example, Zhang et al. reported a high-quality, assembled genome sequence

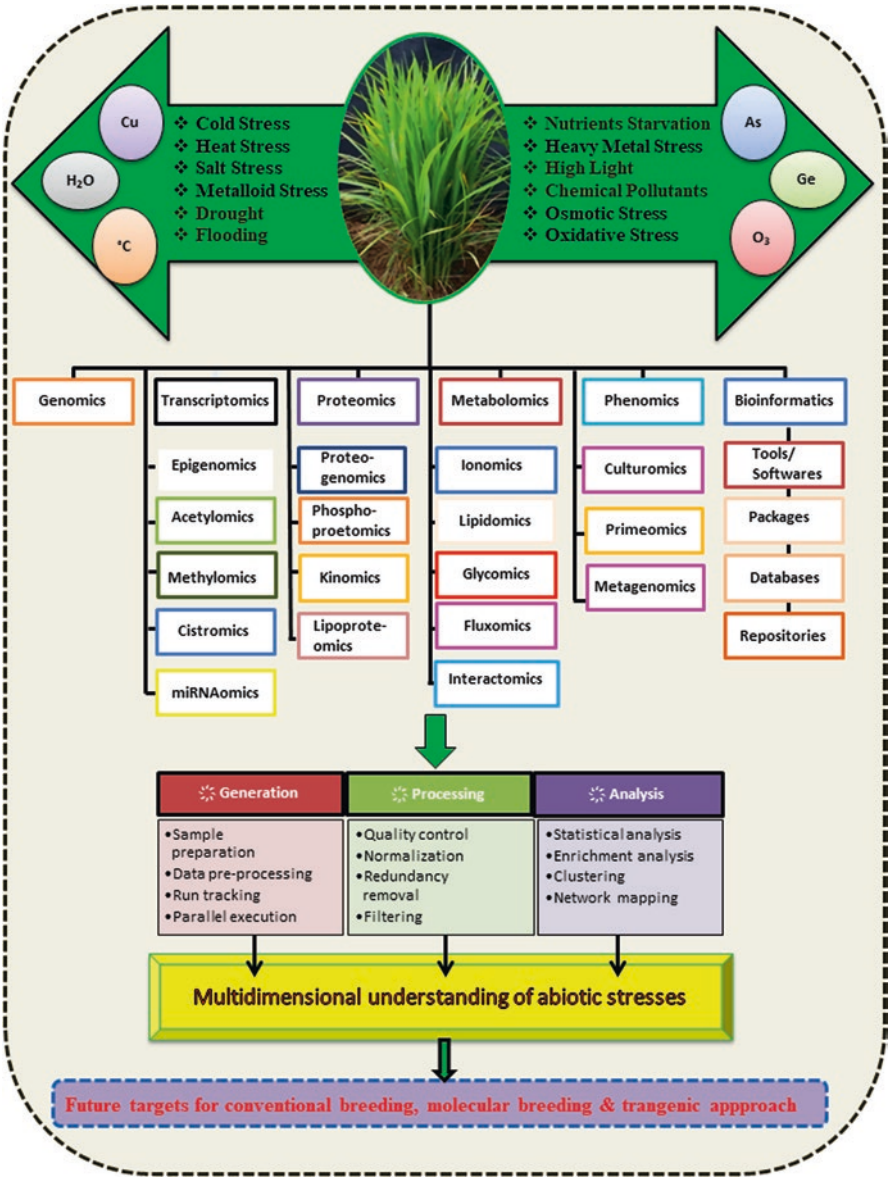

Fig. 1 Omics technologies for abiotic stress tolerance in plant

of Tartary buckwheat using whole-genome shotgun sequencing, genome maps, online available Hi-C sequencing data, and fosmid libraries. They annotated about 33,500 protein-coding genes, revealed whole-genome duplication, and identified the many putative genes related to cold stress, heavy metal stress, and drought resistance (Zhang et al. 2017).

4 Transcriptomics: A Closer Look at Transcripts

The transcriptomics is the branch of "omics technologies" which deals with organism's RNA expression profile in spatial and temporal bases (Duque et al. 2013; El-Metwally et al. 2014a; Shen et al. 2018). Unlike genome, the transcriptome is highly dynamic and changes with age, development stage, nutrient availability, or environment (El-Metwally et al. 2014a). Currently, the RNA profiling is accomplished using RNA sequencing, microarray platforms, digital gene expression profiling, and serial analysis of gene expression (SAGE) (Molina et al. 2011; Duque et al. 2013; Xu et al. 2013; Raney et al. 2014; Li et al. 2017; Leisner et al. 2017; Kreszies et al. 2018). This approach helps in finding the candidate genes which are responsible for phenotypic alterations, stress tolerance by comparing plant under control and stress conditions (Le et al. 2012; Zhang et al. 2014a); prediction of tentative gene functions and providing a better crop productivity (Jogaiah et al. 2013; Agarwal et al. 2014). Similarly, the availability of online databases and archives enables users to perform genome-wide and transcriptome-wide analysis of plant's stress response (Mochida and Shinozaki 2011; Le et al. 2012; Jogaiah et al. 2013; Agarwal et al. 2014; Raney et al. 2014; Alter et al. 2015; Mousavi et al. 2016; Zhang et al. 2018a, b). Rizhsky et al. (2004) used the transcriptomic analysis of Arabidopsis plants under a combination of heat and drought and reported around 770 transcripts level were unaltered. Similarly, they reported an accumulation of at least 53 different unique proteins during the stress combination (Rizhsky et al. 2004). Their results were confirmed in the Arabidopsis (Koussevitzky et al. 2008), sunflower (Hewezi et al. 2008). Additionally, the cytosolic Ascorbate peroxidase1 (APX1) was found to be upregulated during the stress combination (Koussevitzky et al. 2008). Molina et al. (2008, 2011) used NGS and SAGE techniques together to characterize the whole salt and drought-stressed transcriptome in chickpea. The subtractive cDNA suppression hybridization approach was also implied to study transcriptomic profile in plants under stress conditions (Jain and Chattopadhyay 2010).

Similarly, Rasmussen et al. (2013) used large-scale microarray analysis to study the *Arabidopsis thaliana* responses to stresses including high light, salt, heat, and cold. They reported different patterns of transcripts in both individual and combination of stresses. Approximately 7% and 25% of transcripts had a different response to the individual and combination of stresses, respectively. These differentially expressed transcripts were associated with a plant's defense. Around 28% of the total transcripts were involved in the maintenance of photosynthetic machinery. Li et al. (2013) subjected the switchgrass under heat stress conditions and identified around 5350 differentially expressed transcripts using Affymetrix gene chips based transcriptome analysis. Furthermore, they mostly identified probes were related to protein refolding. Under dehydration stress, the RNAseq approach was used for chrysanthemum (Xu et al. 2013). Furthermore, Zhu et al. (2013) studied the changes in the cotton seedlings transcriptome under multiple stress conditions using a comparative microarray analysis technology. Additionally, their work revealed the information about crosstalk of pathways and functional genes under stress.

Prasch and Sonnewald (2013) used transcriptome analysis to understand the effect of heat stress, drought, virus infection, or double or triple combinations on *Arabidopsis* plants. They observed the effect of the stress response is reflected in the transcriptome profile of a plant. Only 11 transcripts expression were found to be altered under all the conditions, namely G-Box binding factor3, Rap2.9 and DEAR1, DREB2A, and two zinc finger proteins. Interestingly, their results confirmed that abiotic stress factors could significantly alter pathogen-related signaling networks, which lead to higher susceptibility of plants. Simlarily, Iyer et al. (2013) subjected *Medicago* plants to single or combination of drought, O_3, and evaluated the effect on the transcriptomic level. The transcripts related to ABA signaling, proline biosynthesis were upregulated in drought subjected plants. However, ozone-stressed plants showed upregulation in the transcripts related to sugars metabolism and phenylalanine ammonia-lyase (PAL) biosynthesis. Under a combination, the jasmonic acid (JA) signaling transcripts were up-regulated. Interestingly, even transcription factors including MYC3 and WRKY were up-regulated. Using RNA-sequencing method, the transcriptomic profile was studied in *Chenopodium quinoa* under drought conditions (Raney et al. 2014). Li et al. used RNA sequencing approach for understanding the effects of heat stress, salt stress, drought, and cold stress on changes in maize leaf transcriptome profile. They reported about 2346, 2019, 1661, and 1841 genes were differentially expressed in each treatment, respectively. These genes were related to transcription, metabolism, signaling using functional annotation approach (Li et al. 2017). Leisner et al. (2017) subjected the soybean plants to low rainfall, ozone stress, and heat stress and reported a significant decline in the stomatal conductance and photosynthesis. Additionally, they studied the effect of these stresses on the seed coat transcriptome using RNAseq analysis. They reported approximately 1576, 148, and 48 genes were differentially expressed under heat stress, ozone stress, and drought, respectively. Muthuramalingam et al. (2017) analyzed the rice response to salt stress, heavy metal stress, and drought by meta-analysis. They reported about 1175 and 12,821 genes are expressed meta-differentially and individually, respectively. They further selected 100 differentially expressed genes and studied their physiochemical properties, transcription factors, and protein–protein interactions. More recently, Shen et al. (2018) assessed the expression levels of HD-Zip genes in tea plant in response to five abiotic stress conditions (heat stress, cold stress, salt stress, ABA, and drought). They reported approximately five, six, nine, six, and three HD-Zip genes were differentially upregulated, respectively. Furthermore, Kreszies et al. (2018) studied the effect of osmotic stress on the transcriptome level in barley roots using RNASeq approach. They observed the upregulation of genes related to suberin biosynthetic pathway (Kreszies et al. 2018). All these data about the differentially expressed genes and their role in signaling pathway can be used to enhance the abiotic stress tolerance.

Muthusamy et al. (2017) analyzed the transcriptional regulation and differential expression levels of heat shock protein 20 (HSP20) family members of wheat under drought, salt, and heat stress. Ruan et al. (2017) performed a genome-wide transcriptome analysis in cassava and predicted about 299 putative members of myeloblastosis (*MYB*) gene family. Additionally, they reported the differential expression

of many MYB genes in cassava leaves subjected to cold and drought conditions. They found that four members of the superfamily respond to ABA treatment. Adding to this, they found that MeMYB2 acts as a negative regulator for drought and cold tolerance using RNAi technology (Ruan et al. 2017). He et al. (2017) identified and evaluated the differential expression pattern of about 17 members of PIN efflux family in stressed cotton plants. Furthermore, they reported these genes to contain salicylic acid and auxin responsive elements in their promoter region. In another instance, Shen et al. (2018) used genomic technology to assess the expression levels of HD-Zip genes in tea plant in response to five different treatments. Recently, Wang et al. identified about 95 grape basic helix-loop-helix (bHLH) genes using a genome-wide analysis and studied the divergence of bHLH family. Additionally, they found around 22 and 17 bHLH genes were induced under osmotic stress and cold stress, respectively. Three other genes were related to secondary metabolite synthesis using GO function annotations. These gene promoters may contain G-box elements which play a role in recognization (Wang et al. 2018).

5 Proteomics: A Key for Understanding Protein Structure, Function, and Regulation

In a wide-ranging term, the proteomics is the quantitative and/or qualitative study of total expressed set of proteins in a given cell, tissue, organ, or organism in spatial and temporal bases (Tyers and Mann 2003; Luan et al. 2018). In the same manner to the transcriptome, the proteome profile is also highly dynamic and changes with age, organ, development stage, nutrient availability, or environmental conditions. The proteomics studies reveal huge information about the set of expressed proteins. Earlier, only the whole proteome were measured in plant stress tolerance; however, later many proteome-related studies including the phosphoproteome, proteogenome, organellar proteome, nuclear proteome, cell wall proteome, also started (Pandey et al. 2010; Helmy et al. 2011, 2012; Nakagami et al. 2012; Duque et al. 2013; Castellana et al. 2014; Cook et al. 2004; Jaiswal et al. 2014; Yin and Komatsu 2016; Wu et al. 2016; Tamburino et al. 2017). Currently, the proteome profiling is accomplished using different types of mass spectrometry (Komatsu et al. 2014; Shao et al. 2014; Luan et al. 2018). In these technologies, the mass and charge of small protein fragments are measured which results from proteases digestion (Nakagami et al. 2012). This generates a standard MS-spectra that is later interpreted to reveal the sequences of peptides and the occurred modification in protein samples (Helmy et al. 2012; Nakagami et al. 2012; Luan et al. 2018). Additionally, many researchers use two-dimensional gel electrophoresis (2-DGE) in plant proteomics (Komatsu et al. 2014; Arentz et al. 2014; Luan et al. 2018).

This approach generates a huge amount of information when used in both genome-wide or sample scale plant stress response studies. Furthermore, it is used to compare the proteome profiles under all optimal, stress and prolonged stress conditions, pinpoint to all the differentially expressed stress tolerant proteins and understand the role of specific proteins in abiotic stress-induced signalling (Hopff et al. 2013;

Yan et al. 2014; Lassowskat et al. 2014; Zhang et al. 2014b; Liu et al. 2015; Kosová et al. 2018). Additionally, the phosphoproteome has received the attention by researchers because the phosphorylated proteins play a major role during abiotic stress conditions (Nakagami et al. 2012; Cheng et al. 2014; Lassowskat et al. 2014; Zhang et al. 2014b; Yin and Komatsu 2015; Tamburino et al. 2017; Luan et al. 2018).

The effect of salt stress on phosphoproteins relative abundance has been studied by Kwon et al. (2006). Tanou et al. (2009) reported the role of post-translational modification in the enhanced tolerance of citrus to salt stress. These data were also supported by Wu et al. (2016). Pandey et al. (2010) studied the extracellular matrix proteome of dehydration stressed rice plants. They revealed alterations in proteins related to signaling, carbohydrate metabolism, ROS scavenging, wall modifiers (Pandey et al. 2010). Many reports in the literature cite about the application of proteomics techniques for understanding the effect of Cd stress in *Brassica juncea* (Alvarez et al. 2009), *A. thaliana* (Semane et al. 2010), *Linum usitatissimum* (Hradilova et al. 2010), *Glycine max* L. (Hossain et al. 2012; Ahsan et al. 2012). Other researchers also evaluated the effect of B (Alves et al. 2011), Al (Duressa et al. 2011) and Cr (Sharmin et al. 2012; Wang et al. 2013). Similarly, Yanguez et al. (2013) studied mRNAs translation efficiency in *A. thaliana* under temperature stress seedlings using genome-wide analysis. Additionally, the proteomic profile of chickpea subjected to cold stress conditions have been evaluated comprehensively by Heidarv and Amiri (2013). Subba et al. (2013) studied the nuclear proteins profile in chickpea subjected to drought conditions. Similarly, other researchers also studied nuclear proteome (Jaiswal et al. 2014). The effect of sublethal hypoxia stress on mRNAs was studied in *A. thaliana* using ribosome footprints mapping (Juntawong et al. 2014). Zhang et al. (2014) studied the leaves phosphoproteome of wheat under drought conditions and reported upregulation of several phosphorylated proteins, transcription factors, transporters, and chaperones. Yin and Komatsu (2015) analyzed the root tips for nuclear phosphoproteome in soybean during flooding and reported around 27 phosphoproteins. Additionally, Yin and Komatsu reported the change in the nuclear proteome of soybean after flooding. They reported the H2, H3, and H4 proteins were differentially regulated indicating profound chromatin remodeling (Yin and Komatsu 2016). Wang et al. (Wang et al. 2016a, b, c) induction of different isoforms of *S*-adenosylmethionine synthetase in soybean under drought and flooding, respectively. The fibrillins proteins are differentially expressed under drought stress (Kosmala et al. 2012; Urban et al. 2017). Santisree et al. (2017) studied the leaf proteome of chickpea. Additionally, they evaluated the effect of different stresses such as heat stress, drought stress, and salt stress on the leaf proteome. They reported about 248, 590, and 797 proteins were differentially regulated, respectively, through comparative label-free quantitative proteomics approach. Tamburino et al. (2017) studied the chloroplast proteome of drought-stressed tomato plants and reported the chloroplast proteins to crosstalk with nuclear signaling proteins.

More recently, Luan exposed two contrasting genotypes of barley to waterlogging conditions and studied the proteome profile of different vegetative organs using 2-DE and tandem MS approaches. They reported a decline in the total biomass, photosynthetic performance in the barley sensitive genotype. Furthermore, they found around 30 and 70 proteins were upregulated in the leaves and roots, respectively.

These differentially expressed proteins were related to energy metabolism and antioxidants in leaves and roots, respectively. Their results highlighted our knowledge about the key players of waterlogging tolerance. This information can be used to enhance the tolerance of crops in future (Luan et al. 2018).

6 Metabolomics in Plant Abiotic Stress

In a wide-ranging term, metabolomics is the fast-growing, advanced branch of omics approach used to study, characterize, identify, detect, and quantify the metabolic profile of cells, tissues, and living organisms under certain environmental circumstances (Collino et al. 2013; Dubery et al. 2013; Kumar et al. 2016; Freund and Hegeman 2017; Parida et al. 2018). The metabolome consists of a broad array of small-sized molecules (molecular mass less than 2000 Da) which exhibits huge diversity in chemical structure and composition. The researchers employ either non-targeted and targeted approaches in their studies for the endogenous metabolites as well as metabolites from exogenous sources (Kosmides et al. 2013; Li et al. 2014). These metabolites include amino acids, peptides, lipids, organic acids, aldehydes, ketones, steroids, vitamins, hormones, and even secondary metabolites. This approach reproduces more thorough data compared to proteomics and transcriptomics (Dos Santos et al. 2017). The advancements in mass spectrometry with liquid chromatography or gas chromatography (LC-MS and GC-MS), high-performance liquid chromatography (HPLC), nuclear magnetic resonance spectroscopy (NMR), direct injection mass spectrometry (DIMS), and other metabolomic techniques have boosted the elucidation of stress tolerance mechanisms as well as metabolite profiling in plants (Wolfender et al. 2013; Parida et al. 2018). This is evident from the fact that in the past decade, various aspects of metabolomics have been used to study plants and their interacting environment. Due to the accuracy, sensitivity, and precision, the metabolomics studies have gain importance in plant sciences research due to mitigating the agricultural losses (Genga et al. 2011) as well as providing knowledge about plant signalling and various regulatory pathways (Carreno-Quintero et al. 2013; Cusido et al. 2014; Shen et al. 2016; Dos Santos et al. 2017; Parida et al. 2018).

In plants, the total metabolite contents are found to be around 250,000 (Kim et al. 2010). Under stress conditions in plants, the total number, concentration, and types of metabolites are significantly enhanced. This alteration in gene expression is directly reflected in the metabolite profiles of plants. Gaining knowledge about the important metabolites which play an essential role in the growth, development, survival, and their modulation upon the onset of various abiotic stresses is highly important. This opened up the scope for the identification of viable metabolomics markers which are important for abiotic stress tolerance of plants (Lafitte et al. 2007; Obata and Fernie 2012; Kumar et al. 2016; Freund and Hegeman 2017; Parida et al. 2018). Various researchers have used the metabolomics approach to study the metabolic profiles in plants under stressed conditions (Urano et al. 2009; Skirycz et al. 2010; Witt et al. 2012; Bowne et al. 2012; Srivastava et al. 2013; Yang et al.

2014; Shen et al. 2016; Muthuramalingam et al. 2018). As a result, it became an indispensable tool in understanding the molecular mechanisms underlying stress responses. Urano et al. (2009) subjected *Arabidopsis thaliana* plants to drought stress and revealed the accumulation of several metabolites, including proline, raffinose family oligosaccharides, gamma-aminobutyrate (GABA), and several tricarboxylic acid (TCA) cycle metabolites. Additionally, they demonstrated that the ABA-dependent transcriptional regulation was responsible for the activation of stress-related metabolic pathways. Skirycz et al. (2010) studied the temporal changes in the profile of proline, erythritol, and putrescine by subjecting *A. thaliana* to mild osmotic stress. They also reported a typical correlation between metabolites and the transcriptional response. Similarly, Verslues and Juenger (2011) revealed osmolytes accumulation during a drought stress response. Caldana et al. subjected *A. thaliana* plants to eight environmental conditions and used metabolome profiling to understand the changes in plant metabolome in response to the environment. They reported accumulation of the photorespiratory intermediates such as glycolate and glycine in the early phase as well as the mid-phase of light stress. In cold stress, they observed an enhancement in the fructose and phenylalanine levels, and a decline in the succinate accumulation. However, they did not give the reason for these overlapped responses (Caldana et al. 2011). Kusano et al. (2011) documented the UV light effect on *A. thaliana* metabolism. They reported major changes in the primary metabolites level in the early phase. Contrastly, they observed an enhancement in the levels of UV-B protectants including phenolics, ascorbate, and flavonoids in the mid and late phases. They concluded reprogramming of the metabolism of carbon toward the production of UV-B protectants. Under dark stress, the function of the different subunits of mitochondrial alternative electron transport pathway was altered (Araujo et al. 2011). Additionally, the levels of branched-chain amino acids (BCAAs) were also elevated under abiotic stress such as salinity, drought, etc. Their findings confirmed the results of the study from the Joshi et al. (2010). These researchers affirmed the function of BCAAs as compatible osmolytes in various plant tissues under stress conditions. The accumulation of amino acids depends upon the desiccation severity. This was confirmed by the amino acid profiling of maize and wheat under water desiccation (Witt et al. 2012; Bowne et al. 2012). In another instance, Colmsee et al. (2012) established a data resource platform namely OPTIMAS-DW to answer different questions of *Zea mays* biology. It can be used to handle different data domains a well as for the integration of metabolomics, transcriptomics, proteomics, ionomics data. Amiour et al. (2012) used the integration of metabolomics, proteomics, and transcriptomics studies to identify key regulating steps in the nitrogen metabolism control. Similarly, Srivastava et al. (2013) documented a study in transgenic *Populus* plant containing superoxide dismutase gene. They applied data processing platform which generated system-level information on ROS metabolism. Yang et al. (2014) focussed on the applications of omics approaches in understanding secondary metabolism. AbdElgawad et al. (2015) reported the enhancement of tocopherol in the maize shoots and a steep decline in the levels of ascorbic acid after subjecting plants to salt stress. Furthermore, Wang et al. (2015) confirmed the enhancement in the proline levels in *Kosteletzkya virginica*

seedlings when exposed to salinity conditions. Shen et al. (2016) reported a rapid decline in the levels of glycolysis pathway related sugars in barley under salt stress. Furthermore, Shin et al. (2016) observed the accumulation of proline in the peach plant when exposed to higher temperatures.

Recently, Sun et al. (2016a, b) assessed the differences in the metabolome of maize after subjecting to different such as heat stress, salinity, and drought. They concluded the effect of individual stresses is different from the combination of stresses based on the metabolomics data. More recently, Khan et al. (2018) assessed the effect of drought on metabolome of sensitive and tolerant chickpea varieties using untargeted metabolic profiling technology. They reported a significant reduction in growth, dry weight, relative water, and chlorophyll content. They reported the most significant enhancement in allantoin and branched chain amino acids; decrease in levels of aromatic amino acids, aspartic acid, and glucosamine (Table 1).

Table 1 List of changes in different metabolites associated with major abiotic stresses

S. No.	Abiotic stress type	Metabolites change(s)	Function(s)	References
1	Heat stress	Amino acids	Antioxidant activity, protein stabilization, signaling	Luengwilai et al. (2012), Chebrolu et al. (2016), Shin et al. (2016)
		Organic acids	Nitrogen cycle	Luengwilai et al. (2012)
		Fatty acid	Cell ultrastructure reconstruction, isoprenoid synthesis	Luengwilai et al. (2012), Mueller et al. (2015)
		Polyamines	Antioxidant activity	Cvikrová et al. (2012)
		Sugars	ROS scavenging, osmoprotectant	Rivero et al. (2014), Chebrolu et al. (2016)
		Flavonoids	Signaling, ROS scavenging, structural integrity	Gill and Tuteja (2010), Chebrolu et al. (2016)
2	Salt stress	Amino acids	Osmoprotectant, nitrogen cycle, carbohydrate metabolism, amino acids synthesis	Joshi et al. (2010), Skirycz et al. (2010), Akçay et al. (2012), Wu et al. (2013), Ni et al. (2015), Chen and Hoehenwarter (2015), Wang et al. (2015)
		Glycolysis metabolites	Osmoprotectant, energy metabolism	Sobhanian et al. (2010), Wu et al. (2013), Chen and Hoehenwarter (2015), Shen et al. (2015)
		Organic acids	Nitrogen cycle	Ni et al. (2015)
		Cyclic acids	Phosphate storage	Zhang et al. (2011), Sung et al. (2015)
		TCA cycle metabolites	Energy metabolism, nitrogen cycle, phosphorus acquisition	Ni et al. (2015), Chen and Hoehenwarter (2015), Pang et al. (2016)

(continued)

Table 1 (continued)

S. No.	Abiotic stress type	Metabolites change(s)	Function(s)	References
3	Drought	Polyols	Osmoprotectant, antioxidant activity	Verslues and Juenger (2011), Warren et al. (2012), Wenzel et al. (2015); de Miguel et al. (2016)
		Organic acids	Membrane integrity, signaling	Wenzel et al. (2015), Alcázar et al. (2014), Lanzinger et al. (2015)
		Sugar alcohols	Osmoprotectant	Sun et al. (2016a, b), de Miguel et al. (2016)
		Sugars	Osmoprotectant	Urano et al. (2009), Shi et al. (2015), Pires et al. (2016), Nakabayashi et al. (2014), Lanzinger et al. (2015)
		Amino acids	Protein stabilization, antioxidant activity, osmoprotectant, signaling	Urano et al. (2009), Joshi et al. (2010), Witt et al. (2012), Bowne et al. (2012), Mao et al. (2013), Shi et al. (2015), Muscolo et al. (2015), Sun et al. (2016a, b), de Miguel et al. (2016), Khan et al. (2018)
		TCA cycle metabolites	Energy metabolism, nitrogen cycle, phosphorus acquisition, secondary metabolism	Urano et al. (2009), Griesser et al. (2015), Sun et al. (2016a, b), de Miguel et al. (2016)
		Phenols	Antioxidant activity	Griesser et al. (2015)
4	Heavy metals	Peptides	Antioxidant activity, metal chelators, photoprotection	Manivasagaperumal et al. (2011), Sytar et al. (2013)
		Amino acids	Osmoprotectant, phytochelatins synthesis, polyamines synthesis	Okem et al. (2015), Begum et al. (2016)
		Phenolics, flavonoids, phytochelatins	Antioxidant, ROS scavenging, structural integrity	Pal and Rai (2010), Okem et al. (2015)
5	Cold stress	Carbohydrate	Cryoprotectant	Caldana et al. (2011), Maruyama et al. (2014)
		Lipids	Membrane stabilization	Degenkolbe et al. (2012)
		Carotenoids and Flavonoids	Energy dissipation, antioxidant activity, UV absorbent	Latowski et al. (2011), Neugart et al. (2016)

Muthuramalingam et al. (2018) used genome-wide based computational metabolomics to study threonine profiling. They identified around 16 genes which modulate threonine levels in abiotic stressed rice plant using in silico expression studies.

7 Lipidomics

Compared to other approaches like metabolomics and genomics, there are fewer studies in the literature which confirm changes in lipid profile and remodeling on exposure to stress (Li et al. 2006; Chen and Thelen 2013; Xie et al. 2015; Pant et al. 2015; Moradi et al. 2017; Zhang et al. 2018a, b). Cold stress brings about many changes in membrane lipids. Burgos et al. (2011) exposed *Arabidopsis* plants to eight different type of stresses and studied the glycerolipid remodeling and saturation profile of fatty acids. Using the lipidomic data from Burgos et al., Szymanski et al. correlated the changes in glycerolipid levels with gene expression (Szymanski et al. 2014). Vu et al. (2014) studied the effect of wounding on changes in lipidomic profile in *Arabidopsis* plants. They also performed a co-occurrence analysis to understand the sorting of different lipids based on pathways. Similarly, Higashi et al. (2015) used *Arabidopsis* plants under heat stress correlated the changes in the lipidome with transcriptomic data. Xie et al. (2015) reported the ceramides accumulation as well as enhancement in fatty-acid unsaturation of lipid bilayer of *Arabidopsis* plants subjected to hypoxic conditions. Narayanan et al. (2016a, b) studied the effect of heat stress, day, and night temperatures on leaf lipid composition of the wheat plant. Tarazona et al. (2015) developed a multiplexed LC-MS lipidomics platform for the better coverage of plant lipidomes. Additionally, they used their own platform to study leaf lipidome of cold or drought treated plants. Their analysis yielded around 23 different classes of lipids. They also reported the accumulation of steryl glycosides, acylated steryl glycosides, and glycosylinositolphosphoceramides in drought-stressed plants.

Natera et al. (2016) studied the effect of salinity on changes in lipid metabolism and composition in the roots of two different *Hordeum vulgare* L. cultivars. They compared both of different genotypes on parameters like fatty acid composition, untargeted, and targeted lipid profiles. Wang et al. (2016a, b, c) used high-resolution EIT-MS to identify about 126 phospholipid molecules in the seedling of *Arabidopsis* under mild light conditions. Spicher et al. (2016) assessed the effect of higher temperature on *Solanum lycopersicum* lipidome. They identified about 791 lipid molecules including membrane lipids, prenylquinones, carotenoids, etc., using the advanced MS technique. The levels of galactolipids, phosphatidyl ethanolamine, prenylquinones, α-tocopherol, and plastoquinone drastically changed under high-temperature stress. They concluded the thylakoid membrane is remodeled with respect to the galactolipids saturation profile and concentrations. Recently, Moradi et al. evaluated the differences in the lipid profile of sensitive and tolerant thyme plants by subjecting under drought conditions (Moradi et al. 2017). More recently, Zhang et al. (2018a, b) evaluated the effect of heat stress on drought primed *Festuca arundinacea* lipidomic profile. They observed primed plants performed better in heat stress conditions compared to non-primed plants.

8 Proteogenomics: A Comprehensive Approach for Elucidating Regulatory Mechanisms

This integrative approach combines the large-scale genomics and transcriptomics data with proteomic data to elucidate the novel regulatory mechanisms (Helmy et al. 2012; Mosa et al. 2017). In proteogenomics studies, the proteomic techniques generate well defined, accurate, and high throughput translation-level data. Therefore, these generated data are mapped back to the genomic and/or transcriptomic data. These mapped back data act as a source for making several predictions for performing large-scale experiments in future (Armengaud 2010; Helmy et al. 2012; Chapman and Bellgard 2017).

In the past years, this approach has been used in elevating our understanding about plant sciences research (Baerenfaller et al. 2008; Castellana et al. 2008, 2014; Helmy et al. 2011; Zhu et al. 2017). Baerenfaller et al. (2008) performed a proteogenomics study in *Arabidopsis thaliana.* They identified around 57 new genes. Furthermore, they annotated hundreds of genes using intensive sampling from *Arabidopsis* under various conditions. Helmy et al. (2011, 2012) developed and expanded a rice proteome database namely OryzaPG-DB. Similarly, Risk et al. (2013) developed another database namely Peppy. Recently, D'Agostino et al. (2016) extended the use of proteogenomics to the plant symbiotic partner *Anabaena*. They analyzed the effect of nutrient depletion and NaCl stress on two different genotypes using the proteogenomic approach. They reported a huge change in protein profile related to transcription, translation, photosynthesis, and metabolism in both conditions (D'Agostino et al. 2016). Recently, Zhu et al. annotated a number of the alternative isoforms of a number of proteins in response to abscisic acid (ABA) treatment using a combination of RNA sequencing (long-read and short-read) and mass spectrometry methodology. Furthermore, they reported about 83.4% of total intron-containing genes undergo alternatively splicing (Zhu et al. 2017). By understanding the proteogenome of plants, the focus of research can be shifted toward increasing the nutritional improvement, total yield, and performance under stress conditions.

9 miRNAomics: For the Better Understanding of the Small RNA Networks

The microRNAs (miRNAs) are a class of small, noncoding RNAs, which act as endogenous posttranscriptional regulators. They play a role in every aspect of signaling (Sharma et al. 2017), development (Hernandez and Sanan-Mishra 2017), and environmental responses (Hernandez and Sanan-Mishra 2017).

The first report about the miRNAs involvement in abiotic stress response came from Jones-Rhoades and Bartel. In *Arabidopsis*, they reported the upregulation of

miR395 in particular during sulfate starvation. This specific miRNA was found to be targeting a transporter and enzymes of sulfate assimilation (Jones-Rhoades and Bartel 2004). Afterward, many researchers also reported the role of other classes of miRNAs in abiotic stress tolerance (Jones-Rhoades and Bartel 2004; Yang et al. 2013a, b; Stief et al. 2014; Cui et al. 2015; Sun et al. 2015; Khaksefidi et al. 2015; Roy 2016; Hivrale et al. 2016; Chauhan and Kumar 2016; Song et al. 2017). Till date, more than 400 miRNAs have been reported in abiotic stresses in plant species from different families including Brassicaceae, Solanaceae, Papaveraceae, Poaceae, Euphorbiaceae, Rosaceae, Amaranthaceae, and Apocynaceae. These *miRNAs respond in a tissue-, stress-, genotype-, and miRNA-dependent manner* (Zhang 2015) *to abiotic stress*. All the major miRNA involved in the abiotic stress response and tolerance are listed in Table 2.

Table 2 List of miRNA families associated to different abiotic stresses

S.No.	miRNA Family name	Abiotic stresses	Reference(s)
1	miR156	Salt stress, drought, heat stress, cold stress, heavy metal stress, UV-B	Stief et al. (2014), Cui et al. (2015), Sun et al. (2015)
2	miR159	Salt stress, heat stress, osmotic stress, ABA hypersensitivity, UV-B	Roy (2016), Hivrale et al. (2016)
3	miR160	Salt stress, heat stress, drought, heavy metal stress, UV-B	Khaksefidi et al. (2015), Hivrale et al. (2016)
4	miR164	Salt stress, heat stress, drought, heavy metal stress, UV-B	Qiu et al. (2016), Hivrale et al. (2016)
5	miR166	Salt stress, heat stress, cold stress, drought, heavy metal stress, UV-B	Hivrale et al. (2016)
6	miR167	Hypoxia, heat stress, cold stress, UV-B, ABA hypersensitivity	Khaksefidi et al. (2015), Hivrale et al. (2016)
7	miR169	Salt stress, drought, heat stress, cold stress, heavy metal stress, ABA hypersensitivity, nitrogen starvation, UV-B	Cheng et al. (2016)
8	miR170	Drought, UV-B	Chauhan and Kumar (2016)
9	miR171	Salt stress, drought, heat stress, heavy metal stress, UV-B	Hivrale et al. (2016), Esmaeili et al. (2017)
10	miR172	Salt stress, drought, heat stress, UV-B, heavy metal stress, cold stress	Khaksefidi et al. (2015), Li et al. (2016)
11	miR319	Salt stress, drought, heat stress, heavy metal stress, cold stress	Zhou et al. (2013), Yang et al. (2013a, b)
12	miR393	Salt stress, drought, heat stress, UV-B, heavy metal stress, cold stress	Hivrale et al. (2016)
13	miR396	Salt stress, drought, heat stress, heavy metal stress, cold stress, alkalinity stress	Hivrale et al. (2016), Song et al. (2017)

Table 2 (continued)

S.No.	miRNA Family name	Abiotic stresses	Reference(s)
14	miR408	Salt stress, drought, heat stress, heavy metal stress	Hajyzadeh et al. (2015)
15	miR444	Nitrogen starvation, phosphate accumulation, salt stress, dehydration, drought, cold stress, heavy metal stress	Song et al. (2017)
16	miR528	Salt stress, heavy metal stress	Bottino et al. (2013), Gentile et al. (2015)
17	miR529	Drought, cold stress, heavy metal stress	Wang et al. (2016a, b, c)
18	miR809	Salt stress, drought	Yang et al. (2013a, b)
19	miR828	Oxidative stress, heat stress	Wang et al. (2016a, b, c)
20	miR2871	Salt stress, cold stress, drought	Hivrale et al. (2016)

These data are based on the currently available literature of *Arabidopsis*, rice, cotton, wheat, rapeseed, barley, bentgrass, sugarcane, and switchgrass

10 Prime-Omics: A Comprehensive Approach to Priming

Plant priming has emerged as a technology over the past decade (Balmer et al. 2015; Hussain et al. 2016; Lal et al. 2018). It is defined as an induced state by which a plant reacts more efficiently, rapidly, and vigorously to the stress conditions (Hussain et al. 2016; Lal et al. 2018). As a result, the germination rate is enhanced adding to better yield, high vigor in crops, forage, and medicinal plants (Lal et al. 2018). There are multiple priming techniques used by researchers all over the world including chemical priming, hydropriming, hormone priming, and nutrient priming (Lal et al. 2018).

Due to the phenomena of priming, many changes occur in the genetic, transcriptome, proteome, and metabolome levels. As a result, the techniques for accomplishing genomics, transcriptomics, proteomics, and metabolomic approaches can be used in priming. There are many reports in the literature citing about the effect of priming on enhanced abiotic stress tolerance (Guan et al. 2009; Srivastava et al. 2010a; Afzal et al. 2012; Sali et al. 2015; Bajwa et al. 2018) (Table 3). Peroxide primed wheat seeds show a higher salt tolerance (Wahid et al. 2007). Akbari et al. (2007) treated wheat seeds with a higher dose of NaCl and observed a reduction in the seed germination. The priming of maize seed with chitosan improved the tolerance at low temperature (Guan et al. 2009). The halopriming also alleviate the harmful effects of drought and salt stress in sugarcane (Patade et al. 2009) and mung bean (Saha et al. 2010). Srivastava et al. (2010a) reported hydro-primed and chemical-primed mustard seeds to exhibit an enhancement in germination rate, total dry weight, and chlorophyll content under salt conditions. Furthermore, they observed the same results in osmotic stress. The supplementation of thiourea in *Brassica juncea* roots enhances salt tolerance (Srivastava et al. 2010b). Anosheh et al. (2011) reported the chemical priming enhanced the tolerance in drought and salt stress in maize. The $CaCl_2$ and KCl seed priming induced salt tolerance in rice cultivar (Afzal et al. 2012). $CaCl_2$ primed wheat seeds showed the enhancement in seedling

Table 3 A general overview of the omics involved in plant's priming and abiotic stress

S.No.	Plant species	Priming	Response ↓/↑	References
1	Wheat	H_2O_2	Salt tolerance ↑	Wahid et al. (2007)
2	Wheat	NaCl	Seed germination ↓	Akbari et al. (2007)
3	Maize	$CuSO_4$, $ZnSO_4$, Na_2SO_4	Salt tolerance ↑	Foti et al. (2008)
4	Maize	Chitosan	Cold tolerance ↑	Guan et al. (2009)
5	Sugarcane	NaCl	Drought tolerance ↑ Salt tolerance ↑	Patade et al. (2009)
6	Mung bean	NaCl	Salt tolerance ↑	Saha et al. (2010)
7	Mustard	H_2O, $CaCl_2$, ABA	Salt tolerance ↑ Osmotic stress tolerance ↑	Srivastava et al. (2010a)
8	Mustard	Thiourea	Salt tolerance ↑	Srivastava et al. (2010b)
9	Maize	Urea, KNO_3	Salt tolerance ↑ Drought tolerance ↑	Anosheh et al. (2011)
10	Rice	$CaCl_2$, KCl	Salt tolerance ↑	Afzal et al. (2012)
11	Wheat	$CaCl_2$	Drought tolerance ↑	Hussain et al. (2013)
12	Sesame	H_2O, $CaCl_2$, Moringa leaf extract	Drought tolerance ↑	Shabbir et al. (2014)
13	Maize	$CaCl_2$, NaCl	Salt tolerance ↑	Sali et al. (2015)
14	Maize	KNO_3	Cold tolerance ↑	Cokkizgin and Bolek (2015)
15	Rice	H_2O, Se, SA	Heat tolerance ↑	Hussain et al. (2016)
16	Rapeseed	H_2O	Salt tolerance ↑ Drought tolerance ↑	Jian et al. (2016)
17	Wheat	PEG	Salt tolerance ↑ Drought tolerance ↑	Mustafa et al. (2017)
18	Rice	H_2O	Desiccation tolerance ↑	Cheng et al. (2017)
19	Quinoa	Saponin	Salt tolerance ↑	Yang et al. (2018)
20	Wheat	Sorghum water extract, Benzyl aminopurine	Salt tolerance ↑	Bajwa et al. (2018)
21	Tall fescue	Drought	Heat stress tolerance ↑	Zhang et al. (2018a, b)

emergence, tillers number, grain traits, and total yield under drought stress (Hussain et al., 2013). Shabbir et al. (2014) evaluated the effect of drought on sesame seeds. Furthermore, they reported the promotive effect of priming on plant growth and performance. Sali et al. (2015) evaluated the effect of salinity stress on germination percentage, carotenoid content, and chlorophyll profile in maize. Furthermore, the priming promoted the metabolic changes and helped in better acclimatization under salinity stress (Sali et al. 2015). The KNO_3 priming of maize seeds improved the germination rate, vigorousity, and cold tolerance (Cokkizgin and Bolek 2015).

Recently, Hussain et al. (2016) compared the transcript profiles of submerged rice seedlings with the control. They revealed the priming alleviated the submergence

harmful effects. Jian et al. (2016) constructed and sequenced the small-RNA libraries from hydro primed embryos. Additionally, they evaluated the effect of salt and drought treatments on the small-RNA libraries. They reported a significant downregulation of six miRNA families. Mustafa et al. (2017) osmoprimed the late sown wheat and reported the enhancement in biological yield and harvest index under higher temperatures. More recently, Cheng et al. (2017) identified many marker genes in dry and imbibed rice seeds using two-dimensional electrophoresis. More recently, Yang et al. (2018) primed *Chenopodium quinoa* seeds with different concentrations of saponin and, then, evaluated the primed seeds germination percentage and rate under NaCl stress. They reported three concentrations of saponin solution alleviated the salt stress effects. More recently, Bajwa et al. (2018) evaluated the effect of sorghum water extract and benzyl aminopurine on wheat plants grown under saline conditions. They sow the chemical-primed and hydro-primed wheat seeds and reported the priming treatments improved the wheat growth under saline conditions.

11 Bioinformatics: Tools for Integrating All Omics Approaches

There are multiple machines used in omics technologies including sequencers, mass spectrometers, etc., which generates an ample amount of data. All this high-throughput data need to be analyzed, visualized, and stored (El-Metwally et al. 2014a, b). Hence, all these approaches are tightly bound to bioinformatics tools, online repositories, platforms, packages, and algorithms that help in analysis, integration, storage, and enable knowledge exchange between the researchers.

There are many computational tools used by day-to-day researchers to analyse the large-scale omics data in a fast, accurate, efficient, and reproducible manner (McDowall et al. 2009; Falda et al. 2012; Chaouiya 2012; Franceschini et al. 2013; Orozco et al. 2013; Yachdav et al. 2014; Henry et al. 2014; Franz et al. 2016). These data visualizing tools include PRIDE Inspector (Wang et al. 2012), Peppy (Risk et al. 2013), Cytoscape web (Lopes et al. 2010), PathwayMatrix (Dang et al. 2015), ReactionFlow (Dang et al. 2015), Integrated Genome Browser (Freese et al. 2016), Hilbert curve (Gu et al. 2016), Cytoscape.js (Franz et al. 2016), Ensembl plants (Bolser et al. 2016) etc.

Furthermore, the generated high throughput data must be sustainably available, processable, and accessible to the plants' researchers (Smalter-Hall et al. 2013; Helmy et al. 2016). Therefore, many databases and repositories are available online for the storage of the biological data (Helmy et al. 2012; Priya and Jain 2013a, b; Yu et al. 2013; Zhao et al. 2014; Cheng et al. 2014; Deborde and Jacob 2014; Liu et al. 2018). Similarly, there are some databases for storing information about plant stress such as QlicRice (Smita et al. 2011), STIFDB2 (Borkotoky et al. 2013; Naika et al. 2013), RiceSRTFDB (Priya and Jain 2013a, b), PSPDB (Kumar et al. 2014), miRBase (Kozomara and Griffiths-Jones 2014), PNRD (Yi et al. 2015), DroughtDB (Alter et al. 2015), PlantPReS (Mousavi et al. 2016), plant ontology (Cooper and Jaiswal 2016), PtRFdb (Gupta et al. 2018), and Stress2TF (Zhang et al. 2018a, b). All the popular tools and available databases used in the plant sciences are listed in Table 4.

Table 4 A general overview of databases and bioinformatics tools used in omics data analysis

S.No.	Databases/tools	Description	URL	References
Tools				
1	PRIDE inspector	To browse and visualize mass spectrometry (MS) proteomics data	http://code.google.com/p/pride-toolsuite/wiki/PRIDEInspector	Wang et al. (2012)
2	Peppy	Proteogenomic search software	http://geneffects.com/peppy	Risk et al. (2013)
3	Cytoscape web	Web-based network visualization tool-modeled	http://cytoscapeweb.cytoscape.org	Lopes et al. (2010)
4	PathwayMatrix	Visualization tool to present the binary relations between proteins	https://github.com/CreativeCodingLab/PathwayMatrix	Dang et al. (2015)
5	ReactionFlow	An interactive visualization tool for causality analysis in biological pathways	https://github.com/CreativeCodingLab/ReactionFlow	Dang et al. (2015)
6	Integrated Genome Browser	Visual analytic platform for genomics	http://bioviz.org/igb	Freese et al. (2016)
7	HilbertCurve	An R/Bioconductor package for high-resolution visualization of genomic data	http://www.bioconductor.org/packages/devel/bioc/html/HilbertCurve.html	Gu et al. (2016)
8	Cytoscape.js	For graph analysis and visualization	http://js.cytoscape.org	Franz et al. (2016)
9	Ensembl plants	Integrating tools for visualizing, mining, and analyzing plant genomic data	http://plants.ensembl.org	Bolser et al. (2016)
Databases				
10	QlicRice	Platform for data mining of abiotic stress responsive QTLs in rice	http://nabg.iasri.res.in:8080/qlic-rice/	Smita et al. (2011)
11	STIFDB2	An updated version of plant stress-responsive database	http://caps.ncbs.res.in/stifdb2	Naika et al. (2013)
12	RiceSRTFDB	Database of rice transcription factors	http://www.nipgr.res.in/RiceSRTFDB.html	Priya and Jain (2013a, b)
13	PSPDB	A database of plant stress-related proteins	http://www.bioclues.org/pspdb/	Kumar et al. (2014)
14	miRBase	Primary repository for published microRNA (miRNA) sequence and annotation data	http://microrna.sanger.ac.uk/	Kozomara and Griffiths-Jones (2014)

(continued)

Table 4 (continued)

S.No.	Databases/tools	Description	URL	References
15	PNRD	A plant non-coding RNA database	http://structuralbiology.cau.edu.cn/PNRD	Yi et al. (2015)
16	DroughtDB	Compilation of plant drought resistant genes and their homologs in nine species	http://pgsb.helmholtz-muenchen.de/droughtdb/	Alter et al. (2015)
17	Plant ontology	A structured vocabulary and database resource that links plant anatomy, morphology, growth, and development to plant genomic data	https://github.com/Planteome?plant-ontology	Cooper and Jaiswal (2016)
18	PlantPReS	A database for plant proteome response to stress	http://www.proteome.ir/	Mousavi et al. (2016)
19	PtRFdb	A database for plant transfer RNA-derived fragments	http://www.nipgr.res.in/PtRFdb/	Gupta et al. (2018)
20	Stress2TF	A manually curated database of TF regulation in plant response to stress	http://csgenomics.ahau.edu.cn/Stress2TF	Zhang et al. (2018a, b)

12 Conclusion

In conclusion, all of these omics approaches, bioinformatics tools, and databases have greatly increased our knowledge with regard to candidate genes, master regulators, proteins, biosynthetic pathways, cross-talk, and biological networks. This has boosted the field of research on plant responses under abiotic stress. However, we are still a step away from understanding the plant response to multiple abiotic stresses in fields as the information generated has to be collated and understood in detail. Additionally, most of the research work has been done only in a few model plants. There is an urgent need to generate data from non-model plant species.

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Genome Editing and Abiotic Stress Tolerance in Crop Plants

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Abbreviations

Cas9	CRISPR-associated protein 9
CRISPR	Clustered regularly interspaced short palindromic repeats
DSB	Double-strand breaks
GE	Genome editing/Genetic engineering
GP	Germination percentage
GR	Germination rate
HDR	Homology directed repair
HR	Homologous recombination
MAPK	Mitogen-activated protein kinase
NHEJ	Nonhomologous end-joining
TAL	Transcription activator-like
TALEN	Transcription activation-like effector nucleases
TrugRNA	Truncated RNA
ZFN	Zinc finger nucleases

1 Introduction

Plants are sessile, so they experience various inescapable abiotic stresses in their ecological habitat. In this era of atmospheric change, abiotic stresses such as salinity (Zhang et al. 2017), drought (Moonmoon and Islam 2017), cold (Liu et al. 2019), high temperature (Gabaldón-Leal et al. 2016), and heavy metals (Shahid et al. 2016)

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are being recognized as the most complex environmental disturbances, which reduce the yield and productivity of crop plants and thus are causing food insecurity throughout the world (Bechtold and Field 2018). Plant responses to the adverse effects of abiotic stresses are dependent on the tissue, organ, genotype, ploidy levels, and crop type (Cramer et al. 2011). Abiotic stress commonly promotes overproduction of reactive oxygen species (ROS), which is a signaling molecule that, depending on its concentration, can be toxic to plants, causing damage to cell membranes, protein structure, lipids, carbohydrates, and DNA, damage that ultimately inhibits physiological and metabolic processes in crop plants (Gall et al. 2015). The current most challenging problem is to provide food security to the growing population of ten billion by 2050 (Jaganathan et al. 2018). Global food production needs to be increased by 60–100% above current levels (FAOSTAT 2016). To cope with unfavorable adverse stress conditions, plants activate their physiological and molecular machinery including stress-resistant genes (Hu et al. 2018; Duan and Kai 2012), transcription factors (Guo et al. 2016), secondary metabolites (Selmar and Kleinwachter 2013), antioxidant enzymes (Pandey et al. 2017), and phytohormones (Sah et al. 2016) to survive under abiotic stress.

Conventional breeding techniques have been applied to improve crop production; however, the use of these techniques for enhancing tolerance toward abiotic stresses has been gradually reduced, as it involves complex inheritance as well as high genotype–environment interactions. In addition, these conventional breeding methods were practically unable to overcome the abiotic stresses resulting from climate change (Mushtaq et al. 2018). Therefore, new techniques are needed for further improvement in crop production to meet the current and future food demand under these ever-changing climate conditions (Mishra 2014). Genetic engineering led to a rapid development of crop plants with enhanced stress tolerance, high nutritional value, and yield, but the use of this technique for crop breeding has several limitations over time. The main disadvantage of this technique is the use of foreign DNA in the plant genome without utilizing the plant's own genetic repertoire to achieve the desirable qualitative and quantitative characters. Gradually this technology lost acceptance among consumers, and its use has been reduced, as it creates several risks to the environment and food safety and unsupported health problems because of its non-specificity and unstable nature, which restricted the use of genetically modified crops (Zhang et al. 2018a, b; Stephens and Barakate 2017; Kamthan et al. 2016). Introduction of genetically modified genes or transgenes in a crop plant has been beneficial for global food security, but such genetically modified crops are largely affected by environmental safety concerns. Overexpression of genes through the promoters can cause growth retardation under normal conditions and reduced fruit/seed numbers. This constitutive stress response pathway by overexpressed genes has diverted plant developmental programs, resulting in crop yield loss and fewer benefits for agricultural crops (Marco et al. 2015).

The recent availability of genome editing tools has avoided the limitations of conventional genetically modified or traditional breeding methods and is developing a new age of crop improvement in the field of abiotic stress-tolerant crops (Waltz 2018; Mishra et al. 2018). In comparison to the transgenic approach, which very

often incorporates the phenotype, genome editing methods have become a vigorous technique in the field of crop breeding by the development of defined mutants with the desired traits (Jaganathan et al. 2018). In contrast to genetic engineering, genome editing technology does not involve integration of any foreign DNA into the host plant; as a result, offspring cannot be discriminated from parental plants (Shanmugavadivel et al. 2019). In the genomic field, sequence-specific genome editing is explained as a collection of advanced molecular techniques that would be specific, and efficient for target modification at genomic loci (Gao 2015).

Four kinds of genome editing tools have been used so far: (1) zinc finger nucleases (ZFN), (2) meganucleases, (3) transcription activator-like effector nucleases (TALENs), and (4) clustered regularly interspaced short palindromic repeats (CRISPR) systems (Jain 2015). These techniques modify genomic sequences by using designer sequence-specific nucleases to make double-strand breaks (DSB). The cellular repair system of the plant fixes the double-strand breaks and allows gene insertion and deletion (INDELS) by using nonhomologous end-joining (NHEJ) and homology-directed recombination (HDR) pathways (Jaganathan et al. 2018). Among all genome editing techniques, CRISPR/Cas9 is modern, popular, and the simplest method in plant research (Ma et al. 2016).

The application of genome editing tools (CRISPR/Cas9, dual sgRNA/Cas9, SRSPR sgRNA, and TALENs) has been implemented in different crops for enhancing abiotic stress tolerance such as drought in maize (Shi et al. 2017) and tomato (Wang et al. 2017; Li et al. 2017), salt tolerance in rice (Bo et al. 2019), cold tolerance in rice (Shen et al. 2017a, b), and heavy metal tolerance in rice (Tang et al. 2017). The applications of genome editing tools expand new opportunities in the field of abiotic stress tolerance and aim to improve crop productivity by developing novel varieties. Here, we have summarized the mechanism, potential application, and future implications of genome editing methods for a prospective view for plants. We highlight the advantages of CRISPR-Cas9 over other genome editing tools by describing recent studies on various plants under different abiotic stresses.

2 Types of Crop Plant Genome Editing Tools

To meet the demands of increasing population as well as extreme climatic conditions, ways to improve crop production are required. Therefore, we need a directed, rapid, and low-cost method to improve crop yield and also to develop multi-stress-resistant crop varieties (Xu et al. 2014). Recently, genome editing has come to light as an alternative to improve plant breeding, crop plants, and reasonable food production (Belhaj et al. 2013). Modification of the target region of the genome using genome editing technology has potential advantages over the traditional method of genetic modification, generally done by random insertion events, which most of the time affects the expression level of the transgene (Forsyth et al. 2016). Genome editing can be defined as the alteration of the target genome to illuminate and control gene functions in plant research (Li et al. 2014). In 1993, the first-ever application

of genome editing was implemented in the production of a transgenic tomato commercially in the United States (USA). Subsequently, several preplanned and specific modifications to the genomes of various plants have been accomplished to upgrade genome editing technology and thereby improve crop breeding methods (Zaman et al. 2018).

In genome editing technology, the genome of an organism itself is modified by knocking out or replacing the targeted gene for desired and selected traits, whereas in transgenic approaches biologically nonexistent foreign genes are introduced to the original genome to develop new characters in the existing species (Mushtaq et al. 2018). To date, many genome editing techniques have been implemented in plant molecular biology. These techniques have enabled researchers to make target regions of genes in a DNA sequence-specific manner (Brooks et al. 2014). Methods using zinc finger nucleases (ZFN), transcription activator-like nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 have been applied to modify the targeted plant genome (Jia and Wang 2014) (Fig. 1). These genome editing technologies generally induce double-strand breaks (DSB) or single-strand breaks (SSB), resulting in mutation of the target regions of the genome. The broken ends are then repaired by nonhomologous end-joining (NHEJ) or homologous recombination (HR) methods. Thus by adopting a gene knockout, knockin, or replacement strategy, site-directed mutagenesis-based genome editing is induced at the target regions of the genome, which results in modification of several morphological, physiological, and enhancement tolerance/resistance

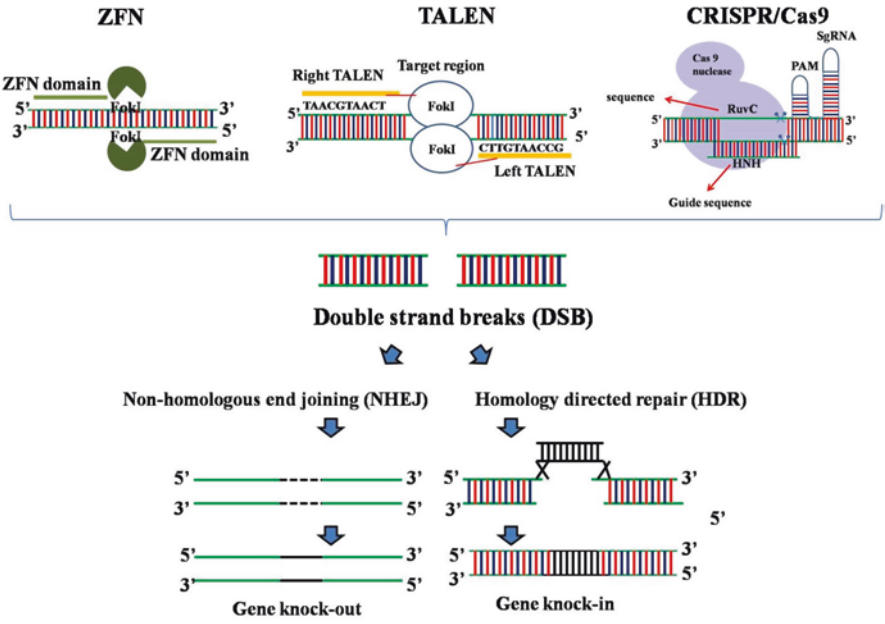

Fig. 1 Schematic representation of generalized genome editing mechanism in crop plants

characters along with the growth and development of different crop plants (Zhang et al. 2017). Modification of the genetic information of plants in an accurate and specific way will not only help us to study the gene function and biological mechanisms but also will help to create various novel phenotypes including enhanced yield and stress-tolerant crops. In this regard, genome editing technology has emerged as an advanced tool for improvement of crop production under abiotic stress (Petolino 2015).

2.1 Zinc Finger Nuclease

Zinc finger nuclease (ZFN) is generally used to cut a target DNA site that is later applied to error-prone nonhomologous end-joining (NHEJ), resulting in mutagenesis of the specific site. ZFNs have been used to modify endogenous genes in a wide spread of organisms and cell types (Urnov et al. 2010; Joung and Sander 2013). Various kinds of genomic alterations such as mutations, deletions, insertions, inversions, duplications, and translocations can be introduced with ZFNs (Fig. 2), which provides researchers with exceptional tools to perform genetic manipulations (Joung and Sander 2013). Fusion of ZFNs consisting of zinc finger protein domains, capable of sequence-specific DNA binding, and a nuclease domain is generally used for identification of protein domains, each recognizing approximately 3 bp DNA (Petolino 2015).

In its first application, ZFN enzymes in plants used a reporter sequence newly incorporated into the plant genome to separate ZFN-derived mutants (Tzfira et al. 2012). Afterward, several site-specific mutations using ZFN constructs were stably integrated into the plant genome (Osakabe et al. 2010; Zhang et al. 2010). Although

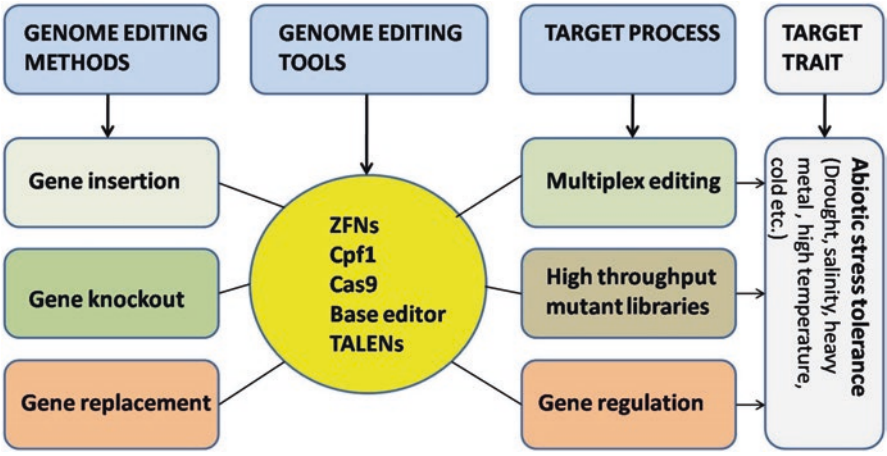

Fig. 2 Schematic depiction of genome editing methods, tools, target process, and target trait in developing abiotic stress-tolerant crop plants

in some cases short deletions count for 80% of the ZFN-induced mutations, in others nucleotide substitutions were 70% of all mutations induced (Lloyd et al. 2005). The design and application of ZFNs include modular design, assembly, and development of zinc fingers against specific DNA sequences, followed by linking of single ZFs in the direction of targeting larger sequences. In recent years, zinc finger domains have been created to identify a large number of triplet nucleotides, which allowed the selection and joining of zinc fingers in a sequence that would permit recognition of the target sequence of interest (Kamburova et al. 2017).

The first ZFN-mediated gene knockouts were implemented in the tobacco acetolactate synthase gene (ALS) known as SuRA for the development of herbicide-resistance plants (Maeder et al. 2008; Podevin et al. 2012). In *Arabidopsis* the ZFN technology has been used to efficiently cleave and stimulate mutations at an endogenous target gene, ABA-INSENSITIVE4 (ABI4). This gene encodes a member of the ERF/AP2 transcription factor family and has a role in regulating abscisic acid (ABA), which controls a number of agronomically important traits, including plant responses to abiotic stress and seed development. This ZFN-based genome modification results in mutation of the target gene ABI4 at a rate of approximately 0.26–2.86% in *Arabidopsis* somatic cells and transmission of the induced mutation in the target gene to subsequent generations (Osakabe et al. 2010).

So far gene modification by ZFN has been successfully implemented in soyabean (Curtin et al. 2013), *Arabidopsis thaliana* (Zhang and Voytas 2011; Qi et al. 2013; Li et al. 2014), maize (Shukla et al. 2009), and tobacco (Townsend et al. 2009; Jia and Wang 2014). However, the ZFN-based technology has a number of limitations from the complexity and high cost of protein domain construction for each particular genome locus and the chances of defective cleavage of target DNA from single nucleotide substitutions or unsuitable interaction between domains. Therefore, the search for new methods for genome editing continued and led to the development of new tools for genome editing: TALENs (transcription activator-like effector nucleases) and CRISPR/Cas9 (clustered regulatory interspaced short palindromic repeats) (Nemudryi et al. 2014).

2.2 Transcription Activator-Like Effector Nucleases (TALEN)

The idea of TALENs development comes from the study of bacteria of the genus *Xanthomonas*. These bacteria are pathogens of crop plants such as rice, pepper, and tomato, causing remarkable economic damage in agriculture, which led to their in-depth study. These bacteria generally secrete effector proteins (transcription activator-like effectors, TALEs) to the cytoplasm of plant cells and affect processes in the plant cell that increases the liability of the cells to the pathogen (Nemudryi et al. 2014). TALENs are generally used to introduce mutation by means of homologous recombination (HR) and nonhomologous end-joining (NHEJ). The homologous donor DNA is used as a template to restore the double-strand break (DSB),

resulting in gene insertion or replacement. Then, the broken ends are joined by NHEJ. Frequently small deletion or insertions are introduced at the junction of the newly rejoined chromosomes (Du et al. 2016) (Fig. 2).

Transcription activator-like (TAL) effectors of *Xanthomonas oryzae* help to reduce the severity of the bacteria *Xanthomonas* by transcriptionally activating the specific rice disease susceptibility (S) gene (Yang et al. 2006; Antony et al. 2010). Therefore, TALEN technology has been applied to edit the specific S gene in rice to counter the virulence strategy of the bacteria *Xanthomonas*. This engineered genome modification results in resistance to bacterial blight, a destructive disease in crops. A combination of TAL effector nucleases (TALENs)—fusion proteins derived from the DNA recognition repeats of unaffected TAL effectors and the DNA cleavage domains of Fok I (Christian et al. 2010; Li et al. 2010; Miller et al. 2010)—have been used to create site-specific gene modifications in plant cells (Mahfouz et al. 2011; Cermak et al. 2011; Li et al. 2012).

TALENs have also been used to modify the genome of the model plant, *Arabidopsis thaliana*. Here TALENs are used to target five *Arabidopsis* genes, namely, ADH1, TT4, MAPKKK1, DSK2B, and NATA2. In pooled seedlings expressing the TALENs, the somatic mutagenesis frequencies ranges from 2% to 15%. However, after modification of the genes by using TALENs, the somatic mutagenesis frequencies rise to 41–73% in individual transgenic plant lines expressing the TALENs. Additionally, a TALEN pair targeting a randomly duplicated gene induced a 4.4-kb deletion in somatic cells (Christian et al. 2013). In potato tubers, cold temperature usually stimulates the accumulation of reducing sugars. At the onset of high temperature, these reducing sugars react with free amino acids to give brown, bitter-tasting products with high levels of acrylamide, a potential carcinogen. To control the accumulation of reducing sugars, RNA interference (RNAi) technology was used to silence the vacuolar invertase gene (VInv). This gene encodes a protein that breaks down sucrose to glucose and fructose. Because RNAi often results in incomplete gene silencing, the transcription activator-like effector nucleases (TALENs) were applied to knock out the VInv gene within the commercial potato variety, Ranger Russet. For this, transiently expressing transcription activator-like effector nucleases (TALENs) are designed to bind and cleave specific DNA sequences in the Vinv locus. TALENs successfully result in complete VInv knockout lines without integrating any foreign DNA. The new potato lines have significantly lower levels of reducing sugars and acrylamide in heat-processed products (Clasen et al. 2015).

In comparison to ZFN, researchers have shown much interest in TALEN as they can be very easily and rapidly designed using a simple protein–DNA code. This protein–DNA code relates modular DNA-binding TALE repeat domains to individual bases at a specific binding site (Joung and Sander 2013). As ZFN and TALEN require considerable time and effort because of the difficulties in protein design, synthesis, and validation, the CRISPR/Cas9 system is widely used for genome editing as it has simplicity, design flexibility, and high efficiency (Wang et al. 2017).

2.3 Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR/Cas9)

The CRISPR locus was first observed in *Escherichia coli* (Ishino et al. 1987). Currently, it is known to be present in about 84% of Archaea and 45% of Bacteria (Grissa et al. 2007). The CRISPR system is an arrangement of short repeated sequences separated by spacers with unique sequences. The CRISPR is generally found on both chromosomal and plasmid DNA. The spacers are commonly derivatives of the nucleic acids of viruses and plasmids (Rath et al. 2015). The CRISPR/Cas9 system consists of a Cas9 endonuclease that is a derivative of *Streptococcus pyogenes*. In this process a chimeric single guided RNA is used to direct Cas9 to a specific DNA sequence in the genome, which results in a DNA double-strand break in the specific locus through Cas9. The DSB is repaired through either endogenous nonhomologous end-joining or through the high-fidelity homology-directed repair (HDR) pathways. NHEJ generally induces small insertions or deletions at the repair junction whereas HDR stimulates programmed sequence correction as well as DNA fragment insertion (Shi et al. 2017).

CRISPR activity is generally regulated by a set of CRISPR associate (Cas) genes, usually found close to the CRISPR. The Cas genes code for proteins essential to the immune response. The CRISPR-Cas mediated defense process functions in three stages. The first stage is called adaptation, which leads to insertion of new spacers in the CRISPR locus. The next step is known as expression, where the system is prepared for action by expressing the *cas* genes and transcribing the CRISPR into a long precursor CRISPR RNA (pre-crRNA). Subsequently, the pre-crRNA is organized into mature crRNA by Cas proteins and accessory factors. In the third stage, the target nucleic acid is identified and eliminated by the co-actions of crRNA and Cas proteins: this is the last stage of CRISPR-mediated action, known as interference (Rath et al. 2015).

The gRNA/Cas9 technology has used for targeting the *Arabidopsis thaliana PDS3* (*PHYTOENE DESATURASE*) gene in *Arabidopsis* mesophyll protoplasts, which are freshly isolated leaf cells without cell walls. The protoplast transient expression system supports highly efficient DNA co-transfection and protein expression. The application of gRNA/pcoCas9-mediated genome editing has also been extended to other plant systems, such as tobacco (*Nicotiana benthamiana*) protoplasts. Significantly higher mutagenesis frequencies were observed, that is, 37.7% and 38.5%, for targets 1 and 2, respectively, by targeting *NbPDS* (ortholog of AtPDS3) at two different sites than in *Arabidopsis*. Interestingly, the gRNA/pcoCas9-induced mutagenesis often led to significant DNA deletions or insertions but only rare single nucleotide (nt) substitutions in tobacco cells as in animal and human cells showing relatively high mutation rates (Li et al. 2013).

This system has also been successfully exploited in rice protoplast cells transformed with Cas9/sgRNA constructs targeting the promoter region of the bacterial blight susceptibility genes, OsSWEET14 and OsSWEET11 (Jiang et al. 2013; Miao

et al. 2013). The CRISPR/Cas9 SSN (C-ERF922) was used to target the OsERF922 gene, ERF, and TF genes in rice to enhance blast disease resistance (Wang et al. 2016a, b). By using the CRISPR/Cas9 system the role of OsMIR528 in rice was identified as a positive regulator in salt stress, targeting miRNAs (Zhou et al. 2017; Shanmugavadivel et al. 2019). Xu et al. (2016) have used the CRISPR/Cas9-mediated multiplex genome editing approach in rice. Here three genes, namely, GW2, GW5, and TGW6, were selected, and mutation in any one of these three genes resulted in significant increase of grain weight, which is considered as one of the most crucial quantitative traits in rice production (Xu et al. 2016).

To enhance the specificity and reduce the off-target effects of CRISPR-Cas system, approaches such as using Cas9 nickase, Cas9n, and dCas9 (mutated version of Cas9), careful design, and gRNAs truncated at the 5′-ends (trugRNAs) have been adopted to create the site-directed modifications in plants (Osakabe et al. 2016; Mushtaq et al. 2018).

So far, genome editing using CRISPR/Cas9 has been successful for various crop plants such as tomato (Wang et al. 2017; Li et al. 2019a, b), rice (Lou et al. 2017), and maize (Shi et al. 2017) for enhancement of drought tolerance. This technology has also been implemented to increase cold tolerance in rice (Shen et al. 2017a, b) and *Arabidopsis* (Li et al. 2016), and it has been applied under salinity and heavy metal stress in rice (Bo et al. 2019; Tang et al. 2017). Studies on the recent application of CRISPR/Cas9 in various crop plants for the enhancement of abiotic stress tolerance with improved morphological, physiological, and yield characteristics are depicted in Table 1.

3 Uses of CRISPR/Cas9 in Enhancing Abiotic Stress Tolerance in Crops

Clustered regularly interspaced short palindromic repeats associated endonuclease Cas9 (CRISPR/Cas9) is an immune system obtained from the microbes (Hryhorowicz et al. 2017). The CRISPR/SgRNA:Cas9 has been successfully implemented in diverse plant families such as Fabaceae (Cai et al. 2018; Jacobs et al. 2015; Du et al. 2016), Poaceae (Kim et al. 2018; Howells et al. 2018; Minkenberg et al. 2016), Malvaceae (Long et al. 2018; Gao et al. 2017), Asteraceae (Ynet and Yilancioglu 2018), and Solanaceae (Anderssona et al. 2018; Brooks et al. 2014). Many research articles discuss the application of the CRISPR-Cas9 system by knocking out a particular reported gene that is which involved in the abiotic stress-tolerant mechanism in plants. The customized CRISPR/Cas9 has become the easiest way of transformation in plants within less time (Belhaj et al. 2015; Bortesi and Fischer 2015). Applications of CRISPR in various crop species under different abiotic stresses are described briefly next.

Table 1 List of target genes manipulated via genome editing in different crop plants for enhancing abiotic stress tolerance

Genome editing methods	Crop plants	Target gene	Stress	Key findings	References
CRISPR/Cas9	Maize (*Zea mays*)	*ARGOS8*	Drought	Drought-tolerant breeding, reduced ethylene response, increase growth and development, and increased grain yield by five bushels per acre under flowering stress conditions	Shi et al. (2017)
	Tomato (*Solanum lycopersicum*)	*SlMAPK3*	Drought	Protects cell membranes from oxidative damage and regulates transcription of stress-related genes, thereby increasing growth and development	Wang et al. (2017)
	Tomato (*Solanum lycopersicum)* Cv. Ailsa Craig	*SlNPR1* mutant	Drought	In comparison to wild type, the *SlNPR1* mutant plants showed reduced drought tolerance, negative regulation of stomatal closure, increased electrolytic leakage, H_2O_2, and MDA content, decreased expression of antioxidant enzymes such as APX, SOD, POD, lower expression level of drought-related genes	Li et al. (2019a, b)
	Rice (*Oryza sativa* L.) Cv. Japonica	*OsSAPK2*	Drought	Enhanced drought tolerance, detoxification of ROS by increasing activities of antioxidant enzymes	Lou et al. (2017)
	Rice (*Oryza sativa* L.)	*OsNAC041*	Salinity	Salt-resistant plants with increased seed germination, growth, decreased level of ROS and MDA accumulation	Bo et al. (2019)
	Rice (*Oryza sativa* L.)	*OsAnn3*	Cold/4–6 °C for 3 days	Tolerance to cold stress, increased survivability up to 81.1% decreased electrical conductivity	Shen et al. (2017a, b)

	Rice (*Oryza sativa* L.) Cv. Huazhan, Cv. Longke 638S	*Osnramp5*	Heavy metals	Lower accumulation of manganese (Mn) and cadmium (Cd) in rice plants, no effect of growth, yield, and agronomic traits	Tang et al. (2017)
	Rice (*Oryza sativa* L.)	*OsPDS, OsMPK2, OsBADH2*	Multiple abiotic stress	Three rice genes showed tolerance capacity against various abiotic stress factors	Shan et al. (2013)
	Rice (*Oryza sativa* L.)	*OsMPK5*	Multiple abiotic stress	Plant exhibited various abiotic stress tolerance and disease resistance	Xie and Yang (2013)
	Rice (*Oryza sativa* L.)	*OsMPK2, OsDEP1*	Multiple abiotic stress	Yield under different abiotic stress conditions	Shan et al. (2014)
	Rice (*Oryza sativa* L.)	*OsDERF1, OsPMS3, OsEPSPS, OsMSH1, OsMYB5*	Drought	Plant showed drought resistance	Zhang et al. (2014)
	Rice (*Oryza sativa* L.)	*OsHAK-1*	Heavy metal stress	Plant showed low cesium accumulation	Cordones et al. (2017)
	Rice (*Oryza sativa* L.)	*OsPRX2*	Heavy metal stress	Potassium deficiency tolerance	Mao et al. (2018)
	Arabidopsis thaliana	*OST2* (OPEN STOMATA 2) (AHA 1)	Osmotic stress	Increased in the stomatal closure in response to abscisic acid (ABA)	Osakabe et al. (2016)
CRISPR sgRNA	Rice (*Oryza sativa* L.)	*OsAOX1a, OsAOX1b, OsAOX1c,OsBEL*	Multiple abiotic stress	Plant exhibited various abiotic tolerance mechanisms	Xu et al. (2015)
Dual-sgRNA/ Cas 9	*Arabidopsis thaliana*	*MIR169a*	Drought	Increased in the yield under drought stress condition	Zhao et al. (2016)
TALEN	Barrelclover (*Medicago truncatula*) v. R108	*MtCAS31*	Drought	Reduced negative effects of drought stress on symbiotic nitrogen fixation. MtCAS31 protects MtLb120-1 from the damage of drought stress	Li et al. (2018)

3.1 Drought

Compared to other abiotic stress factors, water deficit or drought is the most devastating stress affecting plant growth and yield (Zhang et al. 2018a, b). Drought stress normally occurs when the transpiration rate is higher than the uptake of water by the roots (Salehi-lisar et al. 2012). According to a global scale, drought stress decreases cereal production by 9–10% (Lesk et al. 2016). Drought or water deficit hinders plant growth and development by decreasing water uptake of the plant cells with decrease in the cell volume and cell wall size and unfavourably affects many physiological and biochemical responses (Li et al. 2019a, b). Drought stress also interferes with the photosynthetic process by reducing intercellular CO_2 concentration (Ci), chlorophyll *a*/*b* degradation, hydrolysis of chloroplast protein, and reducing of leaf pigments (Liang et al. 2019). Under drought stress plants indemnify by acquiring immobile nutrients, which alter the accumulation of beneficial metabolites such as proline and soluble sugar (Muler et al. 2014). Exposure to drought stress leads to accumulation of ROS, oxidation of amino acids, DNA nicking, lipid peroxidation, etc. (Nezhadahmadi et al. 2013). The causes of drought stress are various relevant conditions such as inadequate rainfall, low moisture quality of soil, evaporation demand, and imprudent water utilization (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Plants that develop tolerance capacity normally limit the number and area of leaves, which results in yield loss under drought stress (Akhtar and Nazir 2013). This stress negatively affects crop yield (Fahad et al. 2017). Daryanto et al. (2016) reported that under drought stress both maize and wheat experienced crop yield reduction as much as 20% and 39%, and also showed fertilization failure during the reproductive stage (Daryanto et al. 2016). Farooq et al. (2017) presented that under drought stress, an important food crop, cowpea, can reduce yield by 34–68% (Farooq et al. 2017). Ten days of drought stress on 35-day-old rice seedlings and 10 days of drought stress at the reproductive stage showed reduction of grain in four cultivars: Swarna Sub1 (46.07%), Nagina 22 (19.71%), NDR 97 (20.32%), and NDR 102 (24.94%) (Singh et al. 2018). Wei et al. (2018) have described that soybean plants on which was imposed drought stress at the vegetative growth stage showed reduction as great as 70–82% (Wei et al. 2018). Thus, application of the transgenic-based approach by introducing TFs to produce genetically engineered plants has reached negative perception because of the limitation of greenhouse trials and the additional cost.

To overcome this issue, nowadays genome editing is the acceptable alternative used in plant breeding (Lamaoui et al. 2018). In the past few years, efficient genome editing technologies have come to light in the research field by the rapid manipulation of DNA sequences and developing drought-tolerant germplasm by editing natural chromosomal context (Shi et al. 2017). Ethylene is responsible for the plant abiotic stress condition, which confers water deficit and high temperature (Müller and Munné-Bosch 2015). *ARGOS8* is a negative regulator of ethylene response that modulates ethylene transduction under drought stress when it is overexpressed in maize plants (Shi et al. 2015). Shi et al. (2017) used CRISPR-Cas9-enabled advanced breeding technology to generate maize lines carrying *ARGOS8* genome-edited variants, which increased maize grain yield and tolerance phenotypes under

drought stress conditions. An RNA-guided Cas9 endonuclease was used to generate DNA double-strand breaks in a site-specific manner for integrating the GOS2 PRO in to the upstream region of *ARGOS8* via homology-directed DNA repair to moderate constitutive expression of *ARGOS8*. The genome-edited plants showed higher expression of *ARGOS8* relative to wild-type (WT) controls. Under field conditions both the ARGOS8-V1/V2 variants showed grain yield of approximately five bushels per acre compared to the wild type. Also, early cessation of grain filling, less grain moisture, plant height, and ear height increased up to 2.6 cm and 3.2 cm in the two variants, respectively (Shi et al. 2017). Wang et al. (2017) aimed to study the function of *SlMAPK3* in tomato plants by using CRISPR/Cas9-mediated *SlMAPK3* mutants to find possible regulating mechanisms for drought tolerance. Both *SlMAPK3* mutants and WT plants were kept under drought stress of 23–25 °C with photoperiod 16:8 h light/dark, withheld from 6-week-old tomato plants for 5 consecutive days, and treated with 25% (w/v) PEG 6000 to analyze drought tolerance and to explore the regulatory mechanism. WT plants showed fewer wilted leaves compared to *SlMAPK3* mutant plants. Ion leakage was 70–83% higher than WT plants, with higher MDA content, more proline content, and H_2O_2 content significantly higher relative to WT control plants. All these elevated contents lead to damage to membranes by accumulating ROS and disturbing membrane integrity and stability. Also, activities of antioxidant enzymes in all mutant lines were significantly lower than in WT. Taken together, all these data reveal that *SlMAPK3* is a positive regulator of drought stress. A control line with expression of *SlMAPK3* is involved in drought response in tomato plants by protecting cell membranes from oxidative damage and modulating transcription of stress-related genes (Wang et al. 2017). Li et al. (2019a, b) used CRISPR-associated protein-9 nuclease (Cas9) technology to predict the function of *SlNPR1* and generated *SlNPR1* mutant tomato plants to compare with the WT tomato plants to analyze physiological and molecular mechanisms under drought stress. *SlNPR1* mutant plants showed seriously wilted leaves, bent stems, lower survival rate, and more stomatal closure compared to WT plants. Electrolyte leakage was 55–63%, and H_2O_2 accumulation was 230–221 $nmol^{-1}$ g^{-1} FW, with higher MDA level compared with WT. Under drought conditions, loss of SlNPR1 function in SlNPR1 mutants led to downregulation of antioxidant enzymes or the antioxidant genes *SlGST*, *SlDHN*, and *SlDREB*. These results suggest that *SlNPR1* might be involved in abiotic stress responses, such as drought stress (Li et al. 2019a, b). The overall studies confirm that by applying CRISPR/Cas9 technologies, there is remarkable potential to improve drought tolerance in important crop plants such as maize and tomato. Thus, this CRISPR/Cas9 technology can be implemented in other crops such as rice and wheat.

3.2 Salinity

Among all stresses, salinity is a vital stress reducing viable agricultural land and the demand for food crops (Gupta and Huang 2014). According to the FAO, salinity stress affects 6% of agricultural land worldwide, which exhibits serious limiting

factors for plant growth and productivity (Parihar et al. 2015). Salinity stress is of two types: (1) hyperosmotic stress and (2) hyperionic stress, which have different effects on plants under salt stress. In hyperosmotic stress, plants lose water from the root system and leaves, which changes various physiological and morphological characters including destroying the ability to detoxify abiotic stress, decrease antioxidant mechanism, impair photosynthetic activity, and decrease stomatal aperture. Under the hyperionic condition, plants uptake high salt that inhibits the intake of essential minerals such as phosphorus (P), potassium (K^+), nitrogen (N), and calcium (Ca^{2+}) (Gupta et al. 2015). This mineral deficiency induces disturbances in osmotic balance and enzymatic activity (Ashraf et al. 2018). Salinity notably affects fruiting, flowering, seed growth, and seed germination (Rai et al. 2013). The yield in crop plants including several growth parameters such as plant height, fresh weight yield, and biomass production are severely affected by salinity stress (Semiz et al. 2012).

The NAC transcription factor family has a key role in altering the number of plant metabolic pathways under abiotic stress such as drought and salinity (Xu et al. 2013; Lee et al. 2017; Shen et al. 2017a, b). Bo et al. (2019) created an OsNAC041 mutant by using the CRISPR/Cas9 method to determine the specific function of rice NAC transcription factor coding gene OsNAC041 under salt treatment. Under 150 mmol/l NaCl treatment, shoots of the wild-type seedling were taller than mutant plants. The wild-type seedling remained alive, whereas almost all the mutant seedlings died. The O_2^- and H_2O_2 levels also revealed a significant increase in ROS accumulation and MDA content in the mutants compared with the wild type.

OsNAC041 mutants affected the membrane protection system by decreasing activities of sediment oxygen demand (SOD), photochemical oxygen demand (POD), and chloramphenicol acetyl transferase (CAT), thereby weakening salt tolerance. These findings provided evidence that OsNAC041 has an important role in salt resistance in rice (Bo et al. 2019). So far, much less work has been carried out on crop plants using genome editing tools to improve salt tolerance. Plant breeders can use CRISPR/Cas9 to improve salt stress tolerance and to understand the physiological responses of plant growth and development.

3.3 Heavy Metals

Heavy metals are nonbiodegradable, with atomic mass greater than 20 and density greater than 5 g/cm^3, and have cytotoxic, genotoxic, and mutagenic effects on living organisms such as plants (Rascio and Navari-Izzo 2011). Toxic heavy metals evoke stress by accumulating ROS, promote DNA damage, or disturb the DNA repair mechanism, and also hinder membrane functional integrity, protein function, and activities (Tamás et al. 2014). Heavy metal pollution can cause crop growth stress, affecting crop production and the quality of crops as well as affecting human health after entering the human body through the food chain (Lei et al. 2015).

Cadmium (Cd) is a heavy metal that is highly toxic for most living organisms (Clemens et al. 2013). A recent survey showed that Cd concentration is high in rice grains. So, controlling Cd accumulation in rice grains is important for food safety and the health of people who consume rice as a daily food in their diet (Jallad 2015). CRISPR/Cas9 has been successfully used to minimize the Cd content in rice grains (Tang et al. 2017). Tang et al. (2017) have developed a low-Cd new Indica rice line by knocking out the gene OsNRamp5 using the CRISPR/Cas9 system. Under toxic conditions of 2.5 μM Cd, the *Onramp5* mutant rice lines showed lower Cd concentration, less than 0.05 mg/kg, compared with the grain of WT plants at 0.33–2.90 mg/kg. Also, low Cd accumulation led to decreased rescue of reduced growth in mutant rice lines relative to WT plants (Tang et al. 2017). However, studies on different crop plants under heavy metal stress and application of CRISPR/Cas9 technology to improve crop tolerances to heavy metals are relatively few.

3.4 Cold Stress

The yield of crops, and their quality and distribution, have been affected by cold stress in various parts of the world. Cold stress generally affects leaf photosynthesis and biomass accumulation, which are the main sources of grain yield (Liu et al. 2019). Yield loss caused by low temperatures is a major restriction on rice cultivation not only in areas at high latitudes or high altitudes but also in tropical countries such as the Philippines and Thailand. Rice plants have a lower threshold temperature (10–13 °C) for cold damage during the early stages of development (germination and vegetative) (Cruz et al. 2013). To enhance cold tolerance in various crops, several transgenic techniques have been applied routinely. The main aim is to identify the novel gene that has the ability to increase cold tolerance (Shen et al. 2017a, b).

Shen et al. (2017a, b) suggested that the rice annexin gene *OsAnn3* was involved in cold tolerance by the knocked-down *OsAnn3* gene in Japonica rice variety Taipai.309 via CRISPR/Cas9 mediated genome editing. Under 4–6 °C for 3 days cold treatment and then return to normal growth conditions, after 10 days wild-type plants showed a survival rate up to 75–81.1%, whereas in T1 mutant plants the survival rate was 55.5%. Electrical conductivity levels increased in the T1 mutants compared to wild-type plants. These results indicated that the knockdown of *OsAnn3* in rice significantly decreases cold tolerance, and also it shows that the presence of *OsAnn3* can enhance plant tolerance under cold stress (Shen et al. 2017a, b). Li et al. (2016) have demonstrated that two *Arabidopsis* glycotransferase genes, *UG79B2* and *UGT79B3*, are involved in cold stress under the regulation of CBF1 by using CRISPR/Cas9 and RNAi technology. Twelve-day-old *Arabidopsis* wild-type, overexpressed *UGT79B2/B3OE* plants and RNAi, Cas9 mutant lines *ugt79b2/b3* were exposed under 4 °C cold conditions. At lower temperature, −12 °C, both *ugt79b2/b3* mutant lines and the wild type turned completely white, with more ion loss, less survivability, whereas overexpressed *UGT79B2/B3OE* plants showed 25% survival rate, less ion leakage, higher antioxidant capacities, and accumulated

anthocyanin for scavenging ROS, which led to enhanced tolerance under cold stress (Li et al. 2016). The use of genome editing tools, especially the CRISPR/Cas9 system, has been able to identify various cold stress-related genes in plants such as rice and *Arabidopsis* through overexpression and mutation.

4 Conclusion

Genome editing is revolutionizing crop breeding to the next generation for its several useful features such as ease of use, accuracy, simplicity, high specificity, and tolerable target effects. Genome editing as an advanced molecular biology technique can produce precisely targeted modifications in any crop plant. Given the availability of a variety of genome-editing tools with different applications, it is important to consider the optimal system for a given species and purpose. With the progress already made in the development of genome-editing tools and the development of new breakthroughs, genome editing promises to have a key role in accelerating crop breeding and in meeting the ever-increasing global demand for food. Moreover, the exigencies of climate change call for great flexibility and innovation in crop resilience and production systems. Application of genome editing tools in improving crop plant tolerance to abiotic stress, yield enhancement, grain quality, nutritional value, and other important agronomic traits will be prominent areas of work in the future. To date, most work in using genome editing technology has been preliminary and needs further improvement to efficiently utilizing the platform that leads to increasing on-target efficacy, and thereby the global food security of the ever-growing population of the world.

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Metabolomic Profiling of Plants to Understand Reasons for Plant Stress Resilience to Abiotic Stress

Prashat G. Rama and T. Vinutha

Large numbers of metabolites are produced by plants. They are of diversified structures and abundance and are important for plant growth, development and environmental response. These diverse metabolites are the chemical base of crop yield and quality, valuable nutrition and energy sources for human beings and live stocks (Hall et al. 2008). These metabolites are generally classified into primary and secondary metabolites. The primary metabolites are indispensable for the growth and development of a plant and secondary metabolites are not essential for growth and development but are necessary for a plant to survive under stress conditions by maintaining a delicate balance with the environment. Primary metabolites are highly conserved in their structures and abundances but secondary metabolites differ widely across plant kingdoms (Scossa et al. 2016). The diversity of plant metabolites and their role in complicated regulatory mechanism necessitates exploring the underlying biochemical nature (Hall et al. 2008). It will be very challenging to study the metabolome of plants because of the complexity of the diverse metabolic characteristics and abundances of molecules. Plant metabolism is disturbed by various abiotic stresses. The reconfiguration of metabolic networks of plant must happen under stress conditions to allow both the maintenance of metabolic homeostasis and production of compounds that ameliorate the stress.

When plants are subjected to unfavourable growth conditions, such as abiotic stress, the plant growth and productivity is retarded. Under most abiotic stress conditions, plant metabolism is disturbed either because of inhibition of metabolic enzymes, shortage of substrate, excess demand for specific compounds or a combination of these factors and many other reasons. So the metabolic network must be reconfigured in a way that essential metabolism is maintained and a new steady state is adopted for acclimatization of the prevailing stress conditions. The metabolic

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reprogramming is necessary to meet the demand for anti-stress agents including compatible solutes, antioxidants and stress-responsive proteins. The reactive oxygen species (ROS) accumulation is another problem which causes oxidation and dysfunction of cellular components and in the worst case cell death. The metabolic flux optimization via the organellar electron transport chains is crucial in order to lessen ROS production. The redox state maintenance in the cell is thus an important task to provide the reducing power required for ROS scavenging. Despite these important roles of metabolic regulation under stress conditions, our understanding of this process currently is fragmented and far from complete.

Despite the fact that metabolomics is downstream of the other functional genomics (transcriptomics and proteomics), the practical size of the metabolome of a species, unlike transcriptome or proteome, cannot be speculated directly by known genomic information via central dogma. Therefore, metabolomics is used to obtain a large amount of valuable information for the discovery of genes and pathways through accurate and high throughput corollary peak annotation via snapshotting the plant metabolome (Tohge et al. 2014). It seems that there is a complicated regulatory network among these small molecules in plants, and by detecting the interactions among these metabolites, metabolomic analysis contributes significantly to the understanding of the relation between genotype and metabolic outputs by tackling key network components (Toubiana et al. 2013). Plant metabolomics has become a powerful tool to explore various aspects of plant physiology and biology, which significantly broadens our knowledge of the metabolic and molecular regulatory mechanisms regulating plant growth, development and stress responses, and the improvement of crop productivity and quality. Plants have evolved a series of adaptive changes at both transcriptional and post-transcriptional levels to encounter various environmental stresses during their developmental processes, resulting in the reconfiguration of regulatory networks to maintain homeostasis (Verslues et al. 2006). Expression of stress responsive genes are activated once the plant receptors are stimulated by stress signals, resulting in subsequent biosynthesis of specialized metabolites to adapt to environmental stresses (Nakabayashi and Saito 2015).

The era of ‘Omics’ has witnessed huge developments in the fields of genomics, transcriptomics, epigenomics, proteomics, metabolomics and phenomics. The information generated by these ‘Omics’ approaches has enhanced the breeding programme precision and speed in developing climate smart and nutrition-rich germplasm for ensuring food security (Parry and Hawkesford 2012). The role of phenomics-based breeding has become more evident in recent years in improving the crop production and productivity (Khush, 2001; Langridge and Fleury 2011; Wang et al. 2017; Xavier et al., 2017). Compared with genomics, transcriptomics and proteomics, metabolomics provide a direct and global snapshot of all the metabolites. Metabolomics is as one of the major breakthroughs in science, paving the way for accurate metabolite profiling in microbes, plants and animals (Heyman and Dubery 2016; van Dam and Bouwmeester 2016; Wuolikainen et al. 2016). Metabolomics is the most complex of all the Omics approaches but received

inadequate attention in the field of crop science, particularly for trait mapping and plant selections. It has the ability to detect a vast array of metabolites from a single extract, thus allowing speedy and precise analysis of metabolites, offering a comprehensive view of cellular metabolites which participate in different cellular events, thus representing the absolute physiological state of a cell.

Metabolomics can be classified as untargeted metabolomics and targeted metabolomics based on the researchers' approach. Untargeted metabolomics, also called as discovery metabolomics, usually involves the comparison of the metabolome between the control and test groups, to identify the differences between their metabolite profiles which may be relevant to specific biological conditions. Targeted metabolomics is a quantitative method for the identification and quantitative analysis of targeted metabolic compounds in organisms. It provides information on the content and composition of metabolites, which are closely associated with the biological activities and can vary dramatically under different physiological conditions. Therefore, metabolomics methods are important for studying the biological function and comparing the metabolic systems of different organisms.

Rapid qualitative and quantitative analyses of metabolic responses of plants to environmental disturbances will not only help us to identify the phenotypic response of plants to abiotic stresses but also they reveal the genetic and biochemical mechanisms underlying the plant's responses to stresses. Metabolomics is a powerful tool by which a comprehensive perspective is gained of how metabolic networks are regulated and has indeed been applied by many researches in recent years. The plant plasticity for the future genetic engineering of stress resistant/tolerant plants can also be better understood. The output largely depends on the methodologies and instrumentations to comprehensively identify, quantify and localize every metabolite. Despite the fact that currently it is not possible to do accurate and exhaustive whole metabolome analysis of a biological sample, the methodologies and instrumentations of plant metabolomics are developing rapidly (Hegeman 2010). Large scale analysis of highly complex mixtures are made possible at present by a series of integrated technologies and methodologies, like non-destructive NMR (nuclear magnetic resonance spectroscopy), mass spectrometry (MS) based methods including GC–MS (gas chromatography–MS), LC–MS (liquid chromatography–MS) and CE–MS (capillary electrophoresis–MS), and FI-ICR–MS (Fourier transform ion cyclotron resonance–MS) (Okazaki and Saito 2012; Khakimov et al. 2014). Metabolomics could be performed in the subcellular level and even in a single cell with assistance from other technologies of sampling (Kueger et al. 2012; Moussaieff et al. 2013; Misra et al. 2014; Sweetlove et al. 2014). These analytical approaches have shown their potential power in plant metabolomic studies in many common plant species including staple food crops such as tomato, rice, wheat and maize for various purposes (Hu et al. 2014; Francki et al. 2016; Rao et al. 2014; Bénard et al. 2015). However, because of the intrinsic limitation of each analytical platform, combined approaches are increasingly used in metabolomics analysis (Figs. 1 and 2).

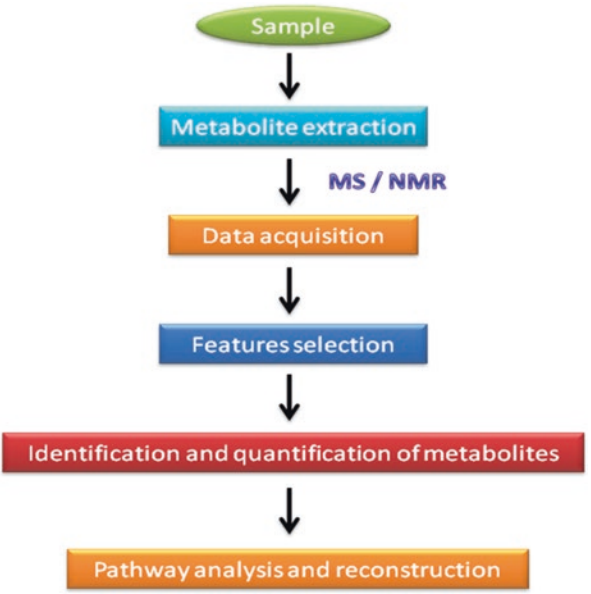
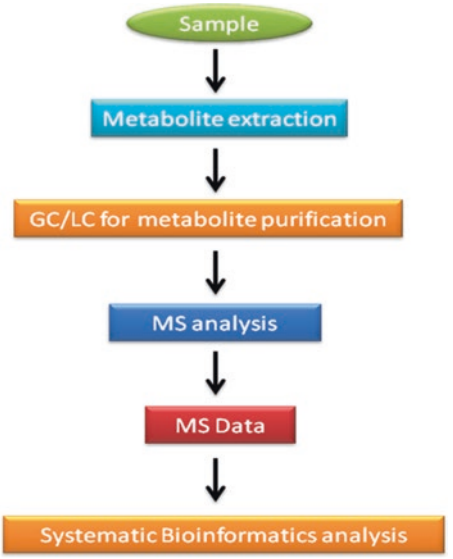


Fig. 1 Steps involved in untargeted metabolomics (Source: https://www.creative-proteomics.com/services/untargeted-metabolomics.htm)

Fig. 2 Steps involved in targeted metabolomics (Source: https://www.creative-proteomics.com/services/targeted-metabolomics.htm)



1 Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography-mass spectrometry is the most commonly used technique for plant metabolomics research. Polar metabolites are derivatised to make them volatile and then separated by GC. The crucial advantage of this technology is that it has long been used for metabolite profiling, and thus there are stable protocols for machine setup and maintenance, and chromatogram evaluation and interpretation (Fernie et al. 2004; Lisec et al. 2006; Halket et al. 2005). The short running time and relatively low running cost are also strong advantages of GC-MS. However the use of GC-MS is limited for thermally stable volatile compounds, so the analysis of high molecular weight compounds (˃1 kDa) difficult. GC-MS facilitates the identification and robust quantification of a few hundred metabolites in plant samples such as sugars, sugar alcohols, amino acids, organic acids and polyamines, resulting in fairly comprehensive coverage of the central pathways of primary metabolism.

2 Liquid Chromatography (LC)-MS

LC does not require prior sample treatment and separates the components in a liquid phase and hence does not have the limitation due to volatilization of compounds. The choice of columns, including reversed phase, ion exchange and hydrophobic interaction columns, provides the separation of metabolites based on different chemical properties. Therefore, LC has the potential to analyse a wide variety of metabolites in plants. The technique becomes more powerful by the recent development of ultra-performance liquid chromatography (UPLC) which has higher resolution, sensitivity and throughput than conventional high-performance liquid chromatography (HPLC) (Rogachev and Aharoni 2012). Electrospray ionisation (ESI) is widely used for ionisation to connect LC and MS. Many types of MS including quadrupole (Q), TOF, qTOF, triple quadrupole (QqQ), ion trap (IT), linear trap quadrupole (LTQ)-Orbitrap and Fourier transform ion cyclotron resonance (FT-ICR)-MS are used depending on the sensitivity, mass-resolution and dynamic range required (Allwood and Goodacre 2010; Lei et al. 2011). The combination of these techniques allows us to identify and quantify a large variety of metabolites even if they have high molecular mass, great polarity and low thermostability.

3 Capillary Electrophoresis (CE)-MS

In capillary electrophoresis, polar and charged compounds are separated on the basis of their charge-to-mass ratio. CE is a more powerful technique than LC with respect to separation efficiency and is able to separate a diverse range of chemical compounds (Ramautar et al. 2009, 2011). ESI is commonly used for ionisation as in

LC-MS, with TOF-MS being the most commonly used detector in CE-MS-based metabolomics studies. This combination provides high mass accuracy and high resolution. The high scan speed of TOF-MS makes this instrument very suitable for full scan analyses in metabolomics. CE-MS requires only a small amount of sample for analysis; only nanolitres of sample are introduced into the capillary. It can produce analysis within seconds with high electric fields and short separation lengths. It is highly preferred in the metabolic analysis of volume-restricted samples. This leads to low concentration sensitivity requiring enrichment of metabolites within the samples (Monton and Soga 2007). Another drawback of CE is the poor migration time reproducibility and lack of reference libraries, which may only be partially overcome by the prediction of migration time (Sugimoto et al. 2010). Since CE and LC can both separate a large variety of metabolites via fundamentally different mechanisms, they are often used in combination to provide a wider coverage of metabolites (Urano et al. 2009; Williams et al. 2007; Soga and Imaizumi 2001). But, the use of CE-MS in plant studies remains relatively rare.

4 Nuclear Magnetic Resonance (NMR) Spectroscopy

Nuclear magnetic resonance spectroscopy is an entirely different analytical technique than that of MS-based techniques being based on atomic interaction. In a strong magnetic field, atoms with non-zero magnetic moment including ^{1}H, ^{13}C, ^{14}N, ^{15}N and ^{31}P absorb and re-emit electromagnetic radiation. The radiation is characterized by its frequency, intensity, fine structure and magnetic relaxation properties, all of which reflect the precise environment of the detected nucleus. Therefore, atoms in a molecule give a specific spectrum of radiation that can be used for identification and quantification of metabolites within a complex biological sample. The sensitivity of this method is much lower than that of MS-based techniques but the structural information content, reproducibility and quantitative aspects can be superior to them. The preparation of the sample is simple and even nondestructive measurement is possible. NMR can further generate kinetic measurements in vivo and examine metabolic responses on the same plant rather than on a set of similar plants (Terskikh et al. 2005). The difference in the sub cellular pHs of the vacuole and the rest of the cell results in distinctive signals from an identical metabolite and thus allows quantification at the subcellular level (Ratcliffe and Shachar-Hill 2005; Eisenreich and Bacher 2007). Thus analysing the metabolite composition of a tissue extract, determining the structure of a novel metabolite, demonstrating the existence of a particular metabolic pathway in vivo, isotope labelling experiment and localising the distribution of a metabolite in a tissue are all possible by NMR. These properties of NMR make it the ideal tool for broad-range profiling of abundant metabolites whilst studying changes in non-annotated profiles is highly useful for metabolite fingerprinting of extensive sample collections (Lommen et al. 1998; Dixon et al. 2006).

The NMR based metabolite detection is based on the magnetic properties of nuclei of atoms under magnetic field. The NMR is a non-destructive method extensively used to identify metabolites with smaller molecular weight (<50 kDa) for diverse applications like metabolite fingerprinting, profiling, metabolic flux and extracting the atomic structural information of compound present in the biological samples (Winning et al. 2009). The drawback of technique is its poor sensitivity owing to a limited coverage of low-abundance biomarkers which poses a major limitation that in turn restricts its extensive use.

Unlike NMR, a wide coverage of metabolome data can be attained by greater sensitivity of MS techniques, leading to the identification of novel metabolic biomarker, and molecules that can facilitate the reconstruction of metabolic pathways and networks. With the advances in the ionization methods such as atmospheric pressure chemical ionization (APCI), electrospray ionization (ESI) and MALDI-TOF, MS has achieved greater accuracy (Issaq et al. 2009). MS is usually combined with chromatography techniques such as gas chromatography (GC), liquid chromatography (LC), capillary electrophoresis (CE), Fourier-transform ion cyclotron resonance (FT-ICR) and field asymmetric waveform ion mobility spectrometry (FAIMS) to enhance the throughput. Despite the low sensitivity and large sample requirement of NMR, its capacity of identifying physical properties of ligands, binding sites on protein, uncovering structures of protein ligand complexes and direct binding of target protein retains its use over MS.

Metabolomics is increasingly becoming common in plant physiology and biochemistry, and to date has been applied to a staggering number of studies. In this chapter we will see the application of metabolomic profiling to understand the reasons for plant stress resilience to abiotic stress.

5 Water Stress

One of the major threats in crop production is the limitation of water and is projected to get considerably worse in the coming years (Cominelli et al. 2009). For this reason considerable research effort has been expended to understand the response to this crucial and common stress. These studies have revealed an important role for metabolic regulation including regulation of photosynthesis and accumulation of osmolytes in the drought stress response (Chaves and Oliveira 2004; Verslues and Juenger 2011). Accumulation of many metabolites including amino acids such as proline, raffinose family oligosaccharides, γ-amino butyrate (GABA) and tricarboxylic acid (TCA) cycle metabolites was observed in *Arabidopsis* leaves under drought condition. These accumulated metabolites are known to respond to drought stress in plants (Urano et al. 2010). Analysis of wheat leaves in response to water deficient conditions, indicated amino acids, organic acids and sugars as the main metabolites changed in abundance upon water deficiency. Bahar *cv*, the drought susceptible spring-wheat cultivar showed increased levels in proline, methionine, arginine, lysine, aromatic and branched chain amino acids. Auxin production was sustained by

tryptophan accumulation via shikimate pathway and glutamate reduction is reasonably linked to polyamine synthesis. But the metabolome of drought tolerant Kavir *cv* was affected only to a lesser extent with only two pathways changed significantly, one of them being purine metabolism (Michaletti et al. 2018). Profiling of soybean leaf metabolites under control, drought and heat stress conditions was conducted in a controlled environment. Analyses of non-targeted metabolomic data showed that in response to drought and heat stress, carbohydrates, amino acids, lipids, cofactors, nucleotides, peptides and secondary metabolites were differentially accumulated in the leaves. The metabolites for various cellular processes, such as glycolysis, the tricarboxylic acid (TCA) cycle, the pentose phosphate pathway and starch biosynthesis, that regulate carbohydrate metabolism, amino acid metabolism, peptide metabolism and purine and pyrimidine biosynthesis, were found to be affected by drought as well as heat stress (Das et al. 2017)

Too much water, in situations like flooding or water-logging of the rhizosphere causes problems because of the reduced oxygen availability (hypoxia/anoxia). ATP has to be produced by fermentation under anoxic conditions, resulting in cytosolic acidification and the accumulation of toxic products. The accumulation of amino acids, alanine, proline and GABA, and the phosphoesters, glucose-6-phosphate and glycerol-3-phosphate, were observed in the analyses of metabolic responses in Arabidopsis roots under anoxic conditions. Changes in the levels of minor sugars and various organic acids were also observed. There is a general tendency for an increase in the levels of the intermediates both of sucrose degradation and the TCA cycle, and in the levels of most amino acids when oxygen is decreased to 4%, whereas they are decreased when the oxygen is further decreased to 1%, indicating the inhibition and reactivation of metabolic activities. Together with the transcriptomic data showing a general downregulation of energy-consuming processes, the results demonstrated a large-scale reprogramming of metabolism under oxygen-limited conditions van Dongen et al. (2009). The accumulation of alanine under anoxic conditions in *Lotus japonicus*, which is highly tolerant to water logging was studied by Rocha et al. (2010). Succinate, alanine and the direct co-substrates for alanine synthesis, glutamate and GABA, were highly accumulated in the roots of *L. japonicus*, whereas the majority of amino acids that are derived from TCA cycle intermediate decreased during water logging. The results are in agreement with the metabolic equilibriums that are expected to drive the metabolic flux from glycolysis, via alanine synthesis and oxoglutarate to succinate, which prevents the accumulation of pyruvate activating fermentation and leading to ATP production by succinyl-CoA ligase.

6 Temperature Stress

Plant cells are seriously damaged by ice formation and dysfunction of cellular membranes when exposed to freezing environments (Guy 1990). Many plant species increase their freezing tolerance when exposed to non-freezing low

temperature by a process known as 'cold acclimation'. The molecular basis of this process has been extensively studied, and the role of particular metabolites including compatible solutes (Wanner and Junttila 1999) and the transcriptional regulatory network has been elucidated (Thomashow 2010; Medina et al. 2011). The metabolomic studies of cold acclimation were first performed by Cook et al. and Kaplan et al. in 2004. Metabolomic changes during cold acclimation in two ecotypes of *Arabidopsis thaliana*, Wassilewskija-2 (Ws-2) and Cape verde islands-1 (Cvi-1), which are relatively freezing tolerant and sensitive, respectively was compared by Cook et al. (2004). Ws-2 plants showed extensive alteration in metabolome in response to low temperature. Seventy-five percent of metabolites monitored were found to increase in cold-acclimated plants including metabolites known to increase in Arabidopsis plants upon exposure to low temperature, like the amino acid proline and the sugars glucose, fructose, inositol, galactinol, raffinose and sucrose. Novel changes like the increase of trehalose, ascorbate, putrescine, citrulline and some TCA cycle intermediates was also found. Considerable overlap was found in the metabolite changes that occurred in the two ecotypes in response to low temperature; however, quantitative differences were evident. Metabolome analysis of Arabidopsis over the time course following the shift to cold and heat conditions was conducted by Kaplan et al. (2004). Surprisingly the majority of heat shock responses were shared with cold shock including the increase of pool sizes of amino acids derived from pyruvate and oxaloacetate, polyamine precursors and compatible solutes. The results of this study were analysed together with following transcript profiling data (Kaplan et al. 2007), which revealed that the regulation of GABA shunt and proline accumulation under cold conditions are achieved by transcriptional and post-transcriptional manners, respectively.

7 Light Stress

Too high light irradiance represents an abiotic stress factor for plants since light is a highly energetic substrate driving photosynthesis that can induce secondary destructive processes at the same time. Metabolite profiling of Arabidopsis leaves for 6 days after transition to high light was conducted by Wulff-Zottele et al. (2010). Most of the metabolites of the glycolysis, TCA cycle and oxidative pentose phosphate pathway were altered in their content, indicating that plants exposed to high light undergo a metabolic shift and enhance the Calvin–Benson cycle to fix more carbon. Elevation of glycine in addition indicated the activation of photorespiratory pathways. The early metabolic response against high light was studied by Caldana et al. (2011) as a part of a more comprehensive study. The photorespiratory intermediates such as glycine and glycolate, were found to be accumulated in the early phase (5–60 min after transition). Interestingly the response during the mid-phase (80–360 min) shares similar properties with low temperature treatment, which includes the accumulation of shikimate, phenylalanine and fructose, and the decrease of succinate; Arabidopsis plants were treated with UV light and the

subsequent metabolic effect of UV light stress was analysed by Kusano et al. (2011). Arabidopsis showed an apparent biphasic response to UV-B stress, characterised by major changes in the levels of primary metabolites, including ascorbate derivatives. By contrast, mid- to late-term responses were observed in the classically defined UV-B protectants, such as flavonoids and phenolics. The results suggested that in early stages of exposure to UV-B, the plant cell is 'primed' at the level of primary metabolism by a mechanism that involves reprogramming of the metabolism to efficiently divert carbon towards the aromatic amino acid precursors of the phenylpropanoid pathway. It also suggested the importance of ascorbate in the short-term response to UV-B.

8 Ion Stress

High levels of salinity in the soil inhibit the growth and development of crops and cause serious problems for world food production (Munns, 2005). Both hyperionic and hyperosmotic stress effects were caused due to high concentrations of NaCl, which results in the decline of turgor, disordered metabolism and the inhibition of uptake of essential ions, as well as other problems in plant cells (Kim et al. 2007; Tester and Davenport 2003). Metabolite profiling of salt-treated *Arabidopsis thaliana* and its relative *Thellungiella halophila* (salt cress), which shows extreme tolerance to a variety of abiotic stresses, like low humidity, freezing and high salinity was studied by Gong et al. (2005). There was a dramatic increase in proline, inositols, hexoses and complex sugars in both the species. The concentrations of metabolites were often several-fold higher in Thellungiella and stress exacerbated the differences in some metabolites. The difference in metabolites between Arabidopsis and Thellungiella under salt and osmotic stresses was assessed for a broader range of metabolites (Lugan et al. 2010). A shift from nonpolar to polar metabolites in both species was observed by the analysis of global physicochemical properties of metabolites, but the shift was much more pronounced in Thellungiella. Such a shift may contribute to keep the water potential during dehydration. The cellular level metabolic response under salinity stress using Arabidopsis T87 cultured cells was studied by Kim et al. (2007). The methylation cycle for the supply of methyl groups, the phenylpropanoid pathway for lignin production and glycine betaine biosynthesis were found to be synergetically induced as a short-term response against salt-stress treatment. The results also suggest the co-induction of glycolysis and sucrose metabolism as well as co-reduction of the methylation cycle as long-term responses to salt stress. Due to the importance of salinity stress in agriculture, there are many metabolomic studies to assess the metabolic effect of salinity in a variety of crop and related plant species including tomato (Shulaev et al. 2008; Johnson et al. 2003), grapevine (Cramer et al. 2007), poplar (Brosché et al. 2005), sea lavender (*Limonium latifolium*; Gagneul et al. 2007) and rice (Zuther et al. 2007).

Heavy metals such as cadmium (Cd), caesium (Cs), lead (Pb), zinc (Zn), nickel (Ni) and chromium (Cr) are major pollutants of the soil causing stress on plants. At inappropriate concentration even the essential nutrients such as copper (Cu), iron (Fe) and manganese (Mn) can cause heavy metal stresses. Generally heavy metals induce enzyme inhibition, cellular oxidation and metabolic perturbation, resulting in growth retardation and in extreme instances can cause plant death (Sharma and Dietz 2009). Increased levels of alanine, β-alanine, proline, serine, putrescine, sucrose and other metabolites with compatible solute-like properties, notably GABA, raffinose and trehalose were found in Arabidopsis plants treated with Cd (Sun et al. 2010). The concentrations of α-tocopherol, campesterol, β-sitosterol and isoflavone (antioxidants) also increased significantly. When taken together these results indicate an important role of antioxidant defences in the mechanisms of resistance to cadmium stress. Transcriptomic and metabolomic analysis of rice roots treated with Cr was conducted by Dubey et al. (2010). Under these conditions proline accumulated to a threefold level than those of the control as did ornithine, which can be used in its synthesis. The content of several other metabolites including lactate, fructose, uracil and alanine increased following exposure to Cr stress. The observations suggest that the modulation of the sucrose degradation pathway involving the three main fermentation pathways was operating as a rescue mechanism when respiration is arrested.

9 Oxidative Stress

Oxidative stress is a key component of most abiotic stresses and a major limiting factor of plant growth in the field (Mittler 2006) which is the result of the overproduction of reactive oxygen species (ROS) in plant cells when plant metabolism is perturbed by various stresses. This consequently leads to oxidative damages of cellular components such as DNA, proteins and lipids (Moller et al. 2007). The metabolic network of plant cells must be reconfigured either to bypass damaged enzymes or to support adaptive responses in order to cope with oxidative stress. Baxter et al. treated, heterotrophic Arabidopsis cells with menadione, which enhances the ROS production via electron transport chains and changes in metabolite abundance, and ^{13}C-labelling kinetics were quantified. Sugar phosphates related to glycolysis and oxidative pentose phosphate pathways (OPPP) were found to be accumulated, suggesting the rerouting of glycolytic carbon flow into the OPPP possibly to provide NADPH for antioxidative effort. In addition the decrease of ascorbate and accumulation of its degradation product, threonate, indicated the activation of antioxidative pathways in menadione-treated cells. The reduced glycolytic activity probably leads to the decrease of levels of amino acids derived from glycolytic intermediates. The decrease of amino acids linked to TCA cycle intermediates and decrease of malate indicated a perturbation of TCA cycle (Baxter et al. 2007).

10 Combination of Stresses

Adverse environmental conditions in nature usually are made of several different factors, where one stress is usually accompanied or followed by another (Král'ová et al. 2012). In order to clearly define the contribution of individual stress, a controlled variable method was introduced and plants were subjected to a single primary stress factor to simplify the system (Chaves et al. 2009). But, in nature, plants often encounter not only one single stress, it will be followed by other stresses. It is most convenient both for experiments and discussion at the single stress level, but the plants are actually subjected to a combination of abiotic stress conditions in their natural habitat. Even some abiotic stresses are already combinations of stresses. For example high salt concentration causes osmotic and ion stresses, and flooding results in hypoxic and shading stresses. Although the metabolic responses of plants under a single abiotic stress have been analysed extensively as discussed, there are only few studies regarding to the effect of stress combinations on plant metabolism. When maize plants are subjected to water stress and salinity stress either separately or at the same time, levels of citrate, fumarate, phenylalanine, valine, leucine, isolecuine in leaves change significantly only under combined stresses, clearly explaining a crosstalk effect in multiple stresses (Sun et al. 2015). Heat and drought stresses becoming big challenges in the era of global warming to sustain grain yields. An experiment on rice floral organ development when subjected to combined stresses, analyses on metabolomics and transcriptomic features indicated that sugar starvation is the determinant of the failure of reproductive success under heat and drought stress in rice (Li et al. 2015). GC–MS profiling combined with transcriptomic analysis in Arabidopsis leaves revealed a synergistic stress response for the joint treatment of darkness and high temperature, which is stopped/lowered by low temperature. Protein degradation occurs rapidly and the amino acid catabolism is the main cellular energy supply in the absence of photosynthesis, as evidenced by the conditional connections between amino acid metabolism and the Kreb's cycle (Caldana et al. 2011). In rice combined cold and dehydration stresses resulted in the upregulation of carbohydrate metabolism associated genes, which are consistent with the buildup of glucose, fructose and sucrose in the aerial parts of the plant (Maruyama et al. 2014). Sugars such as sucrose, raffinose, maltose and glucose frequently accumulate in plant cells when subjected to combined stresses, perhaps protecting plants via osmotic adjustment from oxidative damage that usually follows most stress conditions (Rizhsky et al. 2004; Wulff-Zottele et al. 2010). Combined stresses normally result in a more extreme condition than that of each individual stress alone, and hence has profound effects on central metabolisms such as sugars and their phosphates and sulphur-containing compounds (Caldana et al. 2011; Rizhsky et al. 2004; Wulff-Zottele et al. 2010).

A combination of drought and heat stress was applied to Arabidopsis plants and the metabolic profile was analysed by Rizhsky et al. (2004). The metabolite profile of plants subjected to a combination of drought and heat stress was more similar to that of plants subjected to drought than to that of control plants or plants subjected

to heat stress. High levels of sucrose and other sugars instead of proline was accumulated by the plants subjected to combined stresses, which is accumulated to a very high level in plants subjected to drought but not under stress combination. They concluded that sucrose replaces proline as the major osmo protectant in plants subjected to combined stress because the toxic effect of high level of proline is enhanced under heat stress. The effect of the combination of high light irradiance and S depletion, which can occur in the field simultaneously was analysed by Wulff-Zottele et al. (2010; Buri et al. 2000). The combination of high light and S depletion gives rise to similar metabolic pool modifications such as in high light. Proline was accumulated in a differential time course under high light and stress combination. Other metabolites such as raffinose and putrescine replaced proline during the delay of proline accumulation in the plants subjected to high light and S depletion. The replacement of proline with those sugars is similar to that observed under the combination of drought and heat stress (Rizhsky et al. 2004).

11 Toward the Elucidation of Molecular Mechanisms Underlying Abiotic Stress Tolerance

A wealth of metabolomics data concerning the plant stress response has been accumulated and a large number of metabolic pathways are suggested to be regulated under various abiotic stress. But there are relatively few pathways and metabolites have been experimentally proven to function in abiotic stress tolerance. A metabolite profile does not tell exactly whether the related metabolic pathway is up- or downregulated since both upregulation of upstream reaction and downregulation of downstream reactions can lead to the accumulation of a metabolite. This can be solved by comparing the metabolomic data with those from transcriptomic or proteomic analysis or activities of specific enzymes (Cramer et al. 2011). Gene to metabolite regulatory networks of glucosinolate synthesis and primary metabolism under sulphur- and nitrogen-limited conditions was revealed by Hirai et al. by applying integrated analysis of transcriptome and metabolome data (Hirai et al. 2004).

Successful demonstration of connections between genes and metabolites, elucidating a wide range of signal output from ABA under dehydration (Urano et al. 2009) and the DREB1/CBF transcription factors in response to low temperature was made possible because of integrated analyses of the transcriptome and the metabolome (Maruyama et al. 2014). This approach is proven to be useful to elucidate the regulation of the pathway and also the involvement of transcriptional regulation of the pathway. The studies using proteomics together with metabolomics are relatively rare in the plant stress response field. One example is the study by which showed the importance of starch and raffinose family oligosaccharide metabolism during temperature stress by the metabolomic and proteomic analysis of the starch-deficient Arabidopsis mutant lacking phosphoglucomutase (pgm mutant) was studied by Wienkoop et al. (2008).

To summarise, experiments to date have allowed us to catalogue a vast array of metabolic changes in response to abiotic stress. Without over generalising, since some of the metabolic changes are very well understood at a mechanistic level, our understanding of the causes and effects of these changes remains in some cases is rather negligible. The metabolic changes are considered to be divided into three phases in responses to stress, including a direct effect of environmental changes, transient adaptation to stress conditions and the new steady state established under prolonged stress conditions. Each phase adopts a different duration depending on the type and the severity of the stress. A detailed time course experiment is therefore necessary to distinguish to which phase the metabolic changes are related. It is also very important that the results already obtained should be integrated with those from isotope feeding experiments, comprehensive phytohormone measurements. Better dissection of the plant metabolic regulatory networks and their functions in the responses to complex abiotic stresses can only be achieved by integrated multiple-omics techniques (Caldana et al. 2011; Maruyama et al. 2014; Urano et al. 2010; Kanani et al. 2010). Transcriptomic and proteomic studies will also deepen our understanding of these crucial survival processes. Once obtained such information will provide an immense knowledge and base for various approaches to ensure food security.

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In Vitro Screening of Crop Plants for Abiotic Stress Tolerance

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1 Introduction

Genetic variation is induced commonly through the plant tissue cultures. Some of these variations expressed as phenotypic and cytogenetical modifications in plants regenerated from the callus tissue (Bayliss 1980; Lee and PhilIips 1988). This type of variation called as somaclonal variation by Larkin and Scowcroft (1981). Somaclonal variation has been reported by many researchers and accepted as a real phenomenon among the plantlets regenerated from callus. Most likely, somaclonal variations are originated through the exposure of dedifferentiated tissues to culture cycles (Remotti 1998) or transferring some or all cells from a previous culture to fresh growth medium (subcultures), the intensity of the somaclonal variations vary through the genotype and the genetic base of the species (Karp 1995) and tissue culture conditions. Cell and tissue culture conditions may minimize or maximize the extent of somaclonal variations.

Over the years, many variations in the form of mutations in the genomes of plants have been naturally evolved. Plant breeders took a great advantage of these

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variations for breeding purposes. Thus, wild plants have become a valuable source of variations for plant breeders. However, these variations are limited and their production rate is very low. Thus, plant and crop breeders enhanced the chance of variations using chemicals, ionized radiations and ion beams (Meksem and Kahl 2009). Chemicals such as alkylating agents (e.g., Ethyl methanesulfonate (EMS) produces random mutations in the genome by nucleotide substitution, particularly by nucleotide alkylation. Ionized radiations of neutrons and gamma rays are useful for creation of more changes in the genome. Ion beams such as carbon ions omit nucleotide substitution which could be useful for plant breeding programs. However ionized radiation and ion beams need advanced equipment, unfortunately many laboratories are poorly equipped. Chemicals such as EMS, are usually used in some laboratory to create a variation in the plant genome, however its toxic effects are strongly dangerous for operator. However, creation of somaclonal variations can be appropriate way for acquisition of genetic diversity among crops. This type of variations has many advantages: (1) plant tissue culture creates the mutation in the regenerated plants, (2) materials used in tissue culture media are not toxic for the user such as chemicals (EMS), (3) to create somaclonal variations advanced equipment are not necessary, (4) it is a simple process to create somaclonal variation in vitro in any laboratory, (5) plant breeders can steer the somaclonal variations toward creation of desired traits in in vitro-regenerated plants by using special agents. Random variations in the plant genome are caused due to the use of chemicals, ionized radiation and ion beams whereas, in vitro screening methods can modify a specific trait in plants. Due to the widespread occurrence of abiotic stresses because of climate change. Need arises for plant breeders to improve tolerance of crops to environmental stresses using conventional breeding and gene transformation methods. In the conventional breeding method, much time should be spent creating tolerant progeny against various stresses. In addition, a large volume of field-work should be spent to plant a progeny and elected tolerant progenies to environmental stresses. In in vitro selecting method, potential tolerant progeny screened using special agents. In fact, compounds in tissue culture medium are selected to grow only tolerant offspring. Thus, for plant breeders, this method is much economic and faster compared to conventional methods. By screening, regenerated plantlets will be assessed for their tolerance to environmental stresses in the greenhouse and farm respectively.

2 Screening for Various Abiotic Stress Tolerance

2.1 Cold Stress

Improvement of frost tolerance in winter cereals using conventional breeding has been a slow process which is possibly due to ‘limited genetic variation in the gene pools’ (Limin and Fowler 1993). Molecular methods for this trait have not shown any significant results in winter cereals due to its polygenic control.

As an alternative way to improve cold hardiness of winter barley, a biotechnological approach based on somaclonal variation in tissue culture can be used (Tantau et al. 2004a, b). Different studies have shown the accumulation of proline during cold hardening in many plants including cereals (Dorffling et al. 1993). The level of proline correlates positively with genotype specific frost tolerance in these crops (Tantau et al. 2004a, b). Moreover, proline has protective functions in different plants under abiotic stress such as osmotic (Delanauney and Verma 1993), salinity (Nanjo et al. 1999) and frost stress (Nanjo et al. 1999). Therefore, many researchers believed that any procedure that increases the level of proline in plants should result in an increase in frost tolerance (Tantau et al. 2004a, b).

Successful efforts have been made to increase proline through manipulation of the proline biosynthesis or degrading system by gene engineering to improve stress tolerances. Improvement of salinity tolerance in tobacco, wheat, strawberry, *Brassica napus* and sorghum plants was obtained by overexpression of the key enzyme of proline synthesis, Δ-1-pyrroline-5-carboxylate synthetase, 'P5CS' (Ahmed et al. 2015; Hong et al. 2000; Sawahel and Hassan 2002; Bahramnejad et al. 2015; Kubala et al. 2015; Reddy et al. 2015). Nanjo et al. (1999) improved freezing and salinity tolerance in Arabidopsis by antisense suppression of proline degradation which resulted in overaccumulation of proline.

Besides genetic engineering techniques, based on somaclonal variation and biochemical markers as selection tools, in vitro culture can be used to modify crops for abiotic stress tolerance. In the case of frost tolerance, many studies showed the positive correlation between frost tolerance and proline accumulation. In fact, conventional breeding programs have used proline accumulation as a biochemical marker for increased frost tolerance in some crops (Winkel 1989). Thus, selection of high-proline genotypes may yield improved frost tolerance which can be the first way for screening frost tolerance calli. Van Swaaij et al. (1986, 1987) were the first researchers to increased frost tolerance in potato using hydroxyproline (Hyp), through the in vitro selection. To obtain frost tolerance, embryogenic calli should expose to Hyp as a selection agent to select cell lines with an increased level of proline (Fig. 1). They are assumed to be able to overcome the toxic effect of Hyp. plants will regenerate from selected cell lines and frost tolerance and winter survival in the field are determined in the regenerated plants and their progenies as well as proline levels in the progenies. Crossing experiments can be carried out to confirm the heritability of the trait 'increased frost tolerance' (Tantau et al. 2004a, b).

Many researchers tried to examine HYP as selective agents for screening of cell lines with an increased level of proline (Table 1). Tantau et al. (2004a, b) were plated embryogenic calli derived from anther cultures of the two-rowed winter barley cultivar 'Igri' on solid L3 medium containing the proline analogue hydroxyproline (Hyp), 10–20 mmol/L. A sever degeneration was observed in most calli in the presence of HYP. Hyp resistant calli were distinguished by their lighter color and higher growth rate. From 22,500 anthers exposed to Hyp, 46 Hyp resistant regenerates were selected and then transferred to soil. After 5–10 weeks cultivation at normal growth conditions, they were cold hardened at 2 °C under short day conditions together with control regenerates. Frost tolerance assays revealed that Hyp resistant

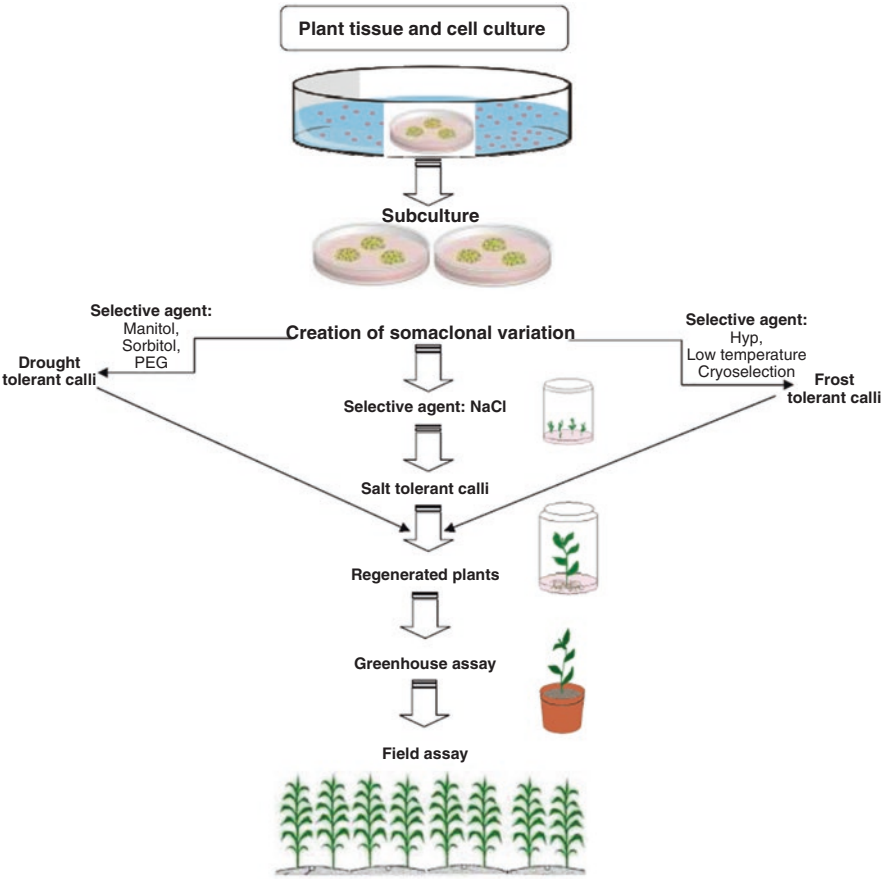

Fig. 1 A schematic diagram illustrating the procedure for development of abiotic stress tolerance in plants

Table 1 List of some plant species showing tolerance to various abiotic stresses through in vitro selection

Plant species	Type of stress	Selecting agents	References
Triticum aestivum	Cold stress	–	Lazar et al. (1988)
Triticum aestivum	Cold stress	Cryoselection (immersion in liquid nitrogen without addition of cryoprotectants)	Kendall et al. (1990)
Triticum aestivum	Cold stress	Hydroxyproline	Tantau and Dörffling (1991)
Triticum aestivum	Cold stress	Hydroxyproline	Dorffling et al. (1993)
Triticum aestivum	Salt stress	NaCl	Karadimova and Djambova (1993)
Triticum aestivum	Cold stress	Hydroxyproline	Dörffling et al. (1997)

(continued)

Table 1 (continued)

Plant species	Type of stress	Selecting agents	References
Oryza sativa	Cold stress	Different temperature regimes	Bertin and Bouharmont (1997)
Solanum tuberosum	Cold stress	Hydroxyproline	Anjum (1998)
Paspalum vaginatum	Cold stress	Different temperature regimes	Liu et al. (2013)
Hordeum vulgare	Cold stress	Hydroxyproline	Tantau et al. (2004a, b)
Oryza sativa	Drought stress	PEG	Biswas et al. (2002)
Solanum lycopersicum	Drought stress	PEG	Aazami et al. (2010)
Oryza sativa	Drought stress	PEG	Wani et al. (2010)
Oryza sativa	Drought stress	PEG	Joshi et al. (2011)
Solanum tuberosum	Drought stress	Sorbitol	Albiski et al. (2012)
Triticum aestivum	Drought stress	PEG	Mahmood et al. (2012)
Pennisetum ciliare	Drought stress	Mannitol	Carloni et al. (2017)
Brassica juncea	Drought stress	Mannitol	Gangopadhyay et al. (1997)
Capsicum annuum	Drought stress	PEG	Santos-Diaz and Ochoa-Alejo (1994)
Daucus carota	Drought stress	PEG	Fallon and Phillips (1989)
Saccharum sp.	Drought stress	Mannitol	Errabii et al. (2006)
Solanum tuberosum	Drought stress	Mannitol	Sabbah and Tal (1990)
Sorghum bicolor	Drought stress	PEG	Smith et al. (1985)
Sorghum bicolor	Drought stress	PEG	Duncan et al. (1995)
Triticum aestivum	Drought stress	PEG	Dorffling et al. (1993)
Triticum aestivum	Drought stress	PEG	Barakat and Abdel-Latif (1996)
Triticum aestivum	Drought stress	PEG	Barakat and Abdel-Latif (1995)
Triticum aestivum	Drought stress	PEG	El-Haris and Barakat (1998)
Triticum durum	Drought stress	PEG	Hasissou and Bouharmont (1994)
Solanum tuberosum	Salt stress	NaCl	Sajid and Aftab (2014)
Maniho tesculenta	Salt stress	NaCl	El-Minisy et al. (2016)
Brassica juncea	Salt stress	NaCl	Jain et al. (1990)
Brassica oleracea	Salt stress	NaCl	Elavumoottil et al. (2003)
Brassica napus	Salt stress	NaCl	Rahman et al. (1995)
Chrysanthemum morifolium	Salt stress	NaCl	Hossain et al. (2007)
Diplachnefusca	Salt stress	NaCl	Nanakorn et al. (2003)
Fragaria×ananassa	Salt stress	NaCl	Dziadczyk et al. (2003)
Glycine max	Salt stress	NaCl	Liu and Staden (2000)
Hordeum vulgare	Salt stress	NaCl	Ye et al. (1987)

(continued)

Table 1 (continued)

Plant species	Type of stress	Selecting agents	References
Helianthus annus	Salt stress	NaCl	Davenport et al. (2003)
Ipomoea batatas	Salt stress	NaCl	He et al. (2009)
Linum usitatissimum	Salt stress	NaCl	McHughen (1987)
Lycopersiconesculentum	Salt stress	NaCl	Kripkyy et al. (2001)
Lycopersicon peruvianum	Salt stress	NaCl	Hassan and Wilkins (1988)
Medicago sativa	Salt stress	NaCl	McCoy (1987)
Medicago sativa	Salt stress	NaCl	Safarnejad et al. (1996)
Nicotiana tabacum	Salt stress	NaCl	Rout et al. (2008)
Oryza sativa	Salt stress	NaCl	Binh and Heszky (1990),
Oryza sativa	Salt stress	NaCl	Basu et al. (1997)
Oryza sativa	Salt stress	NaCl	Shankhdhar et al. (2000)
Oryza sativa	Salt stress	NaCl	Lee et al. (2003)
Saccharum sp.	Salt stress	NaCl	Gandonou et al. (2006)
Solanum tuberosum	Salt stress	NaCl	Sabbah and Tal (1990)
Solanum tuberosum	Salt stress	NaCl	Ochatt et al. (1999)
Solanum tuberosum	Salt stress	NaCl	Queiros et al. (2007)
Triticum aestivum	Salt stress	NaCl	Vajrabhaya et al. (1989)
Triticum aestivum	Salt stress	NaCl	Karadimova and Djambova (1993)
Triticum aestivum	Salt stress	NaCl	Barakat and Abdel-Latif (1996)
Triticum aestivum	Salt stress	NaCl	Zair et al. (2003)
Vigna radiata	Salt stress	NaCl	Hassan et al. (2008)

regenerants were significantly more frost tolerant than the control regenerants. Improved frost tolerance was found also in the progenies R1 to R9, and genotypic segregation in the R1 generation in a 1:2:1 ratio was indicated. A significant increase in proline content was observed in the R2 generation and in subsequent generations ($P \leq 0.001$) which was correlated with increased frost tolerance in the Hyp lines. The results support the hypothesis that proline accumulation in cold acclimated winter barley plants is related to the acquisition of frost tolerance. Moreover, the described biotechnological procedure may be applicable in breeding programs for improved winter hardiness and possibly also for other stress tolerances.

Five hundred hydroxyproline-resistant cell lines were selected from cell cultures of wheat (*Triticum aestivum* L. cv. Koga II) after plating on 10–30 mM hydroxyproline (Hyp) containing solid Gamborg B 5 medium (Tantau and Dorffling 1991). All selected cell lines from 30 mAf Hyp-medium contained increased (up to 17-fold) levels of free proline. Seventy-four cell lines were transferred to Hyp-free medium and sub-cultivated 25 times for 12 months altogether, until 80% still were showing increased proline levels. Fourteen cell lines with increased proline levels were further investigated in liquid media based on their frost tolerance, which was measured by

means of electrolyte leakage. Ten of them showed increased frost tolerance with LT 50 values as low as 2.7 °C below that of the wild type (4.7 °C). Besides increased proline levels and increased percentage of dry weight, the Hyp-resistant cell lines had lower osmotic potentials. Osmotic potentials correlated better than levels of free proline with the increase in frost tolerance.

Dorffling et al. (1993) used immature embryos of a Finnish winter wheat (*Triticum aestivum* L. cv. Jo 3063) for in vitro-selection of hydroxyproline (Hyp) resistant calli plated on solid Gamborg B5 medium containing 10–20 mM Hyp and 2 mg/L 2,4-D (Dorffling et al. 1993). From 6018 embryogenic calli exposed to Hyp in the course of three subcultures, 9 calli proved to be Hyp-resistant and remained viable and embryogenic. The regenerated plants were grown at 18 °C for 6 weeks and then cold hardened at 2 °C for 18 weeks. Their results showed that the mean osmotic potential of the Hyp-resistant cold hardened regenerates was significantly lower than that of hardened controls. At the same time their mean proline content and their mean frost tolerance were significantly higher compared with regenerated controls.

As mentioned above, Dorffling et al. (1993) reported in vitro-selection of proline over accumulating lines of winter wheat (*Triticum sativum* L. cv. Jo 3063) with increased frost tolerance. Then, the improvement of frost tolerance (winter hardiness) under field conditions is confirmed for F_7 progenies of the mutants (Dorffling et al. 2009). Moreover, the mutants accumulated higher levels of glucose, fructose, soluble protein and abscisic acid (ABA) in addition to proline compared to the wild type. This can occur under cold hardening conditions either in growth chamber or field conditions. ABA and proline levels reached to peak when the temperature dropped, whereas carbohydrate levels slowly increased at decreasing temperature. Soluble protein levels also increased during cold hardening, however this showed a sharp decline during frost periods. Increased carbohydrate levels of the mutants were associated with lower osmotic potential values. The differences in carbohydrate, protein and ABA levels between the mutants and the wild type are probably due to pleiotropic effects of the mutation.

In addition to the use of HYP containing medium for selection of frost tolerance calli, some researchers used just low temperature for distinguish of frost tolerance calli. For example, Liu et al. (2013) improved cold tolerance in warm season turf grass species using in vitro selection. Embryogenic calli were subjected to 2 or 6 °C treatment for 90 days for in vitro cold selection of somaclonal variation. Plants regenerated from calli surviving cold treatment (cold-selected) after 45 or 60 days were then exposed to low temperatures [15/10 or 5/3 8 °C (day/night)]. Plant variants derived from cold-selected calli exhibited a significant improvement in their tolerance to low temperature of either 15/10 or 5/3 8 °C (day/night), as manifested by higher turf quality, leaf chlorophyll content, and membrane stability as well as lower levels of lipid peroxidation compared with the control plants. This study demonstrated the feasibility of in vitro selection for cold tolerance in seashore paspalum.

In another study, progeny of 66 plants regenerated from callus cultures derived from immature embryos of Norstar winter wheat were evaluated as seedlings for tolerance to controlled freezing (Lazar et al. 1988). Greater freezing tolerance compared the parent cultivar was observed in both R2 and R3 regenerated families.

LT50 values (predicted temperatures at which mean survival frequencies are 50%) for four families in the R2 generation and three families in the R3 were significantly lower than that of Norstar.

Embryo-derived calli of four rice varieties cultivated at high altitude in Burundi-Facagro 57, Facagro 76, Kirundo 3 and Kirundo 9 were submitted to different temperature regimes (Bertin and Bouharmont 1997). The percentage of regenerating calli greatly varied depending on variety, length of culture and callus temperature treatment. The reduction of regeneration percentages induced by low temperature which was more pronounced in the more sensitive varieties. Regenerated plants (R0) and their progenies in R1, R2 and R3 were cold-screened together with control plants. In all varieties, significantly higher survival rates were obtained in R3 with in vitro plants than with control plants. Such chilling tolerance improvement was not obtained following a massal selection applied during three successive generations onto the control plants. In vitro plants regenerated from calli cultivated either at 25 °C, or at 4 °C, were cultivated at different altitudes in Burundi during two successive generations. For most observed traits, the in vitro plants were characterized by lower means, larger variation and higher maximum values than the control plants. The most chilling-tolerant somaclonal families were most usually characterized by extensive differences in fatty acid composition, chilling-induced electrolyte leakage and chlorophyll fluorescence, compared to the varieties which were derived from.

Kendall et al. (1990) developed a cryoselection protocol that provides freezing-tolerant callus that, in turn, can regenerate plants with enhanced cold hardiness (Kendall et al. 1990). Tolerant calli were selected from spring wheat (*Triticum aestivum* L.) callus by immersion in liquid nitrogen without addition of cryoprotectants. Less than 15% of the calli survived the initial challenge, whereas 30–40% of previously selected calli survived subsequent exposure. Seed progeny from 5 of 11 regenerant (R2) lines tested exhibited significantly enhanced tolerance to freezing at –12 °C. Thus, cryoselection appears to involve at least in part, selection for genetic rather than epigenetic variants. Analysis of one callus line indicated that cryoselection did not induce significant alterations in lipid composition, adenylate energy charge or freezing point. An increase in the soluble sugar component was detected. Changes were also detected in the protein complement of microsomal membrane and soluble protein extracts of cryoselected callus. In all, seven unique proteins ranging from 79 to 149 kDa were identified. The results demonstrated that freezing tolerant callus can be isolated from a heterogeneous population by cryoselection, and factors that contribute to hardiness at the callus level which are biologically stable and can contribute to tolerance at the whole plant level.

2.2 Drought Stress

Drought in agriculture is defined as inadequacy of water availability, including precipitation and soil-moisture storage capacity in quantity and distribution during the life cycle of a crop plant. This restricts the expression of full genetic potential of

the plant (Sinha 1986). Drought is one of the most important environmental stresses that occur in different parts of the world and act as a major limiting factor to prevent the maximum crop yield (Mitra 2001).

Improving drought tolerance and productivity is one of the most difficult tasks for cereal breeders. The difficulty arises from the diverse strategies adopted by plants themselves to combat drought stress depending on the timing, severity and stage of crop growth. Compounding the problem further are the many loci that show efficacy only in a subset of circumstances (Tuinstra et al. 1996; Nguyen et al. 2004).

Breeding of drought tolerance by conventional methods seems to be difficult because the yield heritability is critically low under drought condition due to small genotypic variance or large genotype-environment interaction variances (Blum 1988). The genetic structure and phenotypic expression of a quantitative trait are highly influenced by environmental factors. Thus, one barrier for understanding of inheritance in a quantitative trait is genotype-environment interactions (Breese 1969).

Tissue culture introduces the special methods for selecting individuals in in vitro condition by adding selective agents to the culture medium (Mohamed et al. 2000; Lu et al. 2007), either directly or gradually (Gangopadhyay et al. 1997; Hassan et al. 2004; Mohamed and Ibrahim 2012). The base of this method is creating genetic variations during cell or tissue culture and then recovers of individuals (Biswas et al. 2002; Matheka et al. 2008; Lu et al. 2009; Verma et al. 2013). The agent has been applied at different growth stages: during the callus induction process, during seedling regeneration or all throughout the in vitro culture stages (Biswas et al. 2002; Errabii et al. 2007; Aazami et al. 2010; Verma et al. 2013). Drought stress conditions are usually simulated by adding compounds, such as mannitol, sorbitol or polyethylene glycol (PEG) (Fig. 1) (Leone et al. 1994; Joshi et al. 2011; Mahmood et al. 2012), which reduce the water potential of the medium. The responses of the different explants or in vitro regenerated plants can be influenced by secondary effects, either morphological or physiological, of the compounds used to simulate stress (Hohl and Schopfer 1991; Verslues et al. 1998; Cha-um et al. 2012). For this reason, besides confirming the efficiency of in vitro selection, all selection processes should include an ex vitro assay to determine the exact measure of tolerance to the osmotic agent observed in the laboratory (Remotti 1998).

Many researchers used somaclonal variations to improve drought tolerance of crops using selective agents (Table 1). Carloni et al. (2017) tried to define a protocol for in vitro selection of drought tolerant calli of buffelgrass using mannitol. Buffelgrass is a forage grass that reproduces mainly by apomixis. In species with this reproduction mode, in vitro selection allows the incorporation of alternatives in a breeding program (Carloni et al. 2017). In the embryogenic callus induction medium (IM), the highest values of the variables fresh weight of embryogenic calli, proportion of embryogenic calli and number of regenerated seedlings (NRS) were obtained in the 25 mM mannitol treatment. The remaining concentrations of the osmotic agent (50, 75, 100 and 150 mM) had a negative effect on these variables. In the regeneration medium (RM), NRS was reduced at all mannitol concentrations. When embryogenic calli were induced and seedlings were regenerated maintaining mannitol concentrations in IM and RM, the highest NRS values were recorded at

25 mM mannitol. In vitro regenerated seedlings transplanted to an experimental plot exhibited different morphological characteristics from those of the anther donor plant. ISSR primers detected 22% of polymorphic bands and divergence between 0.20 and 0.37 in in vitro regenerated plants. Finally, water stress assays confirmed that S1 progenies exhibited a differential behavior from that of the parent material. Under 100 mM of mannitol used as selection pressure in IM or in both IM and RM, S1 progenies of two regenerated materials had higher height, fresh weight and dry weight at the end of water stress assay.

2.2.1 Salt Stress

Salt is called as high concentration of soluble salts in the soil. Soil with 4 dS/m ECe or more is approximately equal to 40 mM NaCl and 2/0 MPa osmotic pressure is considered as salty soils. This definition is taken from the ECe that significantly affect crop yield more. NaCl is most abundant salt solution and all plants employ mechanisms to adjust its accumulation (Munns and Tester 2008).

More than 800 million hectares of land worldwide are affected by salinity (Munns and Tester 2008). This amount is more than 6% of the world's land. Most of the land affected by salinity caused by natural factors. This is the result of long-term accumulation of salts during the period when the lands are arid and semiarid (Rengasamy 2002). Apart from the natural salt, an important part of agricultural land that planted recently are salty due to changes in land use for agricultural purposes. Both factors increase the concentration of salts in the root area (Munns and Tester 2008).

Plants vary widely in salt tolerance. Among the cereals, rice (*Oryza sativa*) is the most sensitive and barley (*Hordeum vulgare*) is the most tolerant to salinity. Bread wheat (*Triticum aestivum*) have average tolerance and durum wheat (*Triticum turgidum* ssp durum) are less tolerant to salinity (Munns and tester 2008). Salt tolerance in dicotyledonous have more diversity (Munns and Tester 2008).

Salinity affects the plants in two ways:

1. Osmotic phase, a high concentration of salts in the soil, makes it difficult to uptake the water by root (Munns and Tester 2008). Salts out of the root can have a fast effect on cell growth and the related metabolism. This cause a reduction in leaves growth, new leaves and lateral buds develop slowly so, fewer side branches form (Munns and Tester 2008). The main effect of salinity on barley will emerge as a decrease in the number of tillers. In dicotyledonous species, the main effect of salinity significantly reduces the size of individual leaves or branches (Munns and Tester 2008).
2. Ion-specific phase, salts gradually accumulate within the plant to reach toxic concentration (Munns and tester 2008). This can be started with the accumulation of salt in toxic concentrations in the old leaves. This leaves have less efficient detoxification of salts and eventually die. If the mortality rate of the old leaves become greater than the younger leaves, the growth rate decreases further

(Munns and Tester 2008). Ion stress overcomes the osmotic effect of the ion just in high salinity levels or sensitive species that lack the ability to control the transfer of sodium (Munns and Tester 2008).

Increased salinity is one of the major constraints on crop productivity. Necessity of plant production with increased salt tolerance has been extensively emphasized by increased crop research (Munns et al. 2002; Flowers 2004). In vitro culture techniques are an excellent tool to study the behavior of undifferentiated cells and the bulk plants in ambient stress under control conditions (Sajid and Aftab 2014). The exploitation of somaclonal variation is also potentially quite useful for in vitro selection of cells and tissues against several stresses (Bajaj 1987; Tal 1996). For salt tolerance, different concentration of NaCl can be used to select a salt tolerant calli. So far, different studies have shown a successful in vitro selection of Na^+ tolerant calli for various plants. For salt tolerance, different concentration of NaCl can be used to select a salt tolerant calli (Fig. 1). So far, different studies have shown a successful in vitro selection of Na^+ tolerant calli for various plants (Table 1).

Sajid and Aftab (2014) reported an in vitro direct selection of salt-tolerant callus cultures and subsequent plant regeneration in two potato cultivars (Cardinal and Desiree). Results have shown more than 50% reduction in relative fresh callus mass in the two potato cultivars exposed to 120 mM NaCl. Callus morphology correspondingly changed from off-white to blackish-brown at 120 mM to acutely-necrotic at 140 mM NaCl. Regeneration potential of recurrently-selected callus cultures (100 mM NaCl-treated) on salt-free regeneration medium (MS + 2.64 μM NAA and 1.00 μM TDZ) was not much different as compared to the control (non-selected ones). Regenerated plants from salt-tolerant callus cultures of both the cultivars after selection were transferred to soil and organic matter (50:50, v/v) for climatization in the greenhouse. It was observed that the recurrently selected plants had higher fresh/dry weight and number of tubers compared with the control ones in both cultivars. Likewise the protein, peroxidase (POD), catalase (CAT) and superoxide dismutase (SOD) activities have shown an increasing trend in salt-treated plants of both cultivars. The results from this study highlighted a strong possibility for the selection of salt-tolerant callus lines of potato followed by an efficient plant regeneration and further acclimatization.

In another study, the calli cultures of *Guizotia abyssinica* (niger) cultivars IGP 76 and GA 10 were exposed to different levels of salt treatments (0, 30, 60, and 90 mM NaCl), in order to evaluate growth, physiological, and biochemical responses (Ghane et al. 2014). A significant decrease in relative growth rate and tissue water content of GA 10 calli than IGP 76 under salt-stress conditions was associated with higher sodium ion accumulation. Osmotic adjustment revealed by the osmolytes accumulation was significantly higher in IGP 76 salt-stressed calli as compared to GA 10. The sustained growth and better survival of IGP 76 calli was correlated with lower malondialdehyde content and increased antioxidant activities and higher α-tocopherol content in comparison to GA 10. The higher osmolytes accumulation and presence of better antioxidant system suggested superior adaptation of IGP 76 calli on salt-containing medium for prolonged periods in comparison to GA 10. The regeneration frequency, organogenesis and acclimatization response of the plants derived from

salt-adapted calli was comparatively lower than the plants derived from control calli of IGP 76. The growth, physiological and biochemical characterization of the salt-tolerant regenerated plants exposed to stepwise long-term 90 mM NaCl treatment revealed no significant changes in comparison to the control. Thus, their results suggests the development of an efficient protocol for in vitro selection and production of salt-tolerant plants in self-incompatible crop, and an alternative to traditional breeding programs to increase the abiotic stress tolerance.

In another study, Cassava suspension culture grown on MS media containing 50, 100, 150, 200 and 250 mM NaCl were established from cassava callus cultures were all dramatically induced in response to salt treatment (El-Minisy et al. 2016). The results indicated that the high NaCl concentration of 200 and 250 mM decreased one-fold the viable cell number compared to lower concentrations and control sample. Surprisingly at 50, 100 and 150 mM NaCl higher number of viable cells were found compared to control sample. However, the cell viability in 12 days of NaCl stress showed high tolerance against salt stress and the cell numbers also higher compared to other NaCl concentrations. Ionic status suggested that 200 mM NaCl accumulated less Na^+, Cl^- and Ca^{2+} and maintained better K^+ in comparison to other NaCl stress cell samples. The ion homeostasis data of cassava cell culture under NaCl stress showed that the Na^+ and K^+ accumulation increased very much under lower concentrations of NaCl and gradually decrease in higher concentration. There is a positive relationship between salt tolerance and proline content in *in cassava* cultures up to 200 mM NaCl stress and the highest proline content compared to other treatments. Gel activity assay of superoxide dismutase (SOD), peroxidase (GPX) and total peroxidase (POX) activity increased in tolerant cell lines as compared to control. Analysis of the above enzymes suggests that selected cassava cell lines possessed more efficient scavenging system of reactive oxygen species under 200 mL NaCl. It can be concluded that in cassava suspension culture viability of cell under 200 mM NaCl stress after 15 day will be the perfect time to isolate and identify the intercellular and extracellular protein or/and peptides which could be produced abundantly.

3 Conclusion

Due to the mounting food demand worldwide, plant breeders are seeking fast, low-cost and safe methods for breeding of crops especially tolerant ones against abiotic stresses. In vitro selection can be the appropriate choice for plant breeders because of its advantages mentioned in this review. In vitro selection methods rely on somaclonal variation produced due to mutations in plants regenerated from tissue culture. For creation this type of variation, there is no need for advanced equipment, time and spaces and toxic chemicals for creation of mutations. In addition, plant breeders can direct the somaclonal variations toward creation of desired traits in in vitro regenerated plants by using special agents. It seems that this method will play a more important role for breeding crops in the future.

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Micromics: A Novel Approach to Understand the Molecular Mechanisms in Plant Stress Tolerance

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1 Introduction

Plants being sessile in nature have developed the ability to cope up with different growth habitats and fluctuating climatic conditions by improvising myriad regulatory mechanisms. There are many classes of small endogenous RNA molecules, such as small transfer RNA (tRNA), ribosomal RNA (rRNA), small nucleolar RNA (snoRNA), small interfering RNA (siRNA) and microRNA (miRNA). miRNA and siRNA are biochemically and functionally indistinguishable. Both are 19–20 nucleotides (nt) in length with 5′-phosphate and 3′-hydroxyl ends, and assemble into RISC to silence specific gene expression. MicroRNAs (miRNAs) are small noncoding RNAs with 20–22 nucleotides discovered as the regulatory RNA in *C. elegans*. First plant miRNAs were discovered in Arabidopsis in 2002 and over the past three decades they have been reported in about 120 plant species. A transcribed miRNA acts by different mechanisms like feedback and feedforward loop regulations and has the ability to control its own transcription as well as other genes. A single miRNA may regulate hundreds of mRNAs and in turn may effect a network of interactions. The length of miRNA genes varies from miRNAs to miRNs and from species to species. For example, miRNA genes in plant species are usually longer than in animals. The initial tools like genetic screening for miRNA identification were often time consuming, expensive, and cumbersome. There was a tectonic shift in the technology of the sequencing and computational methods.

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These advances aided in completion of draft genome sequences with less cost. By using bioinformatic software and tools with the combination of the next-generation deep sequencing, miRNA identification and expression studies in plants have increased dramatically. Computational approaches have estimated that organisms probably contain about 1–5% miRNA genes of the total protein-coding genes (Lai 2003; Lim et al. 2003; Lewis et al. 2005). Notably, various miRNAs are now known to play a role in biotic and abiotic stress, which has led researchers to consider them as a promising tool to develop stress-resistant crops.

According to the United Nations reports present world population is at 7.8 billion and expected to reach 9.8 billion by 2050 (The World Population Prospects: The 2017 Revision). Besides population growth, there is an increase in the prosperity across the world. If the present trend continues, because of the richer diets we should double the amount of crops we grow by 2050. Consequently, to feed the ever-increasing world population it is highly important to find solutions to increase the global food production by developing stress tolerant crop plants. Biotic stresses account for up to 30% crop loss worldwide (Bebber and Gurr 2015). To deal with these devastating pathogens and pests, plants have specialized defense mechanisms, which get induced when there is a stress (rewrite). Plants have developed various physiological and molecular mechanisms to deal with abiotic stresses such as drought, salinity, heat, cold and dehydration by minimizing water loss and photosynthesis. The role of the genes were elucidated either through overexpression or through silencing.

In this chapter, we aimed to describe briefly biogenesis of plant miRNAs and different online tools available for discovering and expression profiling of miRNAs based on computational methods and also understanding their role in tolerance mechanism against abiotic and biotic stress.

2 Biogenesis of miRNAs

There are four steps in biogenesis of miRNAs: (1) MIR genes transcription, (2) miRNA precursor processing, (3) miRNA stabilization and (4) RISC formation.

1. *MIR Genes Transcription*

   The genes which are coding for miRNAs are known as MIR genes. The promoters of MIR genes contain typical TATA box motifs and transcription factor binding motifs indicate that transcription MIR genes is regulated by general and specific transcription factors (Xie et al. 2005; Megraw et al. 2006).The first step in biogenesis of miRNAs starts in the nucleus with primary miRNA (pri-miRNA) transcribed from MIR genes by RNA polymerase II (Xie et al. 2005). Pol II activity in MIR transcription is probably subject to phosphoregulation (Hajheidari et al. 2013). pri-miRNAs can be more than 1 kb in length, they can undergo canonical splicing, polyadenylation, and capping. Just like mRNA,

nascent pri-miRNAs are capped at the 5′ end and polyadenylated at the 3′ end, and intron-containing pri-miRNAs are spliced or alternatively spliced (Stepien et al. 2017). The pri-miRNA is processed within the nucleus by a multiprotein complex consisting of DCL1/HYL1/SE called the Microprocessor.

2. *miRNA Precursor Processing*

   The second step involves cleavage of the pri-miRNA into the pre-miRNA, the hairpin structure in the pri-miRNA (Lee et al. 2003). DAWDLE (DDL) is a fork head-associated protein, required for pri-RNA accumulation and recruitment of RNase III family enzyme DICER-like protein 1 (DCL1) to pri-miRNA for downstream processing (Yu et al. 2008). DICER-LIKE1 (DCL1) makes a cut from 15 to 17 nt away from the base of the stem or a bulge or unstructured region within the loop-distal stem. HYPONASTIC LEAVES1 (HYL1) is one of the family member of DOUBLE STRANDED RNA BINDING PROTEINS (DRBs). HYL1 interacts with DCL1 to facilitate efficient and precise miRNA precursor processing (Yang et al. 2014).The resulting precursor-miRNA (pre-miRNA) is further cleaved by DCL1 to produce a 21-nt miRNA/miRNA∗ duplex (Zhu et al. 2013). Alternative processing modes include loop-to-base processing (Bologna et al. 2009). Homodimerization of HYL1 is essential for its functions in miRNA precursor processing (Yang et al. 2014). HYL1 also affects the splicing of some pri-miRNAs and strand selection from miRNA/miRNA∗ duplexes in AGO1 loading (Ben Chaabane et al. 2012). The DCL1 together with HYL1 (HYPONASTIC LEAVES 1) and the zinc-finger protein SE (SERRATE) were required for processing of pre-miRNA into miRNA duplex.

3. *miRNA Stabilization*

   The miRNA/miRNA* duplex is stabilized through 3′-terminal 2′-*O*-methylation by HEN1. The export of miRNAs from the nucleus to the cytoplasm is fundamental for miRNA activity (Köhler and Hurt 2007; Rogers and Chen 2013).The 2-nt 3′ overhang, characteristic of RNase III-mediated cleavage gets methylated by HEN1 (HUA ENHANCER 1), that is recognized by exportin 5, HASTY (HST), is proposed to export the miRNA/miRNA* duplex to the cytoplasm based on the assumption that the duplex is produced by DCL1 in the nucleus (Bollman et al. 2003).

4. *RISC Formation*

   In the cytoplasm, miRNAs are unwound into single strand mature miRNAs by helicase. The miRNA strand with relatively lower stability of base-pairing at its 5′ end act as guide molecule to reach the target mRNA and is incorporated into a ribonucleoprotein complex RISC, whereas the other miRNA strand is typically degraded (Du and Zamore 2005). Once incorporated into RISC, the miRNA directs AGO1 (or AGO10) containing RISCs to its target mRNA for cleavage or translational repression on the basis of sequence complementarity. In cases of perfect or near-perfect complementarity to the miRNA, target mRNA scan be cleaved (sliced) and degraded; otherwise, their translation is repressed (Martinez and Tuschl 2004; Treiber et al. 2012). Therefore, miRNAs control gene expression by regulating mRNA stability and translation (Eulalio et al. 2008).

3 List of Bioinformatics Tools for miRNAs Prediction, Identification and Characterization

miRNA prediction tools	
MiRscan	http://genes.mit.edu/mirscan/
miRank	http://reccr.chem.rpi.edu/MIRank/ MiRank is programmed in Matlab
MirnaFind WebServices	https://mirnafind.fbk.eu/
miRFinderV4.0	http://www.bioinformatics.org/mirfinder/
MirevalV2.0	http://tagc.univ-mrs.fr/mireval
PITA	http://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html
mirplant	https://sourceforge.net/projects/mirplant/
Target prediction tools	
RNAhybrid	https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid
Diana-microT	http://diana.imis.athena-innovation.gr/DianaTools/index.php?r=microT_CDS/index
Rna22	https://cm.jefferson.edu/rna22/
miRecords	http://c1.accurascience.com/miRecords/
TAPIR	http://bioinformatics.psb.ugent.be/webtools/tapir/
miRTar	http://mirtar.mbc.nctu.edu.tw/human/
TargetS	http://liubioinfolab.org/targetS/mirna.html
psRNATarget	http://plantgrn.noble.org/psRNATarget/
MicroTar	http://tiger.dbs.nus.edu.sg/microtar/
MiRNA-EMBL	http://www.russelllab.org/miRNAs/
miRNA database	
MiRBase	http://www.mirbase.org/
miRWalk	http://mirwalk.umm.uni-heidelberg.de/
miRNAMap 2.0	http://mirnamap.mbc.nctu.edu.tw/
PMRD	http://bioinformatics.cau.edu.cn/PMRD/
CSRDB	http://sundarlab.ucdavis.edu/smrnas/
miRNA secondary structure prediction tools	
miRNA Digger	http://www.bioinfolab.cn/
miRNA deep sequencing tools	
mirTools	http://59.79.168.90/mirtools/
miRExpress	http://mirexpress.mbc.nctu.edu.tw/
miRegulome	http://bnet.egr.vcu.edu/miRegulome/
miRspring	http://mirspring.victorchang.edu.au/
R packages	
microRNA	https://bioconductor.org/packages/release/bioc/html/microRNA.html
miRNApath	https://bioconductor.org/packages/release/bioc/html/miRNApath.html
AgiMicroRna	https://bioconductor.org/packages/release/bioc/html/AgiMicroRna.html
mirIntegrator	https://bioconductor.org/packages/release/bioc/html/mirIntegrator.html
miRNAtap	https://bioconductor.org/packages/release/bioc/html/miRNAtap.html
TargetScore	https://bioconductor.org/packages/release/bioc/html/TargetScore.html

continued

ExiMiR	https://bioconductor.org/packages/release/bioc/html/ExiMiR.html
LVSmiRNA	https://bioconductor.org/packages/release/bioc/html/LVSmiRNA.html
MiRaGE	https://bioconductor.org/packages/release/bioc/html/MiRaGE.html
miRcomp	https://bioconductor.org/packages/release/bioc/html/miRcomp.html
miRLAB	https://bioconductor.org/packages/release/bioc/html/miRLAB.html
miRNApath	https://bioconductor.org/packages/release/bioc/html/miRNApath.html
miRNAtap	https://bioconductor.org/packages/release/bioc/html/miRNAtap.html
MmPalateMiRNA	https://bioconductor.org/packages/release/bioc/html/MmPalateMiRNA.html
Roleswitch	https://bioconductor.org/packages/release/bioc/html/Roleswitch.html
ssviz	https://bioconductor.org/packages/release/bioc/html/ssviz.html

4 Role of miRNAs in Plant Abiotic Stress Tolerance

Plants have evolved highly sophisticated molecular machinery to cope up and adapt to the challenging environmental conditions. In addition to various mechanisms, miRNAs-mediated rapid response plays crucial role in plant adaption. Various studies have shown that several miRNAs were downregulated in order to increase their target stress-responsive genes during stress conditions in a range of plant species (López and Pérez-Quintero 2012).

4.1 Drought

miRNAs play significant role in sensing the drought stress and imparting tolerance in plants (Ferdous et al. 2015). Drought-responsive miRNAs and their mechanism in drought tolerance is well established in crop plants including Arabidopsis (Clauw et al. 2016), tomato (Liu et al. 2017), rice (Zhou et al. 2010), maize (Aravind et al. 2017), sorghum (Katiyar et al. 2015) and grasses (Zhou et al. 2013). Thirteen miRNAs expression were up-regulated and six miRNAs were downregulated, under drought stress in Arabidopsis. All these differentially expressed miRNAs also play significant role in key developmental process, suggesting that the existence of tight regulation of plant growth and development and drought tolerance (Ferdous et al. 2015; Muthusamy et al. 2017). Under drought stress conditions, miR166, miR167, miR169, miR383 and miR398 family members displayed differential expression pattern in drought tolerant and drought susceptible genotypes (Katiyar et al. 2015). Balyan et al. (2017) studied the drought tolerance mechanism in the set of rice cultivars comprising drought tolerance and susceptible genotypes and showed the role of Cultivar-specific drought responsive (CSDR)-miRNAs networks involving seven family members (osa-miR159f, osa-miR1871, osa-miR398b, osa-miR408-3p, osa-miR2878-5p, osa-miR528-5p and osa-miR397a) by modulating the Cu and ROS homeostasis. This finding shed a novel insight on

Cultivar-specific drought responsiveness network which can potentially be targeted in breeding programs in regulating drought responsive genes for the development of new drought tolerant genotypes (Lenka et al. 2018).

4.2 Cold

In Arabidopsis, 11 miRNAs (miR156/157, miR159/319, miR164, miR165/166, miR169, miR172, miR393, miR394, miR396, miR397 and miR398) were induced under cold stress (Sunkar and Zhu 2004; Zhou et al. 2008; Liu et al. 2008; Chinnusamy et al. 2010). Song et al. (2017) identified 34 conserved and 5 novel miRNAs family members that showed a differential expression pattern between the cold-stressed and control spikelet samples of wheat. These miRNAs were known to target the floral organ pattern homeotic transcription factors members including ARF, SPB, MYB and MADS-box. Melatonin induced downregulation of *miR159*, *miR858* and *miR8029* increases the cold tolerance ability of *Citrullus lanatus* L. (Li et al. 2016). Melatonin-mediated miRNA downregulation increases the transcript levels of the target cold tolerance genes involved in signaling, protection and detoxification. In tomato, four miRNAs (miR167, miR169, miR172 and miR393) expression were increased immediately under cold stress (Koc et al. 2015). Cold stress-responsive miRNAs target wide range of proteins with diverse cellular function, indicating an intricate regulation molecular network in responses to cold stress (Chinnusamy and Zhu 2009; Chinnusamy et al. 2010; Megha et al. 2018). *Cis*-regulatory analysis in the promoters of cold-responsive miRNAs showed the presence of conserved regulatory elements including ABRE, LTRs, MYB binding sites, and HSE (Liu et al. 2008; Zhou et al. 2008).

4.3 Salt

Salt stress inhibits the plant growth and development. High concentration of salts in the plant cells modulates the ABA synthesis which in turn results in closure of stomata, reduction of photosynthesis activity and increase in ROS (Chinnusamy et al. 2006; Mangrauthia et al. 2013). Several salt-stress responsive genes (transcription factors, transporters, ROS enzymes, etc.) were targeted by the miRNAs (Chinnusamy and Zhu 2003; Mondal and Ganie 2014). The expression pattern of the salt stress responsive genes *NADP-dependent malic enzyme*, *cytochrome oxidase* and *sulfurylase* were modulated by miRNAs (Ding et al. 2009; Mangrauthia et al. 2013). The role of miRNAs in imparting tolerance to salt stress were documented in plants (Ferdous et al. 2015). Ten miRNAs (miR156, miR165, miR319, miR393, miR396, miR167, miR168, miR171, miR152 and miR394) were reported to play a pivotal role in salt tolerance in Arabidopsis and chickpea (Liu et al. 2008; Kohli et al. 2014). In *Populus*, 15 miRNAs targeting the key developmental salt-stress responsive genes regulating auxin signaling, light or circadian rhythms and tissue morphogenesis were

differentially expressed under salt-stress condition (Li et al. 2013). A total of 259 miRNAs were differentially expressed in chickpea under salinity and moisture stress conditions (Khandal et al. 2017). Seventy one miRNAs were differentially expressed under salinity in radish (Sun et al. 2015).

4.4 High Temperature

Heat shock responsive transcription factors *HSFA1b* and *HSFA7b* induce the expression of high temperature responsive miR398 in Arabidopsis (Guan et al. 2013). In rice, miRNA genes belonging to 162 miRNA families were differentially expressed under high temperature stress, 33 families displayed shoot-specific expression, 12 displayed root-specific expressions, and 117 displayed expression in both shoot and root tissues. Seventy-nine miRNAs were differentially expressed under heat stress conditions in wheat. These results suggest the presence of wider role of miRNA mediated regulation in imparting stress tolerance under heat stress conditions (Mangrauthia et al. 2017). Several heat stress responsive genes including ClpATPase (Muthusamy et al. 2016), HSF (Guan et al. 2013) and HSP (Muthusamy et al. 2017) expression were under regulation of miRNA. MiR396b-3p expression were increased under both heat and drought conditions, suggesting a wider scope for utilization in crop improvement programs in developing climate resilient crop plants (Barciszewska-Pacak et al. 2015).

5 Role of miRNAs in Plant Biotic Stress Tolerance

Plants are faced with innumerable biotic stresses caused by pests, parasites and pathogens. Fungi, bacteria, nematodes and viruses are the pathogens primarily accountable for plant diseases and major concern is of their continuous and fast evolution. Plants have different lines of defense to all these biotic stresses and they respond through several morphological, biochemical, and molecular mechanisms and interactions among their respective signaling pathways (Nejat et al. 2017). One of the lines of plants defense in response to biotic stresses through miRNAs by expressing or regulating stress responsive genes and transcription factors strive to mitigate the stress.

In genomics era, the whole genome, transcriptome, proteome and interactome sequencing and analysis has become a baseline for different areas of research. The small RNA sequencing of organisms identified putative and novel RNAs which might be involved in regulatory pathways. Deep sequencing of stress treated and untreated plant samples showed regulation of small RNAs which could be studied further to improve the conventional approaches for development of stress resistant crops. High throughput sequencing of tomato microRNAs in 2011 identified conserved and novel miRNAs expressed in tomato (Zuo et al. 2011), which regulates the expression of genes involved in biotic stresses. miRNAs are explicitly employed by plants in response to pathogenic attacks.

5.1 Viruses

Viruses contain DNA or RNA as a genetic material in either double-stranded or single-stranded form. The viruses affect host transcriptome levels (Reyes et al. 2016) by transferring genomic DNA/RNA into the host genome. The virus utilizes the host machinery to amplify the genomic content and synthesize proteins by using RNA-dependent RNA polymerase and reverse transcriptases (retroviruses). The viruses also affect the miRNA levels which in-turn affects the fate of the target genes (Pradhan et al. 2015).

In soyabean, 12 potential miRNAs were identified and through 5′-RNA-ligase-mediated rapid amplification of cDNA ends (5′-RLM-RACE) analyses showed 9 miRNAs (miR395, miR530, miR1510, miR1514, miR1515, miR1535, miR2109, miR3522 and miR2118-3p) responded to SMV soybean mosaic virus infection (Yin et al. 2013). In tomato, 40 novel miRNAs were identified in response to cucumber mosaic virus (CMV) and functional analysis revealed miRNA related to defense response and photosynthesis (Feng et al. 2014). In watermelon, by using small RNA sequencing RNA technology, 246 novel miRNAs were identified as differentially expressed in response to cucumber green mottle mosaic virus (CGMMV) infection. Further analysis of these miRNAs revealed, these miRNAs influenced wide array of biological functions like cell-wall enhancing, changes in levels of phytohormones, intracellular transport and modulation of different R genes (Sun et al. 2017). miR168 is ubiquitously up-regulated in most of plant-virus combinations. For example in *Malus hupehensis* resistance against *Botryosphaeria dothidea* is conferred by miR168 targeting AGO1 (Yu et al. 2017). Similarly, in rice AGO18 sequesterartion by miR168 confers resistance against viruses (Wu et al. 2015). In response to Mungbean Yellow Mosaic India virus (MYMIV) infection gma-miR5787 maintains AGO homeostasis and targets viral genome in soyabean (Ramesh et al. 2017). In *Vigna mungo* 14 novel and 53 known miRNAs were identified *V. mungo* Mungbean Yellow Mosaic India virus (MYMIV). Among the 53 known miRNAs, induction of miR396 suppresses JA signaling there by activating the SA-mediated pathway (Kundu et al. 2017). Besides SA, auxin also regulates plant-pathogen interactions through two candidates miR160 and miR393. PVX-potyvirus synergistic infections alters miRNAs (miR156, miR171, miR398 and miR168) and targeted mRNA levels in *Nicotiana benthamiana* (Pacheco et al. 2012). Comprehensive genome-wide analyses of miRNA revealed that plants modulate the expression of known, constitutively expressed miRNAs in a spatiotemporal specific manner during viral infection.

5.2 Bacteria

Apart from positive interaction like nitrogen fixation, bacteria also causes diseases through negative interactions. Plant activates pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI), when exposed to flagella or elicitors released from bacterium and further express disease resistance genes in response to Effector triggered susceptibility ETS (Schwessinger and Zipfel 2008).

In Arabidopsis miRNAs were globally profiled in response to infection of *Pseudomonas syringae* pv. *tomato* (Pst) and identified several miRNAs that regulate plant hormone signaling and biosynthesis (Zhang et al. 2011). The involvement of these hormone pathways in against bacterial defense has been well established (Berens et al. 2017). For example, SA signaling pathways regulates the anti-biotrophic pathogen defense in plants while positive regulation of JA triggers and regulates the anti-necrotrophs (Tamaoki et al. 2013) defense *Pseudomonas syringae* and *Xanthomonas axonopodis* induces miRNAs, for example miR160, miR167 and miR390, miR393 all regulate genes involved in the auxin signaling pathway, including different ARFs and F-boxauxin receptors TIR1, AFB2, and AFB3 mRNAs (Zhang et al. 2011; Snyman et al. 2017; Jodder et al. 2017). Evidently, Auxin response factors are the major targets of most of the upregulated miRNAs whilst downregulated miRNAs targets disease resistance genes. In-fact, miR393 involvement in the regulation of auxin signaling pathway was first discovered in anti-bacterial response of *Arabidopsis thaliana* through active contribution in PTI (Zhang et al. 2006). Besides auxin, some miRNAs were identified regulated other hormonal pathways, like miR159 was involved in abscisic acid (ABA) signaling pathway and miR319 was involved in jasmonic acid (JA) signaling cascade (Li et al. 2010; Fahlgren et al. 2007). In Arabidopsis, mi393 and SA pathway act synergistically to provide tolerance to bacterial infections (Chen et al. 2014). Further experiments revealed that miR393 down regulate MEMB12 (SNARE) gene that encodes protein involved in membrane fusion.

5.3 Fungal

In Arabidopsis, miR773 was functionally characterized and found that concomitant upregulation of miR773 target gene METHYLTRANSFERASE 2 (MET2) considerably increased resistance to *Plectosphaerrella cucumerina*, *Fusarium oxysporum* and *Colletototrichum higginianum* infection (Salvador-Guirao et al. 2017). In Rice, a total of 33 potential miRNAs were identified in providing immunity against the Blast Fungus *Magnaporthe oryzae*. Among them miR160a and miR398b were functionally characterized in providing suppression against fungal infection (Li et al. 2014). In cotton, 65 miRNAs were identified as differentially expressed in response to the Verticillium. Among them, Ptc-miR482, Ptc-miR1444 and Ptc-miR1448 were found to specific to cotton cultivars which cleaves the PPO (Polyphenol oxidase) gene in providing resistance (Chi et al. 2014; Tran et al. 2012). In Populus, 74 conserved miRNAs along with 27 novel miRNAs from 37 different miRNA families were identified in response to *Dothiorella gregaria*. Further analysis revealed miR472, miR1447 and miR1448 were targeting the disease resistance gene (Chen et al. 2012).

The change in hormonal pathways is common to all the biotic stresses. In wheat, enhanced auxin-mediated response was observed against powdery mildew infection by miR393 targeting transport inhibitor response 1 (TIR1), i.e., a negative regulator of auxin signaling (Nowara et al. 2010). In case of infection with *Puccinia graminis*

three independent responses (lignin biosynthesis, hormone signaling, and protein biosynthesis) were regulated through eight miRNAs namely miR159, miR164, miR167, miR171, miR408, miR444, miR1129 and miR1138 (Liu and Chen 2009).

In *Brassica* species, 62 novel miRNAs were differently expressed under *Verticillium longisporeum* infection. Among them it was found that miR168 negatively regulates the expression of argonaute1 (AGO1). In most of the fungal infections, pathogens change the expression of DCL1 and AGO1 by overtaking the host machinery and cellular homeostasis. But over the period, plants have acclimatized to the situation and started overexpressing miR162 and miR168 in response to fungal elicitors to maintain the homeotic balance of DCL1 and AGO1 as host derived PTI (Baldrich et al. 2014).

5.4 Nematodes

Over the year, Nematodes have been proved as menace for crop growth, development, yield and productivity. Incidentally, it was in the nematode, *Caenorhabditis elegans* that MicroRNAs (miRNAs) were first discovered (Lee et al. 1993) and subsequently several miRNAs were discovered in response to nematode infection. For example, in Arabidopsis upon the infection of *Heterodera schachtii*, miR161, miR164, miR167a, miR172c, miR396c, miR396a,b, and miR398a were downregulated (Kammerhofer et al. 2015) whereas over expression miR827 silences NLA (Nitrogen Limitation Adaptation), which encodes for ubiquitin E3 ligase enzyme leading to susceptibility to *Heterodera schachtii* (Hewezi et al. 2016). In soyabean, 537 known and 70 putative novel miRNAs were in response to Soybean cyst nematode (SCN) infection of which 60 miRNAs belonging to 25 families were shown to be significantly differentially expressed. After in-depth analysis of these differentially expressed, it was revealed that miR159 and miR399 likely targeting different genes in root during SCN Infection (Tian et al. 2017). In Arabidopsis, miR390/TAS3 discovered as regulatory module for proper gall formation through auxin-responsive factors during infection of *Meloidogyne javanica* (Cabrera et al. 2016). In a recent study more number of gene regulatory modules were identified, i.e., miRNA172/TOE1, miRNA159/MYB33, miRNA390/TAS3-derived-tasiRNAs/ARFs, miRNA319/TCP4 or miRNA396/GRFs during the gall formation (Cabrera et al. 2018).

5.5 Insect Pests

In Chrysanthemum, a total of 303 conserved miRNAs belonging to 276 miRNAs families and 234 potential novel miRNAs were identified. Among them miR159a, miR160a and miR393a (abundant miRNAs) were found to be responsive to the *Chrysanthemum morifolium* and aphid interaction. (Xia et al. 2015). In tea plant,

512 novel miRNAs were identified in response to *Ectropis oblique* feeding. A hypothetical model for miRNA regulatory pathways and their target genes was constructed using the data obtained. This will help to uncover the molecular mechanism involved in stress (Jeyaraj et al. 2017). In most of the cases, pathways were studies in the insect biology and information used for RNAi-based insect control (Xu et al. 2013; Burand and Hunter 2013).

6 Conclusion

Next generation sequencing technologies have enabled to generate voluminous data regarding miRNAs. In combination with the cutting edge computational technologies, researchers able to decipher the role of miRNAs in conferring tolerance to different biotic and abiotic stresses. These findings help to map the detailed molecular mechanism involved in providing the resistance. After considerable meta-analysis, researchers will be enabled to identify conserved pathways and specific pathway. With artificial miRNA (amiRNA) technology emerging as potential tool for gene silencing. The information obtained through different high-throughput sequencing technologies can be useful to construct amiRNAs. With the proper application of genome editing and gene silencing, better varieties could be develop to thrive in adverse conditions and provide good yield.

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MicroRNA as a Tool for Mitigating Abiotic Stress in Rice (*Oryza sativa* L.)

Deepu Pandita and Shabir Hussain Wani

1 Introduction

Rice is a very important crop for mankind with 166 million hectares harvested area. Global rice production in year 2018 was predicted to amount 510.6 million tonnes and total utilization as 509.1 million tonnes wherein food use is 411.7 million tonnes. Man uses 81% of total rice production as food (FAO-RMM 2018) (http://www.fao.org; US Food and Agriculture Organization. Rice is a staple food for the inhabitants of Asian countries. It is estimated that rice production must be increased by 60% to cater to the increase in demand by the year 2020. In certain Asian countries, rice cropping activities got upset by floods or drought during main-crop cycles. In India rice is cultivated in about 44 million hectares by 57% of about 102 million Indian farmers. Rice production in India (2017) was 111.0 million tonnes. One-third of the total calorie needs (2420) are fulfilled through rice. Rice grows in irrigated, rainfed upland and lowlands, deep water and flood prone areas. Rice is a model cereal crop with a relatively small genome size of 430 Mb than other cereals, a huge germplasm collection, massive repertoire of molecular genetic resources and a proficient transformation system. Rice is the first sequenced crop wherein the genome sequence of the two cultivated rice subspecies, *Oryza sativa* L. ssp. *japonica* and ssp. *indica* was elucidated. The sequences of the 12 chromosomes of *O. sativa* ssp. *japonica* cultivar Nipponbare was completed by a consortium of 10 countries under aegis of International Rice Genome Sequencing Project (IRGSP), wherein a map-based complete sequence of the entire genome was acquired through

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hierarchical clone-by-clone sequencing approach (Sasaki and Burr 2000). The sequence of the *O. sativa* ssp. *indica* cultivar was done by a whole-genome shotgun sequencing method (Yu et al. 2002, 2005). These genome sequences are precious resources not only in appreciating the structure and function of the rice plant itself but also in decoding the genome organization of other cereals (Paterson et al. 2004; Devos 2005). By computational gene predictions, 57,000 protein-encoding sequences were inferred from all finished sequences (Yu et al. 2005) out of which a large fraction (13% in chromosome 1 and 18% in chromosome 10) are different categories of transposable elements (TEs) (Sasaki et al. 2002; The Rice Chromosome 10 Sequencing Consortium 2003), suggesting the estimated gene number of around 43,000 (*Arabidopsis* Genome Initiative 2000). Rice grows in complex ecology with a huge number of abiotic physical and chemical factors, which vary both in time and space. Fluctuations of these ecological factors outside of their normal ranges generally have negative biochemical and physiological effects on this plant and hamper their production. Since plants cannot move to escape from their environments, they have evolved very complex pathways in response to environmental stresses in the form of avoidance or tolerance.

This chapter highlights the recent advances in understanding the crucial roles of miRNAs in Rice responses to drought, salinity, heat stress, cold stress, cadmium heavy metal stress and proposes potential strategies of miRNA biotechnology for abiotic stress-regulated rice crop. The rice production enhancement can play a pivotal role in upgrading the economic status of India. The production of rice is not harvested in commensuration to its genetic potential in almost all rice-growing ecosystems because of their sensitivity to diverse abiotic stress factors like heat, drought, salinity, cold, heavy metal in different ecological conditions. The generation of abiotic stress-tolerant rice cultivars therefore emerges as a priority issue. The high-throughput RNA-Seq and transcriptomic technologies have led to identification of hundreds of genes and miRNAs induced under stress conditions for a better understanding of the stress-related mechanisms. The arena of rice molecular biology and biotechnology has progressed appreciably in the past two decades and various transgenic rice plants have already been developed (Grover and Minhas 2000) but the miRNA directed transgenics is a novel field.

2 miRNA and Their Historical Importance

MicroRNAs (miRNAs)are petite, endogenously encoded, single stranded, 18–25 nucleotides in length, non-coding RNAs with significant role in regulation of gene expression at post-transcriptional level by mRNA cleavage, translation repression and DNA methylation (Taylor et al. 2014; Xie et al. 2015). MicroRNAs were first discovered in *Caenorhabditis elegans* (Lee et al. 1993). The miRNA profile of *C. elegans* is probably the most complete, with the number of validated miRNA genes being ~95 (Ambros et al. 2003b; Lim et al. 2003). The first plant miRNA was identified in 2002 in Cinderella of Genetics, that is, *Arabidopsis thaliana*

(Reinhart et al. 2002). The miRNAs are currently known to regulate target genes at post-transcriptional level by binding to complementary miRNA binding-sites on target messenger RNA transcripts (mRNAs) and thus trigger translational repression or gene silencing (Chen 2004; Kim 2005), interaction with signaling pathways of growth regulators (Chen et al. 2011), are involved in tasiRNAs (*trans*-acting small interfering RNAs) biogenesis (Jouannet et al. 2012) and control many biological processes such as growth, development, differentiation, cancer development and progression. The miRNAs are conserved in plant kingdom and play decisive role in plant responses to both biotic and abiotic stresses (Sunkar and Zhu 2004; Xie et al. 2014). The total number of miRNAs in each organism is unknown but is estimated to represent ~1% of the number of coding genes (~250–300 miRNAs in *A. thaliana*) (Sunkar and Zhu 2004; Grad et al. 2003; Lai 2003; Lim et al. 2003; Bartel 2004). This prediction is based on the information that miRNAs are derived from evolutionarily conserved hairpin precursor RNAs. Sixteen years ago, none had even heard of microRNAs and today 38589 hairpin precursor miRNAs, expressing 48885 mature miRNAs from 271 species are registered in release 22 of miRNA database miRBASE (http://www.mirbase.org/) and 10,000 miRNAs from 121 plant species are registered in plant miRNA database (PMRD, http://bioinformatics.cau.edu.cn/PMRD) (Pandita 2018a, b). A database constructed through the Sanger Institute where annotated miRNA sequences are available is (http://www.sanger.ac.uk/cgi-bin/Rfam/mirna/browse.pl and http://www.mir-base.org/index.shtml). The number of miRNAs vary in diverse plant species, for example, *A. thaliana* (199), *O. sativa* (447), *Medicago truncatula* (375), *Zea mays* (170), *Sorghum bicolor* (148), *Vitis vinifera* (137), etc. (Ambros et al. 2003a, b). According to miRBase database release 21, 592 rice miRNA precursor entries are available (Mutum et al. 2016). Mutum et al. (2016), identified 71 putative novel miRNA gene loci in drought-tolerant '*aus*' rice variety Nagina 22 (N22). The study of rice miRNAs was pioneered by the work of Wang et al. (2004) who first identified 20 miRNAs by use of improved cDNA cloning procedure consequential from experimental RNomics. Sunkar et al. (2005) reported a comprehensive study on rice miRNAs 1 year later wherein they identified new miRNAs that were tricky to be predicted by in silico analysis and verified the earlier predicted miRNAs. Sequencing and analysis of small RNA libraries made identification of 14 new miRNAs of 14 families possible, 13 of which were absent in *Arabidopsis*. The sequence complementarity criteria led to prediction of 46 rice genes as putative targets of these new miRNAs. The predicted targets contained transcription factors and genes from various physiological processes (Sunkar et al. 2005). The analysis of plant miRNAs is slightly behind that of animals; still the functional characterization of plant miRNAs is becoming clearer with the passing years. The role of miRNAs in regulation of various developmental processes has been extensively studied, while the emerging roles of miRNAs in plant stress responses are still less discussed. According to Zhang et al. (2005) 25.8% of the *Arabidopsis* Expressed Sequence Tags (ESTs) containing miRNAs were present in stress-induced plant tissues, implying that miRNAs play an imperative role in plant responses to ecological stresses. Several recent studies in different plant species support this hypothesis, but mostly is applicable to *A. thaliana* as a model organism

(Sunkar and Zhu 2004; Jones-Rhoades and Bartel 2004; Achard et al. 2004) the present chapter discusses the role of miRNAs in stress response in rice as a model cereal system. In different plant species, 1511 miRNAs are known to be involved in various abiotic stresses (Zhang et al. 2013b). In rice, *SNP* in the miR156 and miR529 target site of OsSPL14 raises expression of the target leading to fewer tillers, more panicle branching and higher yields in rice (Jiao et al. 2010; Miura et al. 2010; Jeong et al. 2011) suggesting that stress-regulated miRNAs- and miRNA-target sites have a role in future crop improvement efforts. Decoding of all the miRNA targets and their functions will be one of the impending challenges in this promptly promising scientific arena and will act as players of the next generation genetic engineering for augmentation and improvement of crop plants.

3 Plant miRNA Biogenesis

The miRNA biogenesis takes place in multiple steps to form mature miRNAs from *MIRs* (Xie et al. 2015).

3.1 Transcription Stage

There are above 100 *MIR* genes in plants (Nozawa et al. 2012; Yi et al. 2015). *MIRs* genes are intergenic (between two genes) or intragenic (within genes) or clustered (Rogers and Chen 2013). In plants, *MIRs* are only transcribed by RNA polymerase II (Pol II) to generate stem-loop structured pri-miRNA (primary microRNA) with size range of 100 nt to several kilo bases (Kim et al. 2011). It has been documented that introns of plant pri-miRNAs can boost biogenesis of miRNA, thus they are important for proper accumulation levels of mature miRNA (Bielewicz et al. 2013).The transcription of intron containing *MIR* genes is mediated by the coactivators and NOT2s (negative on TATA-less2) (NOT2a and NOT2b homologs of the animal CCR4-NOT complex and VIRE2-INTERACTING PROTEIN2, respectively). This mediator is a conserved protein that advances transcription of protein-coding genes by recalling Pol II to the promoter region of *MIRs* (Kim et al. 2011; Wang et al. 2013). The transcript stabilization occurs by addition of 5′cap and 3′polyadenylate tail (Jones-Rhoades et al. 2006), introns are spliced by STABILIZED1 (STA1) and DAWDLE (DDL) stabilizes pri- miRNAs (Ben et al. 2013; Rogers and Chen 2013).

3.2 Processing (Slicing/Cleavage) Stage

Two sequential RNase III enzyme-mediated cleavages are required to produce mature miRNAs. In the nucleus, RNAse III enzyme DCL1 (DICERLIKE1) and a complex of proteins which includes HYL1 (HYPONASTIC LEAVES1), SE (SERRATE), CPL1

(C-TERMINAL DOMAINPHOSPHATASE-LIKE1), CBC (cap binding complex), DDL, SIC (SICKLE), TGH (G-patch domain protein TOUGH) and NOT2s form D-bodies. Then MOS2 (MODIFIEROF SNC1) along with this complex slices the pri-miRNA twice (Wang et al. 2013; Wen-Wen et al. 2014). During the first slicing, base of the pri-miRNA stem-loop is cleaved to produce a miRNA precursor miRNA (pre-miRNA). Pre-miRNA has a characteristic secondary 'hairpin-like' structure, with high and negative fold-free energy, with size varying between 70 and 400 nt. Then, the pre-miRNA is processed at a second position near the pre-miRNA loop, so loop is removed to generate a miRNA: miRNA∗ (miRNA/miRNA∗) duplex again by DCL1 in plants (Reinhart et al. 2002). The miRNA which is the guide strand has lower thermodynamic stability and miRNA∗ is the passenger strand (Eamens et al. 2009). Most plant pri-miRNAs, for example, miR159 and miR319 are sliced in the stem-to-loop direction (Bologna et al. 2013a, b; Breakfield et al. 2012). Exceptions are Pre-miR172 where the initial cleavage by the DCL1 complex takes place15 nucleotides from the stem base and the loop is removed in the second slicing (Song et al. 2010). And in pre-miR159 and pre-miR319 cleavage occurs near the loop. The precursor after three subsequent cuts by DCL1 at 20–22 nucleotide intervals releases miRNA (Bologna et al. 2009). The final miRNA size produced by Dicer enzymes is determined by the intra-molecular spacing of the RNase III active site and the 3′ overhang binding pocket of the PAZ domain that is supposed to work as a molecular ruler (Macrae et al. 2006). DCL family members form dissimilar small RNAs sizes. DCL1 and DCL4 generate 21 nucleotides; DCL2 produces 22 nucleotides and DCL3 makes 24 nucleotide length of small RNAs (Rogers and Chen 2013). Plant miRNAs mostly have 21 nucleotides (Chen 2010). However, there is a class of miRNA in rice that is 24 nucleotides in length and for biogenesis requires DCL3. The long miRNAs of 24 nucleotide length are incorporated into AGO4 proteins which direct DNA-target gene methylation (Wu et al. 2010). Production of long miRNAs is usually dependent on the spatial or temporal expression of DCL3, indicating probable competition among DCL proteins to slice miRNA precursor under certain conditions. The two-step processing of transforming pri-miRNAs into mature miRNAs is intra nuclear in plants.

3.3 Translocating Stage

The processed miRNA/miRNA∗ duplex is methylated by HEN1 (methyltransferase HUA ENHANCER1) and then transported to the cytoplasm by nuclear shuttle protein, HASTY (HST) an ortholog of exportin 5 (EXP-5) (Zhang et al. 2006).

3.4 Loading Stage

The miRNA strand is selected and loaded into the AGO1 (ARGONAUTE1) component of RISC (RNA-Induced Silencing Complex) (Wen-Wen et al. 2014). AGO is positioned at the core of RISCs and act as a platform for target recognition and silencing

(Yoshikawa et al. 2013). HYL1 itself or alongside DCL1 directionally incorporates the methylated miRNA/miRNA∗ duplex into AGO1, which is accompanied with a complex of HSP9 (HEAT SHOCK PROTEIN900) chaperone and SQN (AGO1-associated proteins SQUINT). The cytoplasmic position of HSP90 indicates that AGO1 loading occurs in this compartment (Iki et al. 2012). The guide strand with the favorable thermodynamic stability at its 5′ terminus is retained by an AGO protein, while the passenger strand (miRNA ∗) is either degraded or in few situations is incorporated into a different RISC (Iki et al. 2010; 2012; Wen-Wen et al. 2014). Under specific conditions, for example, during plant development and under various abiotic stress conditions, miRNA gets replaced by miRNA∗ and then becomes dominantly expressed strand. It indicates that the expression of miRNAs is flexible and can be modified. The RISC complex will guide the cleavage or translational repression of mRNAs complementary to the mature miRNA (Bartel 2004; Zhang et al. 2006). For efficient target slicing by AGO proteins, miRNAs should have a high amount of base complementarity to their targets in plants. Before mRNA cleavage 3′deadenylation or 5′decapping probably occurs by exonucleases in AGO slice processing (Rogers and Chen 2013). Recognition of a target mRNA occurs via the loaded RISC resulting in its degradation/cleavage in plants because of perfect complementarity (Fig. 1) or translational inhibition/repression in animals because of asymmetric or non-perfect complementarity and thus hindrance to ribosome binding. MicroRNAs (intronic and intergenic) in RISC complex repress messenger RNAs which are incompletely complimentary to it that is why single miRNAs can regulate hundreds of genes (Pandita 2018a, b).

4 Role of miRNA in Abiotic Stress Responses in Rice

The stress responsive miRNAs control plant growth and development under stress environmental conditions by adaptation. A number of stress specific miRNAs are identified in model plants under diverse biotic and abiotic stress conditions like salinity (Zhao et al. 2009), drought (Zhao et al. 2013; Ferdous et al. 2015), cold (Zhou et al. 2008), etc. Depending on the tissue and stress type, the *MIR* genes are differentially expressed indicating specific response pathways (Devi et al. 2013; Rogers and Chen 2013). The conserved miRNA families among different plant species also exhibit differential expression patterns in response to same abiotic stress, suggestive of diverse metabolic modifications. The miR160 and miR167 regulate ARFs (auxin response factors) while miR393, miR397b, miR402, miR413 and miR159 get induced by abscisic acid (ABA) hormone signaling pathways suggesting their vital roles in stress responses. miR319 and miR166 are down-regulated by gibberellic acid (GA) (Liu et al. 2009). The miR319 controls jasmonic acid (JA) biosynthesis and senescence in turn regulating the relationship between leaf growth and its senescence (Schommer et al. 2008). These miRNAs consequently control plant growth and development by reprogramming the downstream gene expression under stress conditions (Sunkar et al. 2012). The role of rice miRNA in various stress conditions is explained further in detail.

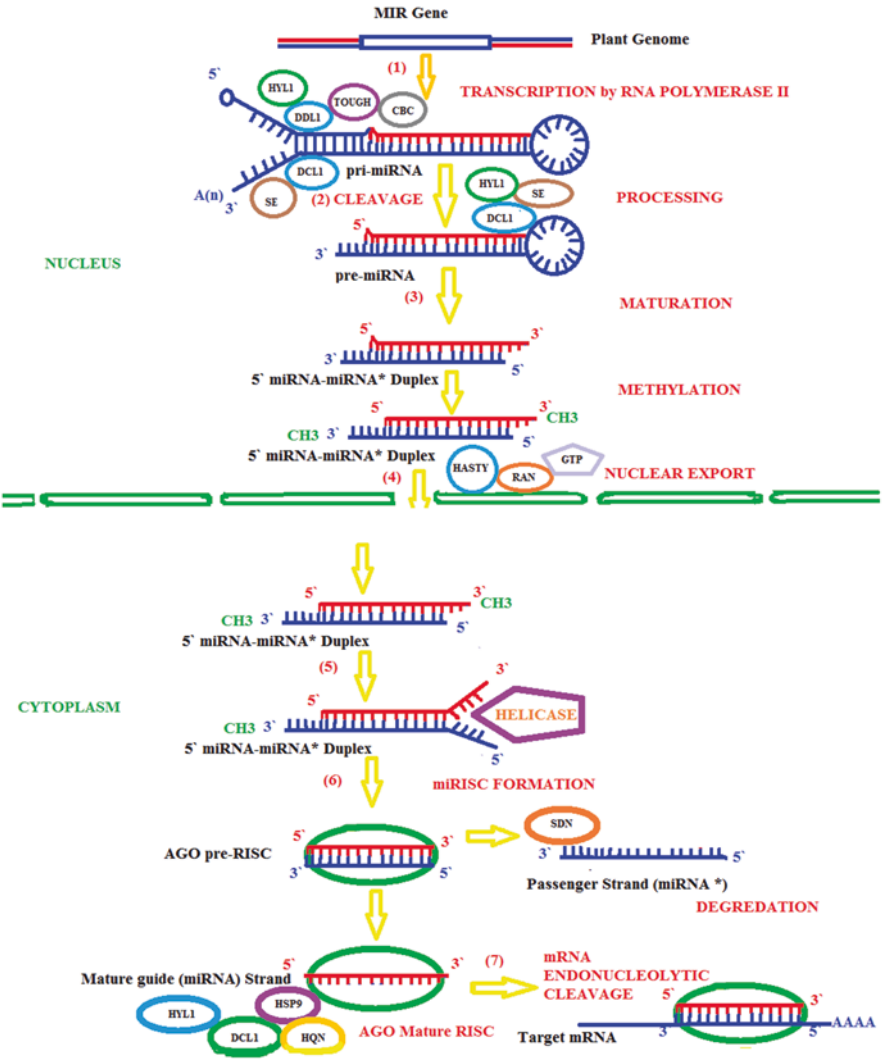

Fig. 1 Schematic model of plant miRNA biogenesis. (1) The miRNA gene undergoes transcription by RNA polymerase II to form primary microRNAs transcript. (2) Then pri-miRNA is processed into precursor microRNAs (pre-miRNA) by DCL1, HYL1, SE, HEN1, TOUGH, DDL and CBC. (3) The pre-miRNA matures into miRNA-miRNA∗ duplex by DCL1, SE, HYL1 which is then methylated by HEN1. (4) HASTY transports the miRNA-miRNA∗ duplex from nucleus into cytoplasm. (5) Helicase unwinds the miRNA-miRNA∗ duplex. (6) The passenger strand undergoes degredation by SDN while guide strand incorporates into AGO to form RISC complex, (7) wherein AGO cleaves the target mRNA strand endonucleolytically

4.1 Drought Stress

Drought is the most destructive abiotic stress which negatively impacts crop yields worldwide. Plants subjected to drought-stress exposure have to re-establish cellular homeostasis by changing gene expression programs at the transcriptional, post-transcriptional and post-translational levels (Zhu 2002; Bartels and Sunkar 2005; Ni et al. 2009). According to recent studies drought alters expression of miRNAs in rice (Zhao et al. 2007; Zhou et al. 2010). During drought stress miR169g in rice, which is one of the miR169family members and targets NF-Y transcription factor family shows up regulation. The dehydration-responsive cis-element (DRE) has CCGAC motif in its promoter region which is the characteristic feature of various drought-induced genes in plants. In silico analysis indicated that two putative DRE cis-elements are present in miR169g promoter (Zhao et al. 2007), further supporting the drought-responsiveness of miR169 in rice. In rice 30 miRNAs have been detected, out of which 16 miRNAs (miR156, miR159, miR168, miR170, miR171, miR172, miR319, miR396, miR397, miR408, miR529, miR896, miR1030, miR1035, miR1050, miR1088 and miR1126) were found to be strongly down-regulated and 14 miRNAs (miR159, miR169, miR171, miR319, miR395, miR474, miR845, miR851, miR854, miR896, miR901, miR903, miR1026 and miR1125) were dominantly up-regulated in response to the drought stress. In rice, miR156, miR168, miR170, miR171, miR172, miR319, miR396, miR397 and miR408 showed opposite expression to that observed in drought stressed *Arabidopsis* (Zhou et al. 2010). In rice, above 3000 genes were found to be influenced by altered expression levels of miR156 members (Xie et al. 2012). The miR168 which targets *AGO1* exhibits differential expression under drought stress in *Arabidopsis* and *O. sativa* (Zhou et al. 2010). In rice, miR319 is up-regulated and miR167 and miR169 are down-regulated in response to ABA (Liu et al. 2009). In rice, os-miR393 levels shows up-regulation under drought stress which in turn reduces the osTIR1 and osAFB2 levels and plant growth. Under drought stress conditions, miR167 is up-regulated in *Arabidopsis*, but miR167 and miR319 are down-regulated in rice by ABA (Liu et al. 2009). During drought stress, osa-miR159f, osa-miR1871, osa-miR398b, osa-miR408-3p, osa-miR2878-5p, osa-miR528-5p and osa-miR397a which are cultivar-specific drought responsive (CSDR)-miRNAs were up-regulated in the flag-leaves of two drought-tolerant rice cultivars, Nagina 22 (N22) and Vandana, but down-regulated in the drought-sensitive cultivars of Pusa Basmati 1 (PB1) and IR64. In Table 1, there are summarized some drought-responsive miRNAs that were proven to be implicated in drought-stress response in rice plants.

4.2 Salt Stress

Salinity is another most significant abiotic stress factor, given the fact that 6% of the land is influenced by salinity (Munns 2010). Our country has about 8.6 million hectares of saline agricultural area including 3.4 million hectares of sodic land (Blumwald and Grover 2006). In addition to the hyper-ionic toxicity, salt stress also

Table 1 Drought responsive miRNA in *Oryza sativa*

miRNA	Target genes	References
miR159	MYB and TCP transcription factors	Jones-Rhoades and Bartel (2004)
miR168	ARGONAUTE1, MAPK	Zhou et al. (2010)
miR169	CBF/DREBs transcription factors (TFs)	Zhao et al. (2007)
miR169g	CCAAT-box binding transcription factor	Zhao et al. (2007)
miR170	SCL transcription factor	Zhou et al. (2010)
miR171	GRAS transcription factors	Liu et al. (2008)
miR172	Floral Homeotic Protein APETALA2, bZIP transcription factor family protein	Jones-Rhoades and Bartel (2004), Zhou et al. (2010)
miR393	Auxin receptors TIR1, AFB2, AFB3	Xia et al. (2012)
miR395	Sulphate transporter	Zhou et al. (2010)
miR396	GRL transcription factors	Liu et al. (2008), Zhou et al. (2010)
miR397	β-fructofuranosidase, Laccases	Cai et al. (2006), Zhou et al. (2010)
miR474	Kinesin, a pentatricopeptide repeat (PPR) family protein	Zhou et al. (2010)

induces hyper-osmotic and oxidative stresses leading to physiological drought conditions that cause reversible inhibition of metabolism and growth or an irreversible damage involving death of the cells (Zhu 2001; Bartels and Sunkar 2005; Chaves et al. 2009; Hasanuzzaman et al. 2013). The altered expression of miRNAs in response to salt stress has been reported in different plant species. The rice miR-169 family consists of 17 members, out of which three members, that is, miR169g, miR169n and miR169o exhibit salt responsiveness (Zhao et al. 2009). Drought-responsive miR169 family members, miR169g and miR169n were also found to be up-regulated under salt stress in rice (Zhao et al. 2009). MIR169g promoter harbors DRE and ABRE (abscisic acid responsive cis-elements) elements. The miR169g up regulation under salinity stress can be due to the salinity-induced osmotic stress activation of DRE and ABRE elements through ABA-dependent and ABA-independent pathways (Zhao et al. 2009). Alternatively, miR169n promoter lacks the DRE element but possesses the ABRE element. Thus, miR169n responsiveness to salt stress could be attributed to ABRE element activation, since ABA is known to amass under both drought and salt stress conditions (Zhao et al. 2009). The up-regulation of miR169 by salt stress is also reported in *Arabidopsis* (Zhao et al. 2009), suggesting that miR169 induction under salt stress is conserved between *Arabidopsis* and *O. sativa*. Interestingly, miR169 selectively down-regulates one of its target genes, Os03g29760, encoding a CCAAT-box binding transcription factor in rice (Zhao et al. 2009). Osa-miR396c down-regulates in ABA-dependent manner under salinity stress and up-regulation of Osa-miR396c results in reduced salt-stress tolerance (Gao et al. 2011). Rice plants show miR408 down-regulation under salinity and drought stress (Mutum et al. 2013), while miR408 up-regulation leads to enhanced tolerance against salinity stress (Ma et al. 2015). The miR393 rice transgenics show reduced tolerance to salt and hypersensitivity to auxin (Xia et al. 2012). In Table 2, there are summarized various salt responsive miRNAs that were proven to be implicated in salinity stress response in rice.

Table 2 Salt responsive miRNAs in *Oryza sativa*

miRNA	Target genes	References
Osa-miR16	Germin-like protein; Ethylene-insensitive3 (EIN3)-Like 1 protein	Sanan-Mishra et al. (2009)
Osa-miR29	Strictosidine synthase precursor	Sanan-Mishra et al. (2009)
Osa-miR2006	Hypothetical protein; Conserved hypothetical protein	Jian et al. (2010)
Osa-miR164	CUC2 No apical meristem (NAM) protein; NAC domain-containing protein; Helicase	Macovei and Tuteja (2012)
Osa-miR169	CBF HAP2-like factor	Zhao et al. (2009)
Osa-miR393	Phytosulfokine receptor precursor; GRF-interacting factor (GIF)	Gao et al. (2011)
Osa-miR394	F-box protein	Barrera-Figueroa et al. (2012)
Osa-miR396	GRL transcription factors; Rhodenase-like protein; Kinesin-like protein B	Gao et al. (2010)
Osa-miR408	S-receptor kinase-like; DEAD-box ATP-dependent RNA helicase	Macovei and Tuteja (2012)
Osa-miR414	Helicase	Macovei and Tuteja (2012)
Osa-miR820	Domains rearranged methylase 2	Sharma et al. (2015)
Osa-miR1866	OsWRKY34; cytochrome P450 72A1	Barrera-Figueroa et al. (2012)
Osa-miR1867	Putative protein; DUF1242 superfamily	Barrera-Figueroa et al. (2012)
Osa-miR2001	Protein GPR107 precursor	Jian et al. (2010)
Osa-miR2003	HEAT repeat family protein; Ribosomal protein S11; NAC domain- protein 90	Jian et al. (2010)
Osa-miR2005	Nitrate and chloride transporter; Phosphate carrier protein	Jian et al. (2010)

4.3 Cold Stress

Cold stress along with chilling (<20 °C) and freezing (<0 °C) has an influence on growth, development and production of crop plants. The physiological and genetic mechanisms of chilling injury and tolerance are well-understood in *Arabidopsis*. To facilitate cold stress adaptation, plants modify their growth and metabolism through gene expression reprogramming (Chinnusamy et al. 2007; Yamaguchi-Shinozaki and Shinozaki 2006). The cold signal perception in plants causes activation of C-repeat/drought-responsive element binding factor (CBF)-dependent and CBF-independent transcriptional pathways that changes the response to cold stress (Achard et al. 2008; Chinnusamy et al. 2010). The microRNA (miRNA)-based gene regulation is critical for coordinating plant responses to cold stress (Khraiwesh et al. 2012; Sunkar et al. 2012; Miura and Furumoto 2013). Contrasting to other cereals, rice especially *indica* subspecies is prone to cold stress resulting in decreased

productivity. According to Yoshida (1981), cold sensitivity varies among germination, vegetative growth and reproductive stages. Cold stress has the potential to affect growth and development of rice during any developmental stage of germination, seedling, vegetative, reproductive and grain maturity (Ye et al. 2009). In rice, 18 cold-responsive miRNAs have been identified (miR156k, miR166k, miR166m, miR167a/b/c, miR168b, miR169e, miR169f, miR169h, miR171a, miR535, miR319a/b, miR1884b, miR444a.1, miR1850, miR1868, miR1320, miR1435 and miR1876) (Lv et al. 2010). Among the cold-responsive miRNAs, six conserved miRNA families (miR-156, miR-166, miR-169, miR-171, miR-319 and miR-444) are acknowledged to play defensive roles against cold stress by targeting transcription factors like squamosa promoter binding proteins, TCP family transcription factors, CCAAT-binding proteins, homeodomain-leucine zipper proteins, scarecrow-like proteins and MADS box proteins (Lv et al. 2010). Four miRNAs (miR-1435 targets Os03g42280 and Os04g44354; miR-1876 targets Os07g41090; miR-1320 targets Os05g47550 and Os06g10980; miR-1884 targets Os06g48240 and Os09g33710, etc.) present in rice but not *Arabidopsis*, respond to cold-stress. So, suggesting that these non-conserved miRNAs may have a species-specific role in cold response (Lv et al. 2010). The miRNA expression may be increased (miR408, miR397, miR393, miR165, miR166, miR169, miR172, miR396), differentially regulated (miR319, miR156) slightly increased (miR398, miR157, miR164, miR394) and decreased (miR167, miR168, miR171) spatiotemporally. Yang et al. (2013a, b) observed that up-regulation of Osa-miR319b and repression of its targets, OsPCF5 and OsPCF8 cause enhancement in cold tolerance (4 °C) after chilling acclimation (12 °C). In rice, miR319 positively regulates cold tolerance by targeting OsPCF6 and OsTCP21 (Wang et al. 2014) and thus acts as a positive regulator of cold stress tolerance. In Table 3, given are some summarized cold responsive miRNAs that were proven to be implicated in cold stress response in rice.

4.4 Heat Stress

Severe heat can spoil yield, grain quality and rice plant growth irreversibly (http://www.irri.org). When temperature exceeds 42 °C, rice yield gets reduced drastically. With global warming and climate change, heat stress is one of the key bottlenecks in rice production and productivity. Many scientific investigations have been done to understand the regulation of heat responsive genes in rice (Mittal et al. 2009; Zou et al. 2009; Mittal et al. 2012). Though, very limited investigation has been done on the miRNAs expression profile under high temperature. Out of 154 heat responsive miRNAs of *A. thaliana*, 55 of them were present in both the species of *O. sativa* and *A. thaliana*. The miR167c and 167d targets genes for heat repeat family proteins and miR396a and miR414 targets code for heat shock 70 kDa protein. The miR414 also targets heat shock protein 81-1 and calmodulin-binding protein. The miR399b and 399e targets heat shock protein binding proteins. The miRNA 413 targets transcription factors (WRKY proteins). The other conserved rice miRNAs miR156, miR157,

Table 3 Cold responsive miRNAs in *Oryza sativa*

miRNA	Predicted target function	Target genes	References
OsmiR156k	Proline synthase gene ROS scavenging gene SPL genes/transcription factors containing the SBP domain, regulate flowering, plant architecture, seed germination, and seedling development	OsP5CS Os01g22249 SPL3, SPL14, and SPL17	Cui et al. (2015) Wang et al. (2014)
OsmiR-164	NAC plant-specific transcription factors		Zhang (2015)
OsmiR-167	ARF transcription factors		Jeong et al. (2011)
OsmiR-168	Argonaute (AGO) proteins		Zhang (2015)
OsmiR-169	Genes encoding NF-Y transcription factors		Zhang (2015)
OsmiR-171	TCP family transcription factors, a putative GAMYB	Os06g01620 and Os04g46860	Lv et al. (2010)
OsmiR319b	DREB1/CBF protein (DREB1A/B/C, DREB2A, TPP1/2) activation and ROS suppression OsPCF6 and OsTCP21 transcription factors		Yang et al. (2013a, b) Zhang (2015) Wang et al. (2014)
OsmiR393	TIR1, AFB2, AFB3, F-box domain, LRR containing protein/MYB family transcription factor		Gao et al. (2011) Barrera-Figueroa et al. (2012)
OsmiR394	F-box domain containing protein		Zhang (2015) Barrera-Figueroa et al. (2012)
OsmiR396	Growth regulating factor TFs, rhodanese-like proteins, kinesin-like protein B		Gao et al. (2010) Barrera-Figueroa et al. (2012)
OsmiR408	Plastocyanin expression and photosynthesis		Sun et al. (2018) Pan et al. (2018) Zhang et al. (2017)
	Copper binding proteins: 10 plastocyanin	Os03g15340, Os03g50160, Os08g37670, Os09g29390	
	and 2 laccase genes		
	E2F family transcription factor Auxin responsive Aux/IAA gene AP2 domain containing protein	Os04g33950 Os01g53880 Os08g42550	
OsmiR-444a	MADS-box proteins, MADS 57 and MADS 27		Lv et al. (2010)
OsmiR-529	SBP-box gene family		Barrera-Figueroa et al. (2012)
OsmiR530-3p	Hairpin-induced protein 1 domain containing protein		Barrera-Figueroa et al. (2012)
OsmiR-809	Glutaredoxin 2, putative, PPR repeat containing protein	3-ketoacyl-CoA synthases	Barrera-Figueroa et al. (2012)
OsmiR812q	Calcineurin B-like (CBL 10) protein interacting protein kinase		Jeong et al. (2011)
OsmiR-1320	Clathrin assembly protein	Os05g47550	Raffaele et al. (2007)
	Fucosyltransferase 7	Os10g36000	
	Remorin	Os06g10980	

(continued)

Table 3 (continued)

miRNA	Predicted target function	Target genes	References
OsmiR1425	Fertility restorer (Rf-1) gene families		Lu et al. (2008) Jeong et al. (2011)
OsmiR-1435	B3 DNA binding domain containing protein	Os03g42280	Mane et al. (2007)
	UDP-glucosyl transferase	Os04g44354	
OsmiR-1850	Actin-2	Os10g36650	Singh et al. (2002)
	Regulatory protein NPR1	Os03g46440	
	Methyltransferase small domain	Os03g63260	
	Pectinesterase inhibitor	Os08g04650	
	Low-complexity proteins	Os10g35810	
	Metal ion binding protein	Os04g47410	
OsmiR-1876	Histone deacetylase 6	Os07g41090	Kawaguchi et al. (2003)
OsmiR-1884b	ATPase	Os06g48240	Sun and Kamiya (1994)
	Beta-glucosidase homolog precursor	Os09g33710	
	Uricase	Os01g64520	
	Transposon protein	Os02g22610	
		Os02g13210	
		Os02g43370	
	Ent-kaurene synthase A	Os04g09900	
	bHLH transcription factor	Os07g35870	Goldgur et al. (2007)
	Abscisic stress-ripening protein	Os01g73250	
	DNA-binding protein	Os04g58730	Gong et al. (2005)
	OsWAK87	Os09g30454	
	Adagio protein 1	Os11g34460	
	Glutathione S-transferase	Os01g72120	
	TMV response-related gene	Os02g09990	Van Dyck et al. (1994)
	ATP binding domain 1 family, member B	Os02g34950	
	Peptidase S16, ion N-terminal	Os03g29540	
	Calmodulin-binding protein	Os03g60890	Singh et al. (2008)
	CP12-2	Os03g19380	
	Win2 precursor	Os11g37950	Dong et al. (2009)
	CHY1	Os12g16350	
OsmiR-2871	GT family protein		Barrera-Figueroa et al. (2012)

miR159 and miR160a targets transcription factors like Squamosa promoter-binding protein-like (SPL-10 11), GA-inducible MYB-transcription factor, MYB transcription factors and auxin response factors (Sailaja et al. 2014). The miR164a/c targets NAC domain which are connected to various abiotic stresses in diverse plant species (Liu et al. 2008). In rice seedlings under heat stress miR1884 was up-regulated in

shoots and down-regulated in roots (Lv et al. 2010). Three microRNAs miR169, miR1884 and miR160 showed differential expression in root and shoot of rice being up-regulated in shoot and down-regulated in root under heat stress. Heat-treated shoot tissue of N22 was compared with control shoot. Here, osa-miR1423a-5p, osa-miR1427, osa-miR2055, osa-miR1863a and osa-miR5072 showed up-regulation, while osa-miR166n-5p, osa-miR2863b and osa-miR396f-3p were down-regulated after short duration stress (SDS), long duration stress (LDS) and recovery (REC). High temperature–treated shoot tissue of the susceptible cultivar Vandana was also compared with control shoot. Here, osa-miR1879, osa-miR394, osa-miR3979-3p, osa-miR408-5p, osa-miR444f, osa-miR531a, osa-miR440 and osa-miR444a5p showed up-regulation after SDS, LDS and REC. In heat-treated root tissue of N22, osa-miR1427 was up-regulated while osa-miR1878 was down-regulated. In heat-treated root tissue of Vandana, osa-miR396c-5p, osa-miR5072, osamiR5082, osa-miR528-5p and osa-miR169i-5p were up-regulated and osa-miR1878 was down-regulated. Majority of miRNAs were up-regulated in root and shoot of Vandana and down-regulated in N22. The miRNAs expressed preferentially in N22 at high temperature were osa-miR1439, osa-miR1848, osa-miR2096, osa-miR2106, osa-miR2875, osa-miR3981, osa-miR5079, osa-miR5151, osa-miR5484, osa-miR5792 and osa-miR5812 which may have a more precise role in heat stress tolerance (Mangrauthia et al. 2017).

4.5 Oxidative Stress

Reactive oxygen species (ROS), that is, superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^-) are generated in metabolically active organelles such as chloroplast, mitochondria and peroxisomes of plant cells (Apel and Hirt 2004). Elevated levels of ROS are often associated both with abiotic stresses, for example, drought, salt, cold, heavy metals and biotic stresses, for example, bacterial and fungal diseases (Mittler et al. 2004). Plants subjected to stress utilize enzymatic superoxide dismutase (SOD), ascorbate peroxidase (APX), and catalase (CAT), glutathione peroxidase and non-enzymatic ROS detoxification pathways to neutralize their harmful effects (Shukla et al. 2008). SODs as first line of defense scavenges the excess superoxide radicals (O_2^-) by converting into hydrogen peroxide (H_2O_2), which is consequently detoxified by peroxidase and catalase (Bartels and Sunkar 2005). Li et al. (2011a, b) identified seven H_2O_2-responsive miRNA families' miR169, miR827, miR528, miR397, miR1425, miR319a.2 and miR408-5p which differential expression pattern in rice seedlings. Of these four miRNAs, miR169, miR397, miR827 and miR1425 were up-regulated while miR528 was down-regulated by H_2O_2 treatments (Li et al. 2011a, b). In rice seedlings, miR529a targets *OsSPL2* and *OsSPL14*, down-regulating their expression in miR529a OE plants and thus fortify plant tolerance to oxidative stress (Yue et al. 2017). Osa-miR528 targets SOD (Cu/Zn superoxide dismutase) and PODs (peroxidases) and plays a role in regulation of these antioxidative enzymes.

4.6 Heavy Metal (Cadmium) Stress

Cadmium (Cd) a non-essential heavy metal is highly toxic to plants. Cd in soil affects the yield and grain quality in rice. However, knowledge of the role of miRNAs in response to Cd stress in rice is still limited. Ding et al. (2011) identified 19 Cd-responsive miRNAs in rice and their target genes were also predicted many of which encode transcription factors of various families. Under Cd stress five miRNA families' miR166, miR171, miR396, miR156and miR444 were down-regulated and their target transcription factor gene expression was up-regulated leading to enhanced Cd tolerance (Ding et al. 2011). The miR166 targets homeo-domain-leucine zipper (HD-Zip) transcription factors, found to be responsible for lateral root development and leaf polarity in *Arabidopsis* (Hawker and Bowman 2004; Jones-Rhoades and Bartel 2004). DCL1 and ARGONAUTE (AGO) proteins get targeted by miR162 and miR168; respectively suggesting that feedback regulation plays a role in miRNA activity in Cd-stressed rice (Ding et al. 2011). Cd-responsive miR192 highly similar to the rice miR819 in sequence acts as a negative regulator of seed germination and was down-regulated under Cd stress. Ding et al. (2013) predicted 44 target genes for eight Cd-responsive miRNAs (miR118, miR59, miR1004, miR361, miR1060, miR192, miR441, miR248) responsible for several physiological processes, antioxidant, defense and detoxification and signal transduction. The first group of target genes was involved in signal transduction, including OsWAK45-OsWAK receptor-like protein kinase and Ras-related protein RHN1. The second group of target genes included ubiquitin system proteins and other proteins involved in the degradation of abnormal proteins. The function of miR192 and ABC transporter in the rice Cd response would be deciphered after studies on Cd accumulation and translocation within the plants (Ding et al. 2013). In Table 4, there are summarized cadmium responsive miRNAs elucidated by Ding et al. (2011) and (2013) implicated in heavy metal cadmium stress response in rice.

5 Genetic Engineering Perspectives of miRNA Applications for Tolerance to Abiotic Stress

MicroRNAs (through second generation RNAi vectors) can be easily employed for the initiation of gene silencing as they target gene. The widespread biotechniques for stress tolerance mitigation through the use of microRNA are: Artificial miRNA (amiRNAs), siRNA directed DNA silencing and transient miRNA (tasiRNA) (Macovei et al. 2012; Zhang and Wang 2015). Out of these, the amiRNAs present a novel and extremely specific gene silencing technology. The premiRNA fold-back structure is the key to proficient tmiRNA processing gave birth to the idea that synthetic or artificial miRNAs can be designed to suppress specific gene expression (Zhang et al. 2006). Various scientific investigations of amiRNAs-mediated gene silencing have been performed on rice (Pérez-Quintero and López 2010).

Table 4 Predicted targets of Cd-responsive miRNAs and their function annotations

miRNA	Target genes	Target function	References
miR118	Os02g32590	Heat shock factor protein 2	Ding et al. (2013)
miR59	Os04g08640	Cadmium tolerance factor	Ding et al. (2013)
	Os04g30010	OsWAK45-OsWAK receptor-like protein kinase	
	Os11g42280	F-box domain-containing protein	
	Os01g09100	OsWRKY10 superfamily of rice TFs having WRKY and zinc finger domains	
	Os03g05740	RAS related protein RHN1	
	Os09g31454	Myb-like DNA	
miR1004	Os06g19980	Myb-like DNA	Ding et al. (2013)
	Os04g46940	Copper-transporting ATPase 3	
	Os02g48990	Phosphatidylinositol transporter/transporter	
	Os10g38580	Glutathione S-transferase GSTU6	
	Os03g63360	Integral membrane protein	
	Os08g09320	Vacuolar protein sorting protein 72	
	Os01g66110	Ankyrin-like protein	
	Os07g27460	Serine/threonine-protein kinase 19	
	Os05g07090	Glutaryl-CoA dehydrogenase, mitochondrial precursor	
miR361	Os12g17900	Ubiquitin protein ligase	Ding et al. (2013)
	Os10g22300	Resistance protein	
miR1060	Os10g30790	4 inorganic phosphate transporter 1	Ding et al. (2013)
	Os10g30770	Inorganic phosphate transporter 1-1	
	Os02g12690	Cytochrome P450 74A4	
miR192	Os09g39910	ATP binding cassette subfamily F member 2	Ding et al. (2013)
	Os12g40920	Light-inducible protein CPRF-2	
	Os06g19990	GPI-anchored protein	
	Os07g03110	F-box domain-containing protein	
	Os10g33700	ATP-binding protein	
	Os01g34620	OsGrx_S15.1 glutaredoxin subgroup II	
	Os03g09210	NADH-ubiquinone oxidoreductase B16.6 subunit	
	Os03g54130	Cysteine protease 1 precursor	
	Os06g08080	Pyrophosphate-energized vacuolar membrane proton pump	
	Os03g50070	Membrane protein	
	Os09g12230	Ubiquitin-conjugating enzyme E2 17 kDa	
	Os11g41860	Ubiquitin-protein ligase	
	Os02g45650	CAAX prenylprotease 1	
	Os06g17390	Auxin-independent growth promoter	
	Os03g62070	IAA-amino acid hydrolase ILR1 precursor	
	Os06g23274	Zinc finger, C3HC4-type family protein	

(continued)

Table 4 (continued)

miRNA	Target genes	Target function	References
miR441	Transposon		Ding et al. (2013)
	Retrotransposon		
miR248	Transposon		Ding et al. (2013)
	Retrotransposon		
miR162a	DCL1	miRNA processing	Ding et al. (2011)
miR528	OsDCL1	miRNA processing	Ding et al. (2011)
miR168b/a	ARGONAUTE protein	miRNA processing; plant development	Ding et al. (2011)
miR166i/e/m/k/g	HD-Zip transcription factors	Lateral root development and leaf polarity	Ding et al. (2011)
miR171g/b/a	Scarecrow-like transcription factors	Floral development	Ding et al. (2011)
miR390	Leucine-rich repeat receptor-like protein kinases	Signal transduction; stimulus response	Ding et al. (2011)
miR396d	Growth regulating factor transcription factors, rhodanese-like proteins, kinesin-like protein B	Flower and leaf development	Ding et al. (2011)
miR156l/k/a	Squamosa-promoter-binding protein transcription factors	Plant phase transition; shoot development	Ding et al. (2011)
miR444b.1	MADS-box transcription factors	Unknown	Ding et al. (2011)
miR1432	EF-hand proteins	Signal transduction	Ding et al. (2011)

Wang et al. (2010) has given a highly efficient novel procedure for easy and rapid construction of artificial miRNA vector in rice in less time with the use of osa-amiR528 in a modified pCAMBIA1300-UR vector. Here the pCAMBIA1300-UR vector was modified by insertion of a 'vector modification fragment' synthesized from the Os-amiR528 precursor by deleting its central miRNA-containing region and creating an AfeI restriction site. This vector modification fragment was then inserted into the destination vector to form a 'Highly Efficient gene Silencing Compatible vector' (HESC vector). AfeI can be used for generating linearized HESC vectors and a blunt-end PCR product of amiRNA sequence cloned into this

site by a single ligation reaction to complete amiRNA vector. amiRNAs can target genes that lack natural targets of endogenous miRNAs and can effectively turnoff one or more genes or alleles with high specificity (Tiwari et al. 2014; Eamens et al. 2014; Canto-Pastor et al. 2015). To create specific favorable features in crops, amiRNAs can be used for silencing the gene expression that regulates dominant agricultural properties. Artificial miRNA constructs were designed from endogenous rice miRNA precursor to target three different rice genes in Nipponbare (*japonica*) and IR64 (*indica*). The target specific gene suppression was achieved in both Nipponbare (*japonica*) and IR64 (*indica*) genotypes (Ossowski et al. 2008). Thus, amiRNAs could be considered one of the most appropriate strategies for generation of rice transgenic plants and improvement of crops for abiotic stress tolerance (Liu and Chen 2010).

Endogenous siRNAs are synthesized from long double stranded RNAs (dsRNAs) which is generated from miRNA-directed cleavage products of non-coding transcripts (known as trans-acting or transient siRNAs (tasiRNAs)), mRNAs encoded by natural cis-antisense target gene pairs (known as natural antisense transcript–derived siRNAs (nat-siRNAs)) and heterochromatin and DNA repeats (Mallory and Vaucheret 2006, Macovei et al. 2012). A group of seven nat-miRNAs (miR444, miR1433, miR1426, miR1425, miR160, miR166 and miR10) are reported in rice (Lu et al. 2008). The natural *cis*-antisense loci with analogous exon-intron arrangements could be one more source of miRNA genes (Lu et al. 2008). According to the natural precursor structures, ath-miR319a, ath-miR172a, ath-miR164b, ath-miR159a and osa-miR528 have been developed (Liu and Chen 2010). The overexpression of OsmiR397 has enhanced rice yield by enhancing grain size and promoting panicle branching (Zhang et al. 2013a, b). The miRNA-mediated approaches provide potent tools for the investigation of gene functions and expand the applicability in producing stress tolerant plants.

6 Conclusion and Future Perspective

Endogenous microRNA expression is regulated both by the development and environment in a spatio-temporal manner. Most plant miRNAs regulate plant growth and development because of target gene nature. The plant growth and development miRNAs—miR156, miR159, miR160, miR164, miR165/166, miR167, miR169, miR170/171, miR172, miR319, miR390, miR393 and miR396—also alter during abiotic stress conditions, suggestive of their role in plant stress responses. Environmental abiotic stress such as drought, cold, heat, salinity, oxidative stress and heavy metal pollution are crucial factors that influence negatively rice growth and productivity. Weak growth rate is a characteristic feature of plants under stress so that the metabolism is lowered and resources get diverted for adaptation (Sunkar 2010). Function of development associated miRNAs in stress responses is still not fully understood, but the inferred evidence suggests that the reduced growth rate during stress seems to be dependent on several miRNAs such as miR160, miR167,

miR390-TasiRNAs and miR393 which regulate auxin perception/signaling and other miRNAs which regulate various transcription factors. So, suggesting that the growth modifications during stress are mediated through miRNAs that control auxin signaling and transcription factors of development. In the past few decades, a great number of stress-regulated genes have been identified. A different level of gene regulation has been uncovered with the knowledge on the role of miRNAs, siRNAs and amiRNAs as components of stress response. Better understanding of the pathways of miRNA-based gene regulation will not only throw light on the molecular mechanisms of plant adaptation to stress, but will also facilitate in formulating approaches for improvement of crop survival and produce under abiotic stress conditions by using biotechnological approaches. The knowledge of the functions of abiotic stress target gene can be used to manipulate the target gene/miRNA itself for improving tolerance to different plant stresses. If the target gene is a negative regulator of stress tolerance, then the miRNA up-regulation can be employed to switch off the negative regulator. If the target gene is a positive regulator of stress tolerance, then miRNA itself can be silenced, but if miRNA has multiple members, then miRNA-resistant form over expression can be designed to surmount the negative regulation of miRNA. The miRNAs-regulated stress responsive genes can help to comprehend the way plants live under acute environmental conditions which can be used for fine tuning the gene expression to produce stress tolerant plants. In spite of many reports introduced in this chapter, there is still lacuna to our understanding of the role of rice miRNAs in various abiotic stress responses. The microRNA machinery pathway in abiotic stress response in rice will become clearer in the coming years if adequate efforts will be directed to this field which is imperative for improving abiotic stress tolerance in rice as well as in other crop plants. It is recommended that in order to overcome abiotic stress conditions in agriculture, the miRNA-based approach is an efficient tool for improving and managing crops in stressful ecological conditions. The most common approaches for augmentation of stress tolerance in rice involve utilization of amiRNAs (artificial miRNA), siRNA-directed DNA silencing and tasiRNA (Macovei et al. 2012; Zhang and Wang 2015) against stress responsive genes.

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Databases: A Weapon from the Arsenal of Bioinformatics for Plant Abiotic Stress Research

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1 Introduction

From early life, plants are considered as a pre-eminent component of every possible food chain (Raubenheimer et al. 2009). Next, to the food, these plants have provided shelter, fibers, timber, oils, drugs, spices, pharmaceuticals, nutraceuticals, etc. to the humans (Cseke et al. 2016; Van Wyk and Wink 2017). However, since the modern humans have overexploited every possible natural resource as well as degraded soil (Brun et al. 2016; Oldeman et al. 2017), this overexploitation combined with perpetually increasing human population has directly overburdened food production (Atkins and Bowler 2016; Barlett 2016; Conijn et al. 2018). There is no doubt that humans have accelerated their food production worldwide in the last few centuries (Grafton et al. 2015; Pilcher 2017). However, the population has increased at an alarming rate. This is even supported by the reports of Population Reference Bureau, 2016 (https://www.prb.org/data). Furthermore, the addition of multiple pollutants, deforestation, industrialization, huge depletion of water resources, desertification, and global warming have worsened the whole scenario (http://www.preservearticles.com). Concomitantly, all these factors have increased the incidence, unpredictability, duration, and severity of environmental stresses to a new level (Debnath et al. 2011; Atkinson and Urwin 2012; Bellard et al. 2012; Vaughan et al. 2018). Prior to biotic stresses, abiotic stresses are the most intimidating stresses which limit the distribution of sessile plants (Mittler 2006; Debnath et al. 2011; Atkinson and Urwin 2012; Gupta et al. 2013; Ramegowda and Senthil-Kumar 2015; dos Reis et al. 2018; Vaughan et al. 2018). These stresses adversely affect plant's growth, development, photosynthesis rate, N-metabolism, nutrient uptake, fruit

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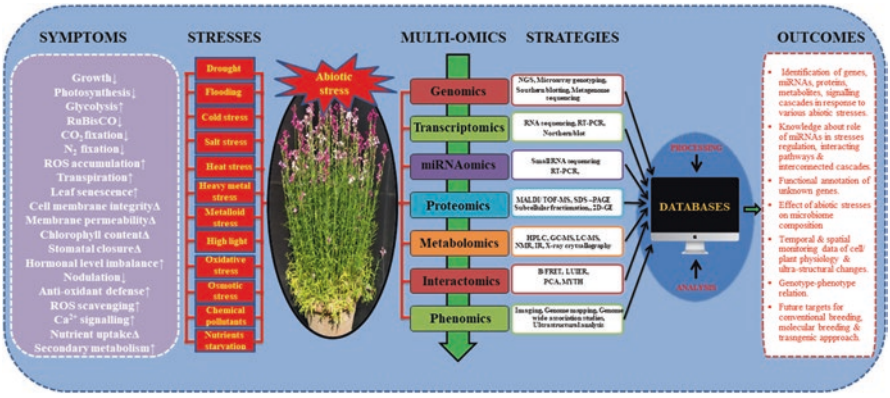

Fig. 1 Relationship among abiotic stresses, omics technologies, and bioinformatics databases

development, survival, and overall productivity (Valentine et al. 2011; Gupta et al. 2013; Ramegowda and Senthil-Kumar 2015; Choudhury et al. 2017; Pandey et al. 2017; dos Reis et al. 2018; Magaña Ugarte et al. 2019) (Fig. 1). Hence, these stresses act as key determinants on achieving the required food production.

These environmental stresses include drought, flooding, heavy metal stress, heat stress, high salinity, dehydration, low temperature, and many more (Fig. 1). It is highly important to understand the responses of various crops, medicinal plants, and non-model plant species to ultimately improve crop productivity under stressful conditions (Pandey et al. 2017). These plants respond, adapt, and acclimatize to these abiotic stresses by making suitable changes at the morphological, molecular, cellular, metabolic, and physiological levels (Grover et al. 1998; Debnath et al. 2011; Gupta et al. 2013; Zhu 2016; Mosa et al. 2017; Pandey et al. 2017; Zandalinas et al. 2018; Magaña et al. 2019). In the twentieth century, it was often difficult to understand the diversity and complexity of the plant's responses to the plethora of abiotic stresses (Debnath et al. 2011). However, nowadays, this has been made possible due to the technical advances in genomics and post-genomic eras (Hamilton and Robin Buell 2012; El-Metwally et al. 2014; Hu et al. 2015; Speed and Balding 2015; Abdurakhmonov 2016; Altpeter et al. 2016; Singh et al. 2018b). These advancements include better nucleic acid−/peptide-sequencing platforms, the decline in rates of sequencing, mass spectrometry technology, and metabolite profiling; completion of several genome sequencing projects, and development of new, accurate analytical as well as statistical methodologies and biotechnological tools (El-Metwally et al. 2014; Reuter et al. 2015; Jain et al. 2016; Jorge et al. 2016; Tyanova et al. 2016; Bauer et al. 2017; Mo et al. 2017; Alaux et al. 2018; Bowne et al. 2018; McCombie et al. 2018; Muthuramalingam et al. 2019; Shang and Huang 2019). As a result, molecular and system biology both complement each other (Moreno-Risueno et al. 2010; Chawla et al. 2011; Debnath et al. 2011; Kudoh 2016; Lavarenne et al. 2018). Furthermore, it is very easy to investigate various intricate topics of plant stress research which were used to be considered unapproachable in

the last century (Tardieu and Tuberosa 2010; Urano et al. 2010; Chawla et al. 2011; Zhu 2016; Choudhury et al. 2017; Hossain et al. 2018; Chérel and Gaillard 2019; Stone 2019).

Furthermore, other comprehensive branches of "omics technologies" such as transcriptomics, miRNAomics, ionomics, proteomics, prime-omics, metabolomics, metagenomics, phenomics, and many more came into the existence in the post-genomic era (Debnath et al. 2011; Mosa et al. 2017; Di Silvestre et al. 2018; Lavarenne et al. 2018; Parida et al. 2018; Saeed 2018; Fahimirad and Ghorbanpour 2019). Along with genomics, these other branches attempt to increase our knowledge regarding the topology of complex regulatory networks, cascades that are activated upon the perception of stress, and change in microbiota profile associated with stress response and adaptation (Chawla et al. 2011; Gupta et al. 2013; Arivaradarajan and Misra 2019). This is evident from the fact that the keywords "omics" and "plant abiotic stress" fetched around 17,600 publications in Google Scholar website (https://scholar.google.co.in) in 2019.

Furthermore, there has been explosive growth in the amount of "biological information" due to the emergence of various "omics technologies" (Vassilev et al. 2006; Fahimirad and Ghorbanpour 2019). This growth has resulted in a parallel emergence of bioinformatic tools, methods, and databases for the storage, visualization, prediction, integration, modeling, analysis, and management of this biological data (El-Metwally et al. 2014; Helmy et al. 2016; Ghosh and Mehta 2017; Liu et al. 2017; Chen et al. 2018; Hu et al. 2018; International Arabidopsis Informatics Consortium et al. 2019; Lo et al. 2019). These databases enable the sustainable exchange of knowledge between the researchers (Smalter et al. 2013; Helmy et al. 2016; Ghosh and Mehta 2017; Liu et al. 2017; Zhang et al. 2018a). With the birth of "big data" in the field of computation and analysis, the importance of these databases has increased to a higher level (Abdurakhmonov 2016; Popescu et al. 2016; Ghosh and Mehta 2017; Kushwaha et al. 2017; Burks 2018; Members BIG Data Center 2019). It will be depicted in the number of integrative databases and sophisticated algorithms in the near future. As a result, we have highlighted the highly updated and curated omics databases which help in improving our crops under a plethora of abiotic stresses.

2 Insights Into the Databases

The biological databases are considered an integral component of the bioinformatics revolution (Baxevanis and Bateman 2015). They are composed of both software and computer hardware and contain biological data from a broad spectrum of "omics technologies" organized in a highly uniform, curated, and efficient manner (Stein 2003; Kushwaha et al. 2017). This is evident from the fact that the keywords "biological databases" fetched around 16,100 publications in Google Scholar (2019). These biological databases enable the users for easy retrieval of biological information based on their query. Additionally, these databases serve for knowledge

Table 1 Summary of the NAR database paper

Sl. No.	Types of databases	Number of databases
1	Nucleotide sequence databases	80
2	Transcriptional regulatory sites and transcription factors	181
3	Protein sequence database	214
4	Structure databases	172
5	Metabolic and signaling pathways	168
6	Gene expression and microarray-based databases	60
7	Proteomics resources/platforms	28
8	Other molecular biology databases	20
9	Organelle databases	20
10	Plant-specific databases	131

The data have been adapted from Oxford academic (Nucleic Acid Research (https://academic.oup.com/nar). Accessed on March 3, 2019

discovery (Kushwaha et al. 2017). These databases are classified as the primary and secondary databases based on information, annotation, and analysis of DNA/RNA/protein/metabolites sequences, structures, and expression profiles (Rao et al. 2008; Kushwaha et al. 2017; Doolittle 2018). Furthermore, they can be divided into different categories based on the type of stored information (Table 1).

3 Genomic Databases

Based on advances in sequencing technologies, plant biology is in the midst of a revolution (Salgotra et al. 2014; Zargar and Rai 2017; Doolittle 2018; Scheben et al. 2018). This tremendous flood of genomic sequences has paved the way for efficient web-based data interfaces for accession as well as submission of information (Kushwaha et al. 2017; Scheben et al. 2018). Furthermore, this is even boosted by the development of algorithms for comparison of multiple sequences and identification of homology in a reliable manner (Romeuf et al. 2010; Prabha et al. 2011; Lee et al. 2012). This has a paramount impact on crop improvement (Chen et al. 2017; Upadhyay et al. 2017; Kumar and Shanker 2018; Das 2019). One of the major indispensables for a boost in crop improvement is the application of computational biology which helps in structuring the NGS data in genomic databases (Kersey 2019).

These genomic databases are archives of genomic data (nucleic acid sequences, protein sequence variants, polymorphic haplotypes, and pathogenic mutations) arranged in a systematic way to be publicly searchable (Kushwaha et al. 2017). It includes gene function, gene sequences, gene structure, attributes, textual descriptions, localization, ontology classifications, mutation effects, and citations. These databases range from generic databases to the species-specific databases (Benson et al. 2004; Leinonen et al. 2010; Lai et al. 2012; Bolser et al. 2016;

Kodama et al. 2017; Doolittle 2018). The list of the prevalent databases used by researchers is enlisted in Table 2.

Currently, GenBank, European Nucleotide Archive (ENA), and DNA Databank of Japan (DDBJ) are the most prevalent generic databases (Benson et al. 2004). GenBank is a publicly available database for available nucleotide sequences along with their protein translations. It is a part of the International Nucleotide Sequence Database Collaboration (INSDC), which embraces the DDBJ (Kodama et al. 2017), ENA (Leinonen et al. 2010) and GenBank at NCBI. Furthermore, Ensembl Plants is a generic database for plants only and present genome-scale information for the perpetually increasing sequenced plant species (Bolser et al. 2016). However, search in these databases is excessively arduous and they provide only information about the genomic sequence and their translated protein.

Some species-specific databases allow comparison of the genome in a particular family like Cucurbit Genomics Database (CuGenDB) (Zheng et al. 2018), Genome Database for Rosaceae (GDR) (Jung et al. 2004), and LegumeIP (Li et al. 2011). While there are many single-species-specific genome databases like TAIR (Lamesch et al. 2011) for *Arabidopsis* sp., MaizeGDB (Schaeffer et al. 2011) for *Zea mays*, and Coffee Genome hub (Dereeper et al. 2014) for *Coffea canephora,* Table 3 enlists the highly curated and updated genomic databases.

Variation in inter-specific genome size is common and thus acts as a taxonomic attribute (Vít et al. 2016). Genome size in Asteraceae Database (GSAD) aspires to provide the genome size data of Asteraceae family members in a user-friendly manner (Garnatje et al. 2011). Furthermore, the investigation of SNPs, functional annotation of genes, and their role in different pathways turned out to be fundamental for an exceptional comprehension of the hereditary premise of plants. Gramene database helps in querying and analyzing functional variations among the plant genome of various important crops. It provides both GUI and programmatic access using Application Programming Interfaces (API) (Tello-Ruiz et al. 2017). Additionally, Phytozome knowledgebase (http://www.phytozome.net) provides data about the sequence, gene structure, and gene family (Goodstein et al. 2011). Other databases which investigate comparative genomics and evolution among the plant genomes are Plaza 4.0 (Proost et al. 2009) and PIECE 2.0 (Wang et al. 2012) (Table 4). Together in a view, these databases revealed several surprising messages about the "pre-history" of crop evolution.

4 Databases for Transcriptomics

Unlike genomics, transcriptomic techniques determine which genes are expressing in a cell, organ, particular growth stage or stress conditions (El-Metwally et al. 2014; Shen et al. 2018). It quantifies whole mRNAs, small RNAs, and noncoding RNAs produced. In this manner, the study of transcriptomics for plants comprehends the profile of differentially regulated genes amid the stress conditions and thus aids in crop improvement (Leisner et al. 2017; Shen et al. 2018; Kreszies et al. 2019).

Table 2 Widely used common databases used by plant researchers

Sl. No	Name	Purpose	URL	Citations	References	Last updated
1.	PDB (protein data Bank)	International repository for three-dimensional structures of proteins	https://www.rcsb.org/	730	Abola et al. (1984)	2019
2.	GenBank	An annotated collection of all publicly available DNA sequences	https://www.ncbi.nlm.nih.gov/genbank/	242	Bilofsky et al. (1986)	Feb 2019
3.	DDBJ (DNA DataBank of Japan)	Comprehensive database for nucleotide sequences	http://www.ddbj.nig.ac.jp	54	Tateno and Gojobori (1997)	2018
4.	NCBI RefSeq	Publicly available curated database for genomics, transcripts, and proteins	http://www.ncbi.nlm.nih.gov/RefSeq/	1455	Pruitt et al. (2005)	Jan 2019
5.	UniprotKB/ Swiss-prot	Comprehensive database for protein sequences and functional information	http://www.uniprot.org/	415	Boutet et al. (2007)	Feb 2019
6.	European nucleotide archive	Nucleotide sequence repository developed in Europe	http://www.ebi.ac.uk/ena	290	Leinonen et al. (2010)	2018
7.	EnsemblPlants	Integrative resource for genome-scale information on plants	http://plants.ensembl.org	46	Bolser et al. (2016)	2018

Table 3 Commonly used genomic databases for plants

Sl. No.	Database	Purpose	Species	URL	Citations	Year	Last updated
1.	TAIR (the Arabidopsis information resource)	Database for genomic data of model organism	*Arabidopsis*	http://arabidopsis.org	446	Huala et al. (2001)	2019
2.	Gramene	Curated resource for genetic, genomic, and comparative genomics for the major crop species	58 reference plant genomes	www.gramene.org	236	Ware et al. (2002)	2019
3.	GDR	Publicly available database for genomics, genetics, and breeding data and data-mining tools for research in Rosaceae	14 species	http://www.rosaceae.org	106	Jung et al. (2004)	2018
4.	MaizeGDB	Central repository for maize sequence, stock, phenotype, genotypic and karyotypic variation, and chromosomal mapping	*Zea mays*	http://www.maizegdb.org/	191	Lawrence et al. (2004)	2019
5.	TropGeneDB	A comprehensive resource for genomic, genetic, and phenotypic information about tropical crops	10 species	http://tropgenedb.cirad.fr	33	Ruiz et al. (2004)	2018
6.	CR-EST (crop expressed sequence tag)	Comprehensive database for the sequence, classification, clustering, and annotation data of crop EST	*Hordeum vulgare, Pisum sativum, Solanum tuberosum, Triticum aestivum, Nicotiana tabacum, Petunia hybrida*	http://pgrc.ipk-gatersleben.de/cr-est/	50	Künne et al. (2005)	2016
7.	SGN (sol genomics network)	Clade-oriented database for Solanaceae species and their close relatives	Six completed genomes, six draft genomes	http://solgenomics.net/	428	Mueller et al. (2005)	2018
8.	ChromDB (the chromatin database)	Database for chromatin-associated proteins	–	http://www.chromdb.org	92	Gendler et al. (2007)	2014

(continued)

Table 3 (continued)

Sl. No.	Database	Purpose	Species	URL	Citations	Year	Last updated
9.	CuGenDB	Web resource for genome and expressed sequences of tag sequences, genetic maps, and transcriptome profiles for cucurbit species	12 species of Cucurbitaceae family	http://cucurbitgenomics.org	1	Zheng et al. (2018)	–
10.	GSAD (genome size in Asteraceae database)	Database for all genome size data for Asteraceae family	1219 species	http://www.asteraceaegenomesize.com/	24	Garnatje et al. (2011)	2018
11.	PGDD (plant gene duplication database)	Database to study homologous genes	47 plant species	http://chibba.agtec.uga.edu/duplication/	305	Lee et al. (2012)	2014
12.	P-MITE (plant miniature inverted-repeat transposable elements database)	Comprehensive web resource for MITEs prevalent in eukaryotic species including plants	41 plant species	http://pmite.hzau.edu.cn/django/mite/	64	Chen et al. (2013)	–
13.	Coffee genome hub	Publicly available database to access genomics and genetics data analysis tools to facilitate translational and applied research in coffee	*Coffea canephora*	http://coffee-genome.org/	36	Dereeper et al. (2014)	–
14.	CottonGen	Curated and integrated database for available genomic, genetic, and breeding data of cotton	*Gossypium*	http://www.cottongen.org	91	Yu et al. (2013)	2018
15.	MethBank	Database to integrate high-quality DNA methylomes across a variety of species	*Oryza sativa, Glycine max, Manihot esculenta, Phaseolus vulgaris, Solanum lycopersicum*	http://bigd.big.ac.cn/methbank	18	Zou et al. (2014)	2017
16.	PGSB PlantsDB (plant genome and systems biology)	Comprehensive database for the comparative analysis and visualization of plants genome data	13 plant genomes	http://pgsb.helmholtz-muenchen.de/plant/index.jsp	25	Spannagl et al. (2015)	–

Table 4 Databases for the study of evolution in plants

Sl. No.	Database	Purpose	Species	URL	Citations	References	Last updated
1.	Phytozome	A comparative and evolutionary hub for plant genome and gene family data and analysis	93 species	http://www.phytozome.net	1858	Goodstein et al. (2011)	2017
2.	Plaza 4.0	Web-accessible resource for comparative, and functional genomics	71 plant species	https://bioinformatics.psb.ugent.be/plaza	250	Proost et al. (2009)	2017
3.	LegumeIP	The database has large-scale genomics and transcriptomics data and provides integrative bioinformatics tools for the study of gene function and evolution in legumes	Six legume species and two outgroup species—*Arabidopsis thaliana* and poplar trichocarpa	http://plantgrn.noble.org/LegumeIP/	69	Li et al. (2011)	2015
4.	PIECE 2.0	A web-based resource that houses intron and exon information of plant genes and also provides insight into the evolution of gene structures in plant species	49 plant species	http://aegilops.wheat.ucdavis.edu/piece	42	Wang et al. (2012)	2016

Currently, there are many databases and online repositories available for the submission and retrieval of transcriptomic data generated by high-throughput technologies. One of the earliest curated and highly updated databases is NCBI GEO which stores microarray data, RNA sequences, and functional annotation (Barrett et al. 2006). The GOA database provides gene ontology annotations to the RNAs as well as proteins from various resources including UniProtKB, Complex Portal, and RNAcentral based on Gene Ontology Consortium (Camon et al. 2003). In similar to the NCBI GEO, Shen et al. (2005) generated the BarleyBase which provides information about microarrays, gene chips, and tool which compares the expression profiles of various homologous genes across the cereals. In addition, Sato and group (2010) generated a database which provides full insights about the entire gene expression profile throughout the life course of rice. In accordance, Makita et al. (2014) also published a database solely devoted to sorghum in the year 2014. One of the important databases is EXPath, an online database that collects tissue-specific and organ-specific microarray expression data for multiple model plants based on various conditions including abiotic stresses. PlantExpress is a transcriptome database for *Oryza* and *Arabidopsis* which provides a platform for gene network analysis with the publicly available microarray datasets of these two. It allows single-species analysis as well as cross-species analysis (Kudo et al. 2017) (Table 5).

In addition to the earlier discussion, there are many other databases available which accounts for the RNA coexpression (Obayashi et al. 2006), plant–pathogen interaction (Wise et al. 2006), phosphorylation sites (Heazlewood et al. 2007), RNA editing events (Picardi et al. 2006; Yura et al. 2009; Chen et al. 2011; Li et al. 2018), and transcription factors (Jin et al. 2013; Bonthala and Gajula 2016; Singh et al. 2017). All the major databases comprising about transcription factors are enlisted in Table 6.

5 miRNAomics-Based Databases

The microRNAomics is a specialized class of "omics technologies" which study the expression profile of small, noncoding miRNAs using high-throughput techniques. These miRNAs act as endogenous post-transcriptional regulators with roles in signaling, development, and environmental responses (Zhang 2015; Hivrale et al. 2016; Sharma et al. 2017; Mishra et al. 2018). Compared to genomic databases, there are very fewer miRNAs-specific databases reported for plant species; however, the number of databases dedicated solely to plant miRNAs is increasing. The list of miRNAs databases includes PMRD (Zhang et al. 2009), TAPIR (Bonnet et al. 2010), mirEX 2.0 (Zielezinski et al. 2015), PmiRExAt (Gurjar et al. 2016), and ARMOUR (Mishra et al. 2018). All the major plant miRNA-based databases are listed in Table 7.

Table 5 Widely used databases for the study of plant transcriptome

Sl. No.	Database	Purpose	Species	URL	Citations	References	Last updated
1.	NCBI GEO	International repository for high-throughput microarray and next-generation sequence functional genomic datasets	–	http://www.ncbi.nlm.nih.gov/geo/	7000	Barrett et al. (2006)	2019
2.	GOA (gene ontology annotation)	Database to provide evidence-based gene ontology annotations to proteins in the UniProtKB	–	http://www.ebi.ac.uk/GOA	162	Camon et al. (2003)	2019
3.	BarleyBase	Web resource for plant microarrays with integrated tools for data visualization and statistical analysis	13 plant species	www.barleybase.org	108	Shen et al. (2005)	2011
4.	ATTED-II (*Arabidopsis thaliana* trans-factor and cis-element prediction database)	Coexpression database for plant species	Seven dicot and two monocot species	http://atted.jp/top_download.shtml	377	Obayashi et al. (2006)	2018
5.	PLEXdb	Expression profiling database for plants and plant pathogens	14 plant species	http://www.plexdb.org	6	Wise et al. (2006)	2011
6.	REDIdb (RNA editing database in plants)	A web-accessible database that annotates RNA editing events	281 species	http://srv00.recas.ba.infn.it/redidb/index.html	40	Picardi et al. (2006)	2018
7.	PhosPhAt (the *Arabidopsis thaliana* phosphorylation site database)	Web resource for phosphorylation sites	*Arabidopsis thaliana*	http://phosphat.mpimp-golm.mpg.de	279	Heazlewood et al. (2007)	2012
8.	RESPOS (RNA editing site on protein 3D structure)	Database for RNA editing sites of plant organelles on protein 3D structures	–	http://cib.cf.ocha.ac.jp/RNAEDITING/	22	Yura et al. (2009)	Aug 2009

(continued)

Table 5 (continued)

Sl. No.	Database	Purpose	Species	URL	Citations	References	Last updated
9.	RiceXPro	Gene expression database to provide an overview of the translational changes throughout the growth of the rice plant in the field	Japonica rice	http://ricexpro.dna.affrc.go.jp/	171	Sato et al. (2010)	Sep 2012
10.	PlantNATsDB	A comprehensive resource of natural antisense signaling in the plant kingdom	69 plant species	http://bis.zju.edu.cn/pnatdb/	57	Chen et al. (2011)	2014
11.	RiceFREND	Database to identify gene modules with similar expression profiles	*Oryza sativa*	http://ricefrend.dna.affrc.go.jp/	66	Sato et al. (2012)	2012
12.	MOROKOSHI	A transcriptome database for sorghum	*Sorghum bicolor*	http://sorghum.riken.jp	42	Makita et al. (2014)	–
13.	EXPath	Web resource that collects and utilizes expression profiles derived from microarray under various conditions to infer metabolic pathways	*Arabidopsis thaliana, Oryza sativa, Glycine max, Medicago truncatula, Zea mays, Solanum lycopersicum*	http://expath.itps.ncku.edu.tw	11	Chien et al. (2015)	2017
14.	PlantExpress	Database for gene expression network analysis with the public microarray data	*Oryza sativa* and *Arabidopsis thaliana*	http://plantomics.mind.meiji.ac.jp/PlantExpress/	3	Kudo et al. (2017)	2017
15.	PED (plant editosome database)	Database of RNA editosome in plants dedicated to curation, integration, and standardization of plant editosome data	1621 species	http://bigd.big.ac.cn/ped	–	Li et al. (2018)	2019

Table 6 Transcription factor databases for plants

Sl. No	Database	Purpose	Species	URL	Citations	References	Last Updated.
1.	AGRIS	A comprehensive resource for gene regulatory studies in Arabidopsis thaliana	*Arabidopsis*	http://arabidopsis.med.ohio-state.edu/	386	Davuluri et al. (2003)	2016
2.	JASPAR	Database of curated, nonredundant TF-binding profiles	–	http://jaspar.genereg.net/	1536	Sandelin et al. (2004)	2018
3.	RARTF (RIKEN *Arabidopsis* transcription factor database)	Web resource which provides information on TFs, their functional motif, full-length cDNAs, pre-mRNA splicing events, and ac/ds transposon-tagged mutants	*Arabidopsis thaliana*	http://rarge.gsc.riken.jp/rartf/	143	Iida et al. (2005)	–
4.	PlnTFDB	The database which provides putatively complete sets of TFs and other TRs in plant species	19 species	http://plntfdb.bio.uni-potsdam.de/v3.0/	313	Riaño-Pachón et al. (2007)	2009
5	DATFAP (database of transcription factors with alignments and primers)	A user-friendly database which provides information about TFs as well as primers designed for real-time PCR	13 plant species	http://cgi-www.daimi.au.dk/cgi-chili/datfap/frontdoor.py	16	Fredslund (2008)	–
6.	GRASSIUS (grass regulatory information services)	Web resource for grasses which provides a resource to relate control of gene expression with their agronomic traits	*Zea mays, Sorghum bicolor, Saccharum spp., and Oryza sativa*	www.grassius.org	185	Yilmaz et al. (2009)	2019
7.	STFIDB (*Arabidopsis* stress-responsive transcription factor database)	A comprehensive collection of biotic and abiotic stress-responsive genes with options to identify probable TFBSs in their promoter	*Arabidopsis thaliana and Oryza sativa*	http://caps.ncbs.res.in/stifdb2/	68	Shameer et al. (2009)	2012
8.	wDBTF (database of wheat transcription factor)	Database of TF sequences of wheat based on homology with rice TFs	*Triticum aestivum*	http://wwwappli.nantes.inra.fr:8180/wDBFT/	25	Romeuf et al. (2010)	–

(continued)

Table 6 (continued)

Sl. No	Database	Purpose	Species	URL	Citations	References	Last Updated.
9.	PlantsTFDB 4.0	Web resource for the functional and evolutionary study of plant transcription factors	165 species	http://planttfdb.cbi.pku.edu.cn/	476	Jin et al. (2013)	2017
10.	PvTFDB	Web-accessible TFs database for *P. vulgaris*	*Phaseolus vulgaris*	http://www.multiomics.in/PvTFDB/	5	Bonthala and Gajula (2016)	–
11.	PpTFDB	Web resource which provides TFs of pigeon pea	*Cajanus cajan* L.	http://14.139.229.199/PpTFDB/Home.aspx	3	Singh et al. (2017)	–

Table 7 Databases for the study of plant miRNAomics

Sl. No.	Database	Purpose	Species	URL	Citations	Year	Last updated
1.	PMRD (plant microRNA database)	Comprehensive database for information on plant miRNAs	121 plant species	http://bioinformatics.cau.edu.cn/PMRD/	297	Zhang et al. (2009)	2013
2.	TAPIR	Publicly accessible web server, designed for the prediction of plant microRNA targets	–	http://bioinformatics.psb.ugent.be/webtools/tapir	159	Bonnet et al. (2010)	–
3.	miRNEST	The database which miRNAs from high-throughput predictions as well as from external databases	522 plant and animal species and 22 viruses	http://rhesus.amu.edu.pl/mirnest/copy/	39	Szczesniak et al. (2011)	2014
4.	PsRobot	A web-based easy-to-use tool dedicated to the identification of smRNAs with stem-loop shaped precursors and their target genes/transcripts	36 plant species	http://omicslab.genetics.ac.cn/psRobot/	146	Wu et al. (2012)	2013
5.	mirEX 2.0	A comprehensive platform for the exploration of microRNA expression data based on quantitative RTpcr and NGS experiments	*Arabidopsis thaliana, Hordeum vulgare,* and *Pellia endiviifolia*	http://www.combio.pl/mirex	25	Zielezinski et al. (2015)	2018
6.	PmiRExAt (plant miRNA expression atlas)	A database which caters plant miRNA expression atlas	Wheat, rice, maize, and *Arabidopsis thaliana*	http://pmirexat.nabi.res.in	10	Gurjar et al. (2016)	2016
7.	ARMOUR (A rice miRNA:mRNA interaction resource)	Publicly available resource developed for providing information on rice miRNA:mRNA interaction	*Oryza sativa*	http://armour.icgeb.trieste.it/login	–	Mishra et al. (2018)	2018

6 Protein Databases

Both genomics and transcriptomics-based studies have increased the information about genes upregulation under abiotic conditions (Zhang et al. 2017a; Wang et al. 2018). However, proteomics-based studies are only a good indicator of predicting the protein's expression level. Using high-performance separation and high-resolution mass spectrometry, we can assess the abundance, protein–protein interactions, PTMs, and cellular localization for a whole set of proteins encoded in spatial and temporal bases (Luan et al. 2018). Abundance in protein levels during abiotic stresses have been already studied in various plants including *Arabidopsis thaliana*, rice, wheat, cotton, soybean, sea buckthorn, and pea.

The publications related to proteomics studies have increased geometrically in the last decade due to the technical advancements (Kosová et al. 2011; Agrawal et al. 2012; Ghosh and Xu 2014; Hu et al. 2015; Tyanova et al. 2016; Zhang et al. 2017a; Kosová et al. 2018; Luan et al. 2018; Wang et al. 2018). The similar increase is reflected in the number of databases for proteins (Consortium 2014; Szklarczyk and Jensen 2015; Chen et al. 2017; Letunic and Bork 2017; Doolittle 2018).

One of the oldest databases devoted to proteomics is AtNoPDB developed by Brown and group (2005). It is a publicly accessible resource which provides information about the proteins identified from *Arabidopsis* cell cultures. The similar type of databases is PPDB (Sun et al. 2008), SALAD (Mihara et al. 2009), MASCP gator (Joshi et al. 2011), and SUBA4 (Hooper et al. 2016). Recently, Hooper and co-workers published an article regarding the curated database which contains all the datasets about subcellular proteins present in *Arabidopsis thaliana.*

In addition to the above databases, there are other databases which contain knowledge regarding protein phosphorylation sites (Gao et al. 2008), molecular interaction (Orchard et al. 2012) and protein–protein interaction (Szklarczyk et al. 2014). The list of the majorly used protein databases is presented in Table 8.

7 Metabolomics

From its first description in the literature of the 1950s, it is considered as one of the earliest "omics," employed toward understanding the holistic picture of metabolites profile under a genetic modification or environmental stimulus (Fiehn 2002; Weckwerth 2003; Dunn and Ellis 2005; Johnson et al. 2016). The term metabolomics originates from metabolites, which is the end product of gene expression as well as part of the regulatory system (Weckwerth 2003; Heyman and Dubery 2016; Bowne et al. 2018). Connecting the above-discussed "omics technologies" with metabolomics (or metabonomics) allow us to deduce the biochemical machinery of plants and open new pathways for metabolic engineering (Hong et al. 2016; Jorge et al. 2016; Bowne et al. 2018; Kera et al. 2018; Kumar and Shanker 2018; Fan et al. 2019). In the last decade, there were many databases reported in the literature

Table 8 Databases for the study of plant proteomics

Sl. No.	Database	Purpose	Species	URL	Citations	Year	Last updated
1.	AtNoPDB	A publicly accessible resource for information on proteins identified in the proteomic analysis of nucleoli isolated from *Arabidopsis* cell culture	*Arabidopsis thaliana*	http://bioinf.scri.sari.ac.uk/cgi-bin/atnopdb/home	77	Brown et al. (2005)	–
2.	PPDB (plant proteomic database)	Integrated resource for experimentally identified proteins	*Arabidopsis thaliana and Zea mays*	http://ppdb.tc.cornell.edu	304	Sun et al. (2008)	–
3.	P3DB	An online repository for plant protein phosphorylation site data	Nine plant species	http://www.p3db.org/	48	Gao et al. (2008)	2014
4.	SALAD (surveyed conserved motif alignment diagram and the associating dendrogram)	Comparative genomics from plant-based-genome datasets	10 species	http://salad.dna.affrc.go.jp/salad/	52	Mihara et al. (2009)	2012
5.	MASCP gator	Online portal for proteomic data produced by the community and unites a large collection of specialized resources to a single portal	*Arabidopsis thaliana*	http://gator.masc-proteomics.org/	83	Joshi et al. (2011)	–
6.	OryzaPG-DB	Proteome database based on shotgun proteogenomics	*Oryza sativa*	https://github.com/MoHelmy/oryza-PG/	48	Helmy et al. (2011)	2012
7.	IMEx	Open-source, open data molecular interaction database populated by data either curated from the literature or from direct data depositions	–	http://www.imexconsortium.org	267	Orchard et al. (2012)	–
8.	STRING v10	The database provides information about protein–protein interactions	–	http://string-db.org	3630	Szklarczyk et al. (2014)	–
9.	SUBA4 (subcellular localization database for Arabidopsis protein)	The manually curated database has published datasets of large-scale subcellular proteomics	*Arabidopsis thaliana*	http://suba.live	44	Hooper et al. (2016)	–

including for metabolomics (Table 1). However, there are very fewer databases devoted to plants in comparison to humans, mouse, and other animals. Some of the curated and popular metabolomics databases are enlisted in Table 9.

8 System Biology Databases

In plants, stress responses start within minutes of stress perception and remain until the very end. During this, many signaling cascades are activated to counter the ill effects of stress; however, it is very challenging to deduce the complex biological system for various stress responses. With the progression in computational biology and advancements in other omics techniques, it has become a little easier to understand these pathways as compared to the last two decades (Cramer et al. 2011; Gupta et al. 2013; Winter and Krömer 2013; Yoshida et al. 2015; Bagati et al. 2018). Nowadays, system biology helps in establishing a correlation between genotype, phenotype, and metabolic pathways involved (Fiehn 2002; Winter and Krömer 2013; Amâncio et al. 2017; Bowne et al. 2018). As a result, there have been a series of databases available for general public including AraCyc (Mueller et al. 2003), Arabidopsis Reactome (Tsesmetzis et al. 2008), PlaNet (Mutwil et al. 2011), AraNet v2 (Lee et al. 2014), Plant Reactome (Naithani et al. 2016), and latest TriForC (Miettinen et al. 2017) (Table 10).

9 Phenomics

Phenomics is a transdisciplinary area for the systematic study of phenotypes (physical and biochemical traits) (Schilling et al. 1999; Kumar et al. 2015) using automated high-throughput and imaging technologies. These phenotypes are affected by development, mutations, and environmental conditions (Furbank and Tester 2011; Kumar et al. 2015; Wani 2018). Due to the advances in high-throughput technologies and bioinformatics, plant phenotyping has become cheap and powerful (Singh et al. 2018a). As a result, we have shifted toward establishing the relation between plant stress responses and plant phenotyping as a tool for improving stress tolerance (Singh et al. 2018a; Mir et al. 2019). Therefore, these advances have paved the way for the databases solely dedicated to phenomics such as RARGE II (Sakurai et al. 2005), AraPheno (Seren et al. 2016), and Planteome (Cooper et al. 2017) (Table 11).

10 Abiotic Stress-Specific Databases

Furthermore, the specialized subclass of databases has been developed to accommodate the comprehensive data for abiotic stress responses (Table 12). These databases include a list of ESTs, genes, cDNA sequences, snoRNAs, smRNAs, miRNAs,

Table 9 Computational resources for the study of metabolomics in plants

Sl. No.	Database	Purpose	URL	Citations	Year	Last updated
1.	ChEBI	Freely available dictionary of molecular entities focused on a small chemical compound	http://www.ebi.ac.uk/chebi/	812	Degtyarenko et al. (2007)	2016
2.	PRIMe	Website to support research and analysis ranging from metabolomics to transcriptomics	http://prime.psc.riken.jp/	188	Akiyama et al. (2008)	2013
3.	AtMetExpress development	Atlas for information about phytochemicals accumulated during the development of model plant *A. thaliana*	http://prime.psc.riken.jp/lcms/AtMetExpress/	144	Matsuda et al. (2010)	Aug 2011
4.	ChemSpider	The free, online chemical database offering access to various properties of almost 25 million unique chemical compounds	http://www.chemspider.com/	455	Pence and Williams (2010)	–
5.	KNApSAcK	Database to describe the relationship between species and their metabolites	http://kanaya.naist.jp/KNApSAcK_Family/	230	Afendi et al. (2011)	–
6.	AtIPD (*Arabidopsis thaliana* Isoprenoid pathway database)	A manually curated database of isoprenoid pathways models and genes	http://www.atipd.ethz.ch	25	Vranova et al. (2011)	–
7.	PMDB (plant Metabolome database)	Structurally and functionally annotated database of metabolites present in plants	http://scbt.sastra.edu/pmdb/	10	Udayakumar et al. (2012)	–
8.	ARALIP (*Arabidopsis* acyl-lipid metabolism)	Comprehensive database for phenotypes, pathways, and enzymes associated with the plant acyl-lipid metabolism mutants	http://aralip.plantbiology.msu.edu	14	McGlew et al. (2015)	2015
9.	PubChem	A public repository for information on chemical substances and their biological activities	https://pubchem.ncbi.nlm.nih.gov	2	Kim et al. (2018)	2019

Table 10 Widely used databases for systems biology in plants

Sl. No.	Database	Purpose	Species	URL	Citations	Year	Last updated
1.	AraCyc	Database for biochemical pathways in *Arabidopsis thaliana*	*Arabidopsis thaliana*	http://www.arabidopsis.org	408	Mueller et al. (2003)	2019
2.	Arabidopsis Reactome	Curated knowledgebase of plant biological pathways	12 species	www.arabidopsisreactome.org	58	Tsesmetzis et al. (2008)	2009
3.	KaPPA-view 4 (Kazusa plant pathway viewer)	Comprehensive database for gene-to-gene and/or metabolite-to-metabolite relationship as curves on a metabolic pathway map	–	http://kpv.kazusa.or.jp/kpv4/	148	Sakurai et al. (2010)	–
4.	PlaNet	Online web resource of whole-genome coexpression networks	Seven species	http://aranet.mpimp-golm.mpg.de	202	Mutwil et al. (2011)	–
5.	FunCoup 3.0	Database for functional couplings between genes and gene networks	*Arabidopsis thaliana, Oryza sativa*	http://FunCoup.sbc.su.se	85	Schmitt et al. (2013)	2017
6.	KEGG (Kyoto encyclopedia of genes and genomes)	Knowledgebase to link genomes to pathways	–	http://www.kegg.jp/	12,252	Kanehisa et al. (2013)	2019
7.	AraNet v2	Comprehensive database for cofunctional gene network for *A. thaliana*	*Arabidopsis thaliana*	http://www.inetbio.org/aranet	62	Lee et al. (2014)	2015
8.	RiceNet v2	Knowledgebase for a network of genes and cofunctional links	*Oryza sativa*	http://www.inetbio.org/ricenet	35	Lee et al. (2015)	–
9.	Plant Reactome	Free, open source for investigating and visualization of plant pathways	79 plant species	http://plantreactome.gramene.org/	22	Naithani et al. (2016)	2019
10.	SoyNet	Database of cofunctional networks for *G. max* and a companion web server for network-based functional predictions	*Glycine max*	www.inetbio.org/soynet	13	Kim et al. (2016)	–
11.	TriForC	Web-accessible database for accessing enzymes involved in triterpenes biosynthesis and their pathways	–	http://bioinformatics.psb.ugent.be/triforc/	3	Miettinen et al. (2017)	–

Table 11 Computational tools for the study of phenomics in plants

Sl. No.	Database	Purpose	Species	URL	Citations	References	Last updated
1.	RARGE II (Riken Arabidopsis genome Encyclopedia)	The freely accessible database which aims to effectively identify the function of genes	*Arabidopsis thaliana*	http://rarge-v2.psc.riken.jp/	81	Sakurai et al. (2005)	2013
2.	AraPheno (Arabidopsis thaliana phenotypes)	Database for population-scale phenotypes for *A. thaliana* inbred lines	*Arabidopsis thaliana*	https://arapheno.1001genomes.org	16	Seren et al. (2016)	–
3.	Planteome	Web-accessible resource to provide integrated knowledge on plant traits, phenotypes, and gene function and expression	95 taxa	http://www.planteome.org	20	Cooper et al. (2017)	2019

Table 12 List of databases related specifically to abiotic stresses

Sl. No.	Database	Purpose	Species	URL	Citations	Year	Last updated
1.	DRASTIC (database resource for the analysis of signal transduction in cells)	The relational database of plant ESTs and genes up- or downregulated in response to stress	73 plant species	http://www.drastic.org.uk/	2	Newton et al. (2002)	2018
2.	Plant snoRNA database	Comprehensive information on snoRNAs in plants	19 plant species	http://www.scri.sari.ac.uk/plant_snoRNA/	89	Brown et al. (2003)	2013
3.	CSRDB (cereal small RNAs database)	Web resource for large-scale datasets of smRNA sequences generated by pyrosequencing	*Zea mays* and *Oryza sativa*	http://sundarlab.ucdavis.edu/smrnas/	99	Johnson et al. (2006)	–
4	Plant stress gene database	It provides information about the genes involved in stress conditions in plants	11 plant species	http://ccbb.jnu.ac.in/stressgenes/frontpage.html	14	Prabha et al. (2011)	–
5.	ASRGDB (*Arabidopsis* stress-responsive gene database)	Literature-curated database for stress-responsive genes	*Arabidopsis thaliana*	http://srgdb.bicpu.edu.in/	24	Borkotoky et al. (2013)	–
6.	PASmiR	Literature-curated web-accessible database to provide characteristics of miRNA molecular recognition in different plant abiotic stresses	33 plant species	http://pcsb.ahau.edu.cn:8080/PASmiR	51	Zhang et al. (2013)	April 2015
7.	UniVIO (uniformed viewer for integrative Omics)	Database to display hormone metabolome and transcriptome data in a single formatted heat map	*Oryza sativa*	http://univio.psc.riken.jp/	24	Kudo et al. (2013)	2012
8.	PNRD (plant noncoding RNA database)	Comprehensive database for plant noncoding RNA	166 plant species	http://structuralbiology.cau.edu.cn/PNRD	83	Yi et al. (2014)	2016
9.	PSPDB (plant stress protein database)	A web-accessible resource that covers 2064 manually curated plant stress proteins	134 plant species	http://www.bioclues.org/pspdb/	12	Kumar et al. (2014)	2018

10.	DroughtDB	Web resource for identification, analysis, and characterization of genes involved in drought stress	*Arabidopsis thaliana and Oryza sativa*	http://pgsb.helmholtz-muenchen.de/droughtdb/	25	Alter et al. (2015)	–
11.	PceRBase (plant competing endogenous RNA database)	Comprehensive database for ceRNA target–target, and ceRNA target–mimic pairs	26 plant species	http://bis.zju.edu.cn/pcernadb/index.jsp	20	Yuan et al. (2016)	2018
12.	PlantCircNet	Database for providing plant circRNA-miRNA-gene regulatory networks	Eight model plants species	http://bis.zju.edu.cn/plantcircnet/index.php	10	Zhang et al. (2017b)	2017
13.	Stress2TF	Literature-curated web resource to document the relationship between transcription factors and stress	41 plant species	http://csgenomics.ahau.edu.cn/Stress2TF	1	Zhang et al. (2018b)	–

TFs, metabolites, hormones, etc., which either downregulate or upregulate specifically under salinity, heat stress, metal stress, drought, and cold stress.

As an established information portal, DRASTIC (http://www.drastic.org.uk/) provides knowledge regarding plant ESTs and genes up- or downregulated in response to various abiotic stresses, biotic stresses, and chemical treatments. The current version gives data on cold, salt, and drought. Based on data from 630+ referred journals, it contains over 33,400 records (Newton et al. 2002).

In the year 2011, the database was developed entitled as "Plant Stress Gene Database" by the Jawahar Lal University, India (Prabha et al. 2011). This site includes stress-related genes and ESTs for various plant species including *Arabidopsis,* rice, wheat, sugarcane, and barley. Stress-related ESTs were also found for *Phaseolus vulgaris*. This portal even enables the user to find the orthologs and paralogs of stress-related proteins.

Another example of the available database is UniVIO (http://univio.psc.riken.jp/) developed on the grants by the Japan Society for the Promotion of Science (Kudo et al. 2013). It is unique in the case as it contains datasets for hormone treatment, mutants, and transcriptome analyses for various rice organs. It is very easy to use for researchers as they have to give inputs like hormone name, IDs (probe, locus), and genes and the data are visualized as heat maps.

Furthermore, the teams from Technische Universität München, Helmholtz Center Munich, and King Saud University collaborated and developed DroughtDB, a prominent database solely for drought (Alter et al. 2015). This portal provides a list of genes, protection factors, transcription factors, and enzymes associated with drought response.

Furthermore, there are other databases which are solely focussed on snoRNAs (Brown et al. 2003), smRNAs (Johnson et al. 2006), miRNA (Zhang et al. 2013), proteins (Kumar et al. 2014), ceRNA (Yuan et al. 2016), circRNA-miRNA-gene regulatory networks (Zhang et al. 2017b), and transcription factors (Zhang et al. 2018b) (Table 12).

With each passing year and advances in high-throughput technologies, the number of databases is increasing like a tree which is depicted in Fig. 2.

11 Conclusion

During the last two decades, there has been a constant increase in the volume of data due to the refinement in sequencing platforms, analytical and computational technologies. As a result, we have just entered the era of big data. Parallelly, the researchers have recognized the value of biological databases for organizing in a structured way. This trend is clearly depicted by the tremendous number of online biological databases, publications, issues, frameworks, sections, and even database-specialized journals around the globe. In this way, these biological databases have established as a prominent weapon from the arsenal bioinformatics. However, these databases

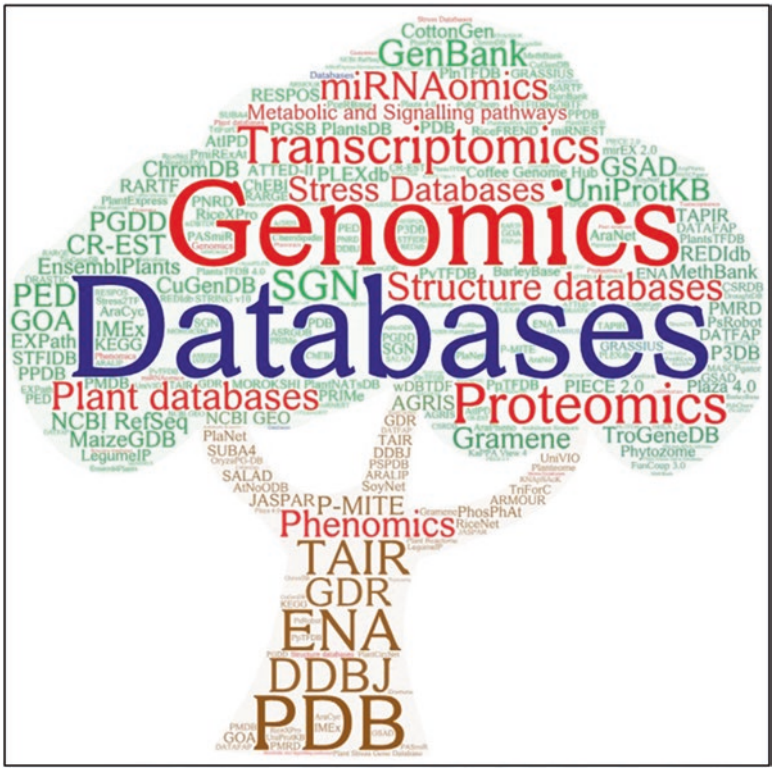

Fig. 2 Increasing tree of biological databases

are currently more biased toward genomics, transcriptome, or metabolomics data, while data for other omics, such as phenome, hormonome, lipidome, and ionome, are scarce. The probable reason is an inclination of "omics technologies" for understanding the intricate levels of various stresses, since the considerable emphasis is currently being placed on enhancing the tolerance against abiotic stresses. Thus, with the considerations, we have endeavored to tabulate all the updated databases on abiotic stress. However, the biggest problem is the time-to-time update which makes multiple databases redundant after some time. For example, Sputnik (http://mips.gsf.de/proj/sputnik/), a database developed by Rudd and colleagues for comparative genomics for plants, had ESTs from 20 species, now become redundant, and is of no use for the scientific community any longer. Other examples include Phytome, Plant Repeat Database, TIGR plant transcript assemblies database, Plants P/Plants T functional genomic database, and many more. So, researchers and bioinformatics community had to make efforts not only in the database development but also in their update. As a result, these updated databases and cloud computing together will help the next generation of scientists in knowledge-driven betterment of crops for abiotic stresses.

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Advances in Functional Genomics in Investigating Salinity Tolerance in Plants

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1 Introduction

Soil salinity has diverse effects on the morphological, physiological, and biochemical characteristics of plants, which results in reduction in yield (Chinnusamy et al. 2005). Salt stress modifies the dimension of morphological parameters that includes root length, leaf area, plant heights, root and branch length, stem diameter, and number of branches and nodes (Yu et al. 2015). Salinity induces morphological and anatomical changes that results in reduction in the dry weight of leaves and roots, root length, root volume, average root diameter, chlorophyll and net photosynthesis, and stomatal conductance (Zhang et al. 2014a; Yan et al. 2015). Photosynthesis is the most important process that is influenced by salt stress by decrease in CO_2 availability, which is induced by diffusion limitations through the stomata and the

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mesophyll (Chaves et al. 2009). Various responses of chlorophyll content have been found in plants (Sudhir and Murthy 2004). Salt stress enhances the accumulation of NaCl in chloroplasts of plants that triggers reduction during photosynthetic electron transport activities in photosynthesis (Sudhir and Murthy 2004). Salt stress inhibits photosynthesis because of water scarcity and lowered carbon substrate (Chaves et al. 2009).

Salinity reduces the growth of plant by three major inhibitory effects namely (1) osmotic effect, (2) ion toxicity, and (3) nutritional imbalance (Ali et al. 2004). Salinity influences seed germination through osmotic effects in beans and wheat (Almansouri et al. 2001; Kaymakanova 2009). In addition, salinity decreases soil water potential due to osmotic stress, it induces ion imbalance in cells due to lower concentrations of K^+, Ca^{2+}, and NO_3^-, and it also causes ion (Na^+ and/or Cl^-) toxicity (Tavakkoli et al. 2011). High concentrations of soil Cl^- is more toxic in reducing growth and yield as compared to Na^+ (Tavakkoli et al. 2010).

Salinity stress causes oxidative stress that further triggers production of toxic reactive oxygen species (ROS). Salinity influences the activity of antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), ascorbate peroxidase (APX), glutathione reductase (GR), guaiacol peroxidase (GPX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDAR), glutathione *S*-transferase (GST), and catalase (CAT). Non-enzymatic antioxidants include phenolics, flavonoids, and tocopherols, which are in active state and scavenge high ROS levels (AbdElgawad et al. 2016). Accumulation of proline in salt-stressed plants decreases osmotic potential in vacuoles in order to maintain chlorophyll level, protein, membrane and subcellular structure, and cell turgor (Cicek and Cakirlar 2002). It plays vital role in scavenging ROS products in salt tolerance (Gharsallah et al. 2016). Glycine betaine (GB) is another osmotic adjustment agent that decreases the ROS concentration and lipid peroxidation in higher plants (Banu et al. 2009). Polyamines also modulate ion channels and activate antioxidant enzymes for osmotic adjustment (Zapata et al. 2017).

Salinity alters the levels of plant hormones such as abscisic acid (ABA), cytokinins, ethylene, and jasmonates that further play a key role in alleviating NaCl-induced salt stress on plant growth and development (Parida and Das 2005). Salinity influences stomatal conductance at the initial stage; afterward the ABA is produced (Munns and Tester 2008). ABA generally increases H^+-pumping under salinity condition in rice (Pons et al. 2013). Cytokinins are antagonists of ABA and have opposite effects in several developmental processes, mainly stomatal opening (Kaya et al. 2009). Ethylene is known as a stress-hormone; ethylene signaling modulates salt response at different levels, including membrane receptors, components in cytoplasm, and nuclear transcription factors (TFs) in the pathway (Cao et al. 2008). The drought stress can be induced by salinity and it influences on redox regulation in plant cells (Uddin et al. 2016). The redox system is important for keeping the cellular homeostasis in environmental stresses (Trachootham et al. 2008). In addition, the late embryogenesis abundant (LEA) proteins are produced by plants to protect themselves from the damage caused by environmental stresses (especially the salt stress) (Bhardwaj et al. 2013).

2 Functional Genomics Approaches for Salt Tolerance

A number of genes were found to be involved in salt tolerance across diverse plant genera. Through functional genomics, transcriptomics, and proteomics approaches the functional significance of some of the major genes have been discovered, which plays a pivotal role in salt tolerance of plants. A number of transgenic approaches have been adopted to over-express genes that are involved in salt-tolerant mechanisms and in addition to that down-regulation of some important genes in transgenic plants also demonstrated salt tolerance. This part of the chapter mostly describes several transgenic methods developed either through over-expression strategies (for enhancing osmoprotectant accumulation, over-expression of TFs, antioxidants and genes involved in ion transport, and heterologous expression of some important proteins involved in salt stress mechanisms) or, using gene-silencing approach for enhanced salt tolerance.

2.1 Over-Expression Strategies for Salt Tolerance in Plants

Over-expression of several genes involved in production of compatible solutes was found to exhibit improved salt tolerance. Those compatible solutes are small, electrically uncharged molecules playing important roles in plant protection as well as membrane and protein stabilization under abiotic stress conditions without hampering normal growth and development (Yancey 1994). Several researches conducted to overcome salt stress using compatible solutes or osmoprotectants and that can be categorized into three groups like over-accumulation of amino acid (e.g., proline), over-production of polyols or sugars (e.g., mannitol, sorbitol, trehalose, etc.), and over-accumulation of quaternary amines (e.g., GB and polyamines).

Several researches were conducted on over-expression of candidate genes for elevated proline accumulation and subsequently improved salt tolerance. A gene, *P5CS* (Δ^1-Pyrroline-5-Carboxylate Synthetase) was found to code a bifunctional enzyme that is responsible for proline synthesis in plants. Over-expression of *P5CS* gene from moth bean (*Vigna aconitifolia*) in transgenic tobacco (*Nicotiana tabacum*) revealed improved salt tolerance possibly due to hyper-accumulation of proline in transgenic plants (Kishor et al. 1995) whereas; over-expression of a mutant *P5CS* (*P5CSF129A*) gene from *V. aconitifolia* in tobacco also documented increased tolerance to salt stress (Hong et al. 2000). Later on, *P5CS* gene was heterologously expressed in different crops like rice, wheat, carrot, etc. by various researchers (Zhu et al. 1998; Sawahel and Hassan 2002; Han and Hwang 2003). Another group isolated *P5CS* gene from *Arabidopsis thaliana* and subsequently over-expressed in potato (*Solanum tuberosum*) and the resultant transgenic potato showed improved tolerance to salinity stress without hampering the tuber yield (Hmida-Sayari et al. 2005). Further study identified two P5CS orthologs (*OsP5CS1* and *OsP5CS2*) from rice and co-expression of those two genes in tobacco depicted

enhanced abiotic stress (including salt stress) tolerance due to over-accumulation of proline in transgenic plants (Zhang et al. 2014b).

A number of researches were conducted to over-accumulate various polyols or sugars in transgenic plants through metabolic engineering. Initially, a mannitol-1-phosphate dehydrogenase (*mtlD*) gene from *Escherichia coli* was transgenically expressed in tobacco and it resulted over accumulation of mannitol and subsequently demonstrated salt tolerance up to 250 mol m^{-3} NaCl (Tarczynski et al. 1993). Further researches transgenically expressed *mtlD* gene in other crops (Chinnusamy et al. 2005). Likewise, another gene sorbitol-6-phosphate dehydrogenase (*Stpd1*) was used by Gao et al. (2001) to accumulate sorbitol in transgenic plant that conferred salt tolerance. Majee et al. (2004) isolated L-myo-inositol-1-phosphate synthase (*MIPS*) gene from halophytic wild rice (*Porteresia coarctata*) and subsequently that gene was heterologously expressed in transgenic tobacco plants. Transgenic tobacco demonstrated tolerance up to 200–300 mM NaCl stress. Trehalose is a non-reducing sugar molecule, which was found to possess osmoprotectant property. Preliminary studies were conducted to isolate trehalose-6-phosphate synthase (*otsA*) and trehalose-6-phosphate phosphatase (*otsB*) genes from *E. coli* and over-expression of those genes in rice as a fusion gene (Garg et al. 2002). Resultant transgenic rice plants showed enhanced trehalose accumulation and thusimproved salt tolerance. Subsequently, Ge et al. (2008) isolated trehalose-6-phosphate phosphatase (*OsTPP1*) gene from rice and over-expressed in rice background. The transgenic plants conferred salt as well as abiotic stress tolerance. Thereafter, another rice gene named trehalose-6-phosphate synthase (*OsTPS1*) was over-expressed in rice and the resultant plants showed over-accumulation of proline as well as trehalose and subsequently tolerance to salt stress (Li et al. 2011). Thus, over-expression of several polyol/sugar accumulating genes was found to show improved salt tolerance in transgenic plants.

The third category of osmoprotectant is quaternary amines and most of researches were conducted to over-accumulate GB compound belonging to this group. Upon exposure to abiotic stresses, GB content is increased in certain plants and that could be used as an osmoprotectant (Khan et al. 2015). Hence, several approaches have been taken to over-accumulate GB content in economically important crops. Initially, two group of scientists heterologously expressed choline oxidase (*codA*) gene, which is responsible for GB synthesis, from *Arthrobacter globiformis* into *Synechococcus* sp. PCC 7942 (Deshnium et al. 1995; Nomura et al. 1995) that was unable to produce GB in natural conditions, but transgenically expressed *codA* gene accumulated GB in that species and subsequently the resultant strain became more salt tolerant. In addition to over-expression in cyanobacteria, several scientists over-expressed *codA* gene in tobacco, *Arabidopsis*, rice and other plants (Hayashi et al. 1997; Jing et al. 2013; Chinnusamy et al. 2005). Another choline oxidase gene (*COX*) was isolated from *Arthrobacter pascens* and used for the generation of salt-tolerant transgenic plants (Sakamoto and Murata 2001; Su et al. 2006). Further researches were conducted to over-accumulate GB in transgenic tobacco plants by over-expressing Betaine-aldehyde dehydrogenase (*BADH*) gene from spinach (*Spinacia oleracea*) and the generated transgenic plants acquired enhanced salt tolerance compared to the untransformed control plants (Yang et al. 2008).

2.2 Transcription Factors

Genetic engineering through over-expression of different TFs was found to have significant salt tolerance in different plant species. Over-expression of a dehydration-responsive element-binding (DREB) protein from *A. thaliana* named *AtDREB1A*, in transgenic tobacco, showed enhanced salt tolerance under controlled condition (Cong et al. 2008) whereas; over-expression of a *Glycine max* DREB gene (*GmDREB1*) in transgenic wheat depicted enhanced salt tolerance compared to wild-type plants under natural field condition (Jiang et al. 2014). Along with DREB, NAC TFs were found to be involved in salt tolerance in different plants. Over-expression of different NAC TFs namely SNAC2, ONAC045, and ONAC022 in transgenic rice documented improved salt tolerance (Hu et al. 2008; Zheng et al. 2009; Hong et al. 2016). *Caragana intermedia* is an extremely salt as well as drought-tolerant desert leguminous shrub. Over-expression of two NAC TFs (CiNAC3 and CiNAC4) from *C. intermedia* into *A. thaliana* caused elevated salt tolerance compared to wild-type plants (Han et al. 2015). Another group of TF (MYB) was also found to be associated with salt stress in different plant species (Roy 2016). An approach taken to functionally characterize OsMYB2, a R2R3 type MYB TF from rice, depicted that over-expression of OsMYB2 improved salt tolerance in transgenic rice plant (Yang et al. 2012). Another study revealed that over-expression of OsMYB48-1 in rice lead to enhanced tolerance to salt as well as drought-stress under simulated condition (Xiong et al. 2014). Although over-expression of a number of TFs documented their importance in salt tolerance, how those TFs regulate the downstream salt stress signaling cascade is still unclear.

2.3 Antioxidants and Detoxification Genes

Antioxidants plays crucial role to cope up the plants from the excessive generated ROS during salt stress as well as other stresses. Plants possess several enzymatic antioxidants like SOD, catalase, and peroxidase along with some non-enzymatic antioxidants including phenol, ascorbic acid, thiol compounds which play crucial role in detoxifying ROS. Over-expression of a cDNA encoding both GST and glutathione peroxidase activity in transgenic tobacco showed faster growth compared to the control tobacco plants under salt-stressed condition (Roxas et al. 1997). MDAR enzyme plays major role for synthesis of ascorbate, an important antioxidant available in plant. Over-expression of *AtMDAR1* gene from *A. thaliana* into tobacco plants (Eltayeb et al. 2007) caused enhanced tolerance of the transgenic tobacco plants to salt stress. In another experiment a catalase gene (*katE*) from *E. coli* was over-expressed in tobacco and upon exposure to salt stress, transgenic plants showed significantly lesser photoinhibition compared to the wild-type plants (Al-Taweel et al. 2007). Very recently a gene named as *Delila* (*Del*), was isolated from snapdragon (*Antirrhinum majus*) and characterized through heterologous expression in

tobacco (Naing et al. 2017). Over-expression of *Del* gene in transgenic tobacco revealed enhanced anthocyanin accumulation as well as elevated antioxidant activity and additionally the transgenic tobacco plants showed improved salt tolerance. Thus, it can be concluded that over-production of several antioxidant enzymes or their combinatory effects might have positive involvement in salt tolerance.

2.4 Genetic Engineering for Ion Transporters

Several transporter proteins were found to possess pivotal role in maintaining osmotic regulation as well as ion homeostasis and recombinant expression of those proteins that depicted improved salt tolerance (Blumwald 2000). Over-expression of a Na^+/H^+ antiporters (*AtNHX1*) in *Arabidopsis* conferred enhanced salt tolerance (up to 200 mM NaCl) as compared to the control plant (Apse et al. 1999). Further studies revealed that over-expression of a vacuolar Na^+/H^+ antiporter in transgenic tomato could successfully produce fruit even at 200 mM NaCl (Zhang and Blumwald 2001). Roy et al. (2014) nicely reviewed several transgenic researches dealing with transporters or proton pumps especially involved in salt tolerance. Likewise, high affinity potassium transporters (*HKT*) were also found to play significant role in salt tolerance (Hauser and Horie 2010). Another study documented that over-expression of a barley transporter (HvHKT2; 1) in barley could reinforce the salt-accumulating mechanism in the transgenic plant (Mian et al. 2011). Salt overlay sensitive gene (*SOS1*) was found to be responsible for producing a membrane protein homologous to Na^+/H^+ transporter (Wu et al. 1996). Over-expression of *AtSOS1* from *Arabidopsis* in transgenic tobacco revealed significant salt tolerance in transgenic plants compared to the untransformed ones (Yue et al. 2012). In a different experiment, another SOS gene (*PtSOS2*) from poplar (*Populus trichocarpa*) was over-expressed in poplar tree and it was found that compared to the untransformed one, in the transgenic tree Na^+/H^+ exchange ratio as well as Na^+ efflux in the plasma membrane were significantly increased along with better ROS scavenging mechanism under salt stress condition (Yang et al. 2015). In this way the functional genomics study of several transporter proteins clearly demonstrated their involvement in salt tolerance.

2.5 Over-Expression of Proteins Having Physiological Significance (LEA, PR, Germin)

LEA proteins were mostly found to be expressed in the seed tissues of the plant and those are expressed at the late stages of the embryo development (Roberts et al. 1993). Over-expression of a barley LEA protein (*HVA1*) in transgenic rice depicted enhanced salt tolerance in transgenic plants (Xu et al. 1996). Later on another LEA protein from wheat (*DHN-5*) was heterologously expressed in *Arabidopsis* and the generated transgenic plants showed enhanced Na^+ and K^+ accumulation as well as

salt tolerance (Brini et al. 2007). Along with the proteins directly involved in salt tolerance or salt stress signaling, how LEA proteins are involved in controlling salt stress in plants has been nicely presented by Bhardwaj et al. (2013).

In addition to that along with LEA proteins, several pathogenesis related (PR) proteins were found to be associated with salt stress. Over-expression of a PR protein (PR-5) from *Arabidopsis* to *S. tuberosum* documented salt tolerance in transgenic plants (Evers et al. 1999). Similarly, heterologous expression of another PR protein (RSOsPR10) in bentgrass showed improved salt tolerance in transgenic plant (Takeuchi et al. 2016). Germin and germin-like proteins (GLPs) are somewhat similar to PR proteins and those proteins are involved in biotic as well as abiotic stress tolerance (Barman and Banerjee 2015). Over-expression of a germin gene possessing oxalate oxidase activity in *S. tuberosum* was found to show enhanced tolerance to salt stress (Turhan 2005).

2.6 Gene Silencing Approaches for Salt Tolerance in Plants

Although over-expression strategies were found to establish several salt-tolerant transgenic lines, there are instances where gene-silencing approaches have also been utilized for metabolic engineering to achieve salt tolerance in plants. Proline dehydrogenase is an enzyme that is responsible for catalyzing proline, an important osmoprotectant. Gene-silencing of proline dehydrogenase gene (*AtProDH*) through antisense RNA technology in *Arabidopsis* conferred constitutive accumulation of proline and improved tolerance to NaCl stress (600 mM NaCl) compared to untransformed as well as vector control plants (Nanjo et al. 1999). Unlike germin of wheat, a rice GPL was found to be negatively involved in salt tolerance. A recent study documented that RNAi-mediated gene-silencing of rice GPL 1 (*OsGLP1*) in rice showed enhanced salt tolerance in transgenic rice at early stages of growth and development as well as in rice calli (Banerjee et al. 2017).

It is clear that since long time several transgenic approaches have been taken either to over-express or down-regulate different target genes to confer salt tolerance. Further research is needed to get robust stress tolerant lines in farmers' field for the future because salt stress is an ever-increasing abiotic stress across the world.

3 Expression Studies Under Salt Stress (Some Case Studies)

The effects of salinity on gene expression have been evaluated in a variety of plants, profiling the global genes help us to understand this complex mechanism. Up-regulation of the *SOS1*, *SOS2*, *SOS3*, *HKT1*, *AKT1*, *NHX1*, *P5CS1*, *HSP90.7*, *HSP81.2*, *HSP71.1*, *HSPC025*, *OTS1*, *SGF29*, and *SAL1* genes cause tolerance in alfalfa genotypes (Sandhu et al. 2017). The *S*alt *O*verly *S*ensitive (SOS) signaling pathway mediates cellular signaling under salt stress, to maintain ion homeostasis

(Ji et al. 2013). It includes SOS3, SOS2, and SOS1. SOS3 is a Ca^{2+} sensor belonging to calcineurin B-like (CBL) protein family, SOS2 act as a serine–threonine protein kinase, belonging to the sucrose non-fermenting 1-related kinase 3 (SnRK3) family and SOS1 is characterized as a plasma membrane Na^+/H^+ antiporter (Sathee et al. 2015). *LEA* genes are a large gene family in plants that are mainly expressed in seeds of rice during salt stress (Gao and Lan 2016). The first category of salt-responsive genes is attributed to plant hormone and calcium signaling pathways and the second category are TFs. TFs including basic leucine zipper (bZIP), WRKY, APETALA2/ETHYLENE RESPONSE FACTOR (AP2/ERF), MYB, basic helix–loop–helix (bHLH), and NAC families will be active in response to salinity (Deinlein et al. 2014). The third category is linked to ion metabolism and transfer (Wang et al. 2018). Energy production and ion homeostasis associated proteins are produced under salt stress (Wang et al. 2009). Ion transporters, protective proteins involved in photosynthesis (D2 protein) and protective compounds (lignin) are activated during salinity (Kosová et al. 2013).

4 miRNA-Mediated Salt-Stress Regulation

Abiotic stress generated by mineral salts is one of the major natural limiting factors affecting crop growth and thus productivity. Salinity stress is a complex phenomena owing the plant species subjected to three types of stress conditions—water stress generated by the osmoticum, mineral toxicity due to the presence of salt and disturbances in the mineral nutrition of the plant. In order to cope up with the detrimental effects of salt stress, plants have developed various elaborate mechanisms to exclude excess salt from their cells or to tolerate salt within the cells (Munns and Tester 2008). Management practices like reclamation of salt, use of salt-tolerant genotypes, method and time of planting can be tailored to mitigate the problem to a considerable extent. In recent years, research works have been initiated to untangle the physiology and molecular mechanism behind the stress tolerance and also to identify the stress responsive proteins and their respective genetic network. Plant resistance to salt stress is controlled by various stress responsive gene complexes (Zhu 2003; De Costa et al. 2007). Precise expression of the genes and their accurate regulation, which is attained by multiple mechanisms at different levels such as transcriptional, post-transcriptional, and post-translational regulations (Mirlohi and He 2016) facilitate plant species to alleviate the problem of salt stress. In the post-transcriptional regulation recent trends are focusing on the role of small non-coding RNAs (sRNAs) categorically microRNAs (miRNAs) in the salinity and other abiotic stress tolerance. Post-transcriptional regulation of genes through small RNAs is known as RNA interference or RNA silencing. MicroRNAs (miRNAs) are short (21–24 nt) RNA molecules that control gene expression at the post-transcriptional level by cleavage of mRNA targets or by inhibition of their translation (Llave et al. 2002; Palatnik et al. 2003; Li et al. 2013). All the plant miRNA genes (MIRs) are originated from the RNA polymerase II (RNAPII) followed by splicing and

tailoring of the long primary RNA transcripts for production of mature and functional miRNA. The processing to pre-miRNA(s) occurs in the nucleus by Dicer Like-1 (DCL1), which then makes a cut of pre-miRNA(s) to liberate the miRNA together with its reverse complement, forming the miRNA–miRNA∗ (or miRNA5p–miRNA3p) duplex (Dolata et al. 2016). The site of this biogenesis process is the cell nucleus involving various protein complexes of RNase III type endoribonuclease family. Like other RNA polymerase II transcripts, the 5′ end of the miRNA primary precursor, is also protected by a specific cap structure that is recognized and bound by the nuclear cap-binding protein complex (CBC) consisting of two subunits: CBP20 and CBP80 (Hugouvieux et al. 2001; Kmieciak et al. 2002; Daszkowska-Golec et al. 2013). Similarly, the 3′ end contains a poly (A) tail after undergoing processing through polyadenylation machinery. After completion of processing the duplex, the miRNA unwound by a helicase in the cytoplasm to release the mature miRNA. Recent genetic and biochemical studies unravel the role of miRNA in the post-transcriptional down-regulation of gene expression through annealing to reverse complementary sequences owing to breakage or translational suppression of the target mRNAs (Fig. 1).

Moreover, these miRNAs encourage DNA methylation thus facilitating gene expression at transcription level. Recent studies reported that during stress, the miRNA could alter the expression of different stress responsive genes thus playing a vital role in plant resistance mechanism. The identification of plant miRNA families (miR156, miR159, miR164, miR171, etc.) began in the year 2000 (Llave et al. 2002;

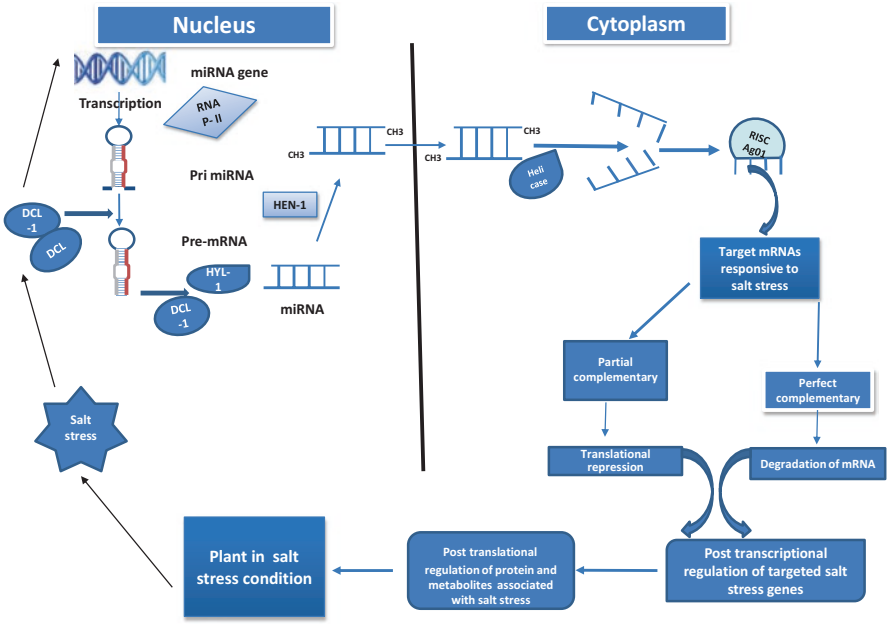

Fig. 1 Schematic illustration of post-transcriptional regulation of miRNA-mediated salt-stress-regulated genes (Source: Mangrauthia et al. 2013; modified by Authors)

Reinhart et al. 2002). Due to difficulties in cloning of miRNA followed by sequencing and prediction using various bioinformatics prediction tools the identification was not satisfactory through classical approaches. The progress of miRNA investigation was accelerated by the development of the next generation sequencing techniques (also called deep sequencing) and complex computational algorithms (Lai et al. 2003; Rajagopalan et al. 2006; Fahlgren et al. 2007; Jagadeeswaran et al. 2010; Rosewick et al. 2013). The first abiotic stress-related miRNAs in plants was reported by Sunkar and Zhu (2004). Later beside the genes several differentially regulated miRNAs have been identified in different plant species like *Arabidopsis*, *Glycine max*, *Glycine soja*, *Gossypium hirsutum*, *Medicago truncatula*, *Nicotiana tabacum*, *Oryza sativa*, *Panicum virgatum*, *Phaseolus vulgaris*, *Populus euphratica*, *Saccharum officinarum*, *Physcomitrella patens*, *Sorghum bicolour*, *Triticum aestivum*, and *Zea mays* under salt-stressed condition. The behaviour of several miRNAs under salt stress is similar to that reported in other species, suggesting a common regulatory mechanism operating widely across species. At present, 8496 miRs from 73 plant species are listed (Kozomara and Griffiths-Jones 2014). It was found that in response to salt stress, miR156, miR158, miR159, mi RNA160, miRNA164, mi RNA165, miRNA166, miR167, miR168, miR169, miR171, miRNA 172, miRNA1b, miRNA319, miR394, miR395, miR396, miR397, miR399 were regulated both in up- and down-regulatory pattern targeting squamosa promoter binding protein, cationic amino acid transporter, SPL TF, MYB TF, auxin response factor, DCL1, NAC family genes, SBP like TFs, APETALA2 like factor, Scarecrow like TF in different crop species like *A. thaliana* (Liu et al. 2008), *Zea mays* (Ding et al. 2009), *Vigna unguiculata* (Paul et al. 2011), *Saccharum* spp. (Bottino et al. 2013), and *Triticum aestivum* (Wang et al. 2014). However, the expression of miR160, miR393, miR4, miR402, miR417, miR482, miR1447, miR1507, and miR2118 were increased in response to salt stress in different crop species targeting F-box protein, protein kinase, APS-reductase, NBS-LRR resistance gene in *Arabidopsis* (Sunkar and Zhu 2004), *P. euphratica* (Qin et al. 2011) while the miR398 was down-regulated in *Arabidopsis*, thus establishing a role for miRNAs in the adaptive response to salt stress (Liu et al. 2008). A better understanding of miRNA-mediated gene regulation under salt stress will certainly help in elucidating the complex network of regulatory molecules, genes, proteins, and metabolites. Most miRNAs regulated the expression of multiple target genes belonging to the same gene family in plants. In response to salt stress, miR156 was found in up-regulated pattern in *A. thaliana* (Liu et al. 2008), *P. euphratica* (Qin et al. 2011), *Gossypium raimondii* (Xie et al. 2014), *P. virgatum* (Sun et al. 2012), and *V. unguiculata* (Paul et al. 2011) targeting squamosa promoter binding-like protein, cationic amino acid transporter, SPL binding protein, etc. However, down-regulation of the same group of miRNA was observed in *Zea mays* (Ding et al. 2009; Kong et al. 2010) regulating the target gene SPL-like TFs in relation to salt stress. The miRNA 158 was associated with salt stress in *A. thaliana* targeting the F-box family protein and pentatricopeptide repeat (PPR) containing protein (Liu et al. 2008). In case of miRNA159 down-regulation were observed in *A. thaliana* (Chen et al. 2012), *Nicotiana tabacum* (Frazier et al. 2011) targeting the MYB TF gene whereas up-regulation were observed *in P. virgatum*.

MiR160 targeted the auxin responsive factor in up-regulated fashion in case of *Vigna*, *Triticum*, and *Gossypium*. With the advancement of genomic techniques, a better understanding of the miRNA-mediated gene regulation targeting the expression of multiple regulatory genes and associated proteins under salt stress can be possible. This can provide a new arena to improve the resistance mechanism in plant under salt stress condition either through conventionally or through transgenic mechanism. It has been found that transgenic rice and *Arabidopsis* constitutively over-expressing osa-MIR396c-reduced salt and alkali stress tolerance compared to that of wild-type plants (Gao et al. 2010). Utilizing various computational and experimental approaches this untapped area can get new dimension regarding discovery of miRNA associated with regulating the expression of salt-stress-related genes. In future this will direct to find out new components regarding salt stress mechanism in plant.

5 Genome Editing for Salt Tolerance

Recently, the clustered regularly interspersed short palindromic repeats (CRISPR)/Cas9 technology has been proved as an efficient nuclease-based technology for genome editing which is the best alternative strategy for genetic engineering without having the conflict related with *Agrobacterium*-mediated or direct gene uptake strategies. This approach opens up new vista for editing each member of a gene family targeting multiple gene of interest without modifying the expression of other genes. The CRISPR are loci with variable short spacers interspersed by short repeats, later transcribed into non-coding RNAs (ncRNA). This ncRNA then forms a complex with the Cas and guides the complex to slice complementary target DNA. This system acting on very small desirable target sequence of 1–20 nt of the target gene need to be altered. The principle of this system is based on the function of an endonuclease, Cas9 protein, having two functional domains—RuvC-like domain and the HNH nuclease domain (Cong et al. 2013). This endonuclease enzyme is driven by a synthetic single guide RNA for recognition of its target sequences and ultimately produces double stranded breaks in the target sequences which consequently trigger DNA repairing mechanism and generate various site-specific genetic alterations through non-homologous end joining (NHEJ) or through homology-directed repair (HDR). These genetic alterations cause aberrations either through insertion or deletion and consequently generate frame-shift mutation thus regulating the expression of the functional domain of the target gene (Gratz et al. 2013; Zhou et al. 2014) (Fig. 2).

The CRISPR/Cas9-based genome editing system is easy to opt and cost-effective therefore having immense potential in future gene editing programme. Sometimes, instead of nuclease-based mechanism this system relies on the principle of nickase activity. In that case, the nuclease activity for one strand has been removed, leaving a nickase activity (Cong et al. 2013). Other recent developments include the use of a disarmed nuclease, lacking nuclease activity, which will competitively bind a

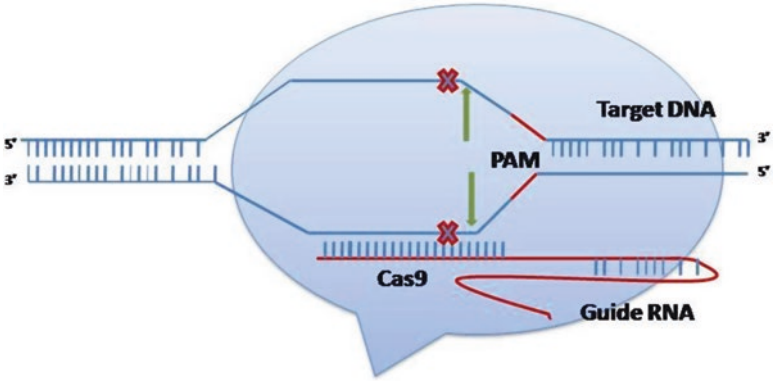

Fig. 2 Schematic illustration of CRISPR/Cas9 technology a new vista for genome editing

defined site to block the access of other molecules such as TFs and down-regulate or turn off the expression of a gene (Gilbert et al. 2013). Similarly, a CRISPR/Cas9 complex can retain transcriptional activators. Further bacterial genera have comparable genes to Cas9, some of which are now being analyzed and developed (Schiml et al. 2014; Zetsche et al. 2015), and further advances are expected to arise from all the variations on this theme (Schaeffer and Nakata 2015). CRISPR/Cas9 is a new emerging concept and has been considered safe for genome editing focusing on biotic and abiotic stress tolerance and creating new metabolites. Recently this system has been utilized in rice for achieving salt stress tolerance following *Arabidopsis*. The Δ 1-pyrroline-5-carboxylate synthetase (P5CS) is the rate-limiting enzyme in proline (Pro) synthesis and is involved in drought and salt-stress-tolerance in plants. In rice, an OsP5CS gene was isolated from a stress-treated commercial rice variety and based on the DNA isolated sequence, four gRNA (guide RNA) constructs were designed for CRISPR-Cas9 editing of the OsP5CS (*Outermost Cell-specific Gene5*) isolated from the commercial rice variety in order to increase the proline accumulation in cells (Jiang et al. 2013). This study laid the foundation for the development of high yielding rice varieties resistant to drought and salinity using genome editing technology. In the near future, this genome editing technology can be proved as a valuable tool in discovering the functions of signaling pathway components. The number of examples is increasing rapidly, and transmission to the next generation has been shown in several cases.

6 Conclusion

A number of studies were conducted on over-expression of candidate genes responsible for subsequently improved tolerance against salinity. Apart from the over-expression approaches that created various salt-tolerant transgenic lines, there were successful gene-silencing methods, utilized for metabolic engineering to attain salt tolerance in plants. Yet, there are ample scopes on use of over-expression ion channel,

antiporter, etc. from stress tolerant plants to salt sensitive crop plants. Nevertheless, a thorough screening is needed to identify if any toxic compound is being produced in the transgenic plants. Functional genomics or biotechnology has a great potential to combat such an ever-increasing salt stress problem to save agriculture.

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Drought Stress in Chickpea: Physiological, Breeding, and Omics Perspectives

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1 Introduction

Legumes are valuable commercial and agricultural crops: among these, chickpea is an annual crop that is generally cultivated on marginal domains with low input resources (Srinivasan 2017). It is a nutritionally rich crop having high protein contents (18–23%), hence known as 'poor man's meat.' Along with protein, it also carries some essential amino acids (leucine, lysine, threonine, isoleucine, methionine) as well as vitamins (e.g., A, K) (Jukanti et al. 2012; Sharma et al. 2013). Some legumes have antinutritional factors, such as trypsin inhibitors (soybean) and vicin (faba bean), whereas chickpea has no specific antinutritional component (William 1987). Nodules that are present on the roots of chickpea fix atmospheric nitrogen through the symbiotic relationship with soil-borne bacteria, particularly *Rhizobium*.

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Based on the symbiosis process, chickpea can achieve up to 76% of its own nitrogen requirements. It is very effective for crop rotation because it has the ability to increase soil fertility for coming crops after it is harvested (Flowers et al. 2010).

Chickpea is cultivated on 931,000 hectares in Pakistan with 359,000 tons of production annually. It occupies about 5% of the rabi-cropped area in Pakistan. India and Australia are the major producers of chickpea: Pakistan ranks third with respect to area and production. Punjab shares about 92% of the chickpea area in Pakistan for cultivation, mainly in the Thal region. The production of chickpea is very low that it is unable to fulfill the requirements of the country, so chickpea must be imported from other countries, particularly from Australia. Pakistan imported 0.450 million tons of chickpea during 2016–2017 (Govt. of Pakistan 2016–2017). Chickpea is classified into two distinct classes ('Desi' and 'Kabuli' types) based on plant type and geographic distribution. The Desi type is primarily grown in India and Pakistan and has small seeds that are brown to black in color. The Kabuli type is mostly grown in temperate regions (e.g., Ethiopia and Syria) and carries large angular seeds varying in color from cream to beige. The Kabuli type is a higher yielder than Desi, and it also has more nutritive value than the Desi type. Interestingly, the Desi type is more tolerant of drought stress as compared to the Kabuli type (Jukanti et al. 2012). Although it is a valuable crop for developing countries, it faces several biotic and biotic stresses; as a result, its production is being reduced while the area remains stagnant. Low production because of the availability of different stresses is the main fact for its cultivation.

Climate change is exerting an adverse effect on crop productivity by shifting the natural growth period. During previous centuries, an increment of 1.2 °C in temperature is recorded as caused by climate change. Moreover, it is expected that it would increase up to 3 °C by 2100 (Patwardhan et al. 2007). During high temperature, the rate of evapotranspiration is increased; consequently, reduction in soil moisture leads to the appearance of drought stress (Chaves et al. 2002). Now, this has become a global phenomenon that can affect the productivity of agricultural crops in advanced as well as developing countries. Globally, 90% of chickpea is cultivated in rain-fed areas where terminal drought stress is the main limiting factor for its growth and production (Srinivasan 2017). Drought stress is the second most important growth-restraining factor after diseases in chickpea (Mohammadi et al. 2011). Transient and terminal drought stress are the most common types of drought, based on the duration of effect. In the short term, shortage of water can affect the plant at any stage of its development and can be remedied by precipitation, whereas terminal drought stress is a long-term stress that creates a constant water deficit condition which hinders the reproductive stage of crop plants. Semi-arid tropics and Mediterranean climates are mostly affected by terminal drought stress (Li et al. 2018).

Drought stress has a severe effect on flowering and seed formation. Throughout the world, terminal drought stress is reducing chickpea yield as much as 40–50% annually (Kumar and Abbo 2001; Ramamoorthy et al. 2017). Oxidative stress is produced by reactive oxygen species (ROS) such as H_2O_2, O_2^-, O^-, and HO^-, which result in the production of a toxic environment for plants. Oxidative stress

deteriorates the normal behavior of different metabolic pathways in the cell. The activity of antioxidants such as superoxide dismutase (SOD), glutathione reductase (GR), catalase (CAT), peroxidases, glutathione peroxidase (GPX), and ascorbate peroxidase (APX) is increased or decreased under drought stress depending upon the tolerance or susceptibility of the plant (Mohammadi et al. 2011). Proline is accumulated in tolerant plants under drought stress to reduce the effect of stress (Dalvi et al. 2018).

In this scenario, the use of omics-based breeding strategies along with conventional techniques is indispensable. In the present era, the availability of next-generation sequencing (NGS) tools provides a comprehensive platform to maximize the use of these omics approaches. The genome of Desi type (ICC 4958) and Kabuli type (CDC Frontier) have been completely sequenced through NGS and their genome assemblies are publicly available (Jain et al. 2013; Varshney et al. 2013b). The sequenced genome was used to develop a genome-wide physical map of chickpea (Varshney et al. 2014a).

Now omics approaches, such as genomics, transcriptomics, proteomics, metabolomics, ionomics, and phenomics, as well as genomic-assisted breeding (marker-assisted recurrent selection and marker-assisted backcrossing), have been applied in chickpea to accelerate the breeding program (Varshney et al. 2014a). RNA-Seq-based differential gene expression is used for the identification of novel genes and associated pathways under different environmental platforms. The availability of diverse germplasm and NGS tools will help the plant breeder to design an appropriated breeding plan to combat drought stress.

2 Impact of Drought on Chickpea

Chickpea is vulnerable to drought stress, so we must face its drastic effect. The effect of drought stress is mainly associated with the stage of the crop as well as the duration of the stressed conditions. All the impacts created by drought stress ultimately result in the reduction of yield and quality as well. Here, some of the prominent effects of drought stress are discussed.

2.1 Effect at Vegetative and Reproductive Phase

There are two main early drastic effects of drought stress, associated with the seedling stage as well as deprived seedling stand in plants (Harris et al. 2002). The effect of drought stress at the time of the vegetative phase is less as compared to the anthesis phase (Mafakheri et al. 2010). The drought-tolerant chickpea variety (Bivaniej) gave more yield as compared to the susceptible variety (Pirouz). The loss of yield was also associated with the phase of stress imposition, either vegetative or anthesis. Application of terminal drought stress, at the time of early stages of pod formation,

was responsible for lower yield by reducing the reproductive growth, biomass, seed yield, and harvest index in chickpea (Pang et al. 2016). Flower abortion and empty pod formation had a substantial effect on yield reduction under the terminal drought environment. Pollen viability was decreased under lower soil moisture level. Historically, it was recorded that the yield and productivity of chickpea remained low under severe dehydration conditions. The normal pollen growth is reduced under drought stress, and consequently the number of sterile pods is increased. Ultimately, the yield is reduced by the increased number of empty pods and reduced seed size under drought stress.

Similarly, the process of seed-filling is disturbed under drought conditions, leading to the development of chickpea seeds of a small size (Kalra et al. 2008). Based on a comparative study of Desi and Kabuli types of chickpea, it was observed that the Desi type had more tolerance against drought stress (Muruiki et al. 2018) because it has a better genetic makeup that is intrinsically inherited. The nodulation process is badly affected by drought stress in chickpea. The number, size, and vigor of nodules are reduced in the presence of drought stress, resulting in inferior nitrogen fixation (Muruiki et al. 2018). The yield potential of chickpea is decreased by abnormal nodule development. Flowering, pod formation, and seed-set are three more sensitive stages in chickpea during drought stress. Roots are the primary outgrowth in plants, responsible for the absorption of water and nutrients from the soil. Roots have a significant effect on the efficiency of transpiration pull, used to extract the water from the soil. If the plant has a deep root system, then it will be good absorber of water, present either at the upper surface or underground in the soil. On the other hand, shallow and dense roots might be good providers of nutrient uptake such as phosphorus, found at the upper surface of the soil (Ramamoorthy et al. 2017).

The reproductive phase is the most critical stage under drought stress, particularly associated with yield potential. At the time of pod-filling, if drought stress occurs, then flower-shedding and pod-abortion will occur, and thus the yield will be reduced by producing fewer seeds with lower seed weight (Pang et al. 2016). Yield losses from drought stress ranged from 40% to 50% in chickpea (Muruiki et al. 2018). The yield potential of chickpea under drought stress was found to be strongly correlated with leaf osmotic potential, leaf water potential, and relative water content (RWC) (Summy et al. 2016). Drought stress had a drastic effect on these parameters, particularly in the susceptible genotypes of chickpea; consequently, the seed yield fell as much as 37.32% in the susceptible genotype, HC-1. The major constraint for the production of chickpea is terminal drought stress, which affects mainly the reproductive stage of the plants. In chickpea, the range of reduction in flower formation, pod formation, and yield was 37–56%, 54–73%, and 15–33%, respectively, under drought stress (Fang et al. 2009).

Moreover, the effect of drought stress on germination and pollen viability was also evaluated by in vitro assessment (Rokhzadi 2014). It was found that in the chickpea genotype (Rupali), the decrease in germination and pollen viability under drought stress was 50% and 89%, respectively. In comparison to in vitro, the rate of pollen reduction was higher, 80%, when exposed to drought stress in vivo. Based on plant

growth regulators, it was also reported that the drought effect was more pronounced during the reproductive stage as compared to vegetative. These results were used to further justify the drastic effect of terminal drought stress in chickpea. So, by seeing the visual effects of drought stress on crop plants, it can be stated that drought stress is the growth-limiting factor, resulting in inferior vegetative and reproductive growth of the plant.

2.2 Effect on Photosynthesis

Photosynthetic machinery is the primary index to observe the health, vigor, and potential of a plant to rescue itself under water deficit conditions. The rate of photosynthesis is reduced under drought stress because of the disturbance of several metabolic pathways. Reduction in photosynthesis causes a reduction in the yield by altering some physiological mechanisms. Thylakoid membrane and chlorophyll pigments are the basic and vital parts of the photosynthetic apparatus. Both these organelles become less efficient under drought stress, resulting in leaf necrosis and reduction in photosynthesis efficiency. Leaf area is also associated with photosynthesis, so reduction in leaf area will result in a lower level of photosynthetic process in the presence of drought stress. Because the production of glucose is decreased, so that a lower amount is available for the plant to use, the production of new leaves is reduced. On the other hand, the rate of leaf abscission is increased, to save the photosynthetic product (glucose) for plant survival (Tas and Tas 2007).

In tolerant chickpea genotypes, the photosynthetic regulatory genes transcribe into β-carbonic anhydrase-5 under drought stress (Das et al. 2016). The interaction of CO_2 with RuBisCO is immersed by a specific combination of RuBisCO and carbonic anhydrase. This combination is used for the effective mechanism of RuBisCO to perform the proper carboxylation process. Carbonic anhydrase can be used as a marker for the selection of drought-tolerant chickpea genotypes in screening experiments. Similarly, at the time of vegetative and anthesis phase, decrease in chlorophyll content, that is, chlorophyll *a*, chlorophyll *b*, and total chlorophyll content was observed under drought stress in chickpea (Mafakheri et al. 2010). The rate of photosynthesis is primarily determined by the resistance of mesophyll under drought stress (Rahbarian et al. 2011). Based on mesophyll tolerance under drought stress, it was shown that the rate of photosynthesis was higher in the drought-tolerant chickpea variety (Bivaniej) as compared to the susceptible one (Pirouz). Similarly, a significant reduction in chlorophyll content, chlorophyll fluorescence, photosynthesis, and PSII-photochemical efficiency (Fv/Fm) was observed in chickpea genotypes under drought conditions at the seedling stage.

Another study also showed that positive osmoregulation and leaf turgor had a significant association with the photosynthetic machinery (e.g., photochemical efficiency of PS-II) under drought stress (Basu et al. 2007b). Leaf turgor and osmoregulation also maintain photosynthetic efficiency by securing normal activity of PS-II under drought stress in chickpea. The effect of drought stress on photosynthesis can

be measured through chlorophyll *a* fluorescence (Kalefetoğlu Macar and Ekmekçi 2009). Increments in the duration of drought stress were responsible for the photoinhibition of PS-II activities in chickpea. There are certain reasons for the reduction of photosynthesis under drought stress; among these, the activation of sucrose-phosphate synthase (SPS) and production of hexose sugars (carbohydrates) are of prime importance (Basu et al. 2007a). When leaf starch starts to decline, carbohydrate and SPS increase, and both these factors are responsible for the accumulation of sucrose. It was experimentally approved that drought-induced alterations were associated with SPS and carbohydrates, which modify the efficiency of water uptake in leaves. So, the rate of osmotic adjustment, photosynthesis, and RWC under drought stress is primarily associated with the SPS and carbohydrate accumulation under drought stress.

2.3 Effect on Water Relationships

Drought stress can be measured by determining water status in the plants, called relative water content (RWC). The lower level of moisture content in plants leads to the reduction in available RWC present in the plants. The genetic makeup of the plants also has a prominent effect on the plants for maintaining their level of RWC under varying levels of moisture. The variation in RWC is produced by the plant ability to obtain water from the soil through the roots. RWC is retained by creating a high water potential gradient, reducing water losses by controlling the stomatal openings, and increasing root length (Omae et al. 2005). RWC is proposed as the best choice for the representation of current water status in terms of genetic variation, based on the genetic association between RWC and production during drought stress. In chickpea, about 85% increment in RWC was recorded in the tolerant chickpea variety (JG-62) as compared with other susceptible varieties under drought stress (Bhushan et al. 2007). The increment in RWC is linked by the accumulation of proline content as well.

The movement and retention of water are controlled by the stomata in plants under stress. Stomatal indices were varied among leaves of well-watered and drought stress environments. Stomatal indices under drought stress were lower in the leaves and vice versa under well-watered leaves (Hamanishi et al. 2012). Drought stress has a significant effect on the opening and closing of stomata (Buchanan et al. 2005). Under drought stress, most often stomatal closure is increased as a result of the biosynthesis of abscisic acid (ABA), the stress hormone, which is also increased. The stomatal opening is closed by stomatal guard cells with the help of ABA, making the leaves turgid. The rate of photosynthesis, as well as water usage, are decreased under drought stress. Ultimately, the normal growth rate of the plant is disturbed by chlorophyll necrosis. Because water is a deficit under drought stress, to overcome the effect of that stress the plant uses water efficiently, that is, water use efficiency. Drought-tolerant chickpea genotypes have high water

use efficiency as compared to susceptible types (Rahbarian et al. 2011). Normally, water use efficiency is increased under drought stress, especially in tolerant plants. The same process was seen in chickpea, in that the water use efficiency was higher in tolerant genotypes (MCC-877 and MCC-392) compared with susceptible genotypes (MCC-448 and MCC-68).

Further, water use efficiency seemed to be increased significantly from seedling stage to early flowering and was reduced quickly during pod filling under drought stress (Basu et al. 2007a). RWC was decreased significantly under drought stress among susceptible genotypes that were unable to counter the effect of drought stress accurately during early growth stages. Terminal drought stress was responsible for the reduction of leaf water potential, that is, −1.00 MPa to −2.25 MPa from prestress level to terminal drought stress level in chickpea. Gradual changes in RWC were observed under certain levels of drought stress; consequently, the osmotic adjustment values were changed significantly in many chickpea genotypes. Drought stress is a reason to limit the RWC in plants by reducing the moisture level in the soil (Zaman-Allah et al. 2011a), whereas the tolerant chickpea genotype has developed the process through which these plants can conserve or save water when a plant needs no more water for its growth and development. It was assumed that the previously saved water will be available for later reproductive stages, that is, flowering and podding in chickpea under drought stress. In contrast to tolerant chickpea genotypes, susceptible genotypes were more prone to drought stress because these were users of more water during early vegetative growth stages. Similar findings were recorded in chickpea for water uptake profile against drought stress (Zaman-Allah et al. 2011b). Tolerant and susceptible genotypes had a clear and distinct type of water profile for drought stress. Root traits, that is, root depth and density, had no clear and distinct criteria among tolerant and susceptible genotypes at the time of the reproductive phase. The main fact about tolerance genotypes was that they conserve water during the vegetative stage equally from a stressed and control environment. That conserved water was used to reduce the canopy conductance; thus, the favor was given to the reproductive stage with a successful completion of the life cycle. Therefore, the temporary pattern of water uptake as adopted by the plant roots is more valuable for the development of drought tolerance as compared to root growth. This process can be used to understand the plant behavior, that is, how the plant can maintain its RWC under terminal drought stress.

2.4 Effects at Molecular and Cellular Level

Several stress-responsive genes, such as ABA-regulatory genes and transcription factors, were identified in some model plants as well as in crop plants (Zhu 2000). Stress tolerance is increased through the regulation of drought-responsive genes, either by direct upregulation of the target genes or by regulating the transcription factors of these stress-responsive genes (Haake et al. 2002).

Cell division has a key role in plant growth and developmental processes. Under drought stress, the normal functioning of cell division is reduced (Taiz and Zeiger 2006). Subsequently, cell membrane stability is reduced, which leads to the reduction of cell growth, and finally, growth is reduced. The movement of water from xylem to extended cells is interrupted under severe water deficit conditions. Reactive oxygen species (ROS), such as H_2O_2 (hydrogen peroxide), O_2^- (superoxide), O^- (singlet oxygen), and HO^- (hydroxyl radicals) are produced in plants under a stressed environment (Rahimizadeh et al. 2007). These ROS are highly toxic to plants and can reduce the quality as well as production potential of crops. ROS produce oxidative stress, damage the normal plant metabolism by altering the cellular changes in membranes, nucleic acids, and proteins. ROS disturb the normal metabolism of the cell through protein denaturation, nucleic acid mutation, and lipid peroxidation (Joseph et al. 2011).

Drought stress has a significant effect on the normal functioning of plants, as cell membrane stability, osmotic regulation, RWC, seedling growth rate, and inhibition retention are reduced under drought stress. Tolerant chickpea cultivars (RSG-143-1, RSG-44, ICC-4958) were found to show lesser effects of drought stress as compared to susceptible cultivars (Pant-G-114) that were unable to avoid the drastic effects of drought (Gupta et al. 2000). Electrolyte leakage under drought stress was altered along with other traits of drought significance. Stomatal conductance, the efficiency of PS-II, and RWC are the main factors that were associated with the tolerance of chickpea plants under drought stress (Pouresmael et al. 2013). Thus, these traits should be characterized ahead of several other factors in deciding selection criteria for the identification of drought-tolerant chickpea plants. ROS affect the electron transport chain, chlorophyll content, PS-II protein (D_1), and some molecules of high energy, for example, nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) (Pagter et al. 2005). When the production of ROS is higher than the antioxidant defense system, cellular function is damaged (Almeselmani et al. 2006).

Chloroplasts damaged by overproduction of ROS ruined the protein–pigment complex as well as the thylakoid membrane (Farooq et al. 2017). The presence of these ROS in excess can cause cell death by denaturing the lipids, proteins, and DNA contents. The defense mechanisms of plants are activated under drought stress in response to ROS. These ROS are considered as a secondary messenger for the activation of the defense system in plants. The capability of roots to absorb water is reduced under drought stress. Similarly, translocation of sap in the phloem tissues is also reduced; consequently, a substantial reduction in plant morphological features such as antioxidant activity and nutrient uptake occurs (Armand et al. 2016). The higher accumulation of H_2O_2 and MDA content in the cell under drought stress is an indicator for drought susceptibility (Kaur et al. 2013). Susceptible chickpea genotypes were found with a higher amount of H_2O_2 and MDA content during drought stress. The tolerant genotype exhibited a higher accumulation of SOD and CAT in the cells to reduce the effect of drought stress.

3 Breeding Strategies for Drought Tolerance

Breeding strategies are used for the identification and development of tolerant varieties. The integration between conventional and nonconventional (omics-based) breeding techniques is the ideal way to accelerate the conventional breeding system for drought stress. The role of these techniques is discussed next.

3.1 Conventional and Mutation Breeding

Conventional breeding has been used in plant improvement, especially for yield and resistance to biotic and abiotic stress. As used historically in agriculture by plant breeders, it includes various methods such as introduction, hybridization, and selection for the improvement of plant architecture. The process of introducing genotypes/plants/groups of genotypes into a new environment, where these were not previously being grown, is known as an introduction. Superior varieties are imported from other countries for the improvement in the varietal developmental program. Selection becomes more effective among diverse germplasms. There are two ways to release variety through the introduction: primary and secondary introduction. If the introduced genotype is released directly for the general cultivation without any changing, this is recognized as primary introduction, for example, semi-dwarf rice and wheat varieties. In contrast, the release of introducing variety for general cultivation after making some modification is based on either selection or hybridization with local varieties is known as secondary selection (Allard 1960). The sharing of germplasm across the world is a way to increase genetic diversity and enhance collaboration among the scientific community. Genetic material is exchanged between different international research institutes, including the International Crops Research Institute for the Semi-Arid Tropic (ICRISAT) and the International Center for Agricultural Research in the Dry Areas (ICARDA) to increase genetic diversity in chickpea germplasm. ICRISAT and ICARDA are the major institutes for the chickpea germplasm collection (Table 1). The center of diversity (e.g., Turkey and Syria for chickpea) has a key role in the adaptation of plants in a changing environment.

After introduction, the other breeding method is hybridization, used to combine desirable genes found among two or more parents. Selection of better parents for novel traits in plant breeding is the first step for the genetic improvement and architecture for crop plants. After selection, it is the prerequisite to make a better combination of these traits, to fix the genetic variation. Hybridization is the basic technique, which is used very commonly in plant breeding to attain the desired combination of genes. Desired traits are transferred into the hybrid progeny and subjected to evaluation for better performance by comparing with their parents. Chickpea is a self-pollinating crop, so the rate of natural cross-pollination is very low. Artificial pollination in the chickpea is difficult because it carries small floral parts that are

Table 1 Chickpea seed banks

Sr. No.	Seed bank	Web link	No. of chickpea accessions
1	International Center for Agricultural Research in the Dry Areas (ICARDA)	www.icarda.org/	13,065
2	International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)	https://www.icrisat.org/	18,963
3	United States Department of Agriculture (USDA)	https://www.usda.gov/	6107
4	Plant Genetic Resources, National Agriculture Research Center, Islamabad (NARC)	www.parc.gov.pk/	2243
5	National Bureau of Plant Genetic Resources India	www.nbpgr.ernet.in/	15,986
6	Seed and Plant Improvement Institute Iran	www.spii.ir/HomePage.aspx?lang=en-US	5600
7	Aegean Agricultural Research Institute Turkey	https://arastirma.tarimorman.gov.tr/etae/Sayfalar/EN/Anasayfa.aspx	2063
8	Biodiversity Conservation and Research Institute (Ethiopia)	www.ebi.gov.et/	1156
9	Uzbek Research Institute of Plant Industry (Uzbekistan)	https://www.genesys-pgr.org	726
10	Bangladesh Agricultural Research Institute (BARI)	http://www.bari.gov.bd/	666
11	Plant Gene Resources of Canada (PGRC)	pgrc3.agr.gc.ca/index_e.html	641
12	Institute of Crop Germplasm Resources, CAAS, Beijing, China	http://www.cgris.net/default.asp	567
13	Agricultural Botany Division (Nepal)	https://www.gfar.net/organizations/agriculture-botany-division-nepal-agricultural-research-council	424
14	National Institute for Agronomic Research (INRA) (Morocco)	www.ias.csic.es/medileg/inram.html	332
15	Leibniz Institute of Plant Genetics and Crop Plant Research (IPK)-Atersleben (Germany)	https://www.ipk-gatersleben.de/en/	310

also more delicate and sensitive as compared to other crops. Handling these flowers is not an easy task: only a 10–50% success rate of artificial cross-pollination is reported in chickpea (Salimath et al. 2007).

Crop wild relatives are the imaginary source of genetic variation. These relatives are found in the different surroundings around the world, mostly in the threatened areas of degradation. The conservation of genetic variation through ex situ or in situ means is very important for the security of wild relatives. To overcome the status of

limited variability, wild relatives were exploited in a breeding program for the development of new varieties with better yield, adaptation, and resistance. Wild relatives are considered as a reservoir of variation for crop plants, that is, a potential source of adaptation that has been declining gradually in the domestic germplasm. For the improvement of genetic variability, intra specific and wide hybridization techniques have been used in cultivated chickpea. The genus *Cicer* carries nine annual and 34 wild perennial species. Among these annual species, only *Cicer arietinum* has been cultivated until now. Information about the genetic relationship of cultivated and wild species is the prerequisite, to study the evolutionary process of cultivated species as well as wild species. Wild species can be used potentially by understanding the crossing compatibilities, cytogenetic affinities, and chemotaxonomic associations between wild and cultivated plants. Methods such as interspecific hybridization, isozymes, molecular markers, and karyotypes have been used for the investigation of the relationship between wild and cultivated species of the genus *Cicer* (Hawkes 1977).

Resistant genes are mostly present in wild species, against biotic and abiotic stresses. The introgression of these resistant genes into the domestic chickpea through breeding is used for the development of tolerant varieties (Hufford et al. 2013). Therefore, the concept of 'crop re-synthesis' was widely used to recover and develop actual resistance in the plant. The development of effective hybrids by using wild relatives also has some restrictions in chickpea. Hybrid breakdown and sterility accompany a diverse group of *Cicer echinospermum* (Kahraman et al. 2017). Differential genetic loci among wild relatives are the source of sterility. The cross between cultivated and wild relatives (*Cicer echinospermum*) was studied in chickpea. The genetic diversity of chickpea can be enhanced via distinct and wide hybridization.

A comparison was made for drought stress between cultivated (*Cicer reticulatum*, *Cicer pinnatifidum*, *Cicer echinospermum*) and wild (*Cicer songaricum*, *Cicer oxydon*, *Cicer anatolicum*, *Cicer montbretii*, *Cicer microphyllum*) chickpea plants (Toker et al. 2007). Perennial species were more tolerant to drought stress than annual and cultivated species of chickpea. *Cicer anatolicum* was used to create drought resistance in cultivated chickpea because it has high affinities for compatibility with cultivated chickpea for the breeding program. Distant hybridization between cultivated and wild chickpea (*Cicer reticulatum*) was effectively exploited for the introgression of genes associated with drought tolerance (Hajjar and Hodgkin 2007).

On the other hand, mutations are the prominent source of de novo variation, particularly in self-pollinated crops. By using mutation breeding, the genetic makeup of chickpea can be diversified with the objective of increasing yield and resistance to biotic as well as abiotic stress. The mutation is used to generate desired treats in crop plants, by using chemical or physical mutagens. Chickpea cultivars were also released as a commercial variety, developed through mutation breeding (Salimath et al. 2007). Mutation breeding is recognized as a beneficial technique for broadening genetic variability and adaptability in self-pollinated as well as cross-pollinated crops.

The reverse genetic approach has a significant role in mutation breeding. In this technique, the development of a nearly isogenic line and mutants is the potential source for functional genomics (Ali et al. 2016). On the basis of evaluation through

induced mutation, *Cicer reticulatum* is documented as a drought-tolerant chickpea accession (Toker et al. 2007; Toker 2009). The Nuclear Institute for Agriculture and Biology (NIAB) is working on chickpea mutation breeding in Pakistan. Desi and Kabuli chickpea varieties of NIAB, developed through mutation, have resistance to biotic and abiotic stresses with high yield. A significant increment in the production, as well as tolerance against biotic and abiotic stresses, were reported (Haq 2009). According to the report of the International Atomic Energy Agency (IAEA), CM-72, -88, -98, and -2000 were developed by using physical mutagens at the rate of 150 Gy, 100 Gy, 300 Gy, and 150 Gy γ-rays, respectively (Table 2). On the other hand, CM-2008 was developed by using a chemical mutagen, 0.2% EMS (ethyl methanesulfonate). These varieties were high yielding and resistant to diseases such as blight and wilt in chickpea (Maluszynski 2001; Lestari 2016). The use of these varieties in a breeding program is associated with the genetic variability of chickpea against stresses. Conventional breeding methods are the basic ways for plant breeding, but omics-based breeding can be used as a supplement to increase the efficiency and worth of conventional breeding by reducing time and targeting exactly the desired genes against drought stress. Thus, the integration between these breeding methods is the key to develop drought-tolerant chickpea accessions.

Table 2 Mutant varieties of chickpea

Sr. no.	Variety name	Year	Country	Improved characters
1	Hyprosola	1981	Bangladesh	Early maturing, higher yielder, more biomass
2	CM-72	1983	Pakistan	Blight resistant and high yielding
3	Kiran	1984	India	Early maturing and salt tolerance
4	Pusa-408	1985	India	Blight resistance, high yield
5	India	1985	India	Wilt resistant and >2 seeds/pod
6	Pusa-417	1985	India	Wilt and pod borer resistant
7	NIFA-88	1990	Pakistan	Earlier maturing, high yield (15–20%) and N_2 fixation
8	Line-3	1992	Egypt	High yielding with profuse branches
9	CM-88	1994	Pakistan	*Ascochyta* and *Fusarium* resistance with high yield
10	NIFA-95	1995	Pakistan	Bacterial blight resistance
11	CM-98	1998	Pakistan	*Ascochyta* and *Fusarium* resistance
12	CM-2000	2000	Pakistan	High yield and resistance to diseases
13	Hassan-2K	2000	Pakistan	High yielding, bacterial blight resistance
14	Binasola-3	2001	Bangladesh	Early maturity
15	BGM-547	2005	India	Bold seed size
16	Pusa-547	2006	India	High yielding, *Ascochyta* and *Fusarium* resistance
17	TAEK-SEGAL	2006	Turkey	High yielding and *Ascochyta* resistance
18	CM-2008	2008	Pakistan	Bold seed and *Fusarium* resistance
19	THAL-2008	2008	Pakistan	*Fusarium* resistance, large seed size
20	Binasola-5	2009	Bangladesh	Early maturing and high yielding
21	Binasola-7	2013	Bangladesh	Tall, greater 100-seed weight
22	Binasola-9	2016	Bangladesh	High yielding and suitable for late sowing
23	Binasola-10	2016	Bangladesh	High yielding and early maturing

3.2 Omics Approaches

Omics approaches are collectively intended for the quantification and characterization of biological molecules, which are translated for various purposes, such as structure, dynamics, and function of different organisms. Different types of omics—genomics, transcriptomics, proteomics, metabolomics, ionomics, and phonemics—have been widely used for the characterization of different responses in plants, under varying environmental conditions. Large-scale genomic resources are produced with the invention of NGS and genotyping technologies such as genomics, transcriptomics, proteomics, and BAC-end sequences (BESs) in chickpea (Varshney et al. 2013a). "Omics" is one of the most imperative fields of science, standardized in recent years. This approach facilitates the identification of novelty genes, proteins, and metabolites against any stress, including drought stress. Functional characterization of the desired genes is also done by using omics approaches (Zargar et al. 2011). Similarly, omics predict the assignments of the genes, proteins, and metabolites, and assess the alterations in plants produced by different environmental conditions (Baginsky et al. 2010). Advances in genomics increased after the postgenomic era as semi-quantitative RT-PCR, real-time polymerase chain reaction (PCR), massively parallel signature sequencing (MPSS), serial analysis of gene expression (SAGE), microarray technologies, and currently NGS-based genome-wide transcriptome analysis via RNA-Seq have become more prominent and comprehensive for the identification of stress-responsive genes in plants (Singh et al. 2015). Although omics-based breeding is a comprehensive and efficient approach for plant breeding, the integration of omics-based breeding strategies with conventional breeding is seen to be effective. These techniques work parallel to each other for the improvement of drought tolerance in chickpea (Fig. 1).

3.2.1 Genomic Resources

Genomic resources have become an effective source for omics studies because of the availability of efficient genomic tools in the recent era. The genome size of chickpea is comparatively small among other legumes, such as faba bean, soybean and lentil. Thus, small genome size, as well as accessibility to NGS, offers a platform for the development of chickpea genomic resources. Plant genomics resources are emerging by the gradual development of scientific technologies in recent years, resulting in the creation of genomic resources publicly for major crops as well as for minor crops. A range of genomic resources can be retrieved through various public databases, such as the National Center for Biotechnology Information (NCBI), having indices of ESTs of different plant species for abiotic stress tolerance. The NCBI database has information vis-à-vis genetic maps, DNA markers, and transcriptome assemblies, available for various crops publicly, as in chickpea. Similarly, SNPs databases as well as some other important genomic resources were developed and notably used to enhance the working efficiency of the breeding program for

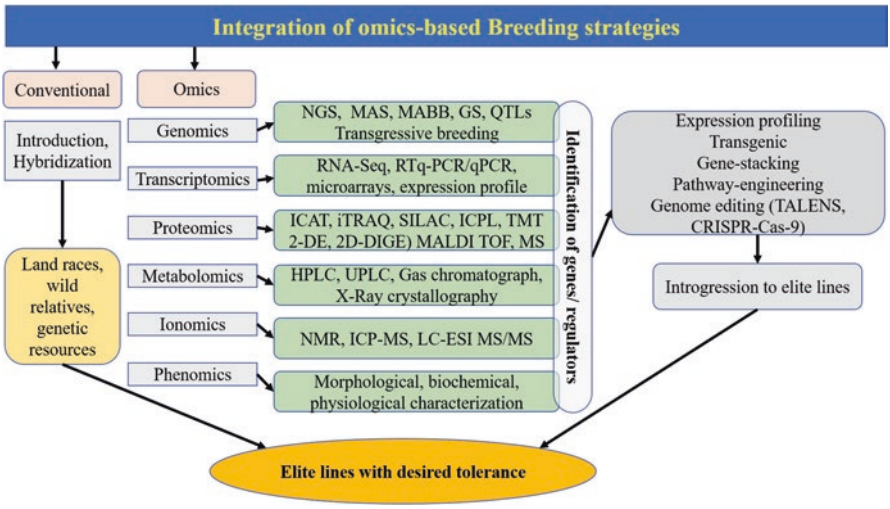

Fig. 1 Integration of omics-based breeding strategies for the development of drought tolerance in chickpea

crop improvements (Table 3). Different genetic applications such as marker-assisted backcrossing, marker-assisted recurrent selection, genomic selection, quantitative trait loci (QTL) mapping, and gene/QTL pyramiding have been massively explored and have an association with genomic techniques. Nevertheless, DNA markers are the more pertinent techniques, used in plant breeding practices, for germplasm characterization, seed purity determination, phylogenetic analysis, F_1 evaluation, and particularly for marker-assisted breeding programs (Collard and Mackill 2007). The reference genomes of chickpea varieties Desi and Kabuli were used for the improvement in assemblage and annotation of the chickpea genome (Gupta et al. 2016; Parween et al. 2015). The whole genome-wide analysis is more effective and easier, as supported by high-throughput whole-genome resequencing technologies. Polymorphisms and InDels were identified in the chickpea genome for drought stress. On the basis of whole-genome resequencing technology, different genomic regions related to nitrogen fixation and yield under drought and heat stress in the field were identified in chickpea (Sadras et al. 2016). Moreover, quick progress was made in the ultradense genetic map: whole genome-wide resequencing, genotyping by sequencing for the creation of novel single nucleotide polymorphisms (SNPs), whole-genome resequencing, mapping, and identification of potential candidate regions on the genome through NGS-based bulk segregation analysis, genome-wide association studies, and epi-genomic properties were attained (Jha 2018; Garg et al. 2016). The genomic region related to drought stress (QTLs) has significant information for the improvement of chickpea genotypes. Several QTLs associated with drought stress tolerance have been identified in chickpea (Table 4).

In this scenario, different studies have been conducted for the identification of QTLs in chickpea. The very first identification of QTLs-linked markers under

Table 3 Chickpea databases

Sr. No.	Database	Web link	Description
1	Chickpea Transcriptome Database (CTDB)	https://www.nipgr.res.in/ctdb.html	Provides information for chickpea transcriptome
2	Legume IP	http://plantgrn.noble.org/LegumeIP	Currently hosts large-scale genomics and transcriptomics data of legumes
3	National center for Biotechnology Information (NCBI)	https://www.ncbi.nlm.nih.gov/search/all/?term=Chickpea	Associated with several databases with biotechnology and biomedicine and major source for bioinformatics tools and services
4	Legume Information System (LIS)	https://legumeinfo.org/organism/Cicer/arietinum_CDCFrontier	Provides access to genetic and genomic information for major legumes
5	Kyoto Encyclopedia of Genes and Genomes (KEGG)	https://www.genome.jp/kegg/	High-level functions, such as the cell, the organism, and the ecosystem, from molecular-level information
6	Cool Season Food Legume Crop Data Base Resources	https://www.coolseasonfoodlegume.org/organism/Cicer/arietinum	Carries three versions of chickpea genome
7	Chickpea Micro-Satellite Database (CicArMiSatDB)	https://cegresources.icrisat.org/CicArMiSatDB/	Have information of SSR from the sequenced genome
8	Chickpea SNP In-Del Data Base (CicArVarDB)	https://cegresources.icrisat.org/cicarvardb/	Deals with variations around specific regions linked with QTLs
9	Chickpea Root Expressed Sequence Tag Database	https://www.icrisat.org//?s=Chickpea+Root+Expressed+Sequence+Tag+Database	Contain information about ICRISAT's root EST database
10	CicerTransDB	http://www.cicertransdb.esy.es/	Concerned with motif architecture, domain, and gene ontology

Table 4 Summary of quantitative trait loci (QTLs) linked with drought stress tolerance in chickpea

Traits	Linkage group	Markers	Phenotypic variation explained (PVE) (%)	Reference
SSR markers				
Grain yield	1, 4	H5A08-TA8, H1B17-TA72	–	Rehman et al. (2011)
Days to flowering	1, 3, 4, 8, 8	H5A08-TA8, TA6-NCPGR12, TA132-GA137, TA159-GA6	15, 22, 5, 8	
Days to maturity	1, 3, 7	H5A08-TA8, TA6-NCPGR12, TA28-CaSTMS25	13, 22, 5–6	
Reproductive period	1, 3, 7	H5A08-TA8, TA6-NCPGR12, A28-CaSTMS25	6, 6, 5–6	
Plant height	1, 4	H5A08-TA8, H1B17-TA2	24, 7	
Harvest index	1, 3	H5A08-TA8, TA6-NCPGR12	13	
Drought tolerance score	1, 3, 7, 8	H5A08-TA8, TA6-NCPGR12, TA28-CaSTMS25, TS12-TA118	13	
Stomatal conductance	7, 7, 3	TA28-CaSTMS25, TA180-H1A10 (290bp), TA125-NCPGR10	7, 8, 9	
Canopy temperature differential	1, 3, 4, 6, 6, 6	H5A08-TA8, TA6-TS58, GA137-TA46, TR7-TA14, TA80-GA21, TA80-GA21, GA21-CaSTMS2	15, 7, 13, 8–13, 8–13, 8–13	
Drought resistance score	3, U, 3, 3, 3, 4, 3	H6C-07, H4D-11, NCPGR-81, H1B-04, H6C-07, H5G-01, H6C-07	23.3, 10.9, 6.4, 6.7, 12.5, 5.1, 15.2	Hamwieh et al. (2013)
100-seed weight	3, 3, 7, 5, 3, 4, 3	NCPGR-50, TR-50s, SCEA19, TAA-58, TS-43, H1H-15, TA-11	17.2, 6.4, 7.1, 5.9, 7.4, 11.8, 7.3	
Pod number	3, 7, 3, 3, U, 4, 3	H6C-07, TAA-55, H3G-09, H1B-04, TS-19, H5G-01, H6C-07	22.7, 9.6, 7.4, 7.3, 5.1, 6.1, 5.3	
Days to flowering	3, 4, 3, 4, U, 3, U, U, 3	H1F-14, H5G-01, H6C-07, H1B-17, TS-19, H4G-07, TS-19, GA-16, H6C-07	5.1, 5.3, 17.7, 7.8, 6.6, 9.7, 6.5, 5.3, 24.2	
Grain yield	3, U, 4, 3, 3, 3	H6C-07, H5E-02, H5G-01, H6C-07, H1B-04, TR-31	12.4, 6.7, 6, 5.3, 5.4, 6.4	
Plant height	3, 2, U, U, 2, U, 7, U, 1, U	TA-179, TA-103, TA-76, TA-76, TA-96, TA-96, TA-76, TAA-55, TA-76, TA-203, TA-113	8.8, 5.9, 5.3, 6.1, 8.5, 5.1, 5.1, 4.8, 7.3, 7.3	
Days to maturity	1, 3, 3, 3, U, 3, U, U, 2, 3, 3, 3, 4, 4, U, 3	TA-203, H6C-07, H6C-07, H6C-07, NCPGR-42, H6C-07, NCPGR-42, TA-113, H2B-061, STMS-21, H6C-07, H6C-07, H1B-17, H5G-01, TS-19, H6C-07	7, 6.6, 13.7, 12.8, 7.9, 7.4, 6.6, 5.5, 5.6, 11.6, 20.3, 6.5, 6.1, 9.1, 10.6	

Harvest index	U, 2, 3, 5, 3, 3, 4, 1, 1	TA-113, TR-58, H6C-07, H1F-21, H6C-07, H6C-07, H5G-01, TA-203, TA-1	11.4, 8.3, 14.4, 6.2, 8.4, 9.1, 6.3, 9.5, 6.3	Hamwieh et al. (2013)
Percentage of empty pods	U, 2, U, 1, U, 2	TA-113, H1O-06, SCOM, TA-1, H5E-02, H1O-06	9.8, 12.5, 5.1, 5.6, 8.3, 7.1	
Empty pods	3, U, 3, 2, 3	GA-119, TS-19, TR-50 s, H1F-22, GA-6	12.7, 6.9, 6.9, 6.2, 5.6	
Seed number	3, U, 3, 4, 3, 3	H6C-07, H5E-02, H1B-04, H1B-17, H3G-09, H6C-07	14, 5.7, 6.5, 5.8, 7.1, 7.5	
Biological yield	U, 7, 3, 3, U	H5E-02, NCPGR-33, H3G-09, TR-31, TS-19	7.6, 5.6, 8.8, 8.6, 7.3	
Root weight	4, 4, 4	ICCM0249, TAA170, STMS11	58.20, 8.20, 58.20	Varshney et al. (2013a)
Root length density	4	NCPGR127–NCPGR21 A	10.90	Varshney et al. (2014a)
Root surface area	6, 4	TA106–H1I16, TAA170–NCPGR21 A	10.26, 16.67	
Shoot dry weight	4	TAA170–NCPGR21 A	17.59	
Plant height	3, 3, 4, 4, 8	TA34–NCPGR49, TA34–NCPGR49, NCPGR127–CPGR21A, NCPGR127–NCPGR21A, NCPGR164–CaM2187	10.00, 10.00, 30.20, 30.20, 14.73	
Days to 50% flowering	4, 8	NCPGR127–TAA170^{A}, NCPGR164–CaM1918	24.49, 26.87	
Days to maturity	6, 8, 4	TA106–CaM0399, NCPGR164–CaM1918, NCPGR127-TAA170A	12.13, 18.83, 19.71	
Pods per plant	4	NCPGR127–NCPGR21	23.18	
Seeds per pod	4	TAA170–NCPGR21 A	42.07	
100-seed weight	1, 4	NCPGR184–ICCM0009b, NCPGR127–NCPGR21 A	10.31, 58.20	
Biomass	4, 8	NCPGR127–NCPGR21 B, NCPGR164–CaM1918	21.32, 10.95	
Harvest index	4, 1, 1	TAA170–NCPGR21 B, cpPb-679915–CaM0393, NCPGR184–ICCM0009b	11.69, 14.36, 10.67	
Yield	1, 4	NCPGR136–CaM0046, TAA170–NCPGR21 A	13.98, 15.72	
Drought tolerance index	1	cpPb-679915–CaM0046	11.23	
SNP marker				
Drought tolerance index	1	cpPb-679915–CaM0046	11.23	Thudi et al. (2014b)
Root length density	4	NCPGR127–NCPGR21 A	10.90	
Root surface area	6	TA106–H1I16	10.26	
Root/shoot ratio	4	TAA170–NCPGR21 A	16.67	

(continued)

Table 4 (continued)

Traits	Linkage group	Markers	Phenotypic variation explained (PVE) (%)	Reference
Root length density	4	ICCM0065–Ca4_11276225	10.65–12.09	Jaganathan et al. (2015)
Root surfacearea	4	Ca4_13840227–NCPGR	11.04	
Root dry weight	4	Ca4_13840227–NCPGR	10.85–13.56	
Shoot dry weight	4	Ca4_13840227–TAA170	13.89–17.59	
Plant height	4	Ca4_12982420–TAA170	10.78–26.91	
Primary branches	8	CaM0812–NCPGR164	10.05–34.57	
Days to 50% flowering	4	NCPGR164–Ca8_3050452	112.92	
Days to maturity	7	NCPGR164–Ca8_3050452	10.11–47.43	
100-seed weight	4	Ca4_13687456–TAA	10.12–60.41	
Biomass	4	NCPGR164–Ca8_3050452	10.11–16.63	
Harvest index	8	NCPGR164–Ca8_3050452	10.14–25.94	
Pods/plant	8	Ca4_13687456–TAA17	10.73–32.34	
Seeds/pod	4	–	11.09–45.40	
Yield	4	–	11.67–18.64	
Drought susceptibility index	4	–	13.00	
Drought tolerance index	4	–	10.10–10.76	
Root length density	4	bin_4_13239546–bin_4_13378761	10.36	Kale et al. (2015)
Root dry weight/total plant dry weight	4	bin_4_13393647–bin_4_13547009	20.09	
shoot dry weight	4	bin_4_13393647–bin_4_13547009	25.22	
Plant height	4	bin_4_13239546–bin_4_13378761	41.76	
Primary branches	8	bin_8_6034209–bin_8_5984553	11.27	
Days to 50% flowering	8	bin_8_6034209–bin_8_5984553	44.76	
Days to maturity	7	bin_7_12870961–bin_7_12856579	45.38	
100-seed weight	4	bin_4_13239546–bin_4_13378761	59.83	

Delta carbon ratio	4	bin_4_13239546–bin_4_13378761	11.90	
Harvest index	8	bin_8_6034209–bin_8_5984553	15.42	
Pods/plant	4	bin_4_13239546–bin_4_13378761	16.66	
Plant vigor	4	Bin_4_13239546-Bin_4_13378761	–	Sivasakthi et al. (2018)
3D-leaf area	1, 3, 4, 4, 4, 6, 5,	Bin_1_16247689-Bin_1_16256900, Bin_3_38153326-Bin_3_38067857, Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_6_59002540-Bin_6_45589823, Bin_5_7175460–Bin_5_5089713	53, 5, 11, 12, 19, 6	
Projected leaf area	4, 4, 4	Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_4_13048918-Bin_4_13097584	8, 9, 11	
Plant height	1, 1, 2, 2, 2, 2, 2, 3, 3, 3, 4, 4, 4, 4, 5, 7, 7, 7, 7, 5, 7, 7, 7, 7	Bin_1_46320971-Bin_1_46550755, Bin_1_4992190–Bin_1_4870121, Bin_2_6254554–Bin_2_9665288, Bin_2_30506636-Bin_2_30527477, Bin_2_30506636-Bin_2_30527477, Bin_2_6254554–Bin_2_9665288, Bin_2_6254554–Bin_2_9665288, Bin_3_33718067-Bin_3_33467733, Bin_3_18480135-Bin_3_18295791, Bin_3_18480135-Bin_3_18295791, Bin_4_18035954-Bin_4_18295099, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_5_31281006-Bin_5_31262177, Bin_7_1934083–Bin_7_1901490, Bin_7_2149821–Bin_7_2075414, Bin_7_12083724-Bin_7_11984393, Bin_7_12083724-Bin_7_11984393	7, 5, 9, 6, 6, 10, 11, 8, 9, 9, 5, 34, 36, 39, 5, 7, 6, 10, 11	
Plant height growth rate	4, 4, 4, 4, 7	Bin_4_3535309–Bin_4_3596208, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_4_13239546-Bin_4_13378761, Bin_7_2149821–Bin_7_2075414	4, 13, 23, 23, 10	
Shoot dry weight	4, 4, 5, 5	Bin_4_13393647-Bin_4_13547009, Bin_4_13393647-Bin_4_13547009, Bin_5_42970924-Bin_5_43185799, Bin_5_24533589-Bin_5_20895327	9, 18, 5, 6	
Specific leaf area	3, 4, 4, 4	Bin_3_33718067-Bin_3_33467733, Bin_4_12586721-Bin_4_12650792, Bin_4_16602407-Bin_4_16662055, Bin_4_12586721-Bin_4_12650792	7, 6, 4, 8	
Specific leaf weight	5, 1, 4, 4, 4	Bin_5_31507607-Bin_5_31449929, Bin_1_9838522–Bin_1_9855212, Bin_4_48635948-Bin_4_48732122, Bin_4_48635948-Bin_4_48732122, Bin_4_13393647-Bin_4_13547009	5, 5, 5, 4, 11	

(continued)

Table 4 (continued)

Traits	Linkage group	Markers	Phenotypic variation explained (PVE) (%)	Reference
Evapotranspiration	4, 4, 5, 6	Bin_4_11992806-Bin_4_12061305, Bin_4_12586721-Bin_4_12650792, Bin_5_41290102-Bin_5_41678447, Bin_6_55893751-Bin_6_54270280	6, 7, 5, 5	
Evapotranspiration rate	3, 4, 4, 4, 7	Bin_3_29064360-Bin_3_28987359, Bin_4_47733094-Bin_4_47796904, Bin_4_10416329-Bin_4_10421332, Bin_4_11210420-Bin_4_11690035, Bin_7_32532047-Bin_7_32710042	8, 5, 8, 11, 5	
Transpiration	4, 4, 5, 1, 1, 2, 3, 7,	Bin_4_12321889-Bin_4_12331005, Bin_4_12321889-Bin_4_12331005, Bin_5_43185799-Bin_5_43439397, Bin_1_45932222-Bin_1_45934626, Bin_1_10100492-Bin_1_10111521, Bin_2_2241242–Bin_2_4309038, Bin_3_29064360-Bin_3_28987359, Bin_7_19452801-Bin_7_16741421	9, 8, 5, 5, 5, 5, 10, 5	
Plant vigor	1, 3, 3, 3, 4, 4, 4, 4	ICCM0009a-STMS21, NCPGR268-NCPGR255, CaM1024-NCPGR49, ICCeM050-TS58s, H4G11-TR20, ICCM0249-NCPGR127, TAA170-NCPGR21, GA24-STMS11	3, 3, 5, 4, 3, 7, 44, 13, 4, 10	
3D-leaf area growth rate	8, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4,	CaM1918-NCPGR170, NCPGR127-TAA170, TAA170-NCPGR21, GA24-STMS11, TAA170-NCPGR21, TR11-GA24, CaM1903-ICCM0249, TAA170-NCPGR21, TR11-GA24, TR11-GA24, TR11-GA24	10, 14, 18, 8, 23, 11, 5, 6, 6, 8, 8	
Projected leaf area	4, 4, 4, 4, 4, 4, 4	CaM1903-ICCM0249, NCPGR127-TAA170, TAA170-NCPGR21, GA24-STMS11, ICCM0249-NCPGR127, TR11-GA24, TAA170-NCPGR21	9, 12, 13, 7, 5, 6, 13	
Leaf area growth rate	4, 7	TAA170-NCPGR21, CaCISP117-STMS12	7, 5	
Plant height	1, 1, 4, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6, 6, 6, 6, 6, 6, 7, 7, 7, 7, 7, 7, 7, 8, 8, 8	ICCM0009a-STMS21, cpPb-490962-cpPb-677672, ICCM0249-NCPGR127, TAA170-NCPGR21, TR11-GA24, ICCM0249-NCPGR127, TAA170-NCPGR21, GA24-STMS11, GA24-STMS11x, TAA170-NCPGR21, TR11-GA24, TA120-CaM0389, TR7-CaM1790, NCPGR202-CaM1760, NCPGR4-TA106, ICCeM051-TR7, NCPGR202-CaM1760, CaM0111-NCPGR99, TA76s-TR24, CaM2155-CaM0111, TA76s-TA142, CaM2155-CaM0111, TA180-ICCM197a, TA76s-TA142, CaM1918-NCPGR170, H1H14-TA25, H1H14-TA25	8, 4, 5, 23, 9, 10, 36, 15, 8, 32, 14, 5, 5, 4, 4, 4, 3, 6, 4, 6, 5, 5, 4, 5, 3, 3, 3	
Plant height growth rate	4, 4, 4, 4, 4, 4, 4, 8, 8	TR11-GA24, TAA170-NCPGR21, TAA170-NCPGR21, GA24-STMS11, ICCM0249-NCPGR127, TAA170-NCPGR21, TR11-GA24, CaM1918-NCPGR170, CaM1918-NCPGR170	9, 11, 20, 12, 4, 25, 14, 4, 5	

R-3D/PLA	1, 1, 4, 4, 4, 4, 4, 4, 4, 4, 4, 6, 6, 6, 6, 6, 6, 7, 7, 7, 7, 7, 7, 7, 7, 7, 7	cpPb-677672-cpPb677690, cpPb-677672-cpPb677690, CaM0507-CaM2093, cpPb-682025-CaM1328, CaM1684-H1A19, CaM0507-CaM2093, cpPb-682025-CaM1328, CaM0507-CaM2093, cpPb-682025-CaM1328, CaM1903-ICCM0249, TAA170-NCPGR21, STMS2-NCPGR93, TA106-H1I16, NCPGR4-TA106, TA106-H1I16, TA106-H1I16, GA26-CaM0399, NCPGR19-CaM2041, cpPb-490874-CaCISP117, TA180-ICCM197a, NCPGR19-CaM2041, CaM1417-ICCM199c, CaCISP117-STMS12, CaM1942-CaM2155, NCPGR19-CaM2041, CaCISP117-STMS12, CaM0111-NCPGR99	6, 5, 11, 12, 13, 11, 9, 15, 13, 10, 5, 4, 8, 6, 7, 10, 10, 6, 10, 4, 6, 7, 7, 4, 6, 9, 5	
Shoot dry weight	4, 4, 4, 4, 4, 4, 4	TR11-GA24, TAA170-NCPGR21, ICCM0249-NCPGR127, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170, NCPGR21-TR11	8, 11, 6, 11, 11, 20, 10	
Specific leaf area	4, 4, 4, 6, 7	TAA170-NCPGR21, ICCM0249-NCPGR127, NCPGR127-TAA170, NCPGR202-CaM1760, ICCM197a-CaM1567	5, 7, 8, 7, 7	
Specific leaf weight	3, 4, 4, 4	TR2-NCPGR10, TAA170-NCPGR21, NCPGR127-TAA170, TAA170-NCPGR21	5, 7, 5, 7	
Leaf area index	2, 4, 4, 4, 4, 4, 4	H1P092-CaM0142, CaM1903-ICCM0249, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170	5, 5, 9, 7, 9, 10, 16	
Evapotranspiration	4, 4, 4, 4, 6, 7	CaM1903-ICCM0249, NCPGR127-TAA170, ICCM0249-NCPGR127, NCPGR127-TAA170, H4H06-NCPGR200, CaCISP117-STMS12	6, 8, 7, 11, 6, 6	
Evapotranspiration rate	4, 4, 4, 4, 4, 7, 7, 7	NCPGR127-TAA170, NCPGR21-TR11, STMS11-cpPb680552, NCPGR21-TR11, STMS11-cpPb680552, TA142-TR24, CaM1567-NCPGR141, TA142-TR24	8, 5, 10, 6, 11, 6, 7	
Transpiration	4, 4, 4, 5, 5, 6, 7, 8, 8	ICCM0249-NCPGR127, NCPGR127-TAA170, cpPb-322921-TA132, cpPb-682328–NCPGR189, cpPb-682328–NCPGR189, CaM1125-H1F21, TA180-ICCM197a, CaM2187–CaM1918, NCPGR164-CaM2187	7, 12, 4, 8, 11, 4, 8, 7, 14	
Transpiration rate	7, 7, 7, 7, 7	NCPGR19–CaM2041, TA142-TR24, NCPGR19-CaM2041, NCPGR19-CaM2041, NCPGR19-CaM2041	11, 7, 10, 10, 10	

drought stress conditions was reported in RILs (recombinant-inbred-lines) of chickpea (Chandra et al. 2004). These RILs were developed from a cross between ICC-4958 (a drought-tolerant chickpea cultivar with deep roots and high biomass) and Annigeri (a drought-susceptible chickpea genotype). Although only 14 SSR markers were used to identify the marker-trait associations for root dry weight, shoot dry weight, and root length, this study described the usefulness of statistical models to identify the QTL-linked markers without a linkage map. Later, another study identified 15 genomic regions for various traits under terminal drought conditions by using 97 SSR markers (Rehman et al. 2011). These regions were identified from a RIL population, developed from a cross of drought-tolerant (ILC 588) and susceptible (ILC 3279) genotypes that were phenotyped for 2 consecutive years across two locations. Stomatal conductance and canopy temperature were the most vital traits associated with drought tolerance. Stomatal conductance and canopy temperature had three and six QTLs respectively and the range of phenotypic variation was 7–15%. These regions can be potentially used in a breeding program for the development of drought-tolerant chickpea genotypes.

Another RIL population of 181 lines developed by a cross of ILC5889 and ILC3279 was used for the identification of drought-linked QTLs by using 77 SSR markers (Hamwieh et al. 2013). The evaluation of these lines was done in ten environments across three different locations under different drought treatment levels. As a result, 93 genomic regions associated with 12 drought-tolerant-related traits (plant height, days to flowering, maturity, etc.) were identified in these RIL populations of chickpea. The QTLs associated with days to flowering had maximum phenotypic variation i.e. 24%. After pooling the data of QTLs obtained from drought and irrigated environments, it was observed that the QTLs of the drought resistance were significantly expressed under drought stress, whereas they had no strong expression under normal well-watered conditions. The very highest contribution from the allele "A" of marker H6C07 was reported as 80% and 29.8% for late planting and drought stress, respectively. That range of the contribution from a single allele is a much higher amount that can be used for the development of drought-tolerant chickpea genotypes.

Another study used 82 different molecular markers (19 ISSR, 28 RAPD, 38 STMS) for the identification of QTLs linked to drought tolerance, by using an intraspecific $F_{2:3}$ population developed from ILC32799 and ICCV2 (Jamalabadi et al. 2013). Among these markers, only 52 were finally mapped on the eight linkage groups. Morphological traits such as plant height, days to flowering, and 100-seed weight were phenotypically evaluated against drought stress. ISSR and RAPD markers exhibited the high segregation distortion as compared with STMS markers. Similarly, 26 of 82 markers were unlinked, and among these markers, the most common were ISSR and RAPD. The phenotypic variation was 32%, 29%, and 51% for QTLs associated with days-to-flowering, plant height, and 100-seed weight, respectively. Similarly, the RIL population of desi chickpea (ICC4958 × ICC1882) was used for the identification of a QTL-hotspot, associated with a deep root system under drought stress (Varshney et al. 2013a). QTL mapping revealed the association of three SSR markers, namely, TAA170, ICCM0249, and STMS11, with the QTL-

hotspot region. That QTL-hotspot region was found in linkage group-4, linked with root traits, and had shown 58.20% explained phenotypic variation.

Advances in genome sequencing techniques, such as NGS, provide easier and cheaper ways to sequence the genome. Thus, QTL mapping by using high-throughput sequencing tools has been shifting toward precise and quick markers such as SNPs. In the chickpea, association mapping was used for the identification of drought-related QTLs by using 300 diverse accessions (Thudi et al. 2014b). The distribution of diversity array technology (DArT) markers was equal across the genome of chickpea. These markers were used to explain the population structure, and three subpopulations were recognized by means of the admixture model in STRUCTURE. Association mapping was performed by using 1872 markers proportionally divided as 36 SSR, 113 gene-based SNPs, 651 SNPs, and 1072 DArTs. Subsequently, 312 marker-trait associations (MTAs) were recognized, the highest number of MTAs being 70, associated with 100-seed weight. The number of identified SNPs was 18, recognized from five different genes and associated with the drought-tolerant traits. The identified MTAs were the significant and potential source for the development of drought tolerant chickpea by improving the traits associated with these MTAs.

Later, a study was conducted by using two populations, ICCrIl03 (ICC 4958 × ICC1882) and ICCrIl04 (ICC 283 × ICC 8261), and subjected to phenotypic screening against drought stress by using 20 different yield- and drought-related traits for seven seasons across five different locations in India (Varshney et al. 2014b). Different type of QTLs, main-effect QTLs (45), epistatic QTLs (973), and drought tolerance-linked QTLs (9), were identified from these populations. One cluster had 48% of robust main-effect QTLs associated with 12 parameters. That cluster was present on the CaLG04, explaining 58.20% of phenotypic variation, and defined as "QTL-hotspot." This genomic region had seven SSR markers associated with drought stress, and the introgression of that genomic region into the chickpea accession would be effective for a breeding program.

On the other hand, bacterial artificial chromosome (BAC) libraries were developed to construct the physical map of chickpea against drought-stress (Varshney et al. 2014a). Two genetic maps, associated with the physical map, were developed by using SSR markers and derived through BAC-end sequencing. Of 337 BES-SSRs, 259 markers were used for the genetic map and integrated into three populations, one inter-specific and two intra-specific mapping populations. The number of identified QTLs was 654 in the QTL hotspot region linked with drought tolerance.

Moreover, this already identified QTL-hotspot on CaLG04 was further explored for the identification of drought-linked genomic regions by using the advanced sequencing tool, genotyping by sequencing (GBS) in a chickpea RIL population (ICC 4958 × ICC 1882) (Jaganathan et al. 2015). The RIL population were phenotyped for 20 drought-related traits within 7 years. Through GBS, data were generated from the parent (ICC-4958, 6.24 Gb, and ICC-188, 25.65 Gb) and RILs population (59.03 Gb), and 828 unique SNPs were identified for the genetic map. A QTL hotspot was found with 49 SNP markers harboring drought tolerance. Cumulatively, 164 main-effect QTLs with 24 unique QTLs were also identified in

the hotspot. The identified SNPs were also converted into cleaved amplified polymorphic sequence (CAPS) and derived CAPS (dCAPS) markers. The markers can enhance the efficiency of marker-assisted breeding in chikcpea.

The same QTL-hotspot (CaLG04) region was further explored by using the two approaches, QTL and gene enrichment analysis among 232 RILs, developed by the single-seed-descent method (ICC-4958 × ICC-1882) within five seasons and across the five locations in chickpea (Kale et al. 2015). QTL identification was done for 17-drought-related traits along with two drought-tolerance indices. A total of 53,523 SNPs were identified from RILs, and these SNPs were used for the construction of a high-density bin map. Gene enrichment analysis based on SNPs associated with the drought-related traits had shown enrichment for 23 genes on the hotspot region. Only 12 genes were common in both approaches and functionally validated by qRT-PCR, resulting in the identification of four promising candidate genes, present in the QTL hotspot.

Quite recently, this QTL hotspot (CaLG04) was further analyzed for the identification of QTLs associated with drought tolerance by using a 232 RILs population (ICC-4958 × ICC-1882) (Sivasakthi et al. 2018). Canopy conductance and plant vigor had 21 major QTLs (M-QTLs), identified with the help of an ultra-high-density bin map. CaLG04 had 13 M-QTLs, linked with canopy conductance, and had favorable alleles from a high vigor parent (ICC-4958). Another M-QTL was also identified on the CaLG03, linked with canopy conductance. Comparative analysis of the QTLs showed that by increasing the marker density, QTL size was reduced while phenotypic variation percentage increased markedly.

Thus, genomic resources are the efficient and comprehensive way to target the genomic regions linked with tolerance to stress, such as drought stress. Identified QTLs can be used in marker-assisted breeding for the development of drought tolerance in the chickpea.

3.2.2 Transcriptomic Resources

Transcriptomics is the study of the transcriptomes, which are generated by the genome, under different environments in the cell, using high-throughput systems such as RNA-Seq and microarray analysis. Comparison of transcriptomes provides a platform for the identification of genes that are differentially expressed in diverse cell populations, or in answer to changed treatments. Transcriptomic resources are the sources used to make the plant expression profile under different environmental conditions, based on their mRNA/cDNA study. Principally, the transcriptomic study was facilitated with the help of the RNA-Seq technique. RNA-Seq is a cost-effective approach, accelerated by the invention of sequencing tools, such as NGS (Wang et al. 2009). Differentially expressed genes and their isoforms and variants such as SNPs, SSR, and InDels can be identified through transcriptomic dissection of genes (Zhao et al. 2014). The use of transcriptomics study in crop plants is a cyclic process that involves the identification of connective genes and linked pathways and provides further support for gene cloning, evaluation, and development of large-scale

genetic markers. Transcriptomic analysis through ESTs is a very old method, used to develop the transcriptome of chickpea under varying environmental conditions.

ESTs were used to compare the responses of chickpea genotypes against drought stress and also subjected to find some up-regulated and down-regulated genes (Gao et al. 2008). cDNA libraries were used to develop clones, and almost 2500 clones were selected randomly from each cDNA library. The selected clones were subjected to sequencing and 92 genes were identified which had differential expressions. Among these genes, the number of up- and downregulated genes were 36 and 56, respectively, under a stressed environment. These upregulated genes were classified into four major groups, metabolism-related genes (7), genetic information-processing genes (1), cellular-processing genes (1), and stress-related genes (27), among their groups. The expression of these genes was also associated with lipid transfer proteins (LTPs), late embryogenesis abundant (LEA) proteins, rubisco-encoding genes, and chlorophyll-binding proteins (*a*/*b*) under drought stress as well. This study provides the platform for understanding of the molecular basis of drought stress in chickpea.

Another study was conducted for the generation and evaluation of ESTs along with gene-based markers in chickpea against drought and salt stress (Varshney et al. 2009). This study identified a total of 20,162 new ESTs, among them 6404 unigenes, portioned as 11,904 and 2595 ESTs against drought and salt stress libraries of root tissues, respectively. Based on 177 SSR markers and 742 genes with SNPs in chickpea, the transcriptomic map of chickpea became comprehensive and more informative. The molecular markers developed from transcriptomic and ESTs data, were used to facilitate direct moving toward the target genes. Similarly, two contrasting chickpea genotypes were used for transcriptomic analysis based on ESTs, obtained from cDNA libraries, and developed from different time points (Jain and Chattopadhyay 2010). A total of 319 ESTs were obtained from different cDNA libraries and were classified into 11 clusters based on their expression profile. Based on higher expression of ESTs under drought stress in tolerant cultivars, 53 ESTs were selected and subjected to further screening analysis. These highly expressed ESTs were involved in protein metabolism, transcription, signal transduction, and cellular organization. These ESTs were the source for improving drought tolerance in chickpea by targeting beneficial genes as identified from a tolerant cultivar.

The suppression subtraction hybridization (SSH) method was used to generate ESTs libraries from the root and shoot tissues of two contrasting genotypes under terminal drought stress by using a dry-down experiment in chickpea (Deokar et al. 2011). Based on the results, a total of 5494 high-quality ESTs were drought responsive. The number of terminal-drought responsive unigenes was 1500. Similarly, 830 unigenes were only expressed in roots under terminal drought stress that showed the presence of genotype-specific expression among contrasting genotypes. On the other hand, pyrosequencing technology was used for the transcriptomic analysis of chickpea under drought stress (Garg et al. 2011). By using this technique, two million high-quality sequences were generated with an average length of 372 bp. Based on de novo assembly, it was clearly indicated that the hybrid assembly of short-read and long-read assemblies revealed better results. More than 4000 SSR markers were

identified and used as functional molecular markers in chickpea. Finally, based on the resultant data, a web resource, namely, the Chickpea Transcriptome Database (CTDB), was developed and made publicly available. So, this study was the source for accelerating the genomic research and breeding programs against drought stress in chickpea.

In parallel, super-SAGE (serial analysis of gene expression) analysis of gene expression was used in chickpea against drought stress by using root tissues (Molina et al. 2008). Super-SAGE is considered as an advanced technique of the SAGE. It is used to create a genome-wide superior-quality transcriptome profile of the chickpea against drought stress. Super-SAGE was used to define cDNA positions by producing 26-bp-long fragments (26-bp tags). Based on this information, mRNA sequencing information was clearly characterized. A total of 7532 UniTags were more than 2.7-fold differentially expressed and 880 were regulated more than eightfold under drought stress as compared to normal irrigated conditions. The genes associated with photosynthesis and energy metabolism were downregulated. On the other hand, transcription factors and signal transduction-related genes were down- and upregulated under drought stress. Moreover, Super-SAGE tags were applied to develope microarrays and probes for RT-PCR, thus overcoming the deficiency of genomic techniques in non-model plants as well.

Quite recently, the cDNA-AFL methodology was used to evaluate the chickpea genotypes for drought stress (Mozafari et al. 2018). About 295 transcript-derived fragments (TDFs) were identified under drought stress. cDNA was subjected to sequencing and classified into different groups related to macromolecule metabolism, signal transduction, cellular transport, cell division, energy production, and transcriptional regulation under different levels of drought stress. Based on transcriptomic results, the genes associated with transcription of mitochondrial chaperone, hydrolases, ribosomal protein S_8, NADPH dehydrogenase, histone deacetylase, calmodulin, histone deacetylase, and chloride channels were significantly affected under drought stress.

Later, the use of microarray for transcriptomic study in chickpea had become common. Leaf and root tissues of chickpea were used for transcriptomic study against drought stress by using an oligonucleotide microarray (Wang et al. 2012). A total of 6164 oligonucleotides spotted microarray was constructed by using 36,301 ESTs as well as 283 sequences of nucleotides. Based on temporal gene expression, the number of differentially expressed unigenes was 2623 and 3969 in root and shoot tissues, respectively. Further, 110 drought-responsive pathways were identified. Similar to other findings, the number of expressed genes under a stressed environment remained greater; in the current study, the number of expressed genes under drought stress was more as compared with normal, 88 and 52 in root and shoot tissues, respectively. More genes were found to be expressed in leaves as compared to roots, linked with different biological activities under drought stress. Another study was conducted to examine the transcriptome dynamics in chickpea using microarray, by applying drought stress and *Ralstonia solanacearum* infection (Sinha et al. 2017). *R. solanacearum* is the chickpea pathogen responsible for wilt disease. The drought-stressed plant was infected with the pathogen for 2 days

(short duration) or 4 days (long duration). The number of differentially expressed genes were 821 and 1039, respectively, under the short and long duration of stressed environment. The pathogen also had a cumulative effect on the drought stress, thus mimicking a combined stress effect. Most of the genes were found upregulated under infection by the pathogen. Real-time PCR was used to validate the microarray results of differentially expressed genes under drought and pathogen stress. This transcriptome is the way to target the resistant and desired genes against these stresses.

After microarray, because of the presence of NGS tools, transcriptomics has been shifted toward RNA-Seq. It also is known as whole transcriptome shotgun sequencing (WTSS), used to show the quantity and quality of RNA exhibited in a biological tissue at a given time point. Principally, it is concerned with the alternative gene spliced transcripts, mutations/SNPs, gene fusion, posttranscriptional modifications, and differential gene expression under varying environments (Wang et al. 2009). So, in chickpea, the transcriptomic profile was made by using root and shoot tissues against drought, salt, and cold stresses (Garg et al. 2015). A total 250 million of excellence reads from stressed and nonstressed tissues were generated. Among the identified transcripts, 11,640 transcripts were seen to be present at least one of the applied stress environments, whereas 3536 transcripts were identified through reference-based transcriptomic assembly, differentially expressed in response to abiotic stresses. Some genes were found to be involved in the regulation of the RNA metabolic process, posttranslational modifications, and epigenetic regulation. The resultant transcriptome profiling of chickpea is the key source of various plant responses to stresses and open avenues to conduct applied and functional genomic studies for improving stress tolerance in chickpea.

Similarly, root and shoot tissues of chickpea were used for RNA-Seq. against drought and salt stress at both stages, vegetative and reproductive (Garg et al. 2016). An Illumina HiSeq 2000 platform was used for sequencing of libraries to generate more than 30 million 100-bp-long paired-end reads for given samples. Differentially expressed genes were identified by using Cuffdiff. There were 4954 and 5545 genes among drought-tolerant and salt tolerant genotypes, respectively. The regulatory network linked with drought and salinity stress tolerance was the key findings of the transcriptomic dynamics. Further, RNA sequencing was also performed to analyze the genes/pathways linked with tolerance/susceptibility against drought stress in chickpea by using two contrasting genotypes: ICC-283 (drought tolerant) and ICC-8261 (drought sensitive) (Badhan et al. 2018). Many genes, such as MYB-related protein, alkane hydroxylase MAH-like, ethylene response, xyloglucan endotransglucosylase, cysteine-rich, BON-1 associated, peroxidase 3, vignain, transmembrane domain, and mitochondrial uncoupling, were upregulated under drought stress in the tolerant genotypes whereas some other genes were downregulated in the sensitive genotypes at the same time point. RNA profiling of the tolerant genotype is a good source for the genetic donor to develop tolerance in the sensitive genotypes.

Similarly, the *Cicer arietinum* Gene Expression Atlas (CaGEA) was presented by using RNA-Seq analysis of chickpea (ICC-4958) under drought stress at different

growth stages (Kudapa et al. 2018). Differentially expressed genes identified from a pairwise combination of samples numbered 15,497. Root development, nodulation, flowering, and seed development processes varied significantly in terms of differential gene expression. The differential gene expression related to drought stress was validated against drought stress present in the QTL hotspot. Moreover, RNA-Seq was used to characterize two Kabuli chickpea genotypes under varying levels of drought stress at the time of early flowering (Mashaki et al. 2018). About 4572 differentially expressed genes were recognized. The number of genes related to drought tolerance was varied according to tissue type; root and shoot carried 261 and 169 genes, respectively. In tolerant genotypes, a gene ontology study was used to further sub-categorize chickpea based on different plant responses: defense response, response to stress, and stimulus–response. Many TFs were recognized, involved in different metabolic pathways, such as flavonoid, proline, and ABA biosynthesis. The QTL hotspot region was also explored for differential gene expression of candidate genes associated with drought stress. Finally, transcriptomic resources are the potential source in plant breeding for the development of drought-tolerant chickpea verities based on their transcriptome profile of drought-responsive candidate genes.

3.2.3 Proteomics

Focus on the application of proteome-wide profiling in plants for the characterization of phenotype has emerged gradually with the advances in genomic tools. In proteomics, the most common techniques are two-dimensional (2-DE) polyacrylamide gel electrophoresis, polyacrylamide gel electrophoresis (PAGE), and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS). The use of liquid chromatography for proteomic analysis is also becoming progressively popular (Komatsu et al. 2013). Omics-based assisted breeding through protein-based markers immensely expands its worth for the improvement of plant breeding. Proteomics simply assist the plant breeder to advance knowledge regarding the investigation and identification of complex stress mechanisms in plants under stress conditions (Eldakak et al. 2013).

Transcriptomic level does not have an exact or constant correlation with the protein functions and their abundance, altered by posttranscriptional modifications. Because of the need to develop the high-throughput proteome, with respect to developing drought-responsive novel proteins in plants, most of the studies to date about drought stress are mostly related to alterations in gene expression whereas very little information about their products has been available until recently. Nevertheless, the worth of drought-responsive genes is incomplete without information about their functions. So, to expose the plasticity of gene expression with respect to their products, proteomics analyses are necessary, because this enables us to visualize the physiological position of the cell by observing protein formation. On the other hand, different factors such as protein abundance, electrophoretic properties, protein abundance, and size are present, which are responsible for the

limiting synthesis of proteins (Westbrook et al. 2006). Proteomics is an important tool for understanding the tolerance mechanisms of the plant under stress conditions. It gives information about which type of protein is formed and what its function is under different environmental conditions.

In chickpea, the cell-wall proteome was developed to recognize the novel functions of extracellular proteins (Chattopadhyay et al. 2006). This proteome was proved as a platform for the comparative studies of these proteins under drought stress. Proteomic analyses discovered some new extracellular-matrix proteins of unknown functions vis-a-vis the existence of several known cell-wall proteins. Moreover, some unknown proteins with known chemical activities were recognized based on the proteome map of chickpea. Another study was carried out for the proteomic profiling of eight commercial varieties of chickpea against drought stress by using electrospray ionization time-of-flight mass spectrometry, the Agilent 1100 Series HPLC system with a Q-STAR Pulsar I mass spectrometer (Bhushan et al. 2007). Based on quantitate image analysis, 163 protein spots were shown that had seemed to be significantly changed according to their intensities by more than 2.5-fold during drought stress. Based on two-dimensional electrophoresis results, a total of 134 differentially expressed proteins were recognized under dehydration stress. A proteome profile revealed the possible functions of some known as well as unknown proteins against drought stress tolerance.

Moreover, in chickpea, the formation of new proteins in response to drought stress was reported in the tolerant genotypes by making the proteomic profile through SDS-PAGE (Patel and Hemantaranjan 2013). Among these proteins, the most common were dehydrin-responsive proteins (DRPs) found in the seeds of chickpea under drought stress. Similarly, the effect of abiotic stresses such as drought, salt, and heat was studied through the leaf proteome in chickpea (Santisree et al. 2017). A total of 590, 797, and 248 regulated proteins were found for drought, salt, and heat stress, respectively. Nitric oxide was applied as a foliar spray, and as a result many proteins were modulated to increase stress tolerance in chickpea. Signaling pathways and regulatory proteins responsible for stress tolerance had been identified in chickpea with the help of proteomic analysis. Various stress-related proteins such as ABRE, MYB, and MYC were recognized in chickpea for drought stress (Hussain et al. 2019).

In chickpea, iTRAQ-based proteomic analysis of mitochondrial proteins, responsible for drought adaptions, was performed (Gayen et al. 2019). A total of 40 drought-responsive proteins were found; their expressions were regulated by drought stress. Various metabolic pathways such as oxidative phosphorylation, pathways of carbon fixation, and the purine-thiamine metabolic network were regulated by differentially expressed proteins. The proteome delivers intriguing insights into the metabolic pathways and provides clues associated with drought tolerance in chickpea. Similarly, two species of chickpea, *C. arietinum* and *C. reticulatum*, were used for comparative physiological and proteomic analysis by exposing drought stress (Çevik et al. 2019). MALDI-TOF/TOF-MS/MS-based quantitate pruritic analysis identified 24 differentially expressed proteins in response to drought stress. *C. reticulatum* had better adaption to drought stress and showed upregulation of the

proteins that were involved in the energy mechanisms and photosynthesis against drought conditions. Moreover, proteins related to glutamine synthetase, sucrose and proline biosynthesis, and cytosolic fructose-bisphosphate aldolase were also upregulated in *C. reticulatum* under drought stress. This study provides clues for targeting the drought-responsive proteins in chickpea that were produced in *C. reticulatum*. Thus, a remarkable development in interrogating proteomes has shown its significance for the identification and evaluation of differential drought responses in plants understress. Although recent technologies have been used in the proteome that make it possible to study the changes in protein expression, yet the proteome profile of crop plants is very new.

3.2.4 Phenomics

Phenomics considers the phenotyping of the plant by using various tools for the measurement of morphological data. The complex traits such as drought tolerance are yet a challenge to measure. The combination of genetic and modern genomic techniques with breeding methodologies and precise phenotyping is considered effective for the understanding of metabolic pathways through which tolerant cultivars can be developed. Phenotyping is the vital phase before the usage of genetic and physiological strategies for enhancing drought tolerance in crop plants (Mir et al. 2012). Phenomics is an important technique that has been used for the identification and dissection of physiological mechanisms related to drought tolerance. Several techniques have been used for phenomics, such as spectroscopy and fluorescent microscopy to measure the rate of photosynthesis and to study photosynthetic processes. Transpiration and temperature profiles are recorded by infrared cameras as well as 3D cameras to record alterations in growth processes (Gupta and Rustgi 2004).

In chickpea, extensive studies on root-related traits were done for identification of drought-tolerant genotypes (Silim and Saxena 1993). The tolerant genotypes seemed to be those with an efficient and long root system as compared to susceptible genotypes under drought stress. Similarly, chickpea germplasm was grown under a low level of soil moisture had adverse effects in the form of terminal drought stress (Kashiwagi et al. 2005). So, the phenomics analysis of roots-related parameters is widely recommended to obtain useful results. It was recorded that the chickpea genotypes with more profuse and deeper root systems can extract more water from the deep water table and are considered as drought-tolerant cultivars. Molecular breeding, genetic dissection, and phenotyping have been used collectively to understand the mechanisms of drought tolerance in chickpea. Different drought-related traits including root, maturity, carbon assimilation, shoot biomass, and seed yield were targeted in chickpea. Phenotypic data were recorded to characterize the germplasm in response to drought stress (Upadhyaya et al. 2012). Moreover, 20 genotypes of chickpea Desi and Kabuli were screened based on indices against drought stress (Khan et al. 2018). Diverse results were obtained between chickpea genotypes. Two genotypes (NKC-5-S-20 and NKC-5-S-17) were found to be more drought tolerant in irrigated as well as rainfed areas. The seed yield of these genotypes remained healthy as compared with that of other genotypes under drought.

4 Genomics-Assisted Breeding

To cope with challenges caused by climate change, genomics-assisted breeding has been adopted successfully by using available genomic tools such as genetic maps and genetic markers. The latest sequencing tools (NGS) have been commonly used to sequence the genome with the contribution of different international institutes. Now, it has become possible to use genomics-assisted breeding for the development of chickpea genotypes to develop either tolerant or high-yielding varieties (Varshney et al. 2017). Genetic diversity, DNA fingerprinting of a plant genome, and the evolutionary relationship between chickpea relatives was studied by using DNA markers (Sudupak et al. 2002). Similarly, AFLP markers were used for the grouping of nine chickpea annual species, and that grouping was similar to RAPD markers (Shan et al. 2005).

Marker-assisted breeding is most commonly divided into two aspects: marker-assisted backcross breeding (MABC) and marker-assisted recurrent selection (MARS). MABC can be used to develop drought-tolerant accessions. Marker-assisted breeding is a rapid and comprehensive molecular breeding approach that is used to isolate the superior individuals and desired marker loci. For meaningful marker-assisted plant breeding, DNA markers should have a few key characteristics, such as quality and quantity, greater reliability, DNA polymorphisms, and low cost for assy designing (Mohler and Singrün 2004). In plant breeding, identification and characterization of QTLs is the key source for meaningful plant breeding to develop drought-tolerant plants. QTLs pyramiding strategy is also a feasible process for developing drought tolerance in plants (Luo et al. 2019).

Several studies based on marker-assisted breeding are reported in chickpea. Similarly, chickpea introgression lines were evaluated for drought tolerance, based on an QTL-hotspot obtained from the donor parent (drought-tolerant) (Sheoran et al. 2018). Based on marker analysis, the introgression lines had that *QTL-hotspot*, exhibited drought tolerance by making a good root system as compared to susceptible genotypes. Presence of the root-linked genomic regions as well as phenotypic resemblance with a recurrent parent was the indication of drought tolerance. Potential lines of chickpea were evaluated from a population of eight parents through multi-parent advanced generation inter-cross (MAGIC) (Samineni et al. 2017). Genetic diversity was created through MAGIC, used to develop promising lines of chickpea against drought stress. Thus, these introgression lines were recognized as drought tolerant as compared with some popular existing cultivars of chickpea.

On the other hand, MARS was exploited to develop elite lines of chickpea against drought stress. Identification of desired genes from genomic resources has become the supreme priority, to target genes related to stress or yield improvement (Samineni et al. 2017). In chickpea, a QTL-hotspot was obtained from a drought-tolerant chickpea line (ICC-4998) and transferred into two widely cultivated and adapted cultivars (JG-11 and Bharati) (Samineni et al. 2015). After transformation of the drought-linked genomic region, 20 introgression lines were developed and evaluated across the three to four locations. Several introgression lines had 10% higher yield than their parents because of better adaptivity under drought stress from a

different location under different environmental conditions, irrigated or rainfed. Moreover, that genomic region also had an influence on the other yield-contributing traits, seed size along with resistance to drought. The resultant chickpea cultivars were thought to be effective for the breeding program against terminal drought stress in chickpea.

To overcome the pyramiding issue of complex traits, an alternative method of marker-assisted selection has been invented during recent years that is most commonly known as 'genomic selection' (Hayes and Goddard 2001). Total information that can be obtained through genetic markers is used to study the breeding worth of the crop plants. The complex traits can be analyzed easily by rendering the pyramiding complex in marker-assisted selection. Genomic selection is the best practice for the assortment of preferred parents for breeding strategies. It can minimize the cost and standard of breeding time cycle for variety development, which is why it has become more popular for plant breeders to hasten the breeding program (Crossa et al. 2014; Hayes et al. 2009). When the population size is large, and the trait has a low range of heritability, then at that point, genomic selection is more effective as compared to phenotypic selection. In chickpea, based on genomic selection, it was revealed that yield was low in rainfed areas as compared to irrigated areas (Jaganathan et al. 2015). Moreover, stress-resistant cultivars with a high potential of production and adaptation were developed through genomic selection in chickpea (Samineni et al. 2017).

A genetic linkage map is required to develop the association between phenotype (single-marker analysis, interval mapping, composite interval mapping) and a marker that confers the targeted genomic regions. Along with basic steps of QTL mapping, different kinds of segregating population have been developed: double haploid, F_2 generation, recombinant inbred line, and near-isogenic line. In this way, the genomic regions contributing to the drought stress were discovered and explored from the genome of plants (Singh et al. 2015), wherein the meta-QTL technique is the way to study the complex QTLs, such as drought-related QTLs, and several populations are screened across the various locations and environments in plants. Canopy conductance and plant vigor were improved through QTLs mapping in chickpea (Sivasakthi et al. 2018): QTLs linked with canopy conductance and plant vigor were transferred from a drought-tolerant chickpea variety to a susceptible variety.

Association mapping identifies QTLs from a diverse panel, based on genome-wide linkage disequilibrium, relevant phenotypes, and forms of genomic variants. In association mapping, there is no need to develop experimental populations resulting from planned crossings. Exotic diverse germplasm is the significant material for association mapping (Mitchell-Olds 2010). In chickpea, 1872 markers were used for 300 diverse chickpea genotypes against drought-stress and market trait associations that were evaluated through association mapping among these genotypes (Thudi et al. 2014a). Similarly, chickpea genotypes were evaluated for drought tolerance by using phenotypic and molecular approaches (Sachdeva et al. 2018). In all, 90 alleles were identified, and polymorphism information content varied from 0.155 to 0.782 per locus. This information was used to detect tolerant and drought-prone genotypes (Sachdeva et al. 2018). In chickpea, four genetic

regions were identified, comprising different SNPs, which indicate the pleiotropic effects of genes under drought stress (Li et al. 2018). Notably, marker-assisted breeding was recognized as more efficient and accurate breeding as compared with conventional breeding. The exploration of germplasm through DNA markers shows profound impacts on conventional breeding. Thus, conventional and omic-based breeding have their relative significance in plant breeding, and the integration between these approaches is helpful for the improvement of drought tolerance in chickpea.

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GM Maize for Abiotic Stresses: Potentials and Opportunities

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1 Introduction

In Africa, Asia, and Latin America, maize serves as the basis of food security but the production rate of maize in most developing countries is very low. Per unit area production of maize in developing countries is about 20% of the average production in developed countries. Over the past 10,000 years, maize has evolved from its wild grass progenitor teosinte (*Zea mays*, *Zea* spp.), which arose in Southwestern Mexico. Maize has become a major food and staple resource through the years of cultivation and substantial selection for traits favoring temperate areas. In many instances, genetic diversity has decreased because of domestication and artificial selection, and favorable genes/alleles have vanished from wild precursors that were formerly associated with environmental stress tolerance. For example, the *ZmWAK* locus, which contained resistance to head smut, has been lost from the

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teosinte ancestry. Therefore, the production rate of maize is limited by many factors, including biotic and abiotic stresses. In tropical and subtropical regions, drought is a highly significant restraint to increasing the production of maize. Fertility of soil is also a major limitation in maize production because less fertilizer is being used and toxic elements are present (Byerlee et al. 2007; Farooq et al. 2015). The changes in the global climate, amalgamation of heat, drought stress, and excess moisture, coupled with susceptibility to rising diseases and insect pests, are increased in some regions, especially in Asia and Africa. Losses from abiotic stresses as well as biotic stresses such as disease, insect pest, and weeds have reduced average yield by more than 30%. It is estimated that annually 54% of yield has been lost to insects, diseases, weeds, and animals in Africa, and 48% in Central and Southern America and 42% in Asia (Oerke 2006). The yield of maize is reduced by insect pests which directly damage stems, leaves, stalks, grains, ears, and tassels: 18% of maize production is lost annually to the stem borers, the most damaging group of insect pests (De Groote 2002).

During the past two centuries, methods of conventional breeding have been used to enhance and improve the production rate as well the quality of grain. These methods have been successful in the form of hybrid technology and increased yield many fold; however, the increasing demands for maize mean that conventional maize breeding methods need addition support to meet the desired production rate. Similarly, quality is also important in providing healthy food to the population (Barampuram and Zhang 2011). To keep pace for increasing yield and grain quality, transgenic technology was introduced in the mid-1980s and now is being improved and used extensively to enhance production and also to improve the grain quality in a short duration of time. Several methods have been used to produce transgenic plants: either the direct method of gene transformation such as biolistic transformation, silicon carbide fibers, electroporation, and native gene transfer, or by indirect methods of transformation such as through *Agrobacterium tumefaciens*-mediated gene transformation (Jones 2009). Biolistic- and *Agrobacterium*-mediated transformation are the two most popular and best studied methods of transformation. Through transgenic technology, a desired gene can be introduced to develop plants with the desired traits such as high nutritional value and resistance against herbicides, drought, and heat stress (Husaini et al. 2010).

1.1 Maize Production in the World

The global output of maize in 2017–2018 was about 1033.75 million tons, 9.8% more than that of 2016–2017 (FAO Report 2018). Worldwide, the maize production rate and areas have changed dramatically from the past 50 years. The global area of maize production has been increased by 50%, from about 100 million hectares to more than 150 million hectares. Especially in developing countries, the cultivated area of maize has been increased, almost doubled from 60 million hectares in 1961 to 120 million hectares in 2010 (Shiferaw et al. 2011).

The yield of maize has been doubled by the use of improved maize genetic architecture in the form of hybrids, fertilizers, water, and pesticides, but it is difficult to meet the needs of increased food production aggravated by climate conditions (Evenson and Gollin 2003). The prime task of the future is to attain sufficient growth in food production in such a way that health, environmental quality, and farming systems are not compromised (Tilman et al. 2002).

1.2 Role of Maize in Food Security

Maize is a major source of food security and nutrition for millions of people all over the world. In 94 developing countries, more than 4.5 billion people obtain 30% of their food calories from maize today. By 2050, in developing countries the requirement of maize will double as the population becomes 9.0 billion (Rosegrant et al. 2009).

Maize is consumed equally by human beings, livestock, and poultry. Maize has a high nutritional composition, as its grain contains starch, vitamins A and B_3, oil, protein, sugar, and fibers. In terms of productivity and industrial products (fermentation and pharmaceuticals), maize is the most important cereal crop in the world. Maize is an ideal staple food because of its low price and the high consumption rate, particularly in those areas where deficiencies of micronutrients are a serious public health problem (Laillou et al. 2012).

For the meat industry, maize is the most important element of feed, particularly in Asia where maize has a high consumption rate from the high demand as poultry and pig feed. Every year, the worldwide demand for maize has been growing at the rate of 6% for livestock feed and is estimated as a vital element of future requirement. In developed countries, 30% of maize used for human consumption whereas 70% is used as feed. Maize is used for many purposes including food and fuel for humans and feed for livestock. The high nutritional value of maize promotes the use of its grain in industries as raw material for many products (Afzal et al. 2009). Maize is grown all over the world: United States, China, and Brazil are the top three maize-producing countries. Maize contains 72% starch, 4% fat, 2.1–27% fiber, 45–70% carbohydrates, and 10% protein: 365 kcal/100 g energy is provided by maize, in comparison to wheat and rice, but it has a lower protein content. Wheat, rice, and maize are considered to be 94% of all cereal utilization as the most important human food sources. Maize can be used to manufacture many products such as starch, beverages, industrial alcohol, glue, and fuel ethanol.

1.3 Role of Maize in Biofuel Production

Maize is used for the production of biofuel, which is mostly used as motor fuel. Maize is the prime feed material used to produce ethanol, and the price of maize has been increased by the high requirements of ethanol production. The demand of

maize has been increased in industry as it used in the bio-energy sector. The demand of maize in fuel production has been increased from the past 10 years. Maize has emerged as the prime source of biofuel because it can be stored without fermenting, which is not the case in competing crops such as sugarcane. Breeders have identified genotypes that are high yielders and more responsive for biofuel production. In USA, 40.5% of the corn-growing area was being used for ethanol production in 2011 (Mumm et al. 2014).

1.4 Seed Quality of Maize

In the world economy and trade, maize has an ascendant position as an industrial grain. Consequently, it is essential to ameliorate the industrial and nutritional characteristics of the grain by acquiring knowledge of the genes that control seed quality traits of protein, starch, oil, and other compounds. The composition of maize grains could be improved for quantity as well as quality of starch, protein, and oil by exploiting genetic variation. The prolific amount of storage protein called "zeins" is present in up to 60% in developing endosperm tissues. The kernel of maize has been extensively used not only for its starch content but also for the oil that accumulates in the embryo. By industrial processing of maize grains, oil is a high-value product and also used as a high-quality source of oil for human consumption. Oil and starch are stored in distinct niches of maize kernel: 85% of oil is accumulated in the embryo and 98% of starch is accumulated in the endosperm (Motto et al. 2012). The grain of maize is highly used for the production of corn flakes, grain cake, cornstarch, lactic acid and acetone, used by many industries such as food and fermentation as well as in the textile industries.

2 Effects of Abiotic Stresses

Such abiotic stresses as heat, high salt content, heavy metal toxicity, or less availability of nitrogen greatly influence crop production (Kellős et al. 2008). Under the climate change regime, these stresses are aggravated further, especially those of heat and drought (Bänziger et al. 2006). Reduction in anthesis to stalking, principally under the drought condition, has been reported for the greater grains which are up to 9.5% per cycle. Unimproved tall tropical germplasm, however, has been accompanied by their short plant height with less barrenness (Edmeades and Tollenaar 1990). The life cycle of every cereal has a phase in which their temperature shifts between the vegetative and the reproductive growth, determining its field utility. Working on climate change-bearing crop varieties is very important in general and temperature-resistant varieties in particular. An important role has been shown by genetic engineering in maize improvement across the world for insect resistance, herbicide tolerance and currently heat tolerance. Maize has the

specificity in its genotypes that grow only in the temperate zone (Khan et al. 2008 and Iqbal et al. 2010), requiring measuring conditions suitable for crops and the water retention potential for sowing in summer and spring seasons.

2.1 Effect of Heat on Maize Grain Quality

The two most important environmental factors that affect the growth of crops, its yield and development, are a shortage of water and heat stress (Prasad and Staggenborg 2009). Increasing temperatures at the crop growing season can disrupt the crop production system. At the growing season, storage temperature can reduce the crop production in two ways as rising temperatures enhance the growth of crops such as maize that reduce the time of grain and maize development by reducing the accomplishment of potential yield. Second, during extreme heat conditions, flowering of maize such as in the silk-tassel stage, pollination is hindered and the development of grain is completely intercepted. The duration of the growth cycle is reduced by the effects of temperature; in particular, the grain-filling stage is the most significant factor that reduces yield at very hot temperature (White and Reynolds 2003).

The yield of maize is a function of the quantity of grain and its weight as there is a strong relationship between grain weight and grain-filling stage. When the duration of filling period and grain-filling rate are optimal, then the full potential weight of the grain is attained. Maximum grain filling is attained at 25–32 °C, a moderate high temperature. High temperature enhances development of the plant but reduces the time period of grain filling. At temperatures higher than 32 °C, production of starch is diminished, affecting the grain-filling rate (Singletary et al. 1994). Above 32 °C, pollen is reduced in their capability to germinate on silk (Basra 2000); as a result, few grains are available for grain filling.

3 Transformation Systems in Maize

Transformation in maize has been tried by all the possible systems. The three major systems that have acquired routine status are biolistic transformation, *Agrobacterium*-mediated transformation, and in planta transformation. These are discussed now.

3.1 Transformation Through a Biolistic Gun

A meta-analysis of 21 (1996–2016) years of field data reported through extensive publications on transgenic maize showed approximately 6% increase in yield compared to near-isogenic lines and 30% reduction in various toxins including

mycotoxins, fumonisin, and thricotecens. This result shows considerable success of the technology when seen in the context of the various controversies that were raised (Pellegrino et al. 2018). To develop transgenic maize for the first time, Coe and Sarkar (1966) injected DNA in newly developed maize seedlings, but no phenotypical change was observed. Transformation of the crop progressed significantly with the development of biolistic technology that abolished the requirement to transfer naked DNA with the capability to integrate DNA across the plant cell wall and could be used for stable transformation. In the biolistic method, metal microparticles are physically coated with the desired gene and accelerated toward the target cell through a gene 'gun' (Sanford 1990) with adequate acceleration to penetrate the cell wall but not trigger cell death. Post bombardment, DNA coated on microparticles is released slowly into the cell and integrated into the genome (Taylor and Fauquet 2002). For the first time, Gordon-Kamm et al. (1990) and Fromm et al. (1990) reported maize transformation by integration of foreign DNA into the embryogenic callus through a biolistic gun and confirmed transgene trafficking to the next generation. Later, several reports on maize transformation showed that the biolistic gun is an efficient technique for successful integration of the transgene into the genome of maize and reproductive results (Brettschneider et al. 1997; Frame et al. 2000).

3.2 Agrobacterium-*Mediated Transformation*

Agrobacterium tumefaciens is a naturally occurring, soil-borne bacterium that naturally infects dicot species and causes crown gall disease. *Agrobacterium* can transfer DNA to dicot as well as monocot species. t-DNA (transfer DNA), an ambulant part of the Ti (tumor-inducing) plasmid, is transferred to the nucleus of the plant cell during *Agrobacterium* infection and integrated into the chromosomes of the plant (Hooykaas and Schilperoort 1992).

The delivery of t-DNA is imparted by genes that are present on another part of the Ti plasmid called the vir region. These virulence genes are not transferred with t-DNA but help in transformation. The t-DNA contains a border sequence, which is a 25-bp direct repeat present on both ends of t-DNA termed the left and right borders, which is recognized and extirpated by particular endonucleases. Phenolic compounds such as acetosyringone, which is produced by wounded cells of a plant, are involved in induction of vir genes that are present on the Ti plasmid, causing a nick on the lower strand of t-DNA through endonucleases. A single-stranded copy of t-DNA is transferred to the plant cell from *Agrobacterium* (Gelvin 2003), covered by a protein that protects it from plant cell nucleases.

For maize transformation, *Agrobacterium tumefaciens* has become the ideal method for delivery of the foreign gene. *Agrobacterium*-mediated transformation was reported for the first time in maize in the early 1990s, and these protocols have since been improving because of the several advantages over other transformation

methods such as stable integration of intact transgenes, stable expression, and inheritance (Dai et al. 2001; Hu et al. 2003; Shou et al. 2004; Travella et al. 2005).

Transformation of maize through *Agrobacterium tumefaciens* depends upon various factors that relate to this transformation system, such as growth stages of explants, the genotypes, cell density and variation of strains, augmentation of phenolic compound, pH of medium, and its composition, and time duration of co-cultivation (Amoah et al. 2001).

3.3 Inplanta Transformation

In Inplanta transformation involves the transformation by reducing or eliminating the tissue culture process to exclude the negative impacts posed by in vitro conditions. In this technique, the reproductive or somatic cells are targeted, and pollination and seed development are accomplished if the reproductive cells are targeted. In the case of somatic cells, the plant is allowed to mature and the seed is harvested. The seed developed is used to obtain the next generation and screening is done by applying selection pressure. The first major report on inplata transformation came from Chumakov et al. (2006), who used maize pistils as target for *Agrobacterium* treatment with the hypothesis that pollen tubes will be going through the silks and make way for the *Agrobacterium* to reach the egg cells. Upon interacting egg cells, the single-celled egg or few-celled embryo is transformed to give rise to transformed seeds that are identified in the next generation through selection pressure. These protocols were modified and improved by many, including the author's laboratory, and Mamontova et al. 2010: Abhishek et al. 2014; Moiseeva et al. 2014, and are now becoming routine.

4 Transgenic Events Commercialized for Abiotic Stresses

The International Service for the Acquisition of Agri-Biotech Application (ISAAA) is an organization that keeps records of genetically modified crops/plants. According to their website there are seven events registered for abiotic stress tolerance, as commercialized. All these events belong to Monsanto. These events cover drought tolerance, herbicide tolerance, water stress, and insect tolerance. Details can be seen in Table 1.

5 Transformation for Abiotic Stress Tolerance

Three major stresses have been addressed extensively through transgenic technology. Some of the experiments conducted are discussed next.

Table 1 Transgenic events commercialized for abiotic stress tolerance in maize (Source, ISAAA)

Introduced gene	Gene source	Product	Function
MON87427 × MON87460 × MON89034 × TC1507 × MON87411 × 59122 (Pyramided Traits)			
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1F	*Bacillus thuringiensis* var. aizawai	Cry1F delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
pat	*Streptomyces viridochromogenes*	Phosphinothricin *N*-acetyltransferase (PAT) enzyme	Eliminates herbicidal activity of glufosinate (phosphinothricin) herbicides by acetylation
cry34Ab1	*Bacillus thuringiensis* strain PS149B1	Cry34Ab1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
cry35Ab1	*Bacillus thuringiensis* strain PS149B1	Cry35Ab1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry3Bb1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
dvsnf7	Western corn rootworm (*Diabrotica virgifera virgifera*)	Double-stranded RNA transcript containing 240-bp fragment of WCR Snf7 gene	RNAi interference resulting in downregulation of function of targeted Snf7 gene, leading to western Corn rootworm mortality
MON87460 (Single Trait)			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
nptII	*Escherichia coli* Tn5 transposon	Neomycin phosphotransferase II enzyme	Allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection

MON87460 × MON88017 (Pyramided Traits)			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry3Bb1 delta-endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
MON87460 × MON89034 × MON88017			
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry3Bb1	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry3Bb1 delta endotoxin	Confers resistance to coleopteran insects, particularly corn rootworm, by selectively damaging their midgut lining
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Hrbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
MON87460 × MON89034 × NK603			
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation

(continued)

Table 1 (continued)

Introduced gene	Gene source	Product	Function
nptII*	*Escherichia coli* Tn5 transposon	Neomycin phosphotransferase II enzyme	Allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection
MON87460 × NK603			
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
nptII*	*Escherichia coli* Tn5 transposon	Neomycin phosphotransferase II enzyme	Allows transformed plants to metabolize neomycin and kanamycin antibiotics during selection
cp4 epsps (aroA:CP4)	*Agrobacterium tumefaciens* strain CP4	Herbicide-tolerant form of 5-enolpyruvulshikimate-3-phosphate synthase (EPSPS) enzyme	Decreases binding affinity for glyphosate, thereby conferring increased tolerance to glyphosate herbicide
MON89034 × MON87460			
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cry1A.105	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry1A.105 protein: comprises Cry1Ab, Cry1F, and Cry1Ac proteins	Confers resistance to lepidopteran insects by selectively damaging their midgut lining
cspB	*Bacillus subtilis*	Cold shock protein B	Maintains normal cellular functions under water stress conditions by preserving RNA stability and translation
cry2Ab2	*Bacillus thuringiensis* subsp. *kumamotoensis*	Cry2Ab delta-endotoxin	Confers resistance to lepidopteran insects by selectively damaging their midgut lining

5.1 Herbicide-Resistant Transgenic Maize

Chlorsulfuron is an extensively used herbicide, but because of its long residual duration in alkaline soils and its effect on shoots, as well as on the growth of further crops, the implementation of chlorsulfuron is restricted to rotation soils of maize and wheat. Haughn and Somerville (1986) acquired a mutant from *Arabidopsis thaliana* that had resistance for the chlorsulfuron herbicide. Mazur et al. (1987) observed that mutation in the *als* gene was the main cause of resistance. In the previous study of maize transformation, maize embryos, calli, and suspension cells were utilized as targets. With the exemption of limited varieties, however, it was difficult for some elite and hybrid lines to produce suspension cells and subsequently regenerated plantlets. Thus, immature embryos as a target for transformation were dependent on greenhouse conditions, and some seasonal deviations and embryogenic calli were dependent on genotype as well. Lowe et al. (1995) bombarded the meristem of immature embryos of hi-bred inbred lines and induced multiple shoot clumps. Although transgenic plantlets were attained, they could not resolve the problem of genotypic dependency in selecting material. Li et al. (2001) developed herbicide (chlorsulfuron)-resistant maize by bombardment of the isolated herbicide-resistant gene *als* from mutant *Arabidopsis thaliana* on an established multiple shoot clump system from shoot tip meristems of maize. Then, herbicide-resistant regenerated plantlets were attained through selection of herbicide (chlorsulfuron), and further, polymerase chain reaction (PCR) and Southern blot analysis were performed to confirm the transformation of transgenes *als* in some regenerants. The spray of chlorsulfuron depicted the transgenic plants and the R1 generation had an affirmative herbicide-resistant trait. Through this protocol, a genotype-free transformation system was established that enabled producing large numbers of transgenic platelets. The prime impetus of this research was not only herbicide-resistant maize plants but also the hope that chlorsulfuron could be used widely in rotation soils of wheat and maize in future.

Weeds compete by divesting other plants of light, water, nutrients, and space. These undesirable plants can produce allelopathic substances that are quite toxic to crop plants. Weeds frequently serve as hosts for several crop diseases and also offer shelter to insects and their diseases. To eradicate these harmful weeds, different kinds of herbicides have been used.

Later, Kim et al. (2009) reported herbicide-resistant hybrid maize lines using type II embryogenic calli as explants through *Agrobacterium tumefaciens* strain C58C1 taking the binary vector pTF102. Glyphosate is nonselective and is an herbicide extensively used throughout the world. 5-Enolpyruvylshikimate-3-phosphate synthase (EPSPS) has always been preferred to target glyphosate to develop transgenic glyphosate-resistant crops (Steinrücken and Amrhein 1980). Glyphosate involves the enolpyruvyl shikimate-3-phosphate (EPSP) synthase enzyme pathway and inhibits it, which in turn interferes with the growth of weed plants. The major benefit of using this herbicide is that its target, the shikimate pathway, is not present in animals; hence, this herbicide is safe for animals, humans, insects, and birds.

In 1980, scientists efficaciously isolated EPSPS from bacteria and plants (He et al. 2001; Wang et al. 2003; Funke et al. 2006; Zhou et al. 2006). The t-DNA vector contained two cassettes of EPSPs, and the bar gene conferred resistance to glyphosate and phosphinothricin, respectively. Northern blot analysis has been performed to confirm the presence of the bar gene in transgenic maize, and through this protocol 0.6% transformation efficiency was obtained.

Later, Wang et al. 2010 integrated glyphosate-resistant gene (EPSPS) in a maize hybrid line through *Agrobacterium tumefaciens* under the observation of several factors that actually affect the transformation efficiency to optimize it. Through this protocol transgenic plants were obtained that could pass the stably expressed gene to further generations. Further, attention has been given to enhance more resistance towards glyphosate by not only isolating new genes but also through optimizing transformation-effecting factors. Therefore, in 2013, Yu Gui-rong et al. reported the glyphosate-resistant gene *2mG2-EPSPS*, which has been isolated from a strain of *Pseudomonas fluorescens*, which exhibited five to tenfold more resistance towards glyphosate, transformed into immature embryos of maize through *EHA105* and *LBA4404* strains of *Agrobacterium tumefaciens* under optimal conditions. Several factors such as usage of different strains, inclusion of L-cysteine, with a long heat and resting duration, were evaluated to enhance the transformation efficiency, such as inclusion of L-cysteine along with heating of the embryos for 3 min before infection-enhanced transformation efficiency and keeping them in resting medium for a longer duration by delaying selection of transgenic embryos, leading to better survival of transgenic calli. Strains of *Agrobacterium tumefaciens* and genotypes of maize are crucial in determining the transformation efficiency. Results suggested that *EHA105* has 65% higher transformation efficiency as compared to *LBA4404*. So, through this protocol, 8.2% transformation efficiency was obtained and from 88 transgenic plants, 66 maize plants exhibited resistance towards glyphosate. Further, PCR and Southern blot analysis confirmed the integration of transgene in the genome of maize. Later, Sun et al. (2015b) transformed *2mG2-EPSPS* (glyphosate-resistant gene) in immature embryos of an inbred line of maize by *Agrobacterium* with little variation in transformation factors such as *Agrobacterium* concentration, which was OD_{600} = 0.6, and a 10-min infection time was reported. From 46 transgenic plants, five showed positive results, and their stable expression in further generations was analyzed through real-time PCR. Ascertainment of novel EPSPS is significant for the production of glyphosate-resistant crops. AM79 is a bacterial gene and AM79 EPSPS can endure a high concentration of glyphosate in *Escherichia coli*, and thus could serve as a good choice for the development of transgenic glyphosate-resistant crops (Cao et al. 2012). In 2015, Ren et al. reported formation of a synthetic *AM79 aroA* gene because the wild-type gene contained numerous motifs that could lead to instability of mRNA of the bacterial gene in transgenic plants. Therefore, synthetic *mAM79 aroA* cloned with plant expression vector pM3301UbiSpAM79 was transformed into immature embryos of maize through *Agrobacterium tumefaciens*. Approximately 79 transgenic plants were obtained, and their PCR analysis exhibited that these plants had an integration of *mAM79*. Results of RT-PCR depicted the high transcription of *mAM79* in transgenic maize,

and these transgenic maize could endure fourfold commercial glyphosate application when sprayed with glyphosate. So, this study confirmed that *mAM79* could be used for generation of transgenic maize against glyphosate.

5.2 Drought-Resistant Transgenic Maize

Maize productivity is mainly affected by drought (Boyer and Westgate 2004; Campos et al. 2004). In plants, water stress is established when the level of transpiration is increased compared to the level of water absorption. Under water stress, photosynthetic activity is reduced by the closing of stomata (Chaves 1991). Seeds and vegetative tissues develop desiccation-tolerant structures where late embryogenesis abundant (LEA) proteins accumulate. Overexpression of LEA protein, in different plants such as *Arabidopsis*, wheat, rice, tobacco, cabbage, or lettuce through transgenic approaches have exhibited an ameliorated phenotype under abiotic stress (Leprince and Buitink 2010; Yang et al. 2010). Amara et al. (2013) reported transgenic maize against water stress by integrating the LEA gene Rab28 along embryogenesis through biolistic transformation. The presence of the gene was confirmed in different cells such as axial and vascular as compared to just the scutellar cells of embryos as previously reported by Niogret et al. (1996) under water stress conditions. The Rab28 gene was constitutively expressed in maize plants, which enabled them to sustain their growth rate and development. Overexpression of LEA proteins under water deficit and salinity enhanced their phenotype by increasing crop stress resistance (Leprince and Buitink 2010; Xiao et al. 2007; Yang et al. 2010).

Drought is one of the most significant environmental stresses, reducing the growth and yield of plants and crops, respectively (Boyer 1982; Passioura 1996). Drought is the major reason of reduction in yield of maize (Maiti et al. 1996). Shortage of water, great variation in weather patterns, and the uncertain nature of drought result in a notable threat to global maize production. In consequence of its complications and hardness, drought has been considered as a “cancer” of plants. Therefore, there is an immense requirement for improving maize drought tolerance through biotechnological techniques.

Earlier studies indicated the activation of oxidative signal cascades from the expression of mitogen-activated protein kinase kinase kinase (MAPKKK), which leads to tolerance against cold, drought, and salinity in tobacco. In 2004, Shou et al. transformed the npk1 gene in maize, under a constitutive promoter through the *Agrobacterium tumefaciens* strain EHA101, to analyze the role of the tobacco MAPKK npk1 gene in crops to improve drought tolerance. Results indicated that the npk1 gene induced a mechanism that protects the photosynthesis process under drought conditions, and in this way transgenic maize maintained its photosynthesis rate compared to nontransgenic plants. Transgenic plants showed an increase in kernel weight and leaf number as compared to negative controls. Thus, this study provides a reference to study the physiological and morphological aspects of transgenic maize under stress conditions.

Phosphatidylinositol-specific phospholipase C (PI-PLC) has a significant role in various physiological processes in plants, incorporating drought tolerance. It has been noted the *ZmPLC1* gene was cloned from maize that encoded PI-PLC and was overexpressed in roots of maize under water stress (Zhai et al. 2005). The transgenic elite inbred lineYe 7922 maize was generated through the *Agrobacterium*-mediated transformation LBA4404 strain by expressing the *ZmPLC1* gene in sense and antisense orientation. Under drought stress, it was found that sense transgenic maize showed a high rate of photosynthesis, high water content, better water adjustment, and higher yield than the wild type. Antisense transgenes showed subservient characters as compared to wild type. It was inferred that enhanced expression of sense *ZmPLC1* ameliorated the drought tolerance of maize (Wang et al. 2008). This finding was the first report to exhibit the role of PI-PLC in plants against drought stress. Later, Omer et al. (2013) conducted an experiment to determine susceptibility of different tropical maize genotypes to *Agrobacterium*-mediated transformation with the drought tolerance gene *NPK1*. It has been observed that plants that have a drought-tolerant gene showed better growth under drought conditions than normal plants. Muoma et al. (2014) transformed *Nicotiana* protein kinase (npk1) in tropical maize lines through the *Agrobacterium tumefaciens EHA101* strain to analyze the role of oxidative signal cascades under stress conditions. PCR and Southern blot analysis were performed to confirm the presence of the *npk1* gene and its copy number, respectively. Further, physiological and morphological variations were assessed in these transgenic lines under drought stress. So, transgenic lines under drought stress have been accredited to lengthen days of maturity an average of 5–8 days as compared to the wild type under absence of any stress. There was no difference in kernel weight among transgenic plants under drought stress and well-watered plants, which showed that the *npk1* gene enabled the transgenic plants to withstand drought stress and also enhanced maize yield (kernel numbers). Overall, a 20–35% improvement in yield of transgenic plants under stress conditions was seen as compared to nontransgenic plants.

However, identification of genetic components that confer resistance to drought in maize is of great significance. For this purpose, Wang and Qin (2017) reported a genome-wide association study (GWAS) of maize at the seedling stage against resistance to drought in a naturally varying population. In maize seedlings, apart from 82 genetic variants, only 42 candidate genes were notably connected to drought tolerance. Five significant single-nucleotide polymorphisms (SNPs) located within the 3′-untranslated region (UTR) of a single gene residing on chromosome 9 encodes vacuolar type H^+ pyrophosphatase, having the same protein sequence homology with *Arabidopsis* AVP1. So, in maize it is designated as zmVPP1. The peak GWAS signal exhibited that ZmVPP1 is significantly related to drought tolerance in maize. Expression of zmVPP1 has been enhanced in the maize inbred line A188 through the *LBA4404* strain of *Agrobacterium*-mediated transformation method; under water deficiency, transgenic lines showed greater grain yield than the wild type. Transgenic maize with a high expression of zmVPP1 exhibited improved drought tolerance that is more probably the result of high photosynthesis efficiency as well as root development.

5.3 Chilling-Resistant Transgenic Maize

Betaine seems to be the interpretative determinant of stress tolerance in plants. Under different stress conditions, the growth and endurance of a diverse variety of plants, incorporating maize, improves with external application of betaine. It reported that with an elevated level of betain, lipid peroxidation of the cell membrane decreased and as a result chilling tolerance was enhanced in maize. Sakamoto and Murata (2002) proposed that under diverse kinds of stress conditions, betain might protect the machinery of protein synthesis and sustained conditions under which the repair processes happened more rapidly as compared to damaging processes. Later, Quan et al. (2004) reported four transgenic elite maize inbred lines by transferring the *betA* gene from *E. coli* through *Agrobacterium tumefaciens* against chilling stress. Betaine concentration in leaves of a few transgenic lines was 208–333% higher than in the wild type in pre-chilling stress, whereas post-stress betaine concentration in transgenic plants was considerably higher than in the wild type. Under chilling stress, less cell membrane damage by maintaining its stability, less cell injury, and no lowered rate of photosynthesis as compared to wild-type maize plants has been observed from betaine synthesis. Therefore, it could be determined that engineering of betaine synthesis is a possible way to enhance chilling tolerance in maize (Table 2).

Extensive studies have been conducted on the development of genetically modified maize plants for biotic and abiotic stress tolerance. Nijmeijer (2013) noted 99 events for insect tolerance and four commercialized events for drought tolerance. One of the possibilities of a large number of transgenic plants not becoming commercialized is the fact that most of the genes improving stress tolerance have alternate targets in the cell. These transgenes result in distorted and agronomically disadvantaged plants; hence, only a few could be commercialized (Nijmeijer 2013).

6 Environmental Protection and Risk Assessment

The possible risks associated with transgenic plants include transgene flow, evolution of resistance in targeted pathogen species, effects on nontargeted species, and health hazards from transgene integration and expression. Maize is a cross-pollinated crop plant, and transgene flow from transgenic to nontransgenic plants is most likely; therefore, specialized biosafety guidelines are recommended to control vertical and horizontal gene transfer. Transgene flow is reported in Mexico, Brazil, and Columbia (Chaparro-Giraldo et al. 2015), and the possible reason was avoidance of recommended biosafety rules. This problem was also seen changing with the social groups: one group of farmers did not care for biosafety guidelines, resulting in gene flow to nontransformed genotypes and landraces, including teosinte in Mexico, whereas other workers did follow the biosafety guidelines and there was no gene flow (Agapito-Tenfen et al. 2017). There is a possibility of developing herbicide-resistant teosinte and other superweeds if biosafety guidelines are not followed. Resistance against all chemical pesticides can be developed by insects according to the

Table 2 Transformation targeted to abiotic stress tolerance

Genotype	Vector	Strain	Trait gene	Agronomic trait	Reference
Hi II and inbred line B73	pSHX002 and pBAR184	*EHA101*	npk1 and phosphinothricin transferase	Drought stress	Shou et al. (2004)
Elite inbred line Ye 7922, TL08, KAT, PTL001, DH01, DLC1	pCUA	LBA4404	*ZmPLC1*	Drought stress	Wang et al. (2008)
Inbred line A188	pSHX004	*EHA101*	npk1	Drought stress	Muoma et al. (2014)
Inbred lines	pSB II	*LBA4404*	ZmVPP1	Drought resistant	Wang and Qin (2017)
Hybrid line	pTF102	C58C1	EPSPs	Glyphosate phosphinothricin resistant	Kim et al. (2009)
78599, Zong 31 and BA	pCAMBIA	*EHA105, LBA4404*	EPSPS	Glyphosate-resistant	Wang et al. (2010)
Inbred line Z31	pM3301UbiSpAM79	*EHA105*	*2mG2-EPSPS*	Glyphosate resistant	Yu et al. (2013)
Inbred line X090 and Z3	p2EPUHLAGN	*LBA4404*	*mAM79 aroA*	Glyphosate resistant	Ren et al. (2015)
Hybrid line Q31 × Z3	*pCDH1300*	*LBA4404*	*Btcry1Ah* and *2mG2-epsps*	Pest and glyphosate resistant	Sun et al. (2015a)
Elite inbred line DH4866	*P35S-als*	*LBA4404*	*betA*	Chilling resistant	Quan et al. (2004)
Inbred lines Qi319 and N10-6	*P35S-als*	Gene-Gun PDS-1000	*als*	Chlorsulfuron resistant	Li et al. (2002)
Hi-II (A-188 × B73)	*pAHC25*	Gene-Gun PDS-1000	Rab -28 gene	Drought resistant	Amara et al. (2013)

principles of evolution; therefore, the Bt corn cultivation was regulated by the United States Environment Protection Agency. As 20% of the seed must have been nontransgenic to provide refuge against evolving insects, and sometimes the rules are not followed by the citizens, so it was seen that 20% of the farmers were planting all Bt seeds in the United States in 2002 (Gewin 2003). Similarly, Dively et al. (2004) reported the unusual behavior of monarch butterfly larvae fed continuously on corn pollen expressing the Cry1AB gene. Allergies and metabolic disorders have been hypothesized over the years by the opponents of transgenic crops; therefore, allergenicity tests have been part of the risk assessment for transgenic plants. According to a report in 2017, approximately 20 proteins were seen with altered expression in transgenic and its counterpart nontransgenic lines under various biotic and abiotic stresses. This report requires the inclusion of molecular analysis of proteins and metabolites in risk assessment studies (Benevenuto et al. 2017).

7 Conclusion and Future Prospects

Because of the necessity for better crop cultivation, there is a growing interest in research for developing good-quality transgenic plants. Maize transformation started about half a century ago, and at present various technologies for gene transfer are available, although the randomness of integration sites as well as low transformation efficiency are yet restraints. *Agrobacterium*-mediated and biolistic transformation are the most efficient techniques because of their simplicity and the stable integration of the gene on the broad spectrum of plant species. An essential definition of transgenic crops is to produce genetic improvement by introducing remote genes, not only from other plant species but also from different bacteria, fungi, viruses, and animals. Using tissue culture technique, the transformed plant cells are regenerated into a complete plant. Regeneration is yet genotype dependent in most cases; therefore, for the past two decades; many efforts have been made to overcome the barrier of genotype dependence so that transformation could also be genotype independent. Inplanta transformation is proving one of the worthy efforts from extensive research during the past 10–12 years. The transformation methods developed over the years have been utilized to enhance various traits in maize with a premise to increase maize productivity, improve nutritional value, and develop resistance against biotic and abiotic stresses to fulfill the requirements of the ever-increasing human population, animal feed, and industrial sectors. However, still there is an immense requirement to explore many pathways to develop crops on a large scale with desirable characteristics that have the competence to combat major environmental restrictions and survive under severe conditions. In coming years, research will be more focused on enhancing not only the quantity of maize through transgenic crops but also towards improving nutritional value. The quality of transgenics plants itself will also be improved by such means as defined integration events, marker-free transgenics, and transgenes pyramided transgenics for quantitatively controlled characters.

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Novel Breeding and Biotechnological Approaches to Mitigate the Effects of Heat Stress on Cotton

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1 Introduction

Cotton belongs to the family Malvaceae, which contains more than 200 genera and about 2300 species. More than 50 species of *Gossypium* are native to Africa, Australia, Central and South America, and Asia (Wendel and Grover 2015). *Gossypium barbadense*, known as Egyptian or American pima cotton, produces 8% of the total cotton in the world. *G. hirsutum* is called upland cotton; native to Mexico and Central America, this species produces 90% of the total cotton in the world (McCarty et al. 2004). Upland cotton is an important fibre crop that is cultivated in more than 80 countries and occupies more than 32 million hectares worldwide. It is an important source of oil and livestock feed (Singh et al. 2007). Pakistan, China, India, and Sudan contain 75% of the total cotton cultivated area in the world, and these countries have the potential to produce cotton at high temperatures. Although cotton is a warm-season crop, this plant does not yield best at a very high temperature. Researchers have reported that high temperature has negative association with boll development and plant growth in *G. hirsutum* (Pettigrew 2004). The reproductive growth of a cotton crop is more sensitive to heat stress than is its vegetative growth, and high temperature exerts a negative effect during the flowering period. High temperature also causes sterility of pollen grains, disruption of plamsa membranes, dropping of flowers, poor retention of bolls, and reduction in the fibre quality (Kakani et al. 2005).

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There are several ways to overcome this problem of the cotton plant but development of heat-tolerant germplasm is one of the best solutions to combat high temperatures. Several projects on cotton breeding are being executed for the development of new and improved upland cotton germplasm resistant to various abiotic stresses (Wahid et al. 2007). Researchers have found a considerable level of tolerance in cotton, but the mean temperature of the Earth is rising continuously, which warns the cotton breeders to identify potential in existing germplasm by using efficient screening techniques based on physiological and molecular parameters. The molecular techniques are also time-saving and reliable (Saranga et al. 2004). Conventional breeding methods along with molecular tools are reliable and efficient methods to breed new lines of crop plants with improved qualities *i.e.* yield and tolerance to abiotic stresses.

2 Effects of High Temperature on Crop Plants

Increase in temperature produces heat stress in both arid and semi-arid regions, and this is becoming an alarming problem for agriculture around the globe (Hall 2000). The global population is ever increasing, so now it is necessary to increase the yield of crop plants. Night temperature is the most important factor reducing per acre yield according to some researchers, with the secondary role played by the day temperature (Willits and Peet 1998). Several studies have been conducted to assess the effect of increased temperatures on various stages of plant development and yield of crops. Knowledge of plant response to elevated temperatures is allowing farmers to grow crops for greater production in a harsh environment and gain justifiable crop yields (Paulsen 1994). In tropical regions, growth and development are damaged by high temperatures in various ways: damage before and after harvesting, sunburn of leaves, scorching of twigs, abscission and senescence of leaves, inhibition of root and shoot growth, discoloration and damage of fruits, and yield reduction of crop plants (Vollenweider and Günthardt-Goerg 2005).

Under heat stress, the response of the plant varies with species and with the degree and duration of temperature. At high temperature, growth of the plant is stopped, but uptake of water from soil is increased, stomata are closed, the internal temperature of the plant is increased, and wilting of leaves takes place. If water is not provided at this stage, wilting of leaves becomes permanent, and ultimately cellular damage or cell death may occur, leading to the catastrophic collapse of cellular organization (Ahuja et al. 2010). Increase in temperature affects the efficiency of enzymes, RNA synthesis, stability of membranes, and proteins that are involved in major physiological processes (Pagamas and Nawata 2008). The effects of heat stress are summerized in the following paragraphs.

2.1 Morphological and Yield Traits

2.1.1 Seedling and Root Growth

The best germination of cotton seed is reported to be at 22–24 °C and the best seedling development is at 26 °C, although in some parts of the world, especially the state of Mississippi of the United States, the optimal range of temperature for germination of seeds and for development of seedlings is 28 °C and 30 °C, respectively. Low temperature also causes many problems in cotton during germination and initial growth stages. Different cotton genotypes exhibit differently for their germination and the development of roots during cool soil temperature conditions (Nathan et al. 2005). In spite of sufficient moisture and nutrient availability, the existence of temperature stress (40/32 °C), plants produce poorly developed and wilted roots. The cotton plant shows its best development at day and night temperature ranges of 30/22 °C and 35/27 °C, respectively (Reddy et al. 1997). In many dry areas of the world, seedlings of the cotton plant have been found to be heat tolerant because this plant provides seed cotton during moist conditions (Burke 2001). The moisture of the soil is depleted quickly at the time of sowing in North Indian and Pakistan regions because of increased temperature and high wind velocity, so the seedlings of cotton varieties grown in these regions are said to be tolerant to high temperature and water deficit conditions (Lather et al. 2001).

2.1.2 Vegetative Plant Growth

When we talk about the growth stages of a plant affected by heat stress, it is the germination stage which is affected significantly. In various cultivated plant species, it is observed that germination percentage, the emergence of plants, normal seedlings, good seedling vigor, and growth of germinated seedlings are greatly reduced during high-temperature conditions (Toh et al. 2008). Reduced seed germination is also reported through induction of abscisic acid (ABA) (Essamine et al. 2010). Although reproductive stages of the cotton crop are more sensitive to heat stress as compared to vegetative stages, high temperatures also cause irreversible damage to the vegetative parts of cotton plants. It has been observed that cotton plants grown at high temperature showed poor growth, very short stature, fewer fruiting branches, and poor crop stand. The vegetative growth of many other crops is also affected adversely under heat stress *e.g.* in wheat, the germination rate is inhibited and burning of embryos takes place at high temperature (45 °C). In rice, the number of tillers, plant height, and biomass are reduced in response to heat stress. In sugarcane, it has been observed that leaf tips and leaf margins are damaged, like rolling and drying of leaves (Omae et al. 2012).

High temperatures can reduce the life period of field crops. The cotton crop completes its life cycle in a short time under heat stress, and the optimal time period required for proper growth of cotton bolls and development of its fibre is reduced, resulting in reduced boll size and poor fibre characteristics. In cereals, a small increase of temperature, 1–2 °C from optimum temperature, is responsible for shortening the time period for grain filling and negatively affects the yield (Nahar et al. 2010).

2.1.3 Square, Flowers, and Bolls

The yield of cotton depends on fruit settings and retention as well as boll weight. In the beginning of fruit development, squares appear, then flowers are developed which finally become the boll. Squares and flowers are aborted at their peak when day and night temperatures are 30 °C and 20 °C for 13 h, respectively (Reddy et al. 1991). Approximately 65–70% of fruiting points, namely, squares, buds, and small bolls of the cotton plant, fell from abiotic stresses, but heat stress is one of the factors in this loss. Pollen grain viability is severely decreased at a temperature above 35 °C; as a result, unfertilized flowers are produced that cannot form bolls (Baloch et al. 2000). Although cotton is a C3 plant, it is more heat tolerant than other plants of this class, but excessive temperature stress, that is, above 36 °C, results in severe losses to the plant (Fisher 1973). All existing squares and flowers were found to be aborted in many cotton cultivars at extreme day temperatures *i.e.* 40 °C, whereas pima cotton was found to be highly susceptible and unable to produce branches.

Retention of few bolls has been observed in cotton plant with increased temperature. Under high temperature conditions, plants also produce poor flowers. Furthermore, both of these traits vary extensively in different years according to environmental conditions and the cultivars. It has been established that abortion of young bolls takes place on exposure to an average daily temperature of more than 28 °C. In the Yangtze Valley of China, many periodic shocks related to temperature, with an air temperature more than 35 °C, usually in the months of July and August when cotton flowering and boll formation are at the peak, result in senescence of leaves, boll abortion, and a reduction in lint yield (Liu et al. 2006). Boll retention in cotton plant is strongly controlled by temperatures regimes. For the maintenance of bolls, 28 °C is the best temperature, and young bolls drop when the mean daily temperature becomes more than the optimum temperature. The main effect of higher temperature is lesser fruit holding, resulting in lower seed cotton yield, poor lint quality, and late crop maturation (Reddy et al. 1999).

2.1.4 Seed Cotton Yield

Yield is the most important trait for the farmer, and it has been observed that seed cotton yield is significantly affected by adverse environmental conditions (Brown et al. 2003). Negative effects on crop yield are observed during water deficit and high-temperature stress conditions (Lewis et al. 2000). It is noted that an increase in

temperature during day and night can produce adverse effects on crop yield. It is suggested that an optimal temperature is necessary for obtaining a good yield from the cotton plant. Optimum temperature is not well defined in the literature because every variety has differential response to temperature. A strong negative relationship between high temperature and yield of seed cotton is observed in the fields of Arkansas (USA) (Oosterhuis 2002). Pakistan and India are two of the four top cotton-producing countries of the world. The cotton belt of Pakistan experiences very high temperatures during the cotton season and similar conditions are observed in India. About 48 °C is observed during the cotton growth in these regions. Although the yield of seed cotton in these two countries is quite low as compared to other cotton-producing countries, the genotypes cultivated in these regions give optimal yield even at this very extreme temperature. It can be assumed that this very high temperature during summer is one of the reasons for low seed cotton yield in these countries.

There is no defined threshold level of heat stress in plants, but the temperature of 32 °C is found to be stressful, causing deterioration of more than 50% of cotton boll development. Furthermore, high temperature results in insufficient production of carbohydrates, reflected by high boll shedding percentage, smaller bolls, lesser lint percentage, and reduced yield production (Oosterhuis 1999). The fibre of the cotton plant is predominantly developed from plant carbohydrates, and decreased amounts of carbohydrates lead to less fibre and reduce ginning turn out. Increase in temperature above the optimum level may also reduce the size of cotton seeds and fibre length. Temperature influences both the rate of elongation of the fibre and secondary wall thickness. The boll maturation period is shortened by increased temperature, and incomplete boll maturation takes place during increased night temperatures. It has been validated that initial stages for elongation of fibre are sensitive to higher night temperature whereas the later stages are less sensitive to increased temperatures (Gipson and Joham 1969).

2.1.5 Quality of Fibre

Cotton fibres are the outgrowth of single epidermal cells. These fibres consist of various carbohydrates with cellulose as the key component. The cotton crop is generally grown for its fibre because the textile industry utilizes these fibres for making cloth. Cotton fibres or mature cotton bolls exposed to sunlight or at high temperature for many days will decrease in fibre quality. As a cotton crop completes its life cycle rapidly under high-temperature stress, thus the time required for fibre elongation and maturity also decreases, resulting in less growth of the epidermal cells with poor deposition of cellulose. The fibre quality parameters are fibre length, fibre strength, and fibre fineness. All these traits are poor in quality under heat stress conditions (Azhar et al. 2009).

Breeding with the purpose of high lint yield is still the primary goal for any cotton breeding program, and improvement in fibre quality has become increasingly important. The presence of heritable variation and the favorable correlation between

the different traits is important for launching any cotton breeding program designed for selection of desired genotypes. Cotton genotypes vary significantly for fibre length, fibre strength, fibre fineness, and fibre uniformity. Heat-tolerant genotypes of cotton provide high and stable seed cotton yield with better quality of fibre than heat-susceptible genotypes. Cotton is extremely responsive to changes in humidity, soil moisture content, and temperature, which affect its yields, the components of the yield, and the fibre properties (Killi et al. 2005).

2.2 Physiological and Biochemical Traits

2.2.1 Viability and Development of Pollen

During heat stress, pollen is adversely affected because it is the most sensitive organ in the plant. The number of fruit points are decreased in response to pollen sterility caused by heat. The yield of the crop depends upon temperature during the development of the pollens (Van Ploeg and Heuvelink 2005). Although cotton is a warmer-season crop, the viability of cotton pollen is greatly decreased under heat stress. The decreased viability of the pollen grains in cotton results in poor boll formation and fewer fruit sets, causing reduced yield. The adverse effects of heat stress on pollen viability are also reported in several crops such as tomato, barley, rice, chickpea, rapeseed, common bean, bell pepper, soybean, and strawberry (Djanaguiraman et al. 2013; Porch and Jahn 2001; Prasad et al. 2006).

High temperatures adversely affect the development of pollen at the early development of the stages (De Storme and Geelen 2014). In several crops, the most sensitive stages are 7–15 days before anthesis and typically at the time of meiosis. However, heat stress does not affect pollen quality components or meiosis stage when the temperature is above optimum level after the release of tetrads has occurred (Ahmed et al. 1992). The development of microspores (4 days before anthesis) and anthesis are the most sensitive stages in peanut (Prasad et al. 2001). Similarly, heat stress in bell pepper during the development of microspore mother cells leads to the reduction of pollen viability while heat stress at the time of lateral developmental stages does not affect pollen viability (Erickson and Markhart 2002).

Major loss of yield in plants has been observed to be caused at the time of flowering by a small increase in temperature because the reproductive stage is the most important and most sensitive stage of plant development (Lobell et al. 2011). Although variations in responses to heat stress have been found in plant species, increase in temperature at the reproductive stage is responsible for reduction in floral buds and increased flower abortion (Sato et al. 2006). Heat stress increases the rate of male sterility by reduced pollen germination, reduced development of the pollen tube, reduced ovule viability, irregularity in positions of stigma and style, reduction in numbers of pollen grains, and decreased fertilization processes (Yun-Ying et al. 2008).

2.2.2 Tapetum and Pollen Germination

The tapetum is the key organ of plants, providing metabolites to the pollen. The growth and development of the tapetum are significantly affected by increases in temperature. A temperature increase leads to the early meiotic phase I and degradation of the tapetum (Oshino et al. 2007). In wheat (Saini et al. 1984), cowpea (Ahmed et al. 1992), and purple false brome (Harsant et al. 2013), degradation of the tapetum has been reported during heat stress. Increase in temperature before anthesis affects the tapetum, causing structural abnormalities. In response, premature degradation of the tapetum occurs (Suzuki et al. 2001).

In plants, germination of the pollen and formation of the pollen tube are sensitive processes adversely affected by increase in temperature. It was found in many crop plants that decreased fruit setting taking place at elevated temperatures was primarily caused by reduction in pollen germination (Hazra and Ansary 2008). Pollen germination may be reduced when pollen development occurs under heat stress. Reduction in pollen germination is observed at 36 °C in such crops as cotton (Kakani et al. 2005), tomato (Vasil 1987), and cucurbit (Jóhannsson and Stephenson 1998). Poor pollen germination also delays fruit development and parthenocarpy (Abdelmageed and Gruda 2009). As a result of temperature increase, pollen germination is reduced and lesser yield is obtained from different plants.

2.2.3 Photosynthesis

In plants, photosynthesis is the most important and heat-sensitive physiological process (Crafts-Brandner and Salvucci 2002). High temperature exerts significant influence on the photosynthetic activity of C3 and C4 plants (Yang et al. 2006). Chloroplasts of plants, especially the photochemical reactions and carbon metabolism of thylakoid membranes and stroma, have been thought to be the primary sites of heat injury (Wang et al. 2009). Increased temperatures change the structural organization of thylakoids with swelling of the grana, so that the plant cannot synthesise its food, and reduction in yield occurs (Ashraf and Hafeez 2004). In leaves of plants, increase in temperature significantly affects stomatal conductance, water status, and intercellular CO_2 concentration (Greer and Weedon 2012). The closing of stomata at high temperature is another reason for decreased photosynthesis, which seriously affects intercellular CO_2 and photosystem II activity. The amount of photosynthetic pigments is greatly reduced by heat shock in plants (Marchand et al. 2005). In sorghum, when the temperature is increased (40/30 °C, day/night), reduction in chlorophyll pigment has been observed (Mohammed and Tarpley 2010). Heat tolerance is positively correlated with the efficiency of plants to assimilate CO_2 and maintain gas exchange under heat conditions (Yang et al. 2006). In plants, photochemistry of photosystems and stomatal conductance were also observed to be reduced when the temperature increased. Other reasons are also responsible for the reduction in photosynthesis and plant yield as a result of heat stress. Reduction in the binding of soluble proteins and damage to subunits of RuBisCo is also caused

by heat stress (Sumesh et al. 2008). Synthesis of starch and sucrose is also affected by increased temperature, causing decreased activity of sucrose phosphate synthase (Djanaguiraman et al. 2009). Increase in temperature is also responsible for lowering of the water potential in leaves and leaf area, so that premature leaf senescence takes place with a negative impact on total plant photosynthesis (Greer and Weedon 2012).

2.2.4 Carbohydrates/Sugar Content

Carbohydrates are a source of energy needed for the development and germination of pollen; they protect the membranes from adverse effects of stress including heat stress. Disruption in the metabolism of carbohydrates by elevated temperatures can affect the nutrient uptake of plants with resulting reduction in crop yield. The effect of heat stress on pollen carbohydrates has been studied in tomato, sorghum, chickpea, cotton, okra, and pepper. Production of fluorescence in the cotton crop is significantly affected at high temperatures and the viability of pollen is also reduced. From heat stress, the flowers show decreased levels of their soluble sugars in both anthers and pollen (Pressman et al. 2002). It is speculated that at the time of high-temperature stress soluble sugars are blocked in the locular fluid and fail to reach the pollens. In contrast to heat stress, during normal conditions of pollen development starch is accumulated in the pollen 3 days before anthesis and is then converted into soluble sugar. During heat stress, the concentration of starch does not reach at the required level, and ultimately the amount of soluble sugar is decreased in mature pollen. These facts indicate that even a minor change in temperature causes a change in sugar transport and metabolism.

2.2.5 Proline

Proline is an amino acid acting as an osmolyte in the cells. It has three vital roles in the plant during stress: maintenance of stability of the cell membrane and prevention of leakage of electrolytes helps optimize the concentration of reactive oxygen species and detoxification of effects of metals by acting as a chelator. It is suggested that a high concentration of proline in the cells during heat stress is desirable for normal growth and functioning of the plant (Rana et al. 2017). The concentration of proline in plants increases in response to different biotic and abiotic stresses such as increase or decrease in temperature, salinity, and pathogen attack. When proline contents in anthers of heat-sensitive and heat-tolerant cultivars of different crop plants were analyzed at 45 °C, it was found that mature pollen of tolerant cultivars had higher proline content than sensitive cultivars (Mutters et al. 1989). Under prolonged heat stress conditions, the expression of genes responsible for proline transfer in cotton is decreased.

2.2.6 Lipids and Polyamine

Lipids have a key role in membrane stability and fluidity during heat stress. The content of ROS in plants increases with a rise in temperature, which causes changes in phospholipids concentration from lipid phosphorylation. High temperature increases unsaturated fatty acids and reduces the quantity of saturated fatty acids. It was reported that the cellular membrane is damaged by an increase in the quantity of unsaturated fatty acids, which enhances membrane fluidity. The unsaturated fatty acids make the membrane more vulnerable to ROS. Pollen viability is correlated with changes in saturation of phospholipids (Djanaguiraman et al. 2013).

Polyamines are considered as a source of tolerance to abiotic stresses such as heat, cold, drought, and high metal content (Fariduddin et al. 2013). They sustain membrane integrity and work as scavengers against ROS (Alcázar et al. 2006). During the period of heat stress, alterations in polyamine content occur and pollen germination is decreased by the reduction of *S*-adenosylmethionine decarboxylase (SAMDC). The blocking of SAMDC translation with cycloheximide could phenocopy the effect of heat stress, which leads to decreasing pollen germination in controlled conditions. Some other proteins have also been reported to affect pollen germination.

2.2.7 Reactive Oxygen Species

Reactive oxygen species (ROS) act as signaling molecules under normal and stress conditions for the activation, upregulation, and downregulation of many genes involved in various metabolic pathways. When the concentration of ROS increases from optimal levels, then these species cause irreversible damage to plants by oxidative stress. High-temperature stress affects metabolic and enzyme pathways by causing the addition of ROS: hydroxyl radical (OH^-), singlet oxygen (O_2^-), and hydrogen peroxide (HO_2^-). These molecules are also responsible for oxidative stress in plants (Asada 2006). The major sites of ROS generation are the chloroplast photosystem I (PSI) and photosystem II (PSII). ROS are produced in different organelles such as mitochondria and peroxisomes (Soliman et al. 2011). There is a direct relationship between the accumulation of ROS and the maximum efficiency of PSII. The optimal quantity of ROS is required for proper functioning of plant cells and tissues. Less absorption of a photon occurs from the thermal damage of photosystems. Under stress conditions, PSI and PSII absorb the intensity of photons; the extra quantity is necessary for the incorporation of CO_2 and is known as extra electrons (Halliwell 2006). During the ROS in photooxidation reactions (flavoprotein, redox cycling), O_2^- is produced by the Mehler reaction in chloroplasts. Singlet oxygen is formed in chloroplasts during the process of photoinhibition and PS II reactions of electron transfer (Karuppanapandian et al. 2011).

3 Plant Response Under Heat Stress

3.1 Post-transcription and Post-translation Gene Regulation

Various post-transcriptional and post-translational gene regularities have been noted when plants experience stress. The expression level of various genes also changes accordingly. These changes make target at a different level to the same transcript. The activity of stress-related genes responds in the form of the transcript as a signal stress including heat stress. Post-transcriptional and post-translational changes can subsequently affect the specific transcripts for integrating with other signals, including the specific type of the main stress, photoperiod, internal growth, or developmental signals and the hormonal pathway. It is revealed from recent studies that there is an interaction between alternative splicing and micro-RNAs (miRNAs). Different kinds of a given miRNA may be produced from the same gene as a result of alternative splicing (AS). It is also reported that modification in the binding sites of miRNA of the target transcript can be done by AS. In heat induction, pre-mRNA splicing has been reported in *Arabidopsis thaliana* by participating in miRNA processing of the intronic miR400, which illustrates the first type of interaction (Yan et al. 2012). It is also reported that miR400 can be co-transcribed with the help of its host gene At1g32583. During heat stress conditions, an event arises in the intron, carrying the miR400 results in the accumulation of miR400 primary transcripts. In *A. thaliana* overexpression of miR400 results in plants hypersensitive to heat stress. Heat induced a negative regulatory mechanism using miR400 expression, resulting in positive effects on the plant during heat tolerance. Several mRNAs, namely, miR842 and miR846, are produced as a result of AS (Jia and Rock 2013).

Some classes of small RNAs are involved in regulation of genes having different biological processes such as plant growth and biotic and abiotic stress responses. Much relevant evidence of the small RNA-based regulatory mechanism has been studied in reaction to heat stress. They make a class of endogenous small noncoding RNAs 20–24 nucleotides in length that act according to sequence pairing of mRNAs of their target genes and obstructing their translation by cleaving them (Axtell 2013). It shows the effect on different stress-related traits such as opening and closing of stomata, root development, osmo-protection, and antioxidant defense, as well as on crosstalk between pathways of auxin and ABA signaling. For example, *miR398* is associated with antioxidant defense. This miRNA controls different genes, such as Cu/Zn superoxide dismutase (CSD). CSD enzymes scavenge on oxidative stress. On exposure to heat, *miR398* is induced by heat shock transcription factor (HSF), thus masking the expression of the targeted genes *csd1*, *csd2*, and *ccs* (Guan et al. 2013). The heat response results in downregulation of several miRNAs. For example, the expression of *miR159* is decreased in bread wheat when exposed to heat stress (Wang et al. 2012). Transgenic wheat plants with overexpressing *miR159* or the *Arabidopsis* double mutant (*myb33 myb65*) are more susceptible to high temperatures.

Several genes are reported in the literature to have a potential role in heat stress response by utilizing genetic screening and genome-wide expression analysis (Yeh et al. 2012). In response to environmental and developmental signals, plants have post-transcriptional mechanisms by encoding miRNAs. Many miRNAs related to the responses of increase in temperature are recognized, and their response has been studied under heat stress conditions. Stress-upregulated miRNAs can downregulate their target genes in thermal stress. Understanding the roles of miRNAs in cellular tolerance, transcriptome homeostasis, and the phenological and developmental plasticity of plants during stress conditions will be helpful in engineering stress-tolerant crops in future (Jenks et al. 2007).

3.2 Hormonal Pathways

Different hormones are produced in plants in response to different environmental signals such as heat stress. One of those phytohormones is auxin, which affects many physiological mechanisms during heat stress conditions. Endogenous auxin is reduced in response to heat stress in developing anthers (Teale et al. 2006), whereas the expression of the *YUCCA* auxin biosynthesis gene was repressed through the increase of temperature and male sterility was caused in barley (Oshino et al. 2007). Salicylic acid (SA) has a role during heat stress for growth and development. SA prevents oxidative damage through detoxification of superoxide radicals, although the capacity of antioxidants prevents membrane damages of the plant. SA can increase heat resistance, improve fertility, and increase yield (Sakata et al. 2010). The antioxidant capacity in rice and *Arabidopsis* is found to be increased after the application of SA. The key stress hormone, abscisic acid (ABA), is responsible for stomatal closure during osmotic stress. ABA is also related to Rboh regulation, and Rboh mediates ABA-induced ROS in the guard cells of leaves. RbohD and RbohF are the major catalytics involved in this process (Miller et al. 2008). A high level of ABA increases growth during the vegetative period and reduces growth during the reproductive period, causing male sterility and a reduction in the rate of seed setting (Todaka et al. 2012).

3.3 Phospholipids Pathways

Many reports are available for the role of phospholipid-based signaling in response to increases in temperature (Zonia and Munnik 2006). Change in the composition of membrane phospholipids is the property of this response. The signals of phosphoinositide (PI) occur in the early events following the onset of heat stress. During subsequent incubation at 40 °C, the metabolism of membrane lipids is not affected significantly. The low level of PI signaling produces stress resistance, and higher

levels can increase damage of the cells. Phosphatidylinositol 4,5-bisphosphate (PIP) and phosphatidic acid are key mediators of pathways of signaling and of cytoskeletal and membrane dynamics organization (Zhu 2002; Staiger and Blanchoin 2006; Mellman et al. 2008). Transfer of phosphate to PI is catalyzed by the PIK enzyme during high temperature. By the activation of PIPK and PDL, heat stress induced the increase of PA and PIP levels. The G protein transduces signals initiated by heat required for PIP and PA accumulation and may be required for activation or localization of PIPK. The role of G protein is based on signaling, which has been identified as a component of heat stress response that is regulated negatively through AIFx (Misra et al. 2007).

3.4 Ubiquitination Pathways

The ability of a plant to survive under abiotic stresses depends upon proteomic plasticity. The ubiquitin-proteasome system (UPS) empowers the plants under heat stress to change the proteome for an effective response (Kurepa et al. 2008). UPS response to specific stress governed by the type of substrate protein such as ubiquitin-dependent dilapidation is a positive regulator which can suppress the various pathways under heat stress conditions. It also promotes the cellular changes that help the plants to acclimatize in stress environments. The negative regulator, ubiquitin ligase, is involved in the modification of regulatory proteins (Chen and Hellmann 2013). It also activates signaling pathways in response to stress stimuli under stress conditions. Many examples of UPS working to attenuate stress signals have been reported, such as degradation of ubiquitin-dependent positive regulation after receiving the stress signal. Maintenance of an optimum level of intensity of a signal and the end of its transduction could permit a plant to retain its normal growth and development when normal environmental conditions are recovered. The gene expression of ubiquitin is upregulated in plants when they are exposed to increased temperature conditions. In fact, overexpression of the ubiquitin gene has been proved to improve plant tolerance during abiotic stresses (Guo et al. 2008). After these findings, the role of many ubiquitin enzymes has been demonstrated in plants. Different E2 coding genes have been reported to regulate under stress conditions. For example, transcription levels of *CUBC*$_2$ *Glycine max* (*Gm CUBC*$_2$; soybean), *Arabidopsis UBC32* (*At UBC32*), and *Arachis hypogaea UBC*$_2$ (*AhUBC*$_2$; peanut) are upregulated by abiotic stress conditions (Cui et al. 2012).

The response of plants during unfavorable environmental conditions is a complex and coordinated process that involves the starting of their signaling network and changes the expression of thousands of genes. By modifying many factors of transcription, the UPS can affect the variations in the expression of genes necessary to lessen the impact of biotic and abiotic stresses. E3 ligases do not allow the transcriptional activity of different transcriptional factors for change from normal conditions, such as the regulation of dehydrated responsive element-binding proteins DREB 2A

through RING type E3 ligase DREB 2A interacting proteins DRIP 1 and DRIP 2. DREB 2A is one of the transcription factors that control the expression of inducible genes during different abiotic stresses (Qin et al. 2008).

3.5 Heat Shock Proteins

Besides biochemical, physiological, and morphological mechanisms, molecular techniques are also being used to understand the basics of abiotic stress tolerance in plants. Plants can tolerate all types of stresses through modifying their gene expression and through coordination of gene expression in various pathways (Vinocur and Altman 2005). Similar to some other abiotic stresses, heat stress upregulates inducible genes and their production increases several fold during heat stress conditions. These genes are commonly referred as "heat shock genes" (HSGs) that encode "heat shock proteins" (HSP), proteins that are necessary for the survival of plants under high-temperature stress (Chang et al. 2007; Prasinos et al. 2004). HSPs are grouped into five different classes: HSP20, HSP60, HSP70, HSP90, and HSP100. HSP20 is also called small heat shock proteins (sHSPs). Because of their thermotolerance in nature, HSP expression could be enhanced through heat treatment and conserved heat shock elements (HSEs), which are present in the promoter region of any HSG. These elements are called *cis*-acting elements and consist of different palindromic nucleotide sequences that assist as recognition and binding sites for HSFs (Nover et al. 2001). In many species of plants, HSFs are expressed constitutively during normal conditions. It is reported that HSPs are present in the cytoplasm as monomer bonded, such as HSP70. When plants are exposed to heat stress, HSP70 is dissociated from cytoplasmic monomeric HSFs and enter into the nucleus where they form a trimer that can bind with HSEs (Lee et al. 1995). HSFs can recruit new transcriptional components, resulting in the expression of genes within seconds during high temperatures. Almost all HSGs have HSFs, and overexpression of HSFs switch on the HSGs, which provide protection to living organisms against high-temperature stress. This system is common in all the eukaryotes, but it is very complicated in plants. All plants have many copies of such genes, at least 17 in tomato, and at least 21 HSGs have been reported in *Arabidopsis*. These genes have been categorized into three different classes (A, B, C) that differ in their linkers, which are flexible and have specific domains (Nover et al. 2001).

HSPs are responsible for protein renaturation, stabilizing denaturized proteins and repairing of broken membranes in plants during heat stress conditions (Török et al. 2001). Protein denaturation occurs in heat stress because reduced cell volume enhances interaction between damaged molecules. These proteins also target nonactive and imperfectly aggregated proteins in the cells, removing them from the cells (dos Reis et al. 2012). These proteins also function primarily for controlling the proper folding and conformation of both structural and functional proteins, that is, cell membranes and enzymes, respectively. They also ensure the normal functioning

of different cellular proteins during high temperatures. NtH SP70-1 is an example of such proteins that are overexpressed under heat stress in the cotton plant and have an important function during high-temperature conditions.

4 Heat Tolerance and Heat Avoidance

4.1 Heat Tolerance

Heat tolerance is defined as the ability of plants to produce optimal yield during conditions of high-temperature stress. Heat stress tolerance is a specific character in similar species. and even different organs, as well as the tissues of a plant, might show a variable degree of tolerance to high-temperature conditions. Development of heat-tolerant genotypes in a crop is one of the major challenges for a plant breeder (Moreno and Orellana 2011). Plants have developed various mechanisms for survival during high temperature. Tolerance mechanisms include osmoprotectants, late embryogenesis abundant (LEA) proteins, and factors involved in signaling pathways, antioxidant defense, and transcriptional control (Wang et al. 2004). Initial stress signals establish the stress-responsive mechanism through the ionic and osmotic process by changes in membrane fluidity: this helps the plant to reestablish and repair damaged membranes and proteins. In heat stress conditions, modifications in the physiological, biochemical, and molecular processes help to produce heat-tolerant genotypes by identification of potential lines from the germplasm.

4.2 Heat Avoidance

During increased temperature conditions, plants show different mechanisms for their survival. These mechanisms include long-term evolutionary phonological and morphological adaptations and short-term avoidance mechanisms such as changing of leaf orientation, transpiration cooling, and alteration of membrane lipid composition. The closing of stomata, increases in stomata density, reduced water loss, and larger xylem vessels are common heat-induced features in plants (Srivastava et al. 2012). Plants growing in a hot climate avoid heat stress by reducing the absorption of solar radiation. This ability is supported by the presence of small hairs, called tomentose, which form a thick coat on the surface of the leaf, the cuticle. In these plants, blades of a leaf usually turn away from light and position themselves corresponding to the rays of the sun. The effects of radiation from the sun can also be decreased by rolling of leaf blades. Those plants that have small leaves avoid heat stress in a better way than plants with larger leaves. Their heat is evacuated more quickly because of the smaller resistance of the air boundary layer. In well-hydrated plants, the leaves are protected from heat stress by increased transpiration rate. The leaf temperature can be decreased from 6 to 15 °C relative to ambient temperature.

Different plant species have different mechanisms that help them to avoid the hottest period of the year, including abscission of leaves, heat-resistant buds, and in desert plants by completing their entire reproductive cycle during the cool season (Fitter and Hay 2012).

5 Screening for Heat Tolerance

Successful breeding programs for heat tolerance always depend upon reliable selection criteria. The first requirement for improvement of a crop involves the characterization of available germplasm for high temperature. It is necessary that dependable and inexpensive screening methods be used to identify agronomic, eco-physiological, and reproductive traits related to stress tolerance. Studies in field conditions have more importance than studies in a controlled environment. Greenhouse and growth chamber studies have also been used to assess the germplasm of cotton for heat stress tolerance. The plants sown in these experiments experience damage to their roots and shoots on exposure to hot conditions, whereas the field-sown plants subjected to increased temperature show more damage to their shoots as compared to roots because of the buffering effects in field conditions (Hall 2004). The basic objective of screening of cotton germplasm and new breeding lines for increased temperature tolerance is to stabilize yield. A reliable selection criteria is required for the valuable breeding program for heat tolerance. Following are some parameters that indicate plant tolerance to high-temperature stress. According to some reports, cell membrane thermostability (CMT) is considered as the most appropriate screening method for determining heat tolerance accessions in germplasm (Blum and Ebercon 1981). Cell membranes are necessary parts of each living cell, acting as a boundary between the environment and the cell protoplasm. These membranes function as a central location for cellular activity and continue their function during stress conditions as well (Raison et al. 1980). The hydrogen bonds of cell membranes are weakened by high temperatures as changes in composition and structure of cell membranes occur. Damage to cell membranes from high-temperature stress modifies their penetrability, which results in the leakage of electrolytes and effects on respiration and photosynthesis. Outflow of electrolytes indicates injury of the cell membrane (McDaniel 1982). It is well known that the static cell membrane has reduced electrolyte outflow, showing heat tolerance, and unstable cell membranes with greater electrolyte leakage indicate heat sensitivity.

Cell membrane thermostability was used for the first time in sorghum for determining heat-tolerant and heat-susceptible genotypes (Sullivan 1972). Later on, this technique was used successfully to determine the degree of heat tolerance in several field crops *i.e*. soybean, wheat, legumes, cowpea, tomato and cotton (Ashraf et al. 1994; Malik et al. 1999; Rahman et al. 2004). Relative cell injury percentage (RCI%) was described as a measure for cell membrane thermostability and could be used to evaluate heat tolerance in *G. hirsutum* (Azhar et al. 2009). It was further described that heat tolerant varieties had stable yield and provide a quality of fibre

superior to that of the susceptible varieties. So, RCI% at the seedling stage could be used as the best screening parameter for cotton germplasm. Loss or decrease of chlorophyll content in plants is associated with a reduction in yield. Genotypes vary significantly under heat stress for their rate of photosynthesis, loss of chlorophyll content, and change in the ratio of chlorophyll *a* to *b* (Reynolds et al. 1994). Okra type leaves are more tolerant to loss of chlorophyll content under high temperature conditions as compared to normal leaves of cotton. Plants with more and stable chlorophyll content are more productive as they produce stable yield under adverse environmental conditions. A higher rate of photosynthesis is observed in plants with more and stable chlorophyll content (Pettigrew et al. 1993). The measurement of leaf chlorophyll content is simply done with chlorophyll meters, available in botanical and agronomic laboratories. Thus, these selection criteria can determine heat-tolerant lines.

A screening technique based on pollen germination can also be used in vitro with the combination of boll retention percentage. Retention of bolls was found to be highly correlated with a maximum number of pollen germination. Some experiments showed the existence of genetic variation for the number of bolls per plant, boll retention, germination of pollen, and pollen tube length: pollen germination, growth of pollen tubes, and their responses to minimum, maximum, and optimum temperatures was noted. This method could be used for differentiating high-temperature-tolerant cultivars from those that are susceptible (Kakani et al. 2005). Length of pollen tube and pollen germination in cotton were found to be decreased during high temperature conditions, with resulting fertilization failure and reduced fruit setting. Along with the aforementioned techniques, other useful screening techniques for high-temperature stress tolerance include pollen viability, proline content, chlorophyll fluorescence, carbon isotopes discrimination, stomatal conductance, canopy temperature, and heat tolerance index (Singh et al. 2007).

6 Breeding Strategies for Heat Tolerance

6.1 Genetic Variation

Genetic variation is the prerequisite for developing heat-tolerant genotypes through breeding. Much information on the diversity of heat tolerance in different crops such as tomato (Abdul-Baki and Stommel 1995), wheat (Ibrahim and Quick 2001), mung bean (Collins et al. 1995), cowpea (El-kholy et al. 1997), rice (Yoshida et al. 1981), and cotton (ur Rahman et al. 2004) is available in the literature. The existence of genetic variation is essential for improving any trait of a crop. Differential behavior of varieties of cotton has been observed in response to temperature. Information for genetic variability for plant height, plant shape, plant color, number of bolls per plant, number of seeds per boll, and seed weight has been recorded. Unfortunately, the present commercial cultivars of cotton have limited genetic variation for most of these traits (Bradow and Davidonis 2000). The presence of genetic

variation in the germplasm of cotton suggests that genetic improvement can be achieved in this species through breeding and selection, provided that the variation is effected through significant genetic components (Khalid et al. 2010). Presence of significant variability in the gene pool is essential for breeding crop plant tolerance against heat stress. It is more important that variation for heat tolerance is available in plant species such as cotton, soybean, legumes, sorghum, cowpeas, tomato, and wheat (Galiba 1997; Ibrahim and Quick 2001).

Genetic variation for heat stress tolerance is available in cultivars of pima cotton, related to the plant ability for setting bolls at lower nodal positions (Feaster and Turcotte 1985). During higher temperature conditions selection has been found effective for developing lines showing good production in a broad range of environmental conditions (Feaster et al. 1980). Similar results have been observed in upland cotton. Genetic variability for photosynthesis, photo-respiration, chlorophyll fluorescence, chlorophyll content, CMT, and other morphological characters exists in the germplasm. Furthermore, other investigations also proved that high genetic variability in pollen germination, pollen viability, development of pollen tube length, and number of bolls is available in upland cotton, which provides the opportunity for the genetic enhancement for heat tolerance. Besides the normal cotton leaf shape, other leaf shapes range from super-okra (highly cleft leaf) to sub-okra (cleft leaf) (Meredith 1984). This variation in the shape of leaves can bring significant changes in plant traits and aid light interception (Wells et al. 1986). It is found that heat and drought tolerance has similar mechanisms (Reynolds et al. 2001). Nevertheless, all the characters concerning heat tolerance have also been found to be related to drought tolerance; the best example is cell membrane thermostability (Blum 2018).

6.2 Gene Action

Gene action for certain traits has been extensively studied in cotton. Some of the results obtained after the study of gene action controlling various traits are discussed here. Plant height, average boll weight, number of bolls per plant, yield of seed cotton, gin turnout, fibre length, fibre strength, and fibre traits are controlled by the additive type of gene action with partial dominance, except for boll weight and gin turnout (Iqbal et al. 2013). Genetic analysis for yield and yield-contributing traits in a diallel analysis of upland cotton genotypes showed that additive dominance was fully adequate for the trait of plant height whereas it was only partially suitable for number of bolls per plant, boll weight, gin turnout, and seed cotton yield (Batool et al. 2013). Genetic analysis of different morphological and physiological traits in six generations (P_1, P_2, F_1, F_2, BC_1 and BC_2) of cotton revealed that plant height, number of bolls per plant, boll weight, gin turnout, fibre length, fibre strength, and fibre fineness are influenced by three types of gene actions, namely, additive, dominance, and interaction. It was also suggested that selection in later segregating generations would help in improving the studied traits (Ahmad et al. 2009).

Inheritance of a number of bolls per plant, seed cotton yield, and height of the main stem was studied in a diallel scheme, indicating that both additive and dominance effects were present in the studied material. Additive and non-additive genetic components could be used through adopting parental matings in an earlier generation for improving the yield-related traits during the crosses of cotton genotypes (Murtaza et al. 2006). In genetic analysis of Egyptian cotton genotypes, it was found that the additive–dominance model was acceptable for demonstrating genetic variability and that it was important for inheriting most of the studied characters. For most studied cases, additive into additive and dominance into dominance were found to be highly significant. Inheritance of all characters was found to be controlled by additive and non-additive types of gene action, but dominant genetic effects had a major role for controlling most studied characters (Abd-El-Haleem et al. 2010). In a complete diallel cross, gene action was studied for number of seeds per boll in F_1 and F_2 populations. The trait was found to be controlled by the additive–dominance model in F_1 whereas F_2 showing partial adequacy (Khan et al. 2007). Six generations (P_1, P_2, F_1, F_2, $BC_1$and BC_2) of cotton were studied to assess the inheritance pattern of various morphological and yield-contributing traits using generation mean analysis. It was also found that number of bolls per plant and boll weight were controlled by overdominance whereas seed cotton yield was controlled by a partial dominance type of gene action (Hussain et al. 2009).

Eight varieties of cotton were hybridized in diallel mating fasion and produced 64 combinations. The inheritance pattern for the traits (number of bolls per plant and boll weight) was found to be influenced by a non-additive over-dominance type of gene action (Murtaza 2006). Plant height, number of bolls per plant, boll weight, and seed cotton yield were studied to determine the inheritance of four parents of cotton. The inheritance of plant height, number of bolls per plant, and boll weight was influenced by the additive type of gene action with partial dominance whereas seed cotton yield was governed by an over-dominance type of gene action; it was suggested that seed cotton yield could be improved by hybrid breeding. It was also found that epistasis has no effect on the aforementioned traits (Latif et al. 2014). The six generations *i.e.* P_1, P_2, F_1, F_2, BC_1, and BC_2 were developed to study the gene action involved in controlling yield, yield components, and quality characters of different crosses of cotton by using generation mean analysis. Significantly high mean values were assessed for number of bolls per plant, boll weight, fibre length, fibre strength, fibre fineness, seed cotton yield, and gin turnout. Additive and dominance genetic effects were significantly high for number of bolls per plant and boll weight for all four crosses, where dominant effects were high than additive effects. Similarly, additive and dominance genetic effects were found significantly high for fibre length in the second cross and fibre fineness in a first and fourth cross, having larger dominance effects than additive effects. In the first cross, number of bolls per plant, fibre length, and fibre fineness, in first and second cross, seed cotton yield and gin turnout, in the fourth cross boll weight, and in the second cross fibre strength, showed that these traits were controlled by dominance and nonallelic interactions (El-Refaey and El-Razek 2013).

6.3 Heritability of Heat Tolerance

Efforts have been made to modify various plant characteristics, such as insect resistance and yield enhancement, by selecting promising plants for the next generation. The primary purpose of this selection is to identify genotypes that can fulfill the food and fibre requirements. Nowadays, increase of yield in stressful conditions is the main breeding objective for plant breeders. The existence of available genetic differences for heat tolerance allows identifying thermo-sensitive and thermo-tolerant genotypes. Heat-sensitive genotypes can be transformed into tolerant genotypes through different breeding methods. There are two types of heritability: broad-sense heritability and narrow-sense heritability. Broad-sense heritability is the ratio of genotypic variance to phenotypic variance and narrow-sense heritability is the ratio of additive variance to phenotypic variance. Cotton breeders rely on traits having narrow-sense heritability. Breeding methods depend upon heritability of certain traits, whereas heritability depends upon environmental and genetic variances (Nyquist and Baker 1991). If a genetic variance has significant contribution for the expression of traits compared to environmental variance then heritability of particular traits will be high (Acquaah 2009). Percentage of heritability indicates that the specific trait will remain stable in later generations, which is important for the breeding aspect. Studies conducted to understand the heritability of thermotolerance in cotton, thermo-tolerant as well as thermo-sensitive cotton genotypes, were analyzed in populations of F_2 generations. Low heritability was found for fruit set, which indicated that the environment has relatively more influence in the inheritance of thermotolerance as compared with the genetic architecture (Hanson et al. 2002). Low heritability might be due to sub-optimal and less robust methods of phenotyping.

6.4 Genetic Engineering

To mitigate the adverse effect of heat stress, various genetic engineering and transgenic approaches are being used by plant scientists (Rodríguez et al. 2005). Constitutive expression of specific proteins has been reported to result from enhanced heat stress (Katiyar-Agarwal et al. 2003). Several studies of the expression of chaperones and manipulation of HSFs resulting in altered gene expression have been described. The genetically modified plants with different degrees of heat tolerance have been developed, but less molecular research work is done on heat tolerance as compared to drought, salt, and cold stress tolerance. Efforts have been made by scientists to utilize HSPs by inducing alterations in transcription factors (*AtHSF$_1$*) in *Arabidopsis*: transgenic *Arabidopsis* was produced for heat stress tolerance. The mitochondrial small HSP (*MT-sHSP*) gene is also utilized for the development of transgenic tobacco (Sanmiya et al. 2004). The *Arabidopsis HSP$_1$01* gene was successfully overexpressed in transgenic rice for enhancing thermotolerance.

Similarly, overexpression of the small heat shock protein *sHSP$_1$7.7* confers heat tolerance in rice (Murakami et al. 2004). Thermotolerance was obtained by transformation of RuBisCo activase. This gene is involved in reversible decarboxylation of RuBisCo, and also protects the photosynthetic apparatus affected by heat stress, which indicates that several technologies could be used for the development of transgenic plants for heat tolerance (Grover et al. 2013).

6.5 Omics Approaches

Several technologies are being used to identify transcription, translation, and post-translation procedures and pathways of signaling that control plant response against biotic and abiotic stresses (Hasanuzzaman et al. 2013). In plants, various "omics" techniques such as metabolomics, proteomics, transcriptomics, and genomics are needed for genetic analysis. These techniques allow the minning of genes involved in various pathways involved in response to high temperature. Regulation of genes is determined by attachment of transcriptional factors, chromatin morphology, and *cis*-regulatory sequences shown by transcript profiling.

A number of genes have been reported with their potential role in heat stress response by utilizing genetic screening and genome-wide expression analysis (Yeh et al. 2012). In response to environmental and developmental signals, plants have post-transcriptional mechanisms by encoding miRNAs. These miRNAs have a specific function, so micromics helps to understand the mechanisms involved in developing tolerance to various stresses. Microarray technique has recently become a reliable tool for the systematic examination of the expression profile of a number of genes related to high temperatures (Liu et al. 2011). Transcripts of 170 cDNAs of tobacco were examined under abiotic stress conditions with or without the stimulus of heat stress (Rizhsky et al. 2002). A large number of unique genes that were not upregulated through increased temperatures or drought conditions have been upregulated later on by using both stresses. Complete genome arrays have also been used in *Arabidopsis* to study transcript alterations in response to higher temperatures (38 °C for 6 h), drought (70% relative water content), and the combined effect of these stresses. It was found that many genes were active under both stresses although some specific genes are active under a particular stress (Rizhsky et al. 2004).

7 Conclusion

Climate change is caused by the increase in greenhouse gases which is the most serious issue for scientists now a days. This change in climate is the primary factor for increases in global mean temperature, drought, irregular rainfall, and floods. Increase in global mean temperature is a great challenge to crop scientists, especially crop physiologists and plant breeders. Because plant growth and production

of yield are greatly influenced by temperature, the current need is to find new sources to cope with these limiting factors and to ensure the food security of the increasing population. Among several field crops, cotton is one of the top crops grown in the tropical and subtropical regions of the world. Although the cotton plant is called "sun-loving," high temperature during its growth period exerts adverse impacts on the yield of seed cotton. This issue must be solved by cotton breeders by developing heat-tolerant lines with enhanced quality parameters to meet the requirements of the textile industry. Plant breeders have developed several heat-tolerant cultivars in the past, but these cultivars are now becoming susceptible to heat stress because the temperature of the earth is steadily rising. Previously, scientists used simple morphological assays to select heat-tolerant lines, but now the need of the time is shifting the research paradigm in cotton breeding. Screening based on only morphological and conventional breeding approaches is not sufficient to develop new cultivars. Currently, scientists have begun using various physiological and biochemical assays along with morphological parameters in breeding programs. Use of molecular markers in plant breeding is also becoming common among cotton breeders. Several genetic engineering and omics approaches are also seeking the attention of molecular plant breeders. It is concluded that the use of these modern approaches along with conventional breeding methods will allow plant breeders to develop heat-tolerant lines more efficiently for the sustainable production of cotton crops under changing environmental conditions.

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Modulation of Proteome and Phosphoproteome Under Abiotic Stress in Plants: An Overview

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Abbreviations

2D-PAGE	Two-dimensional polyacrylamide gel electrophoresis
DIGE	Differential in gel electrophoresis
ESI	Electrospray ionization
ICAT	Isotope-coded affinity tags
IMAC	Immobilized metal affinity chromatography
iTRAQ	Isobaric tags for relative and absolute quantification
LC	Liquid chromatography
LCM	Laser capture micro-dissection
MALDI-TOF	Matrix assisted laser desorption/ionization-time of flight
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MudPit	Multidimensional Protein Identification Technology
PTM	Post-translational modification
SILAC	Stable isotope labelling by amino acids in cell cultures

1 Introduction and Brief Bibliographic Review

Plant growth, development and productivity are severely diminished by abiotic stress factors such as drought, salinity, waterlogging, extreme temperatures and heavy metals (Surabhi 2018). As a consequence to it, physiological and biochemical responses in plants vary and cellular aqueous and ionic equilibriums are disrupted

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(Sreenivasulu et al. 2007). The assessment of potential yield losses by individual abiotic stresses is estimated at 17% (drought), 20% (salinity), 40% (high temperature), 15% (low temperature) and 8% by other factors (Shafiq-ur-Rehman and Ashraf 2005). It has been estimated that 90% of arable land experience different abiotic stresses, singly or in combination (Leopold 1990) under field conditions. Plant responses to abiotic stresses are dynamic and complex, and quite different depending on the type, level, duration of the stress involved, type of tissue and genotype under stress (Cramer et al. 2011). Higher plants have evolved multiple, interconnected strategies that enable them to survive under abiotic stress (Surabhi et al. 2003; Kumari et al. 2007; Surabhi et al. 2008; Veeranagamallaiah et al. 2008; Singh et al. 2010; Witzel et al. 2009, 2010; Surabhi 2018). However, these strategies are not well developed in most agricultural crops (Fig. 1).

Unlike genome which is a static structure inherited from parents and defining plant genotype, changes in plant epigenome, transcriptome, proteome and metabolome shape plant phenotype in response to both developmental stages and for external cues. Plant stress proteomics is a dynamic discipline, aimed at studying plant proteome and protein biological functions in plants exposed to various stress factors (Veeranagamallaiah et al. 2008; Witzel et al. 2009, 2010; Surabhi 2018). The role of proteins in plant stress response is crucial since proteins are directly involved in shaping novel phenotype by adjustment of physiological traits to altered environment.

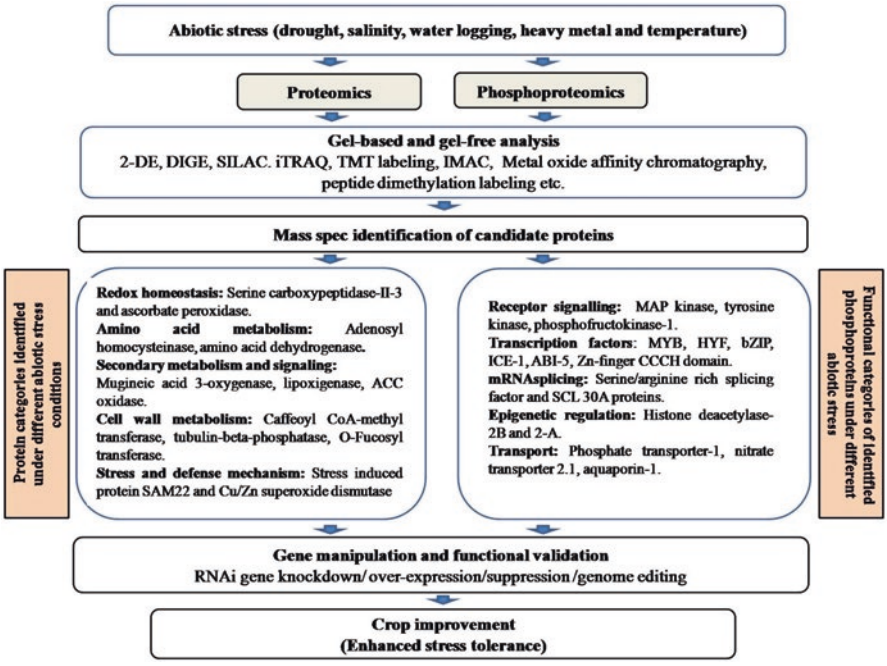

Fig. 1 Schematic representation of the proteomic strategy to study proteome and phosphoproteome modulations under different abiotic stresses in crop plants

Further, the analysis of protein is the most direct approach to define the function of its associated gene, proteome analysis linked to genome-sequence information is a very powerful tool in functional genomics studies (Komatsu et al. 2003). There are several types of proteomes that can be measured under abiotic stress, and each of them can reveal particular information about the expressed proteins. The most common proteomes to be measured in crop/plant or abiotic stress related studies are the whole proteome and the phosphoproteome (Helmy et al. 2012a, b; Witzel et al. 2009, 2010; Surabhi 2018). Phosphorylation is one of the most important post-translational modifications (PTMs) of proteins (Pawson and Scott 1997). Approximately one-third of the proteins are modified by phosphorylation (Hubbard and Cohen 1993). The study of whole proteome and phosphoproteome are the quantitative and/or qualitative profiling of all the expressed proteins and phosphorylated proteins in a given sample, respectively (Nakagami et al. 2012). Through phosphoproteomics, proteins and signalling pathways involved in response to particular stress can be identified (Sugiyama et al. 2008; Lassowskat et al. 2014; Zhang et al. 2014). Both proteome and phosphoproteome can be combined in one study to obtain a holistic understanding of abiotic stress tolerance in plants (Margaria et al. 2013; Yang et al. 2012; Hopff et al. 2013).

In recent years, proteome and phosphoproteome studies were conducted in crop/plants singly or in combination of both (Chitteti and Peng 2007; Margaria et al. 2013; Yang et al. 2012; Hopff et al. 2013) to get an molecular insight under different abiotic stresses such as drought (Atikur et al. 2016; Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Li et al. 2018; Zadraznik et al. 2017; Hu et al. 2015; Bhaskara et al. 2017; Ren et al. 2017), salinity (Mostek et al. 2015; Zhang et al. 2017, 2018; Pi et al. 2018; Witzel et al. 2009), temperature (Guo et al. 2017; Gao et al. 2017; Pi et al. 2017), waterlogging (Pan et al. 2010, 2018; Mustafa and Komatsu 2014) and heavy metal stress (Xue et al. 2015; Chen et al. 2015, Cheng et al. 2017; Zhong et al. 2017). This review highlights some of the recent proteomics and phosphoproteomics studies conducted on crop/plants under different abiotic stresses. In addition, this review briefly discussed about different proteins which were altered in crop/plants under different abiotic stress factors. Finally, functional studies should complement high-throughput proteome analysis and can thus contribute to uncover protein role in plant stress response (Table 1).

2 Summary of Proteome and Phosphoproteome Studies Under Different Abiotic Stresses

Plant stress proteomics has the ability of identifying possible candidate genes that can be used for the genetic enhancement of plants to different stress factors (Cushman and Bohnert 2000; Rodziewicz et al. 2014; Barkla et al. 2016). Proteomics deals with determination, identification, expression profiling, post-translational modifications (PTMs) and protein–protein interactions under stress conditions (Hashiguchi et al. 2010; Nam et al. 2012; Mertins et al. 2013; Ghosh and Xu 2014). Using a proteome

Table 1 Major protein functional categories and proteins identified under different abiotic stress conditions in crop plants

Proteomics		Phosphoproteomics	
Functional categories of proteins	Name of the proteins	Functional categories of proteins	Name of the proteins
Drought stress			
Secondary metabolism (Simova-Stoilova et al. 2015a, b). Protein synthesis (Faghani et al. 2015; Simova-Stoilova et al. 2015a, b; Li et al. 2015). Carbon metabolism (Simova-Stoilova et al. 2015a, b). Glycolysis (Simova-Stoilova et al. 2015a, b; Atikur et al. 2016). Amino acid metabolism (Simova-Stoilova et al. 2015a, b). Adenosine triphosphate (ATP)-synthesis (Faghani et al. 2015; Simova-Stoilova et al. 2015a, b). Protein folding (Simova-Stoilova et al. 2015a, b; Zadraznik et al. 2017). Protein degradation (Simova-Stoilova et al. 2015a, b). Stress response (Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Zadraznik et al. 2017). Photosynthesis (Faghani et al. 2015; Simova-Stoilova et al. 2015a, b). Signal transduction (Li et al. 2015; Atikur et al. 2016). Oxidative stress (Faghani et al. 2015; Atikur et al. 2016; Ghaffari et al. 2013). Energy metabolism (Ghaffari et al. 2013; Paul et al. 2015; Li et al. 2015; Atikur et al. 2016). Transport (Ghaffari et al. 2013; Li et al. 2015). Defense (Faghani et al. 2015, Li et al. 2015). Carbohydrate metabolism (Paul et al. 2015). Cell wall metabolism (Faghani et al. 2015; Zadraznik et al. 2017).	Caffeoyl-CoA-30 methyl transferase and chalcone synthase (Simova-Stoilova et al. 2015a, b). 30-S ribosomal protein S-7 and 40-S ribosomal protein S-17-4 (Li et al. 2015), Protein disulfideisomerase and 60S ribosomal protein L-13 (Faghani et al. 2015), DEAD-box RNA helicase and glycyl t-RNA synthetase (Simova-Stoilova et al. 2015a, b). Methyl malonate dehydrogenase, methylene tetrahydrofolatedreductase (Simova-Stoilova et al. 2015a, b). Glucose-6-phosphate isomerase and phosphofructokinase-1 (Simova-Stoilova et al. 2015a, b), oxaloacetate, malate dehydrogenase (Atikur et al. 2016). Glutamine synthase and cysteine synthase (Simova-Stoilova et al. 2015a, b). ATP-synthase gamma-chain and ATP-synthase mitochondrial (Faghani et al. 2015), ATP-synthase subunit-beta, NADH-unbiquinoneoxidoreductase (Simova-Stoilova et al. 2015a, b). Protein disulphide isomerise,chapperonin 16-Kda (Simova-Stoilova et al. 2015a, b) and UDP-glucose-glycoprotein glycosyltransferase, disulfide dismutase (Zadraznik et al. 2017). Amino peptidase and mitochondrial processing peptidase (Simova-Stoilova et al. 2015a, b).	Stress (Zhang et al. 2014; Hu et al. 2015). Defense and ROS (Zhang et al. 2014; Ke et al. 2009). Signal transduction (Zhang et al. 2014; Umezawa et al. 2013; Ke et al. 2009; Hu et al. 2015; Chen et al. 2017; Sun et al. 2017, Barua et al. 2019). Transport (Zhang et al. 2014; Sun et al. 2017; Yuan et al. 2016). Transcription factors (Zhang et al. 2014; Hu et al. 2015; Sun et al. 2017; Yuan et al. 2016). RNA processing (Zhang et al. 2014). Translational activator (Ren et al. 2017). Post-transcriptional regulation (Hu et al. 2015).	A-20 like-zinc finger and AN-1 zinc finger (Zhang et al. 2014) and Serine pyruvate dehydrogenase (Hu et al. 2015). Glutamate-decarboxylase-1 and phosphorylate glutathione peroxidise (Zhang et al. 2014), Germin like protein-1 (GLP-1) and putative r40-C-1 like protein (Ke et al. 2009). Snrk and Pb-2-C-kinase (Zhang et al. 2014), Phospholipase and phosphoenol pyruvate carboxykinase (Hu et al. 2015), CDPK-kinases and Snrk-2 kinases (Umezawa et al. 2013), Receptor like protein-kinase-1 and serine threonine protein kinase (Hu et al. 2015), CDK-Kinases and MAP-kinases NTF3 (Barua et al. 2019), G-beta subunit like protein kinases (Ke et al. 2009), protein receptor kinase (Chen et al. 2017), CDK-kinases (Sun et al. 2017). S-1-26 protein kinase-A and CDPK-1 kinase (Zhang et al. 2014), Aquaporin and SOS signalling (Sun et al. 2017), Aquaporin NIP-2 and cationic amino acid transporter (Yuan et al. 2016).

Cell growth and division (Li et al. 2015). Cell structure (Li et al. 2015). Transcription factors (Faghani et al. 2015; Li et al. 2015). Protein storage (Li et al. 2015). Metabolism (Li et al. 2015).	Endo-1,3-beta-glucanase and chitinase (Paul et al. 2015), Aldo/keto-reductase (Simova-Stoilova et al. 2015a, b), ascorbate oxidase and reticulin (Zadraznik et al. 2017). Oxygen evolving enhancers proteins ribulose-1,5-biphosphate carboxylase activase (Faghani et al. 2015) and Oxygen-evolving enhancers proteins and psbp domain-containing proteins (Simova-Stoilova et al. 2015a, b). *S*-adenosyl methionine synthase and calmodulin-3 (Li et al. 2015), Cytosolic—ascorbate peroxidase (Atikur et al. 2016). Ascorbate peroxidase hairpin binding protein-1 (Faghani et al. 2015), Peroxy reducing and APX-peroxidase (Atikur et al. 2016), Ascorbate peroxidase-2 and Cu/Zn-superoxide dismutase (Ghaffari et al. 2013). ATP-citrate-lyase and adenosine triphosphate citrate lyase (Ghaffari et al. 2013), Pyrophosphoglycerate adenosine-kinase (Paul et al. 2015), Early-induced protein 22 and glyceraldehydes 3-phosphate dehydrogenase (Li et al. 2015) and mitochondrial dihydrolipoyl dehydrogenase (Atikur et al. 2016). Aldo/keto-reductase and cysteine synthase (Atikur et al. 2016). HSP-21 (Ghaffari et al. 2013), Lipid transfer protein (Li et al. 2015). Pathogenesis related protein (Faghani et al. 2015), Thylakoid ascorbate peroxidase protein CuT A1 (Li et al. 2015). Phosphoglucomutase (Paul et al. 2015). Glyceraldehydes-3-phosphate dehydrogenase fructose bisphosphatealdolase (Faghani et al. 2015), Beta-xylosidase and alpha-aribinofuranosidase (Zadraznik et al. 2017). Tetratricopeptide repeat protein (Li et al. 2015). Cell structure (Li et al. 2015). 14-3-3-protein basic transcription factor 3 (Faghani et al. 2015), Cp31BHv (Li et al. 2015). Cucumisin and GrPE protein (Li et al. 2015). Inorganic phosphatase (Li et al. 2015).	mRNA splicing (Chen et al. 2017; Sun et al. 2017; Barua et al. 2019). Starch biosynthesis (Chen et al. 2017). Protein folding (Sun et al. 2017). Epigenetic regulation (Sun et al. 2017). Pyruvate metabolism (Sun et al. 2017). Water and ion transport (Sun et al. 2017).	MYB1R1 and bHLH (Zhang et al. 2014), 2-amino-ethanethiol dioxygenase like protein and HSP-COP 2 N6 (Hu et al. 2015), Transcriptional adapter ADA-2 and probable nucleolar protein 5-2 (Yuan et al. 2016), bZR1 and HUA1 (Sun et al. 2017), Zinc-finger CCCH domain and TF-PoS F-21 (Barua et al. 2019). CDPK-kinases and Snrk-2 kinases (Umezawa et al. 2013). GCN-1 and eukaryotic translational factor 3-subunit—B (Ke et al. 2009). Splicing factor 3-B-subunit-1 (Zhang et al. 2014), Serine/arginine rich-splicing factor-4 (Chen et al. 2017), serine arginine rich proteins, SCL 30A proteins (Sun et al. 2017) and E-3 Sumo-ligase SIZ 1 protein (Barua et al. 2019). Amlyopectin and amylases (Chen et al. 2017). Ribosomal protein S-6 and S6K-TOR protein (Sun et al. 2017). Histone deacetylase 2-B (Sun et al. 2017). Phosphoenolpyruvate carboxylase-4 and malate dehydrogenase (Sun et al. 2017). NIP-2 and PiP-27 phosphorylase (Yuan et al. 2016).

(continued)

Table 1 (continued)

Salt stress			
Amino acid metabolism (Mostek et al. 2015; Zhang et al. 2017, 2018). Ribosome activation (Zhang et al. 2017, 2018). Energy metabolism (Shen et al. 2016; Wen et al. 2016; Zhang et al. 2017, 2018). Protein folding (Gao et al. 2011, Mostek et al. 2015, Wen et al. 2016, Zhang et al. 2017, 2018). Nucleotide metabolism (Zhang et al. 2017, 2018). Cell division and cell signalling (Zhang et al. 2017, 2018). Membrane trafficking (Zhang et al. 2017, 2018). Glycolysis (Shen et al. 2016; Zhang et al. 2017, 2018). Citrate metabolism (Zhang et al. 2017, 2018). Pentose phosphate pathway (Zhang et al. 2017, 2018). Starch sucrose metabolism (Zhang et al. 2017, 2018). Defense (Witzel et al. 2014; Muneer and Jeong 2015; Wen et al. 2016). Primary metabolism (Witzel et al. 2014). Protein destination and storage (Witzel et al. 2014). Redox homeostasis (Witzel et al. 2014; Gao et al. 2011; Mostek et al. 2015; Shen et al. 2016). Secondary metabolism (Witzel et al. 2014). Carbohydrate metabolism (Gao et al. 2011; Mostek et al. 2015; Cui et al. 2015; Wen et al. 2016). Protein transport (Gao et al. 2011). Carbon metabolism (Gao et al. 2011). Signal transduction (Mostek et al. 2015). Cell wall metabolism (Mostek et al. 2015). RNA splicing (Muneer and Jeong 2015). Transcription regulation (Muneer and Jeong 2015). Starch biosynthesis (Muneer and Jeong 2015). RNA-binding (Muneer and Jeong 2015).	Serine hydroxyl methyl transferase and *S*-adenosyl L-homocysteine hydrolase (Mostek et al. 2015), Adenosine homo-cysteinase (Zhang et al. 2017, 2018). Phosphotransferase and phosphofructokinase-1 (Zhang et al. 2017, 2018). ATP-synthase subunit alpha and gamma (Shen et al. 2016), V-type proton ATPase catalytic subunit A and ATP synthase CF1 beta-subunit (Wen et al. 2016), Pyruvate orthophosphate dikinase (Zhang et al. 2017, 2018). Protein disulphide isomerases (Mostek et al. 2015; Zhang et al. 2017, 2018), CPN-60 alpha-protein (Wen et al. 2016). FAD-binding berberine family (Zhang et al. 2017, 2018). ATP-dependent CLP protease (Zhang et al. 2017, 2018). Coatamer alpha-subunit (Zhang et al. 2017, 2018). Phosphoglycerate kinase and triose-phosphate isomerase (Shen et al. 2016), Triose phosphate isomerase (Zhang et al. 2017, 2018). Pyruvate phosphate dikinase-2 (Zhang et al. 2017, 2018). UDP-glucose dehydrogenase (Zhang et al. 2017, 2018). Glycosyl hydrolases (Zhang et al. 2017, 2018). Germin like protein 12 and pathogenesis related protein 10 (Witzel et al. 2014), NBS/LRR-disease resistance proteins and PR-P10 proteins (Muneer and Jeong 2015), Betaine-aldehyde-dehydrogenase and glutamine synthetase cytosolic isoenzyme (Wen et al. 2016). Lipoxygenase-1 and platidiclipoxygenase-pyrophosphatase (Witzel et al. 2014). Proteasome subunit—alpha type-1 (Witzel et al. 2014). Glutathione transferases (Witzel et al. 2014), Chaperonnin HSP-mutase (Gao et al. 2011), Linolate 9*S*-lipoxygenase-1 and annexintranslationally controlled tumour lipoxygenase-2 (Mostek et al. 2015), Succinyl-CoA-ligase beta-chain and hydroxyacyl glutathione hydrolase (Shen et al. 2016). Mugineic acid 3-dioxygenase (Witzel et al. 2014).	ROS homeostasis (Jun et al. 2010; Pi et al. 2018; Yu et al. 2016). Signal transduction (Jun et al. 2010; Chang et al. 2012; Yu et al. 2016). Cellular regulation (Jun et al. 2010). Post-translational modification (Jun et al. 2010). Energy metabolism (Jun et al. 2010). Carbohydrate metabolism (Jun et al. 2010; Wen et al. 2016). Transport (Hsu et al. 2009; Wen et al. 2016; Chang et al. 2012; Yu et al. 2016). Water channel (Hsu et al. 2009). Protein folding (Hsu et al. 2009). Regulatory mechanism (Wen et al. 2016). Stress (Wen et al. 2016). Defense (Wen et al. 2016). Protein folding (Wen et al. 2016; Yu et al. 2016). Secondary metabolism (Pi et al. 2018). Transcription factors (Pi et al. 2018; Chang et al. 2012; Yu et al. 2016).	Cu/Zn-Superoxide dismutase-1 and glutamate decarboxylase (Jun et al. 2010), ascorbate peroxidase (Pi et al. 2018), glutathione-*s*-transferase and glutathione reductase (Yu et al. 2016). TPX-1 tyrosine kinase and phosphofructokinase-1 (Jun et al. 2010), receptor kinase and CDPK kinase (Chang et al. 2012), MAP-kinases and serine/threonine protein kinase (Yu et al. 2016). Zinc peroxide dismutase-1 (Jun et al. 2010).

Cellular metabolic process (Muneer and Jeong 2015). Nucleotide binding (Muneer and Jeong 2015). Stress (Muneer and Jeong 2015; Wen et al. 2016). ATP-binding (Muneer and Jeong 2015). Glycerol metabolic process (Muneer and Jeong 2015). Ribosome related protein (Cui et al. 2015). Regulatory mechanism (Wen et al. 2016). Protein metabolism (Wen et al. 2016). Photosynthesis (Wen et al. 2016). Cell structure (Wen et al. 2016).	Fructose 1,6-bisphosphatedolase and ribulose-1,6-bisphosphate aldolase (Gao et al. 2011), Citrate synthase and pyrophosphate fructose-6-phosphate phophotransferase (Mostek et al. 2015), Exhydrolase-2-isoform-I and fructokinase-2 (Cui et al. 2015). Putative H^+ transporting ATP-synthase (Gao et al. 2011). Chaperonnin HSP-mutase (Gao et al. 2011). Glutathione *S*-transferase (Gao et al. 2011). Carboxylase 3-phosphoglycerate kinase (Gao et al. 2011). UDP-glucoronic acid decarboxylase and beta-D-glucan-exohydrolase (Mostek et al. 2015). Osmotinvacuolar ATP-ase and thaumatin like protein (Mostek et al. 2015). Maturase (Muneer and Jeong 2015). Transcription elongation factor protein SPT-4 (Muneer and Jeong 2015). Granule bound starch synthase (Muneer and Jeong 2015). S-46 RNase (Muneer and Jeong 2015). Cinnamoyl-CoA-reductase like-1 Muneer and Jeong 2015). Pentatricopeptide repeat containing protein (Muneer and Jeong 2015). Zinc finger nucleases A-20 and AN-1 domain (Muneer and Jeong 2015) Chloroplast—Cu/Zn superoxide dismutase and dehydroascorbatereductase (Wen et al. 2016). 4-coumarate CoA-ligase (Muneer and Jeong 2015). Putative DAK-2-domain containing protein (Muneer and Jeong 2015). Ribosomal protein S-8 and 60S ribosomal protein L-3 (Cui et al. 2015). CP31-BHv (Wen et al. 2016). 50S ribosomal protein L-1 and 50S ribosomal protein L10 (Wen et al. 2016). Fructose-biphosphatealdolase and soluble inorganic pyrophosphatase-1 (Wen et al. 2016). Oxygen evolving enhancer protein-2 (Wen et al. 2016). Ribulose-biphosphate carboxylase and oxygenase (Wen et al. 2016). Enolyl-(acyl carrier protein)-reductase NADH and actin (Wen et al. 2016).		NADH-dehydrogenase-1 (Jun et al. 2010). NADH-dehydrogenase-2 (Jun et al. 2010). Fructose bisphosphate aldolase and NADH dehydrogenase (Jun et al. 2010), Triosphosphate-isomerase and chloroplast fructose bisphosphatealdolase (Wen et al. 2016), sugar transport protein 1 and aquaporin PIP-2 (Chang et al. 2012), PIP-2-1 and NHX-1 protein (Yu et al. 2016). Purine permease (Hsu et al. 2009), ATP synthase CF1 beta subunit and ATP1 synthase (Wen et al. 2016). Aquaporin-PIP-2 (Hsu et al. 2009). FAM-10 protein translase (Hsu et al. 2009). Cp31BHv and Cp31BHv (Wen et al. 2016). Cu/Zn superoxide dismutase and dihydroascorbate (Wen et al. 2016). Chain-A beta glucosidase and 2-cysteine peroredoxin BAS1 (Wen et al. 2016). CPN-60 alpha protein (Wen et al. 2016), 60s ribosomal protein L17 and TRAP (translocon associated protein sub-unit) (Yu et al. 2016). P-Coumanocyl-CoA and alonyl-CoA (Pi et al. 2018). PsbH1 and PsbH2 (Chang et al. 2012), bHLH and SYF1 (Yu et al. 2016), MYBIR and MYB-R2R3 (Pi et al. 2018).

(continued)

Table 1 (continued)

Waterlogging			
Carbohydrate metabolism (Alam et al. 2010; Yin and Komatsu 2017). Energy metabolism (Alam et al. 2010; Yin and Komatsu 2017). Signal transduction (Alam et al. 2010). Programmed cell death (Alam et al. 2010). Redox homeostasis process NAD (Alam et al. 2010; Yin and Komatsu 2017; Oskuei et al. 2017; Kamal et al. 2015; Wang et al. 2015; You et al. 2014; Li et al. 2018). Nitrogen metabolism (Alam et al. 2010). DNA processing (Alam et al. 2010). Post-translational modification (Alam et al. 2010). Cell wall metabolism (Li et al. 2018; Yin and Komatsu 2017; Wang et al. 2015; Khan and Komatsu 2016). RNA metabolism (Li et al. 2018). Protein Transport (Li et al. 2018; Khan and Komatsu 2016; Yin and Komatsu 2017). DNA metabolism (Li et al. 2018). Lipid metabolism (Kamal et al. 2015; Wang et al. 2015; Li et al. 2018). Protein metabolism (Li et al. 2018). Glycolysis (Yin and Komatsu 2017). Defense (Yin and Komatsu 2017). Protein synthesis (Yin and Komatsu 2017). Transcription factor (Yin and Komatsu 2017; You et al. 2014). Glycosylation (Yin and Komatsu 2017). Protein modification (Yin and Komatsu 2017). Biotinylation (Yin and Komatsu 2017). Calcium homeostasis (Yin and Komatsu 2017). Photosynthesis (Oskuei et al. 2017). Cell wall modification (Xue et al. 2015). Phenyl propanoid metabolism (Xue et al. 2015). Lipid catabolism (Xue et al. 2015).	UDP-glucose pyrophosphorylase and carbohydrate-kinases (Alam et al. 2010), *S*-adenosyl methionine synthetase and B-*S*-glucosidase 44 (Yin and Komatsu 2017). Phosphofructokinase and auxin-amidohydrolase (Alam et al. 2010), Enolase and alcohol dehydrogenase (Yin and Komatsu 2017). Receptor kinase and ERK kinase (Alam et al. 2010). Sam synthase and apyrase (Alam et al. 2010). NADH-hydrolase and ascorbate peroxidase (Alam et al. 2010), Ascorbate peroxidase and N-ascorbate dehydrogenase (Yin and Komatsu 2017), Ascorbate peroxidase mono-dehydrate ascorbatereductase (Oskuei et al. 2017), Fructose bisphosphate aldolase-2 (Kamal et al. 2015), PD1 like 16 protein (Wang et al. 2015). Glutamine synthase (Alam et al. 2010). Glucose-6-phosphate/phosphate translocator related protein (Li et al. 2018), mitochondria substrate family carrier protein (Yin and Komatsu 2017), Nucleotidyltransferase and granulin repeat cysteine protease (Khan and Komatsu 2016), Potassium phosphatase beta subunit-A (Wang et al. 2015). AATF-leucine zipper domain protein (Alam et al. 2010). Deoxyhypusine synthase (Alam et al. 2010). O-fucosyl-transferase family protein (Li et al. 2018), Xylosidase (Yin and Komatsu 2017). DNA (Cytosin-5) methyl transferase and RNA recognition motif (Li et al. 2018), Reversibly glycosylated polypeptide 3 (Wang et al. 2015). Oxidoreductase and pyridoxal-5-phosphate dependent enzyme (Wang et al. 2015), Xyloglucanendotransglycosylase (You et al. 2014). Replication factor C2 (Li et al. 2018), Ribosomal LSP-family protein (Kamal et al. 2015). Putative uncharacterized protein (Li et al. 2018).	Energy metabolism (Pan et al. 2018). Sucrose metabolism (Pan et al. 2018). Pentose phosphate pathway (Pan et al. 2018). Transcription factor (Pan et al. 2018). Carbohydrate metabolism (Cho et al. 2016). Transport (Yin et al. 2014; Cho et al. 2016). Signal transduction (Yin et al. 2014; Cho et al. 2016). Protein synthesis (Yin et al. 2014; Cho et al. 2016). Cell wall metabolism (Yin et al. 2014). Nucleotide metabolism (Yin et al. 2014). RNA processing (Yin et al. 2014). Hormone metabolism (Yin et al. 2014). Glycolysis (Yin et al. 2014).	Pyruvate kinase and phosphoenol pyruvate carboxylase (Pan et al. 2018). Glucose-6-phosphate dehydrogenase (Pan et al. 2018). Ribulose-1,5-biphosphate carboxylase (Pan et al. 2018). Serine protein kinase and threonine protein kinase (Pan et al. 2018). F2KP and plastidic acetyl—CoA synthetase (Cho et al. 2016). Proton antiporter and PIP-2 (Cho et al. 2016), transmembrane amino acid transporter family protein (Yin et al. 2014). SNF-kinase-1 (Yin et al. 2014), SnRK1 and MPK kinase-3 (Cho et al. 2016).

Secondary metabolism (Khan and Komatsu 2016). Glycolysis (Khan and Komatsu 2016). Protein transport (Khan and Komatsu 2016). Stress (Khan and Komatsu 2016; Kamal et al. 2015; Wang et al. 2015; Li et al. 2018). Protein synthesis (Kamal et al. 2015). Hormone metabolism (Kamal et al. 2015; Wang et al. 2015). Photosynthesis (Kamal et al. 2015). Nucleotide metabolism (Kamal et al. 2015). Protein degradation (Kamal et al. 2015). Protein activation (Kamal et al. 2015). Nucleotide metabolism (Wang et al. 2015). Cell signalling (Wang et al. 2015; Kamal et al. 2015; Li et al. 2018). Amino acid metabolism (Wang et al. 2015). RNA processing (Wang et al. 2015). Secondary metabolism (Wang et al. 2015). TCA cycle (Wang et al. 2015). Glycolysis (Wang et al. 2015; You et al. 2014). Protein transport (Wang et al. 2015). Protein metabolism (You et al. 2014). Sucrose metabolism (You et al. 2014). Glycolysis (You et al. 2014). Lactate fermentation (You et al. 2014). Ethylene biosynthesis (You et al. 2014). Defense (You et al. 2014). Secondary metabolism (You et al. 2014).	Stress induced protein SAM22 (Li et al. 2018), MLP-like protein (Khan and Komatsu 2016), leucine rich repeats protein (Kamal et al. 2015), HSPs 70 and DNA damaged binding protein (Wang et al. 2015). RAS-GTP transcription activator (Yin and Komatsu 2017), Glutamine synthase (You et al. 2014). Proteasome subunit alpha type and NAG-CoA transferase (Li et al. 2018). Stress induced proteins and SAM-22 (Li et al. 2018). Tubulin beta1 chain (Kamal et al. 2015; RAS-5 and RAS-GTP-binding protein (Wang et al. 2015). Ferritin proteins (Li et al. 2018). ATP-citrate lyase (Yin and Komatsu 2017). UDP-glucose-6-hydrogenase (Yin and Komatsu 2017), Plant intracellular RAS group-LRR-4 and calmodulin binding transcription activator CG-1 (Li et al. 2018), Phospholipase-D and oxophytodienoatereductase (Xin et al. 2016). Eukaryotic translation initiation factor 4-G (Yin and Komatsu 2017). Zinc finger/BTB-domain protein 47 (Yin and Komatsu 2017). Calnexin/calreticulin beta-xylosyltransferase (Yin and Komatsu 2017). Foldase and dnaJ protein (Yin and Komatsu 2017). Class-II-aminoacyltRNA-biotin synthetase (Yin and Komatsu 2017). Calcium transportinATPases (Yin and Komatsu 2017). Carbonic anhydrase-2 and aldolone-type TIM barrier family (Oskuei et al. 2017). Xyloglucanendo-transglucosylase/hydrolases and polygalacturonases (Xue et al. 2015). PAL proteins (Xue et al. 2015). Lipoxygenases and esterases/lipases (Xue et al. 2015). Chalcone flavanone isomerase and beta keto acyl reductase (Khan and Komatsu 2016). MLP-like protein (Khan and Komatsu 2016). Pyruvate protein (Khan and Komatsu 2016).		Translation elongation factor EF1B/ribosomal protein S6 and glutathione-*s*-transferase c-terminal (Yin et al. 2014), 60s acidic ribosomal protein family and ribosomal protein L10 family proteins (Cho et al. 2016). UDP-glucose 6-dehydrogenase (Yin et al. 2014). AMP deaminase (Yin et al. 2014). TUDOR-SN1 protein (Yin et al. 2014). Aluminium induced protein with YGL and LRDR (Yin et al. 2014). Phosphoglucomutase (Yin et al. 2014).

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Table 1 (continued)

	Leucine rich repeat LRR (Kamal et al. 2015). Plant stearoylacyldesaturase carrier protein (Kamal et al. 2015). Ascorbate peroxidase 1 (Kamal et al. 2015), Uridine diphosphate glycosyl transferase (Wang et al. 2015). Pyridine 2 (Kamal et al. 2015). Tubulin-beta-1 chain (Kamal et al. 2015). Regulatory particle triple A1A-protein and 20S proteasome alpha subunit E2 (Kamal et al. 2015). Class-II aminoacyltRNA and biotin synthetases (Kamal et al. 2015). Lipoyl domain protein and ATP-dependent caseinolytic protease (Wang et al. 2015). Pyrophosphorylase (Wang et al. 2015). RAS-5, RAS-GTP binding protein (Wang et al. 2015). RNA binding protein CTC interacting domain II (Wang et al. 2015). GHMP kinase (Wang et al. 2015), Phosphofructokinase and triosphosphateisomerase (You et al. 2014). Dihydrolipoamide acetyl transferase (Wang et al. 2015). Phosphoglucomutase and phosphomannomutase (Wang et al. 2015). Glutathione synthase (You et al. 2014). Glutathione-*S*-transferase and glutathione dehydrogenase (You et al. 2014). Alanine amino-transferase and aminotransferase 3 (You et al. 2014). Sucrose synthase-4 and phosphoglucomutase (You et al. 2014). Lactate dehydrogenase (You et al. 2014). ACC oxidases (You et al. 2014). Thioredoxin and auxin responsive GH3 proteins (You et al. 2014). Flavonoid 3-monoxygenase and phenylalanine ammonia lyase-I (You et al. 2014). Elongation factor (eF-1) alpha-gene and HSP-70 Kda protein (Guo et al. 2017). Glutathione reductase (Guo et al. 2017), Oxalate-CoA-decarboxylase (Lee et al. 2009), ROS scavenging and malate dehydrogenase (Wang et al. 2018). Serine rich splicing factor (Guo et al. 2017).		

Temperature (low and high)			
Protein folding (Guo et al. 2017). Stress (Guo et al. 2017; Lee et al. 2009; Wang et al. 2018). RNA metabolism (Guo et al. 2017). Signal transduction (Guo et al. 2017; Zhang et al. 2017, 2018; Wang et al. 2015; Wang et al. 2018). DNA metabolism (Guo et al. 2017). Transport (Guo et al. 2017; Wang et al. 2015). Regulatory mechanism (Lee et al. 2009). Detoxification (Lee et al. 2009). Energy metabolism (Lee et al. 2009). Amino acid metabolism (Dumont et al. 2011). Glycolysis (Dumont et al. 2011). Pentose phosphate pathway (Dumont et al. 2011). Photosynthesis (Dumont et al. 2011). Nucleotide metabolism (Dumont et al. 2011). Secondary metabolism (Dumont et al. 2011; Wang et al. 2015). Kreb cycle (Dumont et al. 2011). Defense (Dumont et al. 2011; Wang et al. 2015). Post translational modification (Wang et al. 2018). ROS homeostasis (Zhang et al. 2017, 2018; Xin et al. 2016). Transcription factor (Zhang et al. 2017, 2018). Enzyme metabolism (Wang et al. 2015). Protein storage (Wang et al. 2015). Metabolism (Wang et al. 2015). Cell structure (Wang et al. 2015). Cell signalling (Xin et al. 2016). Protein storage (Xin et al. 2016). Calvin cycle (Xin et al. 2016).	Isocitrate dehydrogenase and beta-glucosidase (Guo et al. 2017), MAP kinase and calcium dependent protein kinase (Wang et al. 2018), BRII 1-KD-interacting protein 114 and plastid glutamine synthetaseisoform GS2C (Wang et al. 2015), ZFN like protein (Zhang et al. 2017, 2018). FACT-complex subunit (Guo et al. 2017). Glycotransferase acetyl-CoA-acetyl transferase (Guo et al. 2017), ATP synthase CFI beta subunit and ATP synthase CFI alpha subunit (Wang et al. 2015). Cysteine synthase (Lee et al. 2009). Oxalyl-CoA-decarboxylase and Glyoxalase-I (Lee et al. 2009). Pyruvate orthophosphate dikinase and putative aconitate hydrolase (Lee et al. 2009). Amino acid dehydrogenase (Dumont et al. 2011). Fructose bisphosphate aldolase-2 (Dumont et al. 2011). Ribose-5-phosphate isomerase (Dumont et al. 2011). RuBiscoactivase (Dumont et al. 2011). Nucleotide-disphosphate-kinase-2 (Dumont et al. 2011). Caffeoyl-CoA-O-methyl-transferase (Dumont et al. 2011), SAM synthase (Wang et al. 2015). Malate dehydrogenase (Dumont et al. 2011). Tyrosine kinase (Dumont et al. 2011), 2-cysteine-peroxiredoxin BAS1 and glycine decarboxylase P-subunit (Wang et al. 2015). Ubiquitin E3 (Wang et al. 2018). Chlororespiratory reduction and serine carboxypeptidase II-3 (Wang et al. 2015), Cytochrome P-450 protein and myo-inositol oxygenase 5 (Xin et al. 2016). bHLH and CoP9 protein (Wang et al. 2015). Rubiscoactivase B and Rubiscoactivase small isoform glyceraldehyde-3-phosphate dehydrogenase (Wang et al. 2015). 60-KDa chaperonin subunit beta and alpha putative chloroplast protease (Wang et al. 2015). PS16 proteins (Wang et al. 2015). Actin proteins (Wang et al. 2015). HSPs 90 and peptidylprolyl-cis trans isomerases (Xin et al. 2016). Fructose bisphosphatealdolase and phosphoglycerate kinase (Xin et al. 2016).	Post-translational modification (Pi et al. 2017). Signal transduction (Pi et al. 2017; Gao et al. 2017). Transport (Pi et al. 2017). Sugar metabolism (Gao et al. 2017). Transcription factor (Gao et al. 2017).	Phosphofructokinase and Phosphatase E-3 ligase (Pi et al. 2017). Phosphatidylinositol and MAP-K kinase (Pi et al. 2018), HY5 kinase and MAP kinase (Gao et al. 2017). P-1512 and P-1669 proteins (Pi et al. 2017). Sucrose phosphate synthase and trehalose-6-phosphate synthase (Gao et al. 2017). Serine protein kinase (Gao et al. 2017).

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Table 1 (continued)

Heavy metal stress			
Defense (Kumar and Majeti 2014). Carbohydrate metabolism (Kumar and Majeti 2014; Romeo et al. 2014; You et al. 2014). Cell signalling (Kumar and Majeti 2014). Amino acid metabolism (Kumar and Majeti 2014; Liu et al. 2014; Chen et al. 2015; Cheng et al. 2017). Energy metabolism (Kumar and Majeti 2014; Romeo et al. 2014; You et al. 2014; Cheng et al. 2017). Transport (Kumar and Majeti 2014; You et al. 2014; Xue et al. 2015; Chen et al. 2015). Redox homeostasis (Xue et al. 2015; Chen et al. 2015). Post-translational modification Romeo et al. 2014). Vacuolar dysfunction (Romeo et al. 2014). Cell wall metabolism (You et al. 2014; Cheng et al. 2017; Chen et al. 2015). Stress response (You et al. 2014; Cheng et al. 2017; Kumar and Majeti 2014). Nucleic acid metabolism (You et al. 2014). Signal transduction (Kumar and Majeti 2014; Li et al. 2016; Xue et al. 2015; Romeo et al. 2014). Fatty acid metabolism (You et al. 2014). Secondary metabolism (Liu et al. 2014). Starch and sucrose metabolism (Liu et al. 2014). TCA cycle (Liu et al. 2014). Transcriptional regulation (Chen et al. 2015). Glycosylation (Li et al. 2016). DNA binding protein (Li et al. 2016). Reservoir (Xue et al. 2015). Starch metabolism (Xue et al. 2015). Protein synthesis (Xue et al. 2015). Metabolism (Xue et al. 2015). Photosynthesis (Xue et al. 2015; Cheng et al. 2017).	Peroxidase and aldo-keto-reductase (Kumar and Majeti 2014). 2, 3-bis-phosphoglycerate-mutase-1 (Kumar and Majeti 2014), 2,3-bisphosphoglycerate mutase and succinate dehydrogenase (Romeo et al. 2014; You et al. 2014). Receptor like protein synthase and chalcone flavones isomerase (Kumar and Majeti 2014). Adenosyl-homo-cysteinase (Kumar and Majeti 2014), Peroxidase and ferrodoxin NADP-reductase (Liu et al. 2014), glutamine synthase and putative glutamine synthase (Chen et al. 2015), cysteine synthase and SAM-synthase (Cheng et al. 2017). ATP-synthase subunit beta, mitochondria and ATP-synthase subunit alpha, mitochondria (Kumar and Majeti 2014), NADH-quinoneoxido-reductase (Romeo et al. 2014), malate dehydrogenase and fructose-1,6-bisphosphatase (Cheng et al. 2017). Aquaporin NIP-1 (Kumar and Majeti 2014), Chloroplastic outer envelope membrane (Xue et al. 2015), Translation initiation factor 5A and putative elongation factor (Chen et al. 2015). L-ascorbate peroxidase1and superoxide dismutase (Xue et al. 2015), putative peroxidise and germin like protein-6 (Chen et al. 2015). Glyceraldehyde-3-phosphate dehydrogenase and fructose-bisphophatealdolase (Romeo et al. 2014). Receptor kinase and 2-oxoglutarate dioxygenase (Romeo et al. 2014). ATPase (Romeo et al. 2014). NADP-malic enzyme (You et al. 2014), UDP-arabinopyranose mutase-I (Cheng et al. 2017), Putative caffeoyl-CoA-methyl transferase (Chen et al. 2015).	Signal transduction (Zhong et al. 2017; Lan et al. 2012). Redox homeostasis (Zhong et al. 2017). Transcription factor (Zhong et al. 2017; Lan et al. 2012). Cellular component and molecular function (Zhong et al. 2017). Sugar metabolism (Zhong et al. 2017). Stress and defence (Zhong et al. 2017). Plasma membrane bound transporter (Lan et al. 2012). Carbohydrate metabolism (Lan et al. 2012).	Calmodulin dependent protein phytochelatin synthase (Zhong et al. 2017), 3-phosphoglycerate dehydrogenase (Lan et al. 2012). Mitogen activating kinase (Zhong et al. 2017). RAS kinase (Zhong et al. 2017), Putative pyrophosphatase (Lan et al. 2012). Threonine kinase (Zhong et al. 2017). Sucrose-1-related kinase (Zhong et al. 2017). Superoxide mutase (Zhong et al. 2017). Gln-synthase and phosphatidylinositol kinase (Lan et al. 2012). Calcium calmodulin-dependent kinase (Lan et al. 2012.

Cell wall related protein (Xue et al. 2015). Carbon metabolism (Romeo et al. 2014; Cheng et al. 2017). Transcription factors (Cheng et al. 2017). Protein folding (Cheng et al. 2017).	Alcohol dehydrogenase (You et al. 2014), superoxide dismutase and glutathione-*s*-transferase (Cheng et al. 2017). Transcriptional endoplasmic reticulum ATPase (You et al. 2014). Monoascorbate reductase (You et al. 2014), probable receptor like kinase (Kumar and Majeti 2014), Nucleoside diphosphate kinase and receptor activate protein kinase (Romeo et al. 2014), phosphoglycerate kinase and glutamine synthetase (Li et al. 2016), glucose and ribitol dehydrogenase (Xue et al. 2015). Maturase-K and Xanthine dehydrogenase (You et al. 2014). Putative calcium dependent protein kinase (You et al. 2014). Putative enoyl-acyl carrier protein reductase (You et al. 2014). Chloroplast–NADPH (Liu et al. 2014). Plasma membrane type ATPase (Liu et al. 2014). Glutamine synthase (Chen et al. 2015). Putative oxidase (Chen et al. 2015). CHP-rich zinc finger like protein (Chen et al. 2015). Cal-reticulin-2 (Li et al. 2016). Phosphoglycerate kinase and glutamine synthase (Li et al. 2016). Stress induced protein-1 (Li et al. 2016). Orthophosphate dikinaseRubisco (Xue et al. 2015), Ribulose bisphosphate carboxylase chlorophyll binding protein (cheng et al. 2017). Histone 2A1 protein (Li et al. 2016). Preproglutelingermin-like protein (Xue et al. 2015). ADP-glucose pyrophosphorylase and sucrose synthase 3 (Xue et al. 2015). Protein disulfideisomerase and putative ketol acid reductoisomerase (Xue et al. 2015). Phosphoglucomutase and methionine adenosyl transferase-2 (Xue et al. 2015). Reversibly glycosylated polypeptide (Xue et al. 2015). Aconitase and succinate dehydrogenase (Romeo et al. 2014), phosphoglucomutase and NADH-dehydrogenase iron sulphur protein (Cheng et al. 2017). 30-S ribosomal protein 2 and elongation factor-2 (Cheng et al. 2017). Alpha-amylase/trypsin inhibitor CYP 38 cis isomerase (Cheng et al. 2017).		

approach, the effects of abiotic stress factors on protein abundance have been examined in model, horticultural plants and crop and non-crop species such as Arabidopsis (Guo et al. 2014), rice (Paul et al. 2015, Chen et al. 2015), *Cucumis sativus* and *Solanum tuberosum* (Aghaei et al. 2008), wheat (Gao et al. 2011; Li et al. 2018), barley (Witzel et al. 2009, 2010; Mostek et al. 2015), soybean (Mustafa and Komatsu 2014), stiff grass (Cheng et al. 2017), sunroot (Zhang et al. 2017, 2018), shrubby cinquefoil (Guo et al. 2017), alfalfa (Atikur et al. 2016), Chinese grass (Xue et al. 2015), poplar (Romeo et al. 2014). Similarly, phosphoproteome studies were conducted on different crop/plants such as Arabidopsis (Bhaskara et al. 2017), rice (Zhong et al. 2017), maize (Hu et al. 2015) mulberry (Pi et al. 2017), apple (Ren et al. 2017), banana (Gao et al. 2017) and soybean (Pi et al. 2018).

2.1 Drought

Drought is a widespread environmental stress that limit agricultural productivity worldwide (Carrão et al. 2016). Despite many decades of research, drought stress is continues to be a challenging task to the agricultural scientists in general and plant breeders, in particular (Surabhi 2018). Plant response to drought has become very important in current plant biology research because it causes many changes in the biology of the plant cell, beginning with the stress perception and followed by physiological and molecular changes that promote the acclimation to the stress. Physiological processes like photosynthesis, respiration, water relations, anti-oxidative metabolism and hormonal metabolism are affected by drought (Farooq et al. 2009; Bhargava and Sawant 2013).

2.1.1 Proteome Analysis Under Drought

The proteomic studies of different species under drought stress have been extensively studied to date (Atikur et al. 2016; Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Khan and Komatsu 2016; Li et al. 2018; Zadraznik et al. 2017). The altered level of expression of several protein families such as secondary metabolism, carbohydrate metabolism, energy metabolism, stress response, ROS scavenging proteins, transcription factors, signal transduction, protein folding, hormonal synthesis and cell wall metabolism have been well elucidated from different proteomic studies under drought (Ghaffari et al. 2013; Atikur et al. 2016; Paul et al. 2015; Khan and Komatsu 2016). It has been found that generation of reactive oxygen species (ROS) during drought stress can damage the structures of proteins, lipids and cell membrane integrity which ultimately destroy the plant cell (Atikur et al. 2016).

The higher amount of ROS scavenging proteins in plants increases the resistance mechanism to cope up with particular stress conditions (Atikur et al. 2016; Simova-Stoilova et al. 2015a, b). Some novel proteins which have a key role in generation of drought tolerant plants were found in the form of R40C1, cytosolic ascorbate peroxidase and putative F-box proteins during the proteomic analysis of rice and

alfalfa (Paul et al. 2015; Atikur et al. 2016). Beta-glucosidase which was found to be involved in cell wall modification during proteomic analysis of bean under drought stress showed the highest increase in the abundance during initial and final drought treatment (Zadraznik et al. 2017). Many specialized proteins are differentially expressed in plants during drought, where they have a role as signalling molecules (Atikur et al. 2016), reactive oxygen scavengers (Liu et al. 2017), proteins with responses to pathogen-related (Paul et al. 2015), heat shock proteins, late embryogenesis abundant (LEA) proteins (Liu et al. 2017) and chaperones (Liu et al. 2009; Veeranagamallaiah et al. 2011).

2.1.2 Phosphoproteome Analysis Under Drought

Phosphoproteomic studies were conducted on different plants in response to drought stress (Harb et al. 2010; Hu et al. 2015; Bhaskara et al. 2017; Ren et al. 2017). Functional analysis of maize and wheat proteins during drought stress has revealed that phosphoproteins were involved in signalling pathways or activation of receptor signalling in the form of kinases, protein transport, mRNA processing and transcription factors like bZIP-30, MYB1R1, bHLH and AB15 (Bhaskara et al. 2017; Ren et al. 2017). Phosphoproteome analysis of drought treated wheat and Arabidopsis revealed phosphorylated proteins such as ABA induced SnRK, mitogen activated protein kinases (MAPK) and calcium dependent protein kinases (Umezawa et al. 2013; Ren et al. 2017). Phosphoproteomic studies in crop/plants under drought stress are rather scanty and it requires more focus on this aspect in order to get deeper insights on stress signalling process.

2.2 Salinity

Salt tolerance is a complex phenotype which is controlled by multiple genes. Identifying novel genes, determining their expression patterns in response to salt stress and exploring their functions in stress adaptation are the basis for implementing effective engineering strategies to improve salt tolerance in plants (Cushman and Bohnert 2000). It is estimated that salt stress may affect half of all arable lands by 2050, and will be a major factor responsible for the loss of arable land for the coming decades (Wang et al. 2003a, b).

2.2.1 Proteome Analysis Under Salinity

Salinity induced tissue specific proteome studies were conducted on different crop/plants (Guo et al. 2011, 2014; Mostek et al. 2015; Witzel et al. 2014; Li et al. 2015; Zhang et al. 2017, 2018). While some proteomic studies have focused on the plant response within a few hours of encountering stress (Chitteti and Peng 2007; Li et al. 2015), others have been more interested in studying the response over a

number of days (Guo et al. 2011; Witzel et al. 2014). Functional analysis of proteins identified during salt stress in different plants were involved in protein transport, carbohydrate mechanism, ATP-synthesis, protein folding, detoxification, signal transduction, cell wall modification, energy metabolism, glycolysis, post-translational modification and defence response gives a basic insight into the mechanism of plants to cope with salt stress (Guo et al. 2011, 2014; Mostek et al. 2015; Witzel et al. 2014; Li et al. 2015; Zhang et al. 2017, 2018). During proteomic analysis of salt-sensitive and salt-tolerant barley lines revealed that enhanced salinity tolerance of barley line, that is, DH-187 observed as a result of an increased activity of signal transduction mechanism and cell wall structural changes (Mostek et al. 2015). Majority of the proteins involved in the cell wall metabolism and secondary metabolism were found to be increase in abundance in salt stressed Arabidopsis and cotton roots (Guo et al. 2014; Li et al. 2015). Witzel et al. (2014) have identified some of the new candidate proteins underlying salinity tolerance in barley, such as germin-like, pathogen related and cell-wall modification (β-1,3-glucanase) proteins (Table 1).

2.2.2 Phosphoproteome Analysis Under Salinity

Plants respond to salt stress by triggering phosphorylation cascades to turn on the salt overly sensitive (SOS) signalling pathway (Zhu 2001; Hsu et al. 2009; Jun et al. 2010; Pi et al. 2018). The phosphoproteomic studies were conducted in different plants under salt stress (Hsu et al. 2009; Jun et al. 2010; Pi et al. 2018) and signalling responses and phosphorylation cascades are suggested to function in transmitting and amplifying the extracellular salt stress signals in plants (Jun et al. 2010; Pi et al. 2018). It was found that the growth of *Thellungiella* roots was less inhibited by high-salinity stress than Arabidopsis and also *Thellungiella* roots have higher abilities to limit the Na influx than Arabidopsis because of expression of specific Na/K antiporter (Hsu et al. 2009; Jun et al. 2010). Five novel membrane proteins, that is, AHA1, STP1, patellin-2 and probable receptor kinase were identified in salt treated Arabidopsis plant (Hsu et al. 2009). Three MYB proteins were found to be differentially phosphorylated upon salt treatment in soybean and it was reported that over-expression of the GmMYB173S59D and GmCHS5 resulted in the enhancement of salt tolerance mechanism (Pi et al. 2018). The above-mentioned investigations suggested the power of proteomic and phosphoproteomic approaches in identifying functional proteins responsive to salt stress in plants. However, our understanding of salt stress responsive proteins in different tissues of crop plants is still far from complete.

2.3 Waterlogging

Waterlogging is defined as prolonged soil saturation with water at least 20% higher than the field capacity (Aggarwal et al. 2006). It is a major problem of utmost importance as it limits the growth and yield of many crops in humid areas.

Globally, approximately 10% of irrigated farmlands suffer from frequent waterlogging; however, values up to 20% occur in specific regions such as Eastern Europe and the Russian Federation (FAO 2002; Alam et al. 2010). During 1993, approximately 20 million acres of corn and soybean were inundated in the mid-western United States leading to heavy economic loss, as estimated by United State Department of Agriculture, National Agricultural Statistics Service (Suszkiw 1994). The deleterious effects associated with hypoxia and anoxia include a decrease in cellular energy charge, drop in cytoplasmic pH, and the accumulation of toxic metabolites and reactive oxygen species (ROS) which are responsible for the slowed growth and reduced yield of many agriculturally important crops (Subbaiah and Sachs 2003; Surabhi 2018).

2.3.1 Proteome Analysis Under Waterlogging

Several proteomic studies on crop/plants in responses to waterlogging (flooding) stress revealed that it affects the proteins involved in several metabolic pathways such as cellular processes, defence mechanism, secondary metabolite synthesis, protein storage and amino acid metabolism (Ahsan et al. 2007; Komatsu and Hossain 2013). Earlier studies revealed that waterlogging treatment of maize seedlings drastically altered the profile of total protein synthesis. In an anaerobic environment, 20 proteins, which account for more than 70% of the total translation, are selectively synthesized (Sachs et al. 1980). A proteomic examination of the soybean cell wall found that flooding induces a suppression of lignification through a decrease in the expression of proteins involved in ROS scavenging (Komatsu et al. 2010). In another study, it was revealed that accumulation of glycoproteins localized in the secretory pathway decreased under flood stress in soybean. Further, some novel proteins, that is, 3-β-hydroxylases, glutamyl t-RNA reductase, cysteine proteases, auxin-amidohydrolase and coprophyrinogen oxidase were identified in soybean during flooding stress (Ahsan et al. 2007; Komatsu and Hossain 2013).

2.3.2 Phosphoproteome Analysis Under Waterlogging

The effect of flooding on soybean has been extensively studied because soybean is a flood-in tolerant crop, whose growth and grain yield are significantly reduced under flooding stress (Nanjo et al. 2010, 2012). Comparative gel-free proteomics and gel-based phosphoproteomics techniques were used to investigate early responses to flooding stress in the roots and hypocotyls of soybean seedlings (Nanjo et al. 2010). De-phosphorylation of proteins involved in protein folding and synthesis was found to be one of the early responses. Different studies have suggested that the translational or post-translational control of proteins involved in protein folding and synthesis during flooding induces an imbalanced expression of proteins involved in several metabolic pathways, including carbohydrate metabolism, which may cause flooding-induced injury to the seedlings. Recently, gel-free mass spectrometry-based

proteomics techniques was used to compare protein phosphorylation states in the root tips of flooded soybean seedlings (Nanjo et al. 2012). A comparison of the proteins identified through phosphoproteomic and quantitative proteomic analyses revealed six proteins affected by flooding and showed changes in both abundance and phosphorylation status, including those involved in energy generation, protein synthesis and cell structure maintenance (Nanjo et al. 2010, 2012). It was concluded that protein phosphorylation is likely to play a major role in the regulation of pentose phosphate pathways, photosynthesis activities, pyruvate metabolism and ROS production which together contribute to stable energy supply that enhances flooding tolerance in *Kandelia candel*. Some novel phosphoproteins were identified in *Kandelia* during flood stress, that is, GSP, GxxSP and RSxS (Pan et al. 2018). Phosphoproteomic studies on different crop/plants under waterlogging are rather scanty. It requires attention to explore the specific set proteins expressed under waterlogging in order to utilizing them for crop improvement programs.

2.4 Temperature

The effects of global warming will not be limited to rising mean annual temperatures around the globe. There will also be a remarkable increase in both frequency and amplitude of severe temperature events, resulting in more extreme hot and cold days, more frequently (Neilson et al. 2010).

2.4.1 Proteome and Phosphoproteome Analysis Under High Temperature

When subjected to a high-temperature stress, plants generally respond through alterations in cell structure, cell membrane permeability, cell osmotic adjustment and photosynthetic activity (Dias et al. 2010). Guo et al. (2017) have studied proteomic changes in *Potentilla fruticosa* leaves after subjecting plants to 42 °C heat stress for 3 days, using isobaric tags for relative and absolute quantification (iTRAQ) coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). They identified 35 up-regulated and 23 down-regulated proteins after the heat stress treatment. Those differentially abundant proteins were involved mainly in protein synthesis, protein folding and degradation, abiotic stress defence, photosynthesis, RNA process, signal transduction and other functions. Further, 58 proteins were categorized based on their sub-cellular localization mainly in the chloroplast envelope, cytoplasm, nucleus, cytosol, chloroplast, mitochondrion and cell membrane. In another study, Xu and Huang (2008) have reported that, upon imposition of heat stress, 70 protein spots were altered in at least one species. Both moderate and severe heat stress caused down-regulation of majority of proteins than up-regulated, and thermal *Agrostis scabra* roots had more up-regulated proteins than *Agrostis stolonifera* roots. Further, the mass spectrometry studies led to the identification of corresponding sequences of 66 differentially expressed protein spots. The results

suggested that up-regulation of sucrose synthase, glutathione *S*-transferase, superoxide dismutase and heat shock protein stress-inducible protein (Sti) may contribute to the superior root thermo tolerance of *A. scabra*. In addition, two isoforms of fructose-biphosphate aldolase were highly phosphorylated under heat stress as revealed by phosphoproteomic analysis, and thermal *A. scabra* had greater phosphorylation than *A. stolnifera*, suggesting that the aldolase phosphorylation might be involved in root thermo tolerance (Xu and Huang 2008).

Chen et al. (2011) have studied phosphoproteome of rice leaves after exposing plants to heat stress, and their study revealed 10 differentially expressed proteins. Analysis of the biological processes revealed that three of the variable phosphoproteins are involved in the Calvin cycle, two are part of hydrogen peroxide catabolism, two participate in ATP synthesis-coupled proton transport, one is involved in microtubule-based movement and one in cellular metabolic processes; the others have unknown functions. Heat stress induced the dephosphorylation of ribulose bisphosphate carboxylase (RuBisCo) and the phosphorylation of ATP synthase subunit-ß. This modification decreases the activities of these enzymes, but the functional significance of other phosphorylation events remains to be examined. Characterization of different candidate proteins expressed under high-temperature stress provides valuable information on their functional role and also scope for further utilization of the proteins/genes for developing high-temperature tolerant plants (Xu and Huang 2008; Guo et al. 2017; Surabhi 2018).

2.4.2 Proteome and Phosphoproteome Analysis Under Low-Temperature Stress

Low temperature, as an extreme environment, is responsible for 30–40% yield reduction in temperate growing areas (Thakur et al. 2010). The plants exposed to low-temperature stress reported to shift the thermodynamic equilibrium, when there is an increased likelihood that non-polar side chains of proteins become exposed to the aqueous medium of the cell, which can directly affect the stability and the solubility of many globular proteins. This leads to a disturbance in the stability of proteins or protein complexes, and, therefore, to a disruption of metabolic regulations. The investigation of proteome expression in different plants under chilling stress and identification of some novel proteins could be useful for better understanding the molecular basis of low-temperature stress responses in plants.

Hashimoto et al. (2009) have identified 12 number of cold stress responsive proteins from the rice root plasma membrane using a 2D-PAGE-based proteomic approach. The identified proteins were such as receptor-type protein kinase, GPI-anchored protein, leucine-rich repeat transmembrane protein kinase, water channel protein, plasma membrane integral protein, lipid transfer protein, phosphate transporter and MAP 3 K like protein kinase. In addition, cold shock protein-1 was significantly decreased in plasma membrane of rice under cold stress.

Two pea lines (*Pisum sativum* L.) with contrasted behaviours towards chilling and subsequent frost were studied by Dumont et al. (2011). Following a chilling

period, the Champagne line showed tolerant to frost, whereas, Terese line remains sensitive. Fifteen-root proteins were identified and these proteins were related to chilling response or cold acclimation. Altogether, the investigation revealed that cold acclimation is a very complex biological process that might be linked to genetic variability within the two pea species (Dumont et al. 2011).

In rice roots, a total of 27 up-regulated proteins were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry or electro spray ionization-tandem mass spectrometry (ESI-MS/MS), after subjecting plants to chilling stress treatment (Lee et al. 2009). In their study, a group of novel proteins were identified including acetyltransferase, phosphogluconate dehydrogenase, NADP-specific isocitrate dehydrogenase, fructokinase, PrMC3, putative alpha-soluble N-ethylmaleimide-sensitive factor (NSF) attachment protein and glyoxalase-1, in addition to the previously identified cold-stress responsive proteins. The identified proteins are involved in several cellular processes, including energy production and metabolism, vesicular trafficking and detoxification. Gene expression at mRNA level of some selected proteins revealed that transcription levels are not always concomitant to the translational level.

Phosphoproteomics analysis using LTQ-Orbitrap with mass spectrometry have elucidated the molecular mechanism of chilling (4 °C) tolerance in mulberry leaves (Pi et al. 2017). The result showed that 427 differentially expressed phosphoproteins were detected after 6 h of chilling, while a total of 611 phosphoproteins which were found to be significantly changed during 48 h of chilling injury. Several groups of phosphoproteins were identified in the form of protein kinases (CKII) which were responsible for the proteomic changes during chilling injury and also found to be involved in the signal transduction, protein modifications and translation process. Two phosphorylation proteins BpSIZ1 and BpICE1 found to be involved in transcription factors such as CBF/DREB during chilling stress was identified (Pi et al. 2017).

A comparative phosphoproteomic profiling of cold-sensitive Cavendish and relatively cold-tolerant Dajiao under cold stress was conducted to identify the differentially expressed proteins in banana (Gao et al. 2017). The study revealed that five phosphoproteins were differentially expressed and kinesin proteins showed a difference between the two cultivars of banana during cold stress (Gao et al. 2017). Western blot analysis showed that T31 phosphoproteins were increased, while MKK2 was decreased in Daojiao during cold stress. In case of Cavendish, MKK2 was increased, while T31 was not detected during cold stress (Gao et al. 2017). Identification of chilling related pathways and novel phosphopeptides in plants would broaden the insight into chilling response.

2.5 Heavy Metal Stress

Heavy metal pollution of air and agricultural soils is one of the most important ecological problems worldwide. Although many heavy metals occur naturally in the earth's crust at various levels, the problem arises when they are released in excess

into the environment due to natural and/or anthropogenic activities (Singh et al. 2016). Large areas of land have been contaminated with heavy metals due to the use of pesticides, fertilizers, municipal and compost wastes, and also due to heavy metal release from smelting industries and metalliferous mines (Yang et al. 2005). The annual toxicity of all toxic metals mobilized exceeds the combined total toxicity of radioactive and organic wastes produced every year from all sources (Nriagu and Pacyna 1998).

2.5.1 Proteome Analysis Under Heavy Metal Stress

Liu et al. (2014) have utilized recently developed 6-plex Tandem Mass Tag (TMT) for relative and absolute quantitation methods to achieve a comprehensive understanding of Cu tolerance/detoxification molecular mechanisms in *Elsholtzia splendens* root cell wall, for the first time. An LC-MS/MS approach was followed to analyse the Cu-responsive cell wall proteins and polysaccharides. The majority of 22 up-regulated proteins was involved in antioxidant defence pathway, cell wall polysaccharide remodelling and cell metabolism process. Changes in polysaccharide amount, composition and distribution could offer more binding sites for Cu ions. Further, the 33 down-regulated proteins were involved in signalling pathway, energy and protein synthesis.

In another study by Chen et al. (2015) have investigated the differences in Cu-binding protein expression between Cu-tolerant and Cu-sensitive rice varieties using a new IMAC method. In total, 27 differentially expressed Cu-binding proteins were identified, out of which 16 proteins were not previously identified as Cu-IMAC-binding proteins either from plants or animals (Chen et al. 2015). These novel Cu-binding proteins were of four main types, proteins involved in antioxidant defence and detoxification, putative pathogenesis-related proteins, putative cold-shock domain proteins and eukaryotic translation initiation factors.

Kumar and Majeti (2014) have studied Pb-stress effects on *Talinum triangulare* Jacq. (Willd.) after exposing the plants for 7 days and proteomic study was performed for control and 1.25 mM Pb-treated plants to examine the root protein dynamics in the presence of Pb. Twenty-three major proteins showed increased abundance, of which three proteins are new (appeared only in 1.25 mM Pb). Functional categorization of identified proteins under 1.25 mM Pb-stress have given a very clear indication about their involvement in root architecture, energy metabolism, reactive oxygen species (ROS) detoxification, cell signalling, primary and secondary metabolisms, and molecular transport systems.

The seedlings of 'Sour pummelo' (*Citrus grandis*) and 'Xuegan' (*Citrus sinensis*) were irrigated for 17 weeks with 2 μM (control) or 600 μM (Mn-toxic) MnSO4 (You et al. 2014). Two-dimensional gel electrophoresis (2-DE) subsequent analysis yielded 11 up-regulated and 42 down-regulated protein spots from Mn-toxic *C. sinensis* roots, and 25 up-regulated and 14 down-regulated protein spots from Mn-toxic *C. grandis* roots. This indicates more remarkable metabolic flexibility in *C. sinensis* roots than in *C. grandis* ones. They found important differences in

Mn-toxicity-induced changes in root protein profiles as well as root metabolic responses between the two species, especially in these proteins involved in protein biosynthesis and degradation, nucleic acid metabolism, carbohydrate and energy metabolism, and stress responses. The abundance of proteins related to nucleic acid metabolism, glycolysis and cell transport increased in non-tolerant *C. grandis* roots in response to Mn-toxicity, and decreased in tolerant *C. sinensis* roots (You et al. 2014) (Table 1).

2.5.2 Phosphoproteome Analysis Under Heavy Metal Stress

Zhong et al. (2017) have studied Cd stress effect on rice seedlings using an iTRAQ-based quantitative phosphoproteomic approach. They identified 2454 phosphosites, associated with 1244 proteins, and a total of 482 of these proteins became differentially phosphorylated under Cd stress. Number of proteins which were affected at 100 μM Cd^{2+} was sixfold higher than in 10 μM treatment. Functional analysis of the proteins which were differentially phosphorylated under stress revealed that a significant number was involved in signalling, stress tolerance and reactive oxygen species metabolism, in addition transcription factor related proteins were identified (Zhong et al. 2017). Currently, proteome and phosphoproteome analysis under heavy metal stress in crop plants is infancy and more attention is required to get deeper molecular insights of heavy metal stress tolerance in crop plants.

3 Combined Proteomics and Phosphoproteomic Studies Under Different Abiotic Stress in Crop Plants

Significant amount of proteome work has been conducted on crop/plants under different abiotic stresses. However, phosphoproteome studies in plants dealing with abiotic stresses or combined proteome and phosphoproteome studies are rather scanty. One biochemical manifestation common to all stresses is specific, regulated protein phosphorylation. It is universally accepted that a major part of the signal linking is the environmental perception of the stress at the cell surface to the nucleus, where response proteins can be translated, Protein phosphorylation is generally transmitted by protein kinase cascades (Kersten et al. 2009). A few kinase-mediated signalling pathways have been elucidated (e.g. Asai et al. 2002) in the model plants *Arabidopsis thaliana* (van Bentem and Hirt 2007; Pitzschke et al. 2009) and rice (Chen and Ronald 2011). A picture of the complexity of these signalling pathways, with all their cross-talk and branch points, is beginning to emerge. Since these pathways rely principally on post-translational modification to transmit their signal, their elucidation is well served by a proteomic approach.

Guo et al. (2014) have conducted two-dimensional gel-based proteome (coomassie brilliant blue R-350 stain) and phosphoproteome (Pro-Q diamond stain)

studies coupled with mass spectrometry to investigate salt stress induced alterations in protein profiles in the model plant, Arabidopsis roots. Non-synchronous differences were found between total proteins and phosphorylated proteins. Ten differential spots were common between 28 differential total protein spots and 13 differential phosphoproteins spots. The identified proteins are involved in binding, catalysis, signal transduction, transport, metabolisms of cell wall and energy, and reactive oxygen species (ROS) scavenging and defence (Guo et al. 2014).

Chitteti and Peng (2007) have investigated differential expression of proteins after imposing salinity stress for 24 h in rice roots. They have utilized both SYPRO ruby and Pro-Q diamond stain to study proteome and phosphoproteome fractions, respectively. Thirty-one differentially regulated proteins revealed by SYPRO ruby and 28 differentially regulated putative phosphoproteins revealed by Pro-Q diamond stain were identified using mass spectrometry. Seven proteins displayed differential expression whether the gel was stained by Pro-Q diamond or SYPRO ruby stain. The other differentially regulated proteins were specific either to Pro-Q diamond or SYPRO ruby stain, suggesting, necessity of conducting proteome and phosphoproteome studies in order to obtain holistic view of plant response to abiotic stresses (Chitteti and Peng 2007).

In another study, Lv et al. (2014) have conducted combined proteome and phosphoproteome study on *Brachypodium distachyon* leaves, after imposing salt stress. A total of 80 differentially expressed protein spots corresponding to 60 unique proteins were identified. Phosphopeptide purification was carried using TiO_2 micro-columns and LC-MS/MS for phosphoproteome analysis to identify phosphorylation sites and phosphoproteins. A total of 1509 phosphoproteins and 2839 phosphorylation sites were identified. Among them, 468 phosphoproteins containing 496 phosphorylation sites demonstrated significant changes at the phosphorylation level. Of the 60 unique differentially expressed proteins, 14 were also identified as phosphoproteins. Many proteins and phosphoproteins, as well as potential signal pathways associated with salt response and defence, were found, including three 14-3-3s (GF14A, GF14B and 14-3-3A) for signal transduction and several ABA signal-associated proteins such as ABF2, TRAB1 and SAPK8. Based on different studies, it is clear that the overlapping between proteome and phosphoproteome within different studies under varying stress conditions were found minimal. Therefore, it necessitates conducting both proteome and phosphoproteome in each study to identify metabolic and signalling proteins, respectively, under abiotic stress in crop plants.

4 Mass Spectrometry in Proteomic and Phosphoproteomic Studies

The technology of choice for proteomics is mass spectrometry (MS) including several approaches such as liquid chromatography–mass spectrometry (LC-MS/MS), ion trap–mass spectrometry (IT-MS) and matrix-assisted laser desorption/

ionization–time of flight mass spectrometry (MALDI-TOF-MS) (Komatsu and Hossain 2013; Shao et al. 2015). However, it is necessary to choose the appropriate instrument for the purpose as there is no MS that can be useful for all fields of proteome analysis. MALDI-TOF/MS is often used for high-throughput identification of the protein by peptide mass fingerprinting (Witzel et al. 2009, 2010). In the analysis of amino acid sequence and post-translational modification, MS/MS such as ESI-IT and ESI-Q-TOF/MS are used. These technologies are basically used in measuring the mass and charge of small protein fragments (or 'peptides') that result from protein enzymatic digestion with special enzymes called proteases, such as trypsin (Helmy et al. 2012a; Nakagami et al. 2012). The output of a standard MS-based proteomic analysis is a set of peptide fingerprints called MS spectra. MS spectra require another layer of interpretation to reveal the peptide sequences associated with each of them, the protein of each peptide and the modification occurring in each protein after being translated (Tyers and Mann 2003; Helmy et al. 2012a, b; Nakagami et al. 2012).

Proteomic and phosphoproteomic investigation was carried on plants under different abiotic stress conditions by using different mass spectrometry platforms such as MALDI-TOF/MS (Atikur et al. 2016; Paul et al. 2015), LC-MS/MS (Zadraznik et al. 2017; Li et al. 2018; Guo et al. 2017), LC-ESI-MS/MS (Ren et al. 2017; Zhang et al. 2017, 2018), nano-LC-MS/MS (Oskuei et al. 2017; Zhang et al. 2017, 2018; Li et al. 2018; Wang et al. 2018), nano-LC-ESI-Q-TOF-MS/MS (Pan et al. 2018), nano-LC-ESI-LIT-MS/MS (Romeo et al. 2014) and nano-RPLC-MS/MS (Pi et al. 2018). Despite these technological innovations and advancements, the analysis of a full proteome is still a challenging task, mainly because of the high complexity of protein samples (Bachi and Bonaldi 2008; Surabhi 2018). To overcome this difficulty, several separation techniques such as multi-dimensional chromatography, MudPit (Washburn et al. 2001) or specific enrichment/depletion techniques, tandem affinity purification (Gavin et al. 2002) and equalizer beads (Guerrier et al. 2008) can be applied prior to mass spectrometric analysis. These approaches increase the proteome coverage and the dynamic range of large-scale proteomics analysis.

4.1 Gel-Based Proteomic and Phosphoproteomic Analysis in Plant Abiotic Stress

2-DE coupled with MALDI-TOF-MS or ESI-Q-TOF-MS/MS are the most common technique used in the abiotic stress-related proteomic studies. 2-DE resolves proteins on the basis of isoelectric point (pI) and molecular mass (Mr) (Roy et al. 2011). The separated protein spots can then be stained, with coomassie brilliant blue, silver nitrate, or SYPRO Ruby (Robinson et al. 2011), among others. When combined with advanced MS techniques, 2-DE allows hundreds of proteins to be characterized in a single polyacrylamide gel (Magdeldin et al. 2014), including the position of the protein spot (pI and Mr) on the gel. This capability of 2-DE has allowed for analysis of post-translational modifications (PTMs) of proteins. Two-dimensional gel-based proteomic and phosphoproteomic analysis were conducted on plants

under different abiotic stresses such as drought (Atikur et al. 2016; Paul et al. 2015; Simova-Stoilova et al. 2015a, b), salt (Chitteti and Peng 2007; Jun et al. 2010; Guo et al. 2014; Wen et al. 2014; Witzel et al. 2014; Mostek et al. 2015), heavy-metal (Romeo et al. 2014; You et al. 2014), low-temperature (Lee et al. 2009) and water-logging stress (Alam et al. 2010). The DIGE technique was developed to improve the reproducibility of 2-DE and to overcome gel-to-gel variation (Unlu et al. 1997). Each protein sample is labelled at a lysine residue with different fluorophores, such as CyDye2, CyDye3 and CyDye5 (Beckett 2012), prior to mixing and separation on the same gel, and the abundance of the same protein in different samples can easily be determined by using these fluorophores (Magdeldin et al. 2014). This technique reduces the number of gels needed for one experiment and is able to detect as little as 150 pg of a single protein with a linear response in protein concentration of over five orders of magnitude. Differential-in-gel electrophoresis (DIGE) performed in different plants in response to several abiotic stress such as salt (Gao et al. 2011) and heavy-metal stress (Kumar and Majeti 2014; Chen et al. 2015; Xue et al. 2015; Cheng et al. 2017). The relatively high cost of DIGE equipment, software and consumables, however, has limited its use. Despite the successes of 2-DE, the method has many limitations (Robinson et al. 2011). For example, 2-DE can separate only 30–50% of the entire proteome, depending on the tissue, and it is unable to separate all the proteins present in a complex sample (Beckett 2012).

The low-abundance proteins with physiological relevance, including regulatory and signal-transducing proteins or phosphoproteins, are also rarely detected on traditional 2-DE gels, because the large amount of highly abundant proteins masks their detection (Roy et al. 2011). For instance, ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO), which accounts for a large percentage of total plant protein, hinders absorption of low-abundant proteins on the immobilized pH gradient (IPG) strips and results in poor detection and identification of these proteins on 2-D gels and by MS (Beckett 2012). Different staining technique have applied for visualization of proteins and phosphoproteins which were differentially expressed under different abiotic stress such as colloidal coomassie blue R-250, R-350 and G-250 (Chen et al. 2015; Paul et al. 2015; Simova-Stoilova et al. 2015a, b; Xue et al. 2015; Atikur et al. 2016; Cheng et al. 2017) and SYPRO-ruby staining (Chitteti and Peng 2007). The Pro-Q diamond in gel stain was found to be useful method for direct visualization of the putative phosphoprotein spots expressed under different abiotic stress such as salt (Chitteti and Peng 2007; Jun et al. 2010; Guo et al. 2014; Liu et al. 2014) and drought (Yuan et al. 2016).

4.2 Gel-Free Proteomics and Phosphoproteomics Analysis in Plant Abiotic Stress

In iTRAQ (Isobaric Tags for Relative and Absolute Quantitation), samples are labelled at peptide level and it is an LC-based gel-free method. All the proteins present in requisite amounts will be systematically quantified and identified in iTRAQ

method, and at the end it provides a more comprehensive map of the protein content of a sample (Alvarez et al. 2009). iTRAQ labelling overcomes some of the limitations of 2-D gel-based techniques and also improves the throughput of proteomic studies. This technique has a high degree of sensitivity, and the amine specific isobaric reagents of iTRAQ allow the identification and quantitation of up to eight different samples simultaneously (Ross et al. 2004; Aggarwal et al. 2006; Zieske 2006). iTRAQ can identify proteins outside the pH range of commonly used gels and distinguish between proteins that would co-migrate on a gel, whereas DIGE resolves only soluble proteins included in a pH range of 3–11 (Alvarez et al. 2009). iTRAQ-based proteomics and phosphoproteomic analysis have conducted in plants in response to different abiotic stresses, that is, drought (Hu et al. 2015; Ren et al. 2017; Sun et al. 2017), salt (Zhang et al. 2017, 2018; Pi et al. 2018), heavy metal (Lan et al. 2012; Zhong et al. 2017) and high temperature (Guo et al. 2017; Wang et al. 2018). Recent advancement in LC-MS-based quantitative techniques such as isotope-coded affinity tags (ICAT) (Gygi et al. 1999), stable isotope labelling by amino acids in cell cultures (SILAC) (Schutz et al. 2011), and isobaric tags for relative and absolute quantification (iTRAQ) (Alvarez et al. 2009) showed advantages for relative quantification of proteins or peptides on a large scale. Advances in these techniques and in the MS field can allow the analysis of complex proteomes at organ/tissue and whole plant levels in different crops. This technological advancement in gel-free proteomics could further expand our scope of understanding of abiotic stress sensing mechanisms in plants.

Immobilized metal ion affinity chromatography (IMAC) is a common separation platform used prior to MS analysis for large-scale identification of protein phosphorylation sites from complex samples (Nühse et al. 2003). Typically, phosphopeptides are bound by immobilized metal ions through metal-phosphate affinity interactions, and non-phosphorylated peptides are removed by washing. The phosphopeptides can be released from the solid support by phosphate or alkaline elution. Several metal ions were employed for IMAC, and each metal ion has distinct strengths and weaknesses (Zhou et al. 2008). Among these metal ions, Fe^{3+} is the most common metal ion used in the IMAC approach; however, its specificity is insufficient for comprehensive phosphoproteome analysis (Kinoshita et al. 2004). IMAC-based phosphoproteomics analysis has been conducted in *Arabidopsis thaliana,* banana, rice and chickpea in response to salinity (Hsu et al. 2009), low-temperature (Gao et al. 2017) and heavy metal stress (Lan et al. 2012; Chen et al. 2015). Metal affinity chromatography (TiO_2)-based phosphoproteomic studies were conducted on banana and *Ammopiptanthus mongolicus* under low-temperature (Gao et al. 2017) and drought (Sun et al. 2017). IMAC and LC-MS/MS-based phosphoproteomics analysis on *Arabidopsis thaliana* during salt stress has revealed that level of phosphopeptides on five membrane proteins such asAHA1, STP1, Patellin-2, probable inactive receptor kinase (At3g02880) and probable purine permease-18 showed at least twofold increase in comparison to control in response to 200 mM salt-stress (Hsu et al. 2009).

5 Conclusion

Investigating the molecular events occurring in stress responses using gel-based and gel-free phosphoproteomic studies will enhance our understanding of the biological processes in crop plants. Recent advancement in proteomic methodologies, such as multi-dimensional protein fractionation (MudPit), SILAC, ICAT, iTRAQ, IMAC, DIGE and high-resolution tandem mass spectrometry, has facilitated a more accurate comparison of crop stress responses and can detect more deferentially abundant proteins than prior analysis. Sensitive proteomic approaches are capable of identifying low-abundance proteins (especially transcription factors and regulatory proteins) involved in the initial stress response in crops. Currently, majority of the crop proteomic changes often analysed after several hours, even days after a stress onset. A focus on early responsive proteins is required in order to identify regulatory and signalling proteins. Combined proteome and phosphoproteome analysis of the response of plants to stress at the protein and phosphoprotein level, together with physiological measurements, will assist in identifying the novel proteins and pathways that are crucial for stress tolerance. Further, proteomics has identified a vast number of proteins that participate in the growth of plants or their adaptation to environmental stresses. Functional analysis of those proteins will contribute to the development of high-yielding crops through artificial manipulation of the basic life phenomena of plants or through the assessment of their stress tolerance. In addition, integration of proteomics result with findings from other large-scale 'omics' and bioinformatics applications will surely facilitate the establishment of molecular networks underlying abiotic stress response and tolerance in crop plants.

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Unravelling the Complex Networks Involved in Plant Stress Tolerance Through Metabolomics

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1 Introduction

In response to the external stimuli, plants undergo a variety of molecular cascades that affect the metabolome as a whole resulting in activation of some specialized compounds called metabolites which help them to acclimatize to the changing environment. These compounds leading to the acclimatization are basically antioxidants that act as osmoprotectants during cell damage because of stress. Apart from these, several by-products are also formed along with signal transduction molecules. When plant sensed a change in environmental condition, the signal transduction pathways initiate and activate different protein compounds that reinstate homeostasis (Mittler et al. 2004). To gain comprehensive knowledge of plant response to abiotic stress, researchers are embracing ionomic profiling, transcriptomic, proteomic and metabolomic analyses. Recently, metabolomics has been proposed as a complementary approach to the genomics-assisted selection for crop improvement (Fernie and Schauer 2009; Kliebenstein 2009). A few methylation quantitative trait loci (mQTLs) have already been identified in *Arabidopsis*, tomato and *Populus* and have been shown to have intermediate hereditability (Schauer et al. 2008; Ruan and Teixeira da Silva 2011). The integration of QTL mapping with gene expression and metabolite profiling showed a complex relation among them (Wentzell et al. 2007). A deep dissection of the biochemical pathways in plants facing abiotic stressing

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conditions requires integrative and comprehensive analyses in order to identify all the simultaneous metabolic responses and, more importantly, to be able to link these responses to specific abiotic stress.

2 What Are Plant Metabolites?

A substance essential to the metabolism of a particular organism or to a particular metabolic process is called metabolite (Irchhaiya et al. 2014). Metabolites are the intermediates and products of metabolism, and the term metabolite is usually restricted to small low molecular weight organic compounds with typically involved in a biological process as substrate or product or synthesized by organisms using enzyme-mediated chemical reactions/metabolic pathways. Metabolites have various functions including fuel, structure, signalling, stimulatory and inhibitory effects on enzymes, the catalytic activity of their own (usually as a cofactor to an enzyme), defence and interactions with other organisms (Tiwari and Rana 2015). Plants are particularly biochemically rich as compared to many other species and there are around 200,000 metabolites across the plant kingdom and somewhere between 7000–15,000 within an individual plant species (D'Auria and Gershenzon 2005), but these are likely to be underestimated because it is difficult to detect low abundance molecules due to the complexity of the biological system. Therefore, the regulation of gene expression, the action of gene products and the metabolic networks resulting from catalytic proteins must make fundamental contributions to the remarkable diversity inherent in living systems (Dixon et al. 2006).

Metabolites perform both essential functions like growth, development and maintenance of cellular functions (primary metabolites) and specific functions (secondary metabolites).

2.1 Primary Metabolites

Primary metabolites comprise many different types of organic compounds including, but not limited to, carbohydrates, lipids, proteins and nucleic acids. Primary metabolites are essential to growth and development and found universally in the plant kingdom as they have functions in fundamental metabolic pathways (glycolysis, the Krebs cycle and the Calvin cycle) enabling a plant to synthesize, assimilate and degrade organic compounds. Primary metabolites include energy-rich fuel molecules (sucrose and starch), structural components (cellulose), informational molecules (DNA, RNA and chlorophyll pigment) besides their role as precursors for the synthesis of secondary metabolites. Although primary metabolites involved in central metabolism can be used to determine nutritional and growth status of an organism.

2.2 Secondary Metabolites

Secondary metabolites are considered as end products of plant metabolism which are accumulated by plants in specialized cells at particular development stage, generally in smaller quantities compared to primary metabolites. Secondary metabolites are variously distributed in the plant kingdom and their functions are specific to the plants in which they are found. These are often coloured, fragrant or flavourful compounds and they typically mediate the interaction of plants with other organisms such as plant-pollinator, plant-pathogen and plant-herbivore. Secondary metabolites largely fall into three classes of compounds alkaloids, terpenoids and phenolics However, these classes also include primary metabolites, so whether a compound is a primary or secondary metabolite is a distinction based not only on its chemical structure but also on its function and distribution within the plant kingdom.

Secondary metabolite profiles may better reflect the differentiation of species and their complex response to environmental factors and other organisms and also extremely important for most organisms to defend themselves from stressful environments or predators (Roessner and Bowne 2009).

3 Metabolomics

Metabolomics is the comprehensive, qualitative and quantitative profiling of all the small molecules in cells, tissues or whole organisms at a specific point in time (Oliver et al. 1998; Clish Clary 2015; Newgard Christopher 2017). Metabolomics is essentially comprehensive non-biased, high-throughput analyses of complex metabolite mixtures typical of plant extracts (Hong et al. 2016) and can be seen as another level of information encoded by the organism but more subject to manipulation by its environment (Seger and Sturm 2006). Metabolomics is a relatively new approach aimed at improved understanding of these metabolic networks and the subsequent biochemical composition of plants and other biological organisms (Dixon et al. 2006). Metabolomics is bridging the gap between genotype and phenotype (Fig. 1) by providing a more comprehensive view of how cells function, as well as identifying novel or striking changes in specific metabolites (Roessner and Bowne 2009). Since metabolites are so closely linked to the phenotype of an organism, metabolomics can be used for a large range of applications, including phenotyping of genetically modified plants and substantial equivalence testing, determination of gene function and monitoring responses to biotic and abiotic stress (Roessner and Bowne 2009). Metabolomics represents the interface between genetic predisposition, environmental influence and invaluable to understand the function of genes and the complexity of biological systems (Barchet 2007). It deals with the identification and quantification of the metabolites present in biological systems with molecular weights less than 1500 Da although could be occasionally wider in the range

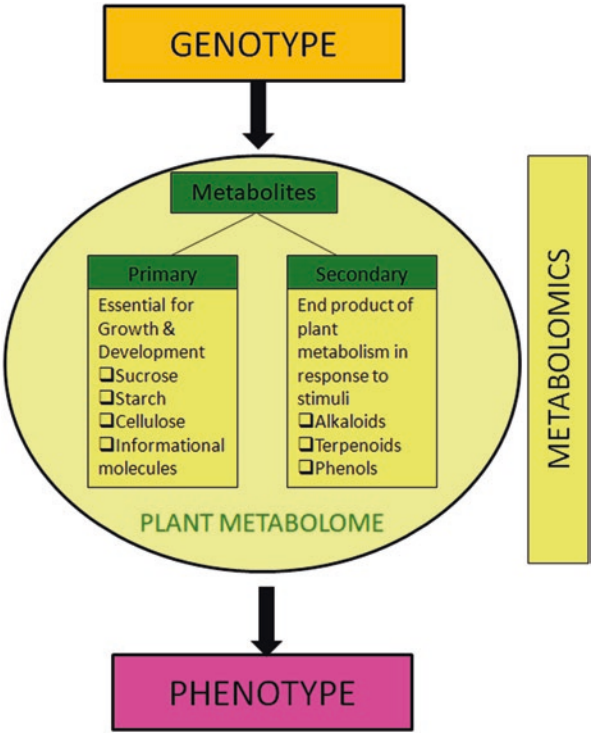

Fig. 1 Metabolomics, its components and relation with genotype and phenotype

30–3000 Da (Estrada et al. 2016). It is a powerful approach which best represents the molecular phenotype because metabolites and their concentrations directly reflect the underlying biochemical activity and state of cells or tissues. The non-invasive nature and its close link to the phenotype makes it an ideal tool for the pharmaceutical, preventive healthcare and agricultural industries. Metabolomics information have potential to assist in the deeper understanding of the complex interactive nature of plant metabolic networks and their responses to environmental and genetic change as well as provide unique insights into the fundamental nature of plant phenotypes in relation to development, physiology, tissue identity, resistance, biodiversity etc. (Hall et al. 2002). Currently, metabolomics research is being applied to myriad different uses, from plant science (in studies relating to biomass accumulation, environmental stress resistance and secondary metabolite production) to medicine (Hall 2006; Meyer et al. 2007; Barchet 2007). Metabolomics has contributed significantly not only to the understanding of plant physiology and biology from the view of small chemical molecules that reflect the end point of biological activities but also to the attempts to improve plant behaviour under both normal and stressed conditions during past decades (Hong et al. 2016). Its results could be the basis of genetic improvement based on the participation of some chemical compounds involved in the resistance in some plant species (Estrada et al. 2016).

4 Metabolites Mediated Plant Stress Tolerance

A vast metabolic diversity exists in plants and the biosynthesis, concentration, transport and storage of primary and secondary metabolites are greatly affected by stresses (Velázquez and Hernández 2013). In higher plants, a wide variety of secondary metabolites are synthesized from primary metabolites that have application in plant stress physiology for adaptation against various stresses (Ramakrishna and Ravishankar 2013), and proper activation of early metabolic responses helps cells to restore chemical and energetic imbalances crucial to acclimation and survival (Velázquez and Hernández 2013) (Table 1). To combat environmental stress, plants execute various mechanisms like scavenging of reactive oxygen species (ROS), production of antioxidants, maintenance of membrane stability and accumulation or adjustment of compatible solutes and various plant metabolites (polyamines, flavonoids, jasmonic acid (JA), methyl-jasmonate, glycine betaine etc.) (Ramakrishna and Ravishankar 2013). However, the production of these compounds is often low (<1% dry weight) and depends greatly on the physiological and developmental stage of the plant (Ramakrishna and Ravishankar 2013). Stresses such as pathogen attack, UV-irradiation, high irradiation, wounding, nutrient deficiencies, temperature and herbicide treatment often increase the accumulation of phenylpropanoids (Dixon and Paiva 1995).

Table 1 Effect of abiotic stress on the expression of different metabolites in various crops

Crop	Effect of stress on metabolites	References
Wheat	Increased levels of proline, tryptophan, leucine, isoleucine and valine	Bowne et al. (2012)
Barley	Increased levels of flavonoids and phenols	Ahmed et al. (2015)
Maize	Enhanced levels of proline, malate, p-coumarate and caffeate; reduced levels of ferulate	Alvarez et al. (2008)
	Reduction in quinic acid and pyruvic acid; increased levels of histidine, putrescine, proline and phenylalanine	Witt et al. (2012)
	Accumulation of soluble carbohydrates, proline, shikimate, serine, glycine and aconitase; decrease in leaf starch, malate, fumarate and 2-oxoglutarate	Sicher and Barnaby (2012)
	Change in levels of citrate, fumarate, phenylalanine, valine, leucine and isoleucine	Sun et al. (2015)
	Increased levels of Methyl Jasmonate (MeJA); decreased levels of cinnamic acid (CA) and Jasmonic Acid (JA)	Benevenuto et al. (2017)
Rice	Lower levels of sucrose and myo-inositol in anthers; higher levels of galactinol and raffinose	Li et al. (2015)
	Upregulated expression of intercellular sugar transport regulation gene (CSA)	Zhang et al. (2010)
	Enhanced expression of MST8 and INV4	Li et al. (2015), Zhang et al. (2010)
	Enhanced sugar content	Fumagalli et al. (2009)

(continued)

Table 1 (continued)

Crop	Effect of stress on metabolites	References
Soybean	Differential expression of aspartate, 2-oxoglutaric acid, myo-inositol, pinitol, sucrose and allantoin	Silvente et al. (2012a, b)
	Altered expression of daidzin, daidzein, glycitin, syringic acid, formononetin, genistein and genistin	Das et al. (2017a, b)
	Enhanced expression of tocopherols, phenyl propanoids, flavonoids and ascorbate precursors	Chebrolu et al. (2016a, b)
	Increased levels of amino acids	Komatsu et al. (2011)
	Synthesis of compatible solutes, ROS scavengers, induction of plant hormones	Lu et al. (2013)
Groundnut	Increased expression of β-*d*-galactofuranoside, *d*-glucopyranose, stearic acid, 4-ketoglucose, 1-threonine, hexopyranose, gulose, 2-*o*-glycerol-α.*d*-galactopyranoside and serine	Raval et al. (2018)
Chickpea	Altered levels of sugars, sugar acids, sugar phosphates and organic acids	Dias et al. (2015)
Alfalfa	Accumulation of antioxidants, osmolytes and organic acid	Song et al. (2017)
Lentil	Reduction in levels of threonic acid, ornithine, asparagines, alanine and homoserine	Muscolo et al. (2015)
Pea	Increased levels of proline, valine, threonine, homoserine, myo-inositol, γ-aminobutyrate (GABA) and trigonelline	Charlton et al. (2008)
Lotus japonicus	Increase in the levels of amino acids, sugars and polyols; decrease in organic acids	Sanchez et al. (2012)

Abiotic environmental stresses such as drought, salinity and low temperature are major limitations for plant growth and crop productivity. In plants, tolerance to various stresses is achieved through osmoprotectants, (sugars, amino acids and ammonium compounds), scavenging of reactive oxygen species (ROS) generated in response to abiotic stresses and hormone metabolism (auxin, cytokinin, ethylene and abscisic acid) involved in the regulation and production of secondary metabolites and osmoprotectants (Jain 2013). Accumulation of certain organic solutes known as osmoprotectants is a common metabolic adaptation which protects proteins and membranes against damage by high concentrations of inorganic ions as well as protects the metabolic machinery against oxidative damage (Rathinasabapathi 2000). Correlation between amino acid accumulation (mainly proline) and stress tolerance is well proven, and in response to various stresses like salinity, freezing, heavy metal toxicity and drought proline serves as an osmoprotectant, a cryoprotectant, a signalling molecule, a protein structure stabilizer and an ROS scavenger (Verslues et al. 2006; Verbruggen and Hermans 2008). Water deficiency stress induces abscisic acid accumulation in plant tissues and promotes transpiration reduction via stomatal closure, thus minimize water losses and diminish stress injury. Besides, abscisic acid regulates expression of many stress-responsive genes including the late embryogenesis abundant (LEA) proteins, leading to a reinforcement of drought stress tolerance in plants (Velázquez and Hernández 2013). Carbohydrate metabolism plays an important role in the stress tolerance conditions

as it is directly linked to photosynthetic performance, and during the stress period plants use starch and fructans as a source of energy instead of glucose to maintain cell turgor, stabilizing cell membranes and preventing protein degradation; high amounts of non-reducing disaccharides (trehalose), oligosaccharides (raffinose and stachyose) also accumulate in different plant species in response to a broad range of abiotic stresses like drought, salinity or extreme temperatures and cause reduction in oxidative membrane damage and ROS scavenging, whereas accumulation of sugar alcohols like mannitol or sorbitol has been linked to stress tolerance (Arbona et al. 2013). Phenolics, including flavonoids, anthocyanins, lignins etc., are the most important class of secondary metabolites in plants and play a variety of roles including tolerance to abiotic stresses. High-temperature stress induces production of phenolic compounds (flavonoids and phenylpropanoids); anthocyanins, a subclass of flavonoid compounds, are greatly modulated in plant tissues by prevailing temperature (low-temperature increases and elevated temperature decreases their concentration in buds and fruits); carotenoids protect cellular structures in various plant species irrespective of the stress type; plants capable of emitting greater amounts of isoprene generally display better photosynthesis under heat stress, thus there is a relationship between isoprene emission and heat-stress tolerance (Wahid et al. 2007). Plants have evolved complex mechanisms for adaptation to osmotic and ionic stresses caused by high salt by accumulation of compatible solutes such as proline, glycine, betaine, polyols, sugar alcohols and soluble sugars, and lowering the toxic concentration of ions in the cytoplasm by restriction of Na^+influx or its sequestration into the vacuole and/or its extrusion (Haghighi et al. 2012). Nutrient stress also has a marked effect on phenolic levels in plant tissues (Chalker-Scott and Fnchigami 1989).

Plants are continuously exposed to the attack of invasive microorganisms such as fungi or bacteria and also viruses, and to combat these stresses plants develop different metabolic and genetic responses, whose final outcome is the production of either toxic compounds that kill the pathogen or deter its growth, and/or semiotic molecules that alert other individuals from the same plant species (Arbona and Gómez-Cadenas 2016). Plants produce many antimicrobial secondary metabolites. Two major classes with a demonstrated or proposed role in resistance to plant pathogens are phytoanticipins, or preformed inhibitors, which are present constitutively in plants and phytoalexins, which are synthesized only in response to pathogen attack. Available evidence is consistent with both phytoanticipins and phytoalexins being important in defence in some disease interactions (Walton 2001). Plants produce terpenes, phenolics, nitrogen and sulphur containing compounds with a prominent function in the protection against predators and microbial pathogens due to their toxic nature and repellence to herbivores and microbes (Mazid et al. 2011). Plants contain preformed peptides, proteins and secondary metabolites (phenolics, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates), and the multicomponent defence response induced after the pathogen attack requires a substantial commitment of cellular resources, including extensive genetic reprogramming, because the induced expression of a large number of defence related genes is essential for plants to counter pathogen attack (Lattanzio et al. 2006). Plant produce

preformed metabolites or phytoanticipins that are converted into toxic molecules upon pathogen perception and toxic metabolites or phytoalexins that are produced only upon pathogen attack (Arbona and Gómez-Cadenas 2016). Phytoalexins are low-molecular-weight antimicrobial compounds that accumulate in plants as a result of infection or stress and the rapidity of phytoalexin accumulation is associated with resistance in plants to diseases caused by fungi and bacteria, although the genetic information for phytoalexin synthesis is found in susceptible and resistant plants (Kuc 1995).

4.1 Metabolites and Stress Tolerance in Cereals and Millets

Plant responses to abiotic stresses have been studied extensively, especially in model plants, which are now shifting towards cereal plants like rice, maize, wheat, barley and others. Studies made in the recent past have shown some light on explaining functions of many key genes, proteins, metabolites and molecular networks involved in plant responses to salinity, drought, heat, cold, heavy metals and other abiotic stresses. Using a targeted Gas Chromatography-Mass Spectrometry (GC-MS) approach, analysis of 103 metabolites in wheat was done under drought stress, and increased levels of amino acids, including proline, tryptophan, leucine, isoleucine and valine, were reported (Bowne et al. 2012). Metabolic adjustments in response to adverse conditions are transient and depend on the severity of the stress. In addition, a recent study revealed that secondary metabolism is also involved in the plant's tolerance to the combinatorial drought and salinity stresses, in which the tolerant Tibetan wild barley (XZ25 and XZ16) displays transcriptomic alterations in the levels of secondary metabolism pathway genes and lower DNA damage, as compared with control barley cv CM72, together with an increase of flavonoids and phenols (Ahmed et al. 2015).

Metabolite based profiling study in maize showed that primary damages of salt stress are associated with the osmotic component. Alvarez et al. (2008) noted enhanced ranks of proline, malate, p-coumarate and caffeate by means of an Liquid Chromatography-Mass Spectrometry (LC-MS) method for maize xylem sap; though, ferulate reduced under adverse environmental conditions. Witt et al. (2012) found a reduction in quinic acid and pyruvic acid and increased level of histidine, putrescine, proline and phenylalanine in the leaves of maize crop in response to drought treatment. Pelleschi et al. (2006) reported that the QTL designed for invertase activity shows mapping near Ivr2, an invertase-encoding gene. Additionally, co-location between the sucrose-P synthase and ADP-glucose pyrophosphorylase has been reported in young maize plants exposed to drought. Furthermore, these studies provide new basis for the discovery of unique methods that would help in drought tolerance of plants (Oksman-Caldentey and Saito 2005). In a 17-day time course experiment in maize subjected to drought stress, GC-MS metabolic analysis revealed changes in concentrations of 28 metabolites. Accumulation of soluble carbohydrates, proline and eight other amino acids, shikimate, serine, glycine and

aconitase, was accompanied by the decrement of leaf starch, malate, fumarate, 2-oxoglutarate and seven amino acids during the drought treatment course. However, as the water potential became more negative, between the 8th and 10th days, the changes in some metabolites were more dramatic, demonstrating their dependence on stress severity (Sicher and Barnaby 2012). A significant change in the levels of six metabolites (citrate, fumarate, phenylalanine, valine, leucine, isoleucine) has also been reported when maize plants were subjected to water and salinity stress either separately or concurrently (Sun et al. 2015). Proteomic profile and key metabolic compounds were analysed in comparison to the non-GM near-isogenic variety under the same experimental conditions aiming at exploring molecular responses of GM maize variety to drought and herbicide stress conditions (Benevenuto et al. 2017). MeJA (Methyl Jasmonate), CA (Cinnamic acid) and JA (Jasmonic acid) showed significantly different levels between the GM and its non-GM near-isogenic variety under both control and stress conditions. MeJA levels increased in the GM and GM D samples, while significantly lower levels were observed for CA and JA. Opposite regulation of JA and MeJA was an unexpected result since they are synthesized in the same main octadecanoid pathway (Benevenuto et al. 2017). Moreover, these compounds play important roles together to activate plant defence mechanisms in response to biotic and abiotic stresses, such as drought, low temperature and salinity (Cheong and Choi 2003).

As global warming is approaching, heat and drought stresses become big challenges to sustain grain yields. A recent work on rice floral organ development provided mechanistic understandings of the responses of rice floral organs to combined stresses, in which integrative analyses on metabolomics and transcriptomic features of floral organs revealed that sugar starvation is the determinant of the failure of reproductive success under heat and drought stress in rice (Li et al. 2015). Heat-sensitive (Moroberekan) anther has lower levels of sucrose and myo-inositol but higher level of galactinol and raffinose, while heat-tolerant (N22) anther has lower abundances of glucose-6-P and fructose-6-P (Li et al. 2015). Consistent with metabolomic changes in anther, Moroberekan rice has significantly up-regulated expression of the intercellular sugar transport regulation gene Carbon Starved Anthers (CSA) (Zhang et al. 2010), while N22 rice shows the enhanced expression of MST8, a sugar transporter gene, and INV4, a cell wall invertase gene (Li et al. 2015; Zhang et al. 2010) investigations. Fumagalli et al. (2009) studied the metabolite profile of two different cultivars of rice under salinity (150 mM) and showed enhanced sugar contents during salinity stress in both of the cultivars. Their results also exhibited that salt stress reformed the accumulation of various metabolites in rice, which have a vital role in salt tolerance. Chen et al. (2014) sequenced 529 rice accessions and generated 6.4 million Single Nucleotide Polymorphism (SNPs), responsible for 840 metabolites, which finally led to the identification of 36 candidate metabolite modulating genes with potential physiological and nutritional importance.

Millets are an important staple crop of the developing world especially semi-arid tropical regions of Africa and Asia. Millets are generally resilient to extreme climatic changes and can act as model cereal to study the metabolic profile during extreme conditions to reveal the mechanism involved in stress tolerance. While foxtail

millet has been suggested to be a model crop for studying switch grass genome (Doust et al. 2009), not much work has been done in studying metabolic responses to abiotic stress in millets. There has been a single report of plant metabolite study by Kim et al. (2013), where they have used gas chromatography coupled with time-of-flight mass spectrometry (GC-TOFMS) to determine the diversity among *Panicum miliaceum* genotypes in terms of primary metabolite activity and phenolic acid content.

4.2 Metabolites and Stress Tolerance in Legumes

The legumes such as chickpea, common bean, cowpea, groundnut, pigeon pea, soybean, lentils and other food legumes are important sources of protein for human and animal nutrition. However, the production of legumes is constrained due to several abiotic stresses including drought and salinity. Metabolome analysis has been extensively applied in the model legume species, as well as in other crop legumes to examine stress signalling pathways, cellular and developmental processes and nodule symbiosis. The *Leguminosae* is a class of plants known to contain several characteristic secondary compounds such as isoflavonoids, and the dynamics of primary compounds in *Leguminosae* have unique patterns compared to other plant species.

Soybean is an important source of high-quality protein and oil. The soybean crop requires adequate water all through its growth period to attain its yield potential, and the lack of soil moisture at critical stages of growth severely impacts the productivity. Silvente et al. (2012a, b) conducted 1H Nuclear Magnetic Resonance (NMR)-based metabolic profiling analysis to assess the effects of water stress in drought tolerant and sensitive genotypes of soybean. The results demonstrated critical differences in physiological responses and in the metabolic pathways that were affected by water stress in soybean plants. Six metabolites in leaves (aspartate, 2-oxoglutaric acid, myo-inositol, pinitol, sucrose, allantoin) and two in nodule (2-oxoglutaric acid and pinitol) were affected differentially in the genotypes when drought was imposed at the vegetative stage in the nodulated soybean plants. Recently, Das et al. (2017a, b) conducted a study with a goal to identify leaf metabolites under heat stress condition. This study showed that drought, as well as heat stress, affects metabolites associated with various cellular processes, such as starch biosynthesis, glycolysis, the pentose phosphate pathway, the tricarboxylic acid (TCA) cycle, and that regulate carbohydrate metabolism, peptide metabolism, amino acid metabolism, and purine and pyrimidine biosynthesis. Computational analysis predicted additional compounds which indicated the possibility of other metabolites that could also be important under drought and heat stress conditions. Metabolomic profiling demonstrates that keeping up with sugar and nitrogen metabolism is of prime significance, along with phytochemical metabolism under drought and heat stress conditions. Heat stress affects the synthesis of phytochemicals, such as daidzein, daidzein, glycitein, syringic acid, formononetin, genistein and genistin.

Chebrolu et al. (2016a, b) studied the impact of heat stress during seed development on soybean seed metabolome. Seed development in soybean is a temperature-sensitive process that is much more vulnerable than vegetative tissues to abiotic stresses. High temperatures during soybean seed development frequently result in seed with poor germination and decreased economic value. Global metabolite profiles were compared between seed from heat-tolerant and heat-susceptible genotypes. A total of 275 seed metabolites were analysed and genotype-specific differences and temperature specific differences were identified. A diverse sets of antioxidant metabolites, including tocopherols, phenylpropanoids, flavonoids and ascorbate precursors, were found to be enriched in the seed of the heat-tolerant genotype. The abundance of these metabolites indicated that these compounds are more likely responsible for the tolerance to high temperatures during seed development. In soybean, excess water due to flooding can also affect the plant growth and productivity. Komatsu et al. (2011) used Capillary Electrophoresis-Mass Spectrometry (CE-MS) and identified 81 metabolites related to the mitochondria under flooding stress in roots and hypocotyls of soybean which showed that the glycolysis-related metabolites, TCA-cycle-related metabolites and amino acids increased, while Adenosine Triphosphate (ATP) decreased.

Salinity is the second most important abiotic stress that affects germination, crop growth and productivity. Salinity stress directly affects the normal growth, development and reproduction of a plant, and therefore the primary metabolites involved in these processes. Lu et al. (2013) studied the mechanism of salt tolerance in soybean based on metabolomics approach. The study indicated that the salt tolerance of soybean is mainly based on the synthesis of compatible solutes, the induction of reactive oxygen species (ROS) scavengers, the modification of cell membranes and the induction of plant hormones. Recently Li et al. (2017) conducted metabolomics analysis of semi-wild, wild and cultivated soybeans seedling roots under salt stress condition using gas chromatography-mass spectrometry analysis. The salt tolerance of wild soybean was highest, while that of cultivated soybean was the lowest. Wild soybean's salt tolerance ability was mainly due to increases in nitrogen metabolism and antioxidant metabolism of secondary metabolites. In addition, carbon metabolism is also important in resisting neutral salt, and the resistance to alkaline salt stress is also dependent on the enhancement of the TCA cycle.

To gain comprehensive knowledge of plant response to abiotic stress, researchers are embracing proteomic, transcriptomic, ionomic profiling and metabolomic analyses from various crop and model legumes. In recent years, several web-based tools which support metabolomics applications and mass-spectrometry-based metabolite profiling have appeared. Joshi et al. (2012) developed Soybean Knowledge Base (SoyKB) which is a comprehensive web resource for soybean translational genomics. It is useful for integrating, mining and visualizing soybean metabolomic data, including the identification and expression of various metabolites across different experiments. It incorporates GC-MS and LC-MS-based metabolite-profiling data dynamically linked to metabolite information from other public metabolomic databases.

Groundnut is another important legume and oilseed crop. Raval et al. (2018) conducted metabolomic analysis of groundnut genotypes under varying temperature conditions. Results indicate that at low-temperature most of the metabolites were synthesized at higher concentrations at the pegging stage, while at the higher-temperature conditions metabolites were found to be accumulated at the pod filling stage. This study shows that Beta-*d*-galactofuranoside, *d*-glucopyranose, stearic acid, 4-ketoglucose, 1-threonine, hexopyranose, gulose, 2-*o*-glycerol-alpha.*d*-galactopyranoside and serine can be used as biomarkers for high-temperature stress tolerance. Higher accumulation of phenolics during low temperature suggested a crucial role of phenolics in low-temperature stress tolerance in groundnut. A higher accumulation of caffeic acid, salicylic acid, cinnamic acid and vanillic acid during high-temperature stress at the pegging stage implied their critical role in heat-stress tolerance in groundnut.

In chickpea, metabolites were examined to study the adverse impact of salinity. Dias et al. (2015) developed and validated a GC-QqQ-MS method to quantify 49 primary metabolites from four major classes (sugars, sugar acids, sugar phosphates and organic acids) and applied it to the tissues of salt tolerant and sensitive chickpea cultivars. Differences between two chickpea cultivars following salt stress involve metabolites associated in carbon metabolism and in the TCA cycle, as well as amino acid metabolism. Larger differences in sugar levels were noted in tissues of the sensitive cultivar. During the salt stress conditions, amino acid levels were depleted in flowers of the tolerant cultivar, while amino acid levels increased in flowers of the sensitive cultivar. Song et al. (2017) conducted metabolomics profiling to study the alkaline salt stress in alfalfa. Alkaline salts stress causes more severe morphological and physiological damage to plants than neutral salts due to differences in pH. In this study, alfalfa roots were treated with alkali and samples were analysed for various metabolites. Metabolic profiling revealed that Rhizobium-nodulized plants accumulated more antioxidants, osmolytes, organic acids and metabolites that are involved in nitrogen fixation. Analysis revealed that Rhizobium-nodulized alfalfa plants exhibited a distinct metabolic profile associated with alkali putative tolerance relative to non-nodulized alfalfa plants. Muscolo et al. (2015) studied the phenotypic and metabolic responses to drought and salinity stress in contrasting lentil accessions. Metabolic differences in the stress tolerance of the different genotypes were related to a reduction in the levels of tricarboxylic acid (TCA) cycle intermediates. The relevant differences between the salinity tolerant and sensitive genotypes were related to the decrease in the threonic acid level. In this study, ornithine and asparagine were identified as markers of drought stress and alanine and homoserine as markers of salinity stress. Charlton et al. (2008) studied the responses of the pea leaf metabolome to drought stress using nuclear magnetic resonance spectroscopy. The metabolites proline, valine, threonine, homoserine, myoinositol, γ-aminobutyrate (GABA) and trigonelline (nicotinic acid betaine) were present at significantly higher concentrations in drought-stressed plants under all growth conditions.

Lotus japonicus is a model legume and has been analysed using various metabolomic approaches. Sanchez et al. (2012) used a non-targeted metabolomic approach

to explore plastic systems responses to drought stress in model and forage legume species of the Lotus genus. Using GC coupled with electron impact ionization (EI)-TOF-MS (GCEI-TOF-MS), this study reported a gradual increase in most of the soluble molecules profiled, reflecting a global and progressive reprogramming of metabolic pathways. The comparative analysis between Lotus species revealed conserved and unique metabolic responses to drought stress. Only a few drought-responsive metabolites were conserved among all species. In *Lotus japonicus*, a general increase in the levels of many amino acids, sugars and polyols, while a decrease in most organic acids was observed. *Medicago truncatula* is another model legume forage crop and is an ideal candidate to study the molecular mechanisms conferring drought resistance in plants. Zhang et al. (2014) conducted metabolite profiling of drought-stressed plants which revealed the presence of 135 polar and 165 non-polar compounds in roots and shoots. The study shows that myo-inositol and proline have striking regulatory profiles indicating involvement in Medicago drought tolerance. A deep dissection of the biochemical pathways in legumes facing abiotic stressing conditions is required to identify all the simultaneous metabolic responses and to link these responses to specific abiotic stress. To integrate the information from genome to phenome, metabolomic approaches have an important intermediary bridge-building role.

5 Conclusion

Plant metabolites can be a key factor to study and analyse plant response to various stresses as they are more adaptable to changes as compared to transcriptome and proteome, thus the effect of even a minute change in the external stimuli can be recognized while studying a plant metabolome. Though it is an expensive venture, it can lead to the identification of a subclass of metabolites for a particular growth stage and stress a plant is exposed to at a given time.

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Omics Approaches for Cold Stress Tolerance in Plants

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Abbreviations

2D-DIGE	2D differential gel electrophoresis
ADH	Alcohol dehydrogenase
APETALA2	Ethylene responsive factor
AtCBF3	Arabidopsis thaliana C-repeat binding factors
CA	Cold acclimation
Cbf	C-repeat binding factors
CE-MS	Narrow electrophoresis coupled to mass spectrometry
Coda	Choline oxidase A
Cor	Cold-regulated genes
CT	Cold tolerant
DA	Deacclimation
DaCBF4	Deschampsia antarctica C-repeat binding factors
DHNs	Dehydrin proteins
DREB	Dehydration responsive element binding protein
ERD10	Early responsive to dehydration proteins

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FLAs	Fasciclin-like arabinogalactan protein
FT	Freezing tolerance
FT-ICR/MS	Fourier change particle cyclotron reverberation mass spectrometry
GABA	Gamma-aminobutyric acid (GABA)
GB	Glycine betaine
GB	Glycine betaine (GB)
GC-MS	Gas chromatography coupled to mass spectrometry
HSPs	Heat shock proteins
ICAT	Isotope-coded affinity tags
ILs	Introgression lines
iTRAQ	Isobaric tags for relative and absolute quantitation
LC-MS	Liquid chromatography coupled to mass spectrometry
LC-PDA/MS	Liquid chromatography- photodiode analysis coupled to mass spectrometry
LeCBF1	Lycopersicon esculentum C-repeat binding factors
LRR	Leucine-rich repeat protein kinase
MAS	Marker assisted selection
MDR1	Multidrug resistance 1
MS	Mass spectrometry
MS	Mass spectrometry
NMR	Nuclear magnetic resonance
OsiSAP1	Zinc finger protein of rice cultivars
PCR	Polymerase chain reaction
PDR8	Pleiotropic drug resistance 8
PEG	Polyethylene glycol
PLDδ	Phospholipase Dδ
PM	Plasma membrane
PSI, PSII	Photosystems I, II
PTMs	Post-translational modifications
QTL	Quantitative trait loci
RAP	Ethylene responsive factor
RG	Rootstock grafted
ROS	Reactive oxygen species
RuBisCO	Ribulose bisphosphate carboxylase/oxygenase
SILAC	Stable isotope labeling by amino acids in cell culture
SSR	Simple sequence repeats
STN7	Serine/threonine protein kinase
SYT1	Synaptotagmin
WCS120	Wheat cold stress responsive gene
WT	Wild type

1 Introduction

As plants are sessile, they are always exposed to various stresses, both biotic and abiotic, during their lifetime. Abiotic stresses, which mainly include drought, salt, temperature (low/high), flooding and nutritional deficiency/excess, are attributed to 50% crop losses (Ahmad et al. 2016). These abiotic stresses hamper crop growth and yield to a great extent (Bita and Gerats 2013). The ability to change is the key to adaptation of plants, which have developed highly sophisticated and effective mechanisms to counteract environmental cues and such adaptations also include changes in several proteins at pre- and post-transcriptional and translational levels (Lin et al. 2016). Cold temperature acts as a major constraint that affects growth, productivity and distribution of plants (Zaynab et al. 2017). Crops in temperate zones experience temperatures that range from 0 to 15 °C during their growing seasons (Megha et al. 2014). It is estimated that 42% of the total land area on the earth experiences periodic low temperatures (Gharechahi et al. 2016). Cold temperatures are very harmful to plants. They induce changes in proteins involved in carbohydrate metabolism, photosynthesis, stress-related proteins among other processes, protein folding and degradation, as well as reactive oxygen species (ROS) scavenging and biosynthesis of compatible solutes (Shi et al. 2014). Plants may experience chilling stress when they are exposed to temperatures from 0 to 15 °C (non-freezing temperatures) or freezing stress when exposed to temperatures below 0 °C (Miki et al. 2019). Chilling stress may hamper plant growth and development by reducing water absorption resulting in cellular desiccation, inducing alteration in metabolites leading to an oxidative stress, inhibiting cellular metabolism, perturbing gene transcription and finally causing cell death. Freezing temperatures, on the other hand, leads to cellular dehydration and extracellular ice formation and imbalances in plasma membranes, leading to the formation of inverted hexagonal-phase membrane structure (Shi et al. 2018). The changes in metabolic profile of crops help them to adapt and resist freezing stresses when air temperature decreases and day length shortens, which is called cold acclimation (Zhao et al. 2015). Various genes/gene products play important roles in inducing cold stress tolerance in crops, some of which are shown in Table 1. Comprehensive changes in photosynthesis and carbohydrate metabolism, protein biosynthesis, folding and degradation, as well as reactive oxygen species (ROS) scavenging and biosynthesis of compatible solutes and stress-related proteins, are aimed at minimizing the harmful effects of low temperatures and gaining a sufficient level of freezing tolerance (Preston and Sandve 2013).

2 Genomics Approaches for Resistance Against Cold-Induced Stress

One of the most common environmental stresses that affects growth and development of plant and reduces its productivity is cold temperature (Shinada et al. 2014). Within the temperature range of 0–10 °C, many plants of tropical or subtropical

Table 1 Genes/gene products involved in inducing cold stress tolerance in crops

Gene(s)/gene product	Cellular role	References
cor15a Cold-regulated gene	Promotes freezing tolerance	Sowemimo et al. (2019)
cbf1 CRT/DRE-binding factor	Transcription factor	Park et al. (2018)
dreb1 and dreb2 DRE-binding protein	Transcription factor	van Buer et al. (2019)
WCS120/COR39 CCGAC sequences like CRT/DREs in its promoter	Low-temperature-regulated gene	Cheng et al. (2019)
Coda Choline oxidase A	Glycine betaine biosynthesis	Zuther et al. (2018)
Coda Choline oxidase A	Glycine betaine biosynthesis	Cai et al. (2018)
DREB1A (CBF3) DRE-binding protein	Transcription factor	Zhang et al. (2018)
CBF3 DRE-binding protein	Transcription factor	Ma et al. (2018)
CBF1/DREB1b DRE-binding protein	Transcription factor	Zhang et al. (2019)
DREB1A (rd29A) DRE-binding protein	Stress-inducible promoter	Liu et al. (2018)
OSISAP1 Zinc-finger protein	Transcription factor	Barrero-Gil and Salinas (2018)

origin suffer damage (Goodstal et al. 2005). Susceptible crop species are affected by cold and they show different symptoms of chilling injury, such as chlorosis, necrosis and growth retardation, or are ultimately killed by non-freezing low temperatures. Plants from temperate regions are acclimatized to cold due to their constant exposure to cold and are considered to be chilling tolerant. Their constant exposure to chilling, non-freezing temperatures can also increase their freezing tolerance. Cold acclimation provides selective advantage to crop plants in temperate regions and positively influences their survival and distribution. However, plants in tropics and subtropics are not acclimatized to chilling stress. Cold tolerance is a very complex trait controlled by many genes and regulated by chill in atmosphere (Sanghera et al. 2011). It is, therefore, important to develop cold-tolerant varieties. As the underlying trait is complex in nature and regulated by many genes, integrated molecular approaches can assist in the development of cold-tolerant varieties.

Tomato is sensitive to both chilling and freezing temperatures, and low temperature (10 °C or below) inhibits tomato growth. Temperature below 6 °C causes irreparable damage to tomatoes. Several quantitative trait loci (QTLs) for shoot turgor maintenance (stm) under root chilling have been identified in an interspecific backcross population derived from crossing chilling-susceptible cultivated tomato (*Lycopersicon esculentum*) and chilling-tolerant wild *L. hirsutum*. Major QTL for

enhanced chilling tolerance is located on chromosome 9 (stm9) of the *Lycopersicon hirsutum*. Marker-assisted backcross breeding was used for introgression of the *L. hirsutum* allele at the QTL on chromosome 9 in cultivated tomato (*Lycopersicon esculentum*) (Goodstal et al. 2005). In another study conducted by Shinada et al. (2014), pyramiding of quantitative trait loci (QTLs) using marker-assisted selection (MAS) was carried out to improve cold tolerance at the fertilization stage (CTF) in the case of rice. Cold stress tolerance at the reproductive stage is an important parameter of spikelet fertility and thus stable yield per plant. CTF is a quantitatively inherited trait and three QTLs controlling CTF, namely, *qCTF7*, *qCTF8* and *qCTF12*, were identified using backcrossed inbred lines derived from a cross between rice cultivar Eikei88223 (vigorous CTF) and Suisei (very weak CTF). Using MAS pyramiding, QTLs controlling CTF levels were pyramided utilizing an F_3 population derived from a cross between Eikei and Suisei. These novel QTLs for CTF imparted cold tolerance in combinations between *qCTF7* and *qCTF12* and between *qCTF8* and *qCTF12*. In rice, cold damage (below 15 °C) at the seedling stage results in poor seedling establishment and greatly reduces yield. Advanced backcross between a japonica cultivar, Xiushui 09, and an indica breeding line, IR2061, revealed QTLs affecting cold tolerance (CT) at seedling stage. A total of four QTLs (*qSRS1*, *qSRS7*, *qSRS11a* and *qSRS11b*) for CT were identified on chromosomes 1, 7 and 11. Marker-assisted selection (MAS) holds a great potential for introgression of these QTLs imparting cold tolerance from resistant varieties to susceptible varieties (Li-rui et al. 2012). Liu et al. (2016) made a cross between a cold-sensitive cultivated *Solanum lycopersicum* L. XF98-7 and a cold-tolerant wild *Solanum pimpinellifolium* LA2184 and derived a RIL (recombinant inbred line) population. Genome mapping using simple sequence repeats (SSRs) helped in identification of QTLs conferring cold tolerance in tomato. The QTLs qCI-1-1, qCI-2-1, qCI-3-1 and qCI-9-1 were located on chromosomes 1, 2, 3 and 9, respectively. Marker-assisted selection serves as a means of indirectly selecting the trait of interest and promoting development of a new tomato variety tolerant to chilling stress. Booting stage of rice is more sensitive to cold stress than seedling stage. Cold stress at booting stage affects pollen survival, seed set, grain filling and ultimately yield. Therefore, identification of cold-tolerant QTLs for the booting stage is essential. Zhu et al. (2015) developed interconnected breeding (IB) populations using Huanghuazhan (HHZ) as the recurrent parent and eight diverse elite indica lines as donors to identify stably expressed QTLs for cold tolerance at the booting stage. Six QTLs for cold tolerance on the chromosomes 3, 4 and 12 were identified, among which QTL qCT-3-2 showed stable cold tolerance over years. Raharinivo et al. (2016) identified QTLs for cold tolerance at the seedling stage in rice from a breeding population derived from the cross between Chomrongdhan, a donor parent tolerant, and Vary borty, a susceptible parent. Four QTLs on chromosomes 2 and 10 were identified that conferred cold tolerance. Three QTLs *qSdGwth14-10-1*, *qSdGwth14-10-2* and *qLfGwth14-10-1* located on chromosome 10 conferred cold tolerances for seedling growth and leaf growth at 14 day after recovery and one QTL *qSdVig0-2-1* located on chromosome 2 was identified for seedling vigor after

recovery. Information and materials developed can be utilized for developing cold-tolerant rice cultivars by marker-assisted selection (MAS). In wheat, the major Freezing tolerance QTLs named Fr-1 and Fr-2 and the major vernalization gene *VRN-1* are located on the homologous group 5 chromosomes. A cluster of cold-responsive CBF transcriptional activators was mapped at Fr2 locus and several minor freezing tolerance (FT) QTLs have been identified on several other wheat chromosomes (2B, 4B, 4D, 6A, 7A) (Sutka 2001). Wainaina et al. (2018) identified two QTLs for cold tolerance at the booting stage on chromosome 8 (*qCTB-8*) and chromosome 10 (*qCTB-10*) and three QTLs for heading date (*qHD-4, 7 and 11*) in a rice cross of a Japanese tolerant variety, Hananomai, and a NERICA parent, WAB56-104. Identified QTLs can be introgressed in different cold-sensitive varieties through MAS to enhance their tolerance. Liang et al. (2018) identified 17 QTLs for cold tolerance (CT) in 84 cold-tolerant introgression lines (ILs) selected from five BC_2 populations. Among them, three were large-effect CT QTLs (*qCT4.6*, *qCT6.6* and *qCT11.5*) and the remaining were *qCT1.2* (RM532), *qCT2.4* (between RM29 and RM341), *qCT3.5* (between OSR13 and RM7), *qCT3.12* (RM85), *qCT4.2* (RM518), *qCT4.6* (between RM303 and RM317), *qCT6.6* (between RM3 and RM439), *qCT9.7* (between RM278 and RM160) and *qCT11.5* (between RM457 and RM21). Yu et al. (2018) identified one major QTL, *qSCT8*, and one QTL, *qSCT4.3*, on chromosomes 8 and 4 for cold tolerance at the seedling stage using an *Oryza sativa* × *O. rufipogon* backcross inbred line population. In the subpopulation, three QTLs, *qSCT4.1*, *qSCT4.2* and *qSCT12*, were detected on chromosomes 4 and 12.

3 Transcriptomic Approaches for Cold Stress Tolerance in Plants

3.1 Introduction

Transcriptomics is a prominent field of study related to functional genome of an organism. It deals with quantification of the total set of transcripts or a specific subset of it present in a particular cell type and transcript abundance in a specific developmental stage (Imadi et al. 2015). The main objective of transcriptomics is to catalogue all the transcripts to determine transcriptional status of genes, 5′ and 3′ end sites of genome, post-transcriptional modifications and splicing patterns. Moreover, it quantifies the modulations in gene expression levels during various stress conditions and developmental stages (Wang et al. 2009). Different technologies have been used to study transcriptome that include hybridization-based approaches, sequence-based approaches and RNA sequencing (Wang et al. 2009). RNA sequencing is the most recent approach to study transcriptome. It is a recently developed deep sequencing technology and does not have a reference genome to gain useful information about the transcripts (Strickler et al. 2012).

3.2 Transcriptomics: A Key to Understanding Cold Stress Responses in Plants

Low temperature stress is one of the major environmental stresses affecting plant yield, quality and distribution. A comprehensive understanding of molecular mechanisms through which plants respond to low temperature is of fundamental importance to plant biology. Transcriptomics can be better employed to study cold stress responses in plants. Nineteen microRNA genes of 11 microRNA families in *Arabidopsis thaliana* were identified that were upregulated in response to cold stress. A further analysis of their promoter sequence shows the prevalence of some stress regulatory *cis*-elements (Gupta et al. 2013). These cold-responsive microRNA genes directly or indirectly affect different signalling pathways during the period of stress. In order to understand the gene network controlling tolerance to cold stress, Lee et al. (2005) performed an *Arabidopsis thaliana* genome transcript expression profile using Affymetrix GeneChips that contained ~24,000 genes. A total of 939 cold-regulated genes with 655 upregulated and 284 downregulated were statistically determined. A large number of early cold-responsive genes encode transcription factors that likely control late-responsive genes, suggesting a multitude of transcriptional cascades. In addition, many genes involved in chromatin level and post-transcriptional regulation were also cold regulated, suggesting their involvement in cold-responsive gene regulation. A number of genes important for the biosynthesis or signalling of plant hormones are regulated by cold stress, which is of potential importance in coordinating cold tolerance with growth and development. They compared the cold-responsive transcriptomes of the wild type with the inducer of CBF expression 1 (ice1), a mutant defective in an upstream transcription factor required for chilling and freezing tolerance. The transcript levels of many cold-responsive genes were altered in the ice1 mutant not only during cold stress but also before cold treatments. This study provides a global picture of the *Arabidopsis* cold-responsive transcriptome and its control by ICE1 and will be valuable for understanding gene regulation under cold stress and molecular mechanisms of cold tolerance. Significant progress has been made in the past decade in elucidating the transcriptional networks regulating cold acclimation.

Cold stress induces the expression of APETALA2/ETHYLENE RESPONSE FACTOR family of transcription factors, that is, CBFs (C-repeat binding factors, also known as dehydration-responsive element-binding protein 1s or DREB1s), which can bind to *cis*-elements in the promoters of *COR* genes and activate their expression. Analyses of transgenic plants have shown that ectopic expression of CBFs is sufficient to activate the expression of *COR* genes and induce cold acclimation even at warm temperatures (Chinnusamy et al. 2007). Transgenic expression of *Arabidopsis* CBFs in different plant species was able to enhance chilling/freezing tolerance, and, conversely, the ectopic expression of *CBFs* from other plant species could enhance the freezing tolerance of transgenic *Arabidopsis* (Yamaguchi-Shinozaki and Shinozaki 2006). Microarray analyses of transgenic *Arabidopsis* plants ectopically expressing *CBFs* revealed a constitutive expression of downstream

cold-responsive transcription factor genes *Rap 2.1* and *Rap 2.6* that might control subregulons of the CBF regulon (Fowler and Thomashow 2002). Thus, CBFs play a pivotal role in gene regulation during cold acclimation in evolutionarily diverse plant species. However, CBF regulons from freezing-tolerant and sensitive plant species can differ as evident from microarray analysis of transgenic tomato and *Arabidopsis* plants overexpressing LeCBF1 and AtCBF3, respectively (Zhang et al. 2004). The reason why winter plants exhibit significant genotypic differences in constitutive freezing tolerance is poorly understood. Transcriptome and metabolome analyses in *Arabidopsis* accessions differing in constitutive freezing tolerance suggest that the CBF pathway might also have a crucial role in constitutive freezing tolerance (Hannah et al. 2006).

Byun et al. (2018) investigated the molecular mechanism of Antarctic adaptation of *Deschampsia antarctica* by identifying and characterizing *D. antarctica* C-repeat binding factor 4 (DaCBF4), which belongs to monocot CBF group IV. The transcript level of DaCBF4 in *D. antarctica* was markedly increased by cold and dehydration stress. To assess the roles of DaCBF4 in plants, a DaCBF4-overexpressing transgenic rice plant (Ubi:DaCBF4) was generated and its abiotic stress response was analysed. Ubi:DaCBF4 showed enhanced tolerance to cold stress without growth retardation under any condition compared to wild-type plants. The genes responsible for the improved cold tolerance in rice were screened by selecting differentially regulated genes in both transgenic rice lines because the cold-specific phenotype of Ubi:DaCBF4 was similar to that of Ubi:DaCBF7 (Byun et al. 2015). By comparative transcriptome analysis using RNA-seq, 9 and 15 genes were identified under normal and cold stress conditions, respectively, as putative downstream targets of the two *D. antarctica* CBFs. Hence, results suggested that Antarctic hairgrass *DaCBF4* mediates the cold-stress response of transgenic rice plants by adjusting the expression levels of a set of stress-responsive genes in transgenic rice plants. Thus, such Transcriptional factors which regulate expression of a set of selected downstream target genes will be useful for genetic engineering to enhance the cold tolerance of including rice.

4 Proteomic Changes in Response to Cold Stress

4.1 Introduction

Plants when exposed to cold temperatures experience myriad of changes at physiological, biochemical and molecular levels (Sasaki and Imai 2012). Cold-induced changes in expression of specific proteins have been observed in cold-tolerant plant species. Different cold-response proteins separated by one-dimensional SDS-PAGE and identified by specific antibodies or two-dimensional SDS-PAGE (2DE) combined with mass spectrometry (MS) help to identify differentially abundant proteins during cold treatment in several crops such as barley, soybean, *Arabidopsis thaliana*, rice, wheat and tobacco (Gharechahi et al. 2014; Lee et al. 2009; Gai

et al. 2011; Kosova et al. 2013; Hlavackova et al. 2013; Nakaminami et al. 2014; Yan et al. 2006).

Proteomic approaches help in unraveling stress-inducible proteins and thus aid in dissection and understanding of pathways associated with crop physiological and stress responses (Zhang et al. 2017). Understanding of such stress pathways can be implemented into biotechnological applications for improving stress tolerance in plants. The 'omics' technologies, which essentially include metabolomics, proteomics and genomics, are being put to use to dissect key proteins, metabolites and novel genes involved in stress signalling (Cramer et al. 2011) (Fig. 1). Proteomics is a science that focuses on the study of proteins: their roles, structures, localization, interactions, expression profile, post-translational modifications (PTMs), and other factors under stress and non-stress conditions (Yates et al. 2009). In the wake of cold stress, notable changes in protein expression levels have been observed with differential abundance and expression (Koehler et al. 2012; Grimaud et al. 2013; Xu et al. 2013; Chen et al. 2015; Zhang et al. 2017). Proteomic studies of different organs as well as subcellular compartments under stress are conducted to infer the responses of plant cells to abiotic stresses in different organs. Plants respond to cold

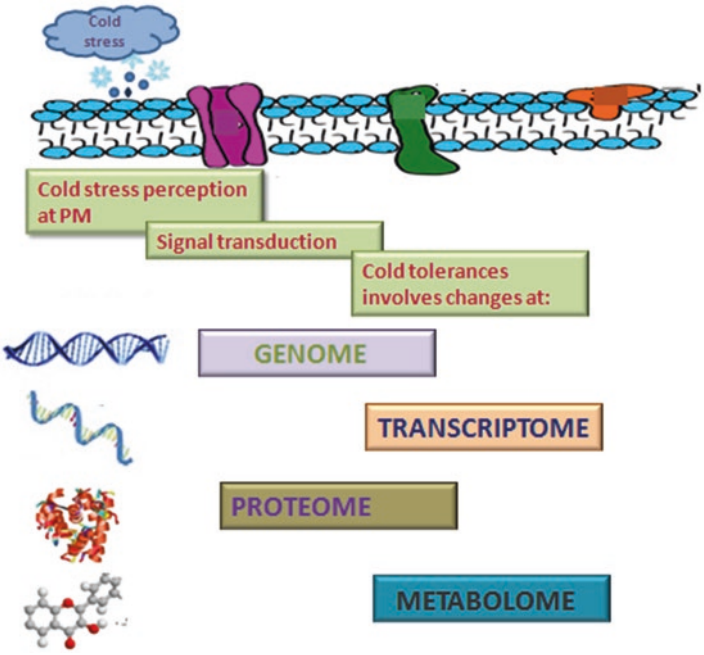

Fig. 1 Cold signals are perceived primarily at the plasma membrane, which, through a series of secondary signalling molecules, transduces the signal to the nucleus. Transcription of cold-responsive genes and transcriptional factors may confer cold tolerance to plants. Transcripts and proteins sometimes may undergo different pre- and post-transcriptional changes or post-translational changes that result in altered transcriptome, proteome, or metabolome associated with cold tolerance in crop plants

by bringing about structural and compositional modifications of compatible solutes in various subcellular compartments via changes in the transcriptome and metabolome (Gharechahi et al. 2016).

Proteomics acts as a central link between gene expression and metabolism as the proteins encoded by transcripts undergo different modifications, such as acetylation, biotinylation and phosphorylation, that may regulate their cellular function and distribution differently. Protein profiling can be done through 'high-throughput mapping', which involves separation of all proteins by 2D electrophoresis followed by their identification, 'differential comparison' or 'protein interaction mapping'. Protein profiling can be achieved via gel-based techniques such as 2D gel electrophoresis, 2D differential gel electrophoresis (2D-DIGE) and gel-free approaches, include isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantitation (iTRAQ), stable isotope labeling by amino acids in cell culture (SILAC), and so on (Yates et al. 2009). Protein isolation by TCA/acetone method or phenol/SDS method is generally carried out and sometimes a polyethylene glycol (PEG) mediated pre-fractionation method is used to remove RuBisCO and their derivatives (Damerval et al. 1986; Hurkman and Tanaka 1986). Comparative proteomic approaches have already been applied to analyse changes in cold-sensitive proteins in different cold-tolerant cultivars, such as meadow fescue (leaf), pea (leaf and chloroplast), perennial ryegrass (leaf), strawberry (crown) and winter wheat (leaf) (Kosmala et al. 2009; Bocian et al. 2011; Dumont et al. 2011; Koehler et al. 2012; Grimaud et al. 2013; Xu et al. 2013; Chen et al. 2015). Proteins involved in energy metabolism, photosynthesis, reactive oxygen species (ROS) scavenging, storage, protection from stress, regulation of the cell cycle and plant development in wheat and barley showed differential abundance between stress-tolerant and stress-sensitive genotypes (Kosova et al. 2014; Chen et al. 2015).

4.2 Plasma Membrane Proteomics

Plasma membrane (PM) acts as the primary site of freezing injury. Protein profiles and specifically lipid raft composition of PM act as an important determinant of freezing tolerance in crop plants (Ruelland et al. 2009). Increased activity of certain proteins like P-type ATPase, disassembly of microtubules and accumulation of several dehydrin family proteins occur on the plasma membrane (Ishikawa and Yoshida 1985; Abdrakhamanova et al. 2003; Kosová et al. 2007). These have been also confirmed by semi-proteomic analysis such as 2D electrophoresis. Comparative proteomic analysis revealed that several PM proteins play an important role in cold acclimatization such as overexpression of phospholipase Dδ (PLDδ) or deficiency in PLDα. Several dehydrin families mitigate freezing injuries in *Arabdiopsis*. Synaptotagmin-1 (SYT1) reseals PMs disrupted by extracellular ice crystals (Yamazaki et al. 2008; Takahashi et al. 2013a, b). PM lipid profile changes significantly in response to cold in cold-tolerant varieties. Specific lipid classes and increase in levels of unsaturated phospholipids enhance the cryostability of the PM.

Comprehensiveness of the proteomic approaches with the help of rapid advance in analytical techniques using mass spectrometry, advanced shotgun proteomics, nano-LC using a LTQ Orbitrap XL mass spectrometer has enhanced our knowledge regarding PM proteome (Li et al. 2012; Takahashi et al. 2013a, b, 2016; Abdallah et al. 2012).

Miki et al. (2019) successfully identified 873 PM proteins responsive to cold acclimation and de-acclimation treatment by conducting shotgun proteomics with label-free semiquantification on plasma membrane fractions of *Arabidopsis* leaves during cold acclimation (CA) and de-acclimation (DA). A comprehensive understanding of PM protein profile in response to rising temperature was gained. This study revealed that global cold-acclimation-responsive proteins return to non-acclimation levels following de-acclimation. This change in protein profile following de-acclimation tends to allow plants to restart normal growth and development. However, levels of certain representative cold-acclimation-responsive proteins were maintained following de-acclimation, which may suggest their role in guarding against the threat of sudden temperature drop. Significant changes were observed in PM proteome during CA in *Arabidopsis* along with decrease in the proportions of transporters and alterations in the activities of several transporters such as ATPases and aquaporins. The study found a number of low temperature-induced proteins such as low-temperature-induced 29 (LTI29, At1g20450.1), cold-regulated 78 (COR78, At5g52310.1), temperature-induced lipocalin (TIL, At5g58070.1), proteins LTI29 and COR78 (dehydrin family proteins) and LTI29 (membrane- and protein-protective hydrophilic protein), COR78, TIL, GPI-anchored lipid transfer protein (LTPG1, At1g27950.1), blue copper-binding protein (BCB, At5g20230.1), SVL2 (At1g66970.1), AIR12 (At3g07390.1), β-1,3-glucanase putative plasmodesmata-associated protein (BG_PPAP, At5g42100.1), a glycoprotein (At5g19240.1) and FLAs (At2g45470.1, At5g44130.1) and SVL2, AIR12 and FLA8 (cell structure-related proteins). A protein, PIP1D, that act as negative regulator and whose abundance decreases during cold acclimation was also identified. PIP1D belongs to a group of proteins known as PIPs, which are freezing-related proteins that function as water transporters and might be involved in rehydration kinetics during freezing recovery processes. Cytoskeletal proteins such as tubulins and actins acted as CA-downregulated proteins. Some stable proteins showing a CA response but no significant changes in abundance during the CA and DA processes are ERD4 (At1g30360.1), SYT1 (At2g20990.1), SHV3 (At4g26690.1), SVL1 (At5g55480.1) and SKU5 (At4g12420.1), PM-localized ATP-binding cassette (ABC)-type transporters, two patellins (PATLs) PATL1 and PATL2 (sec14-like proteins that bind to phosphoinositides and play a role in cell plate formation), ten leucine-rich repeat protein kinase (LRR) family proteins (cell-surface receptor kinases) and two ABC transporters, that is, pleiotropic drug resistance 8 (PDR8, At1g59870.1) (associated with hypersensitive-like cell death) and multidrug resistance 1 (MDR1, At3g28860.1). These proteins can be used as control markers for studies of CA and DA mechanisms.

Chen et al. (2015) conducted studies on freezing-tolerant and freezing-sensitive cultivars of alfalfa to reveal the difference between them at proteomic levels.

Results revealed that proteins to chilling were related to photosynthesis, protein metabolism, energy metabolism, stress and redox, and other proteins were mobilized in an adaptation to chilling stress. The relative abundance of the cytochrome b6-f complex iron-sulfur subunit, oxygen-evolving enhancer protein, chlorophyll A/B binding protein was altered in cold-resistant genotype. In yet another study, levels of 38 plasma membrane proteins were altered in *Arabidopsis* 3 days after cold acclimation. These proteins include early responsive to dehydration proteins (ERD10 and ERD14) (Kosová et al. 2007). Another novel protein plant synaptotagmin-1 (SYT1) that is believed to be involved in resealing the freeze-fractured membranes imparts freeze tolerance (Reddy et al. 2001). Clathrins and dynamin-related proteins accumulate in the microdomain during cold acclimation. They are associated with the clathrin-dependent endocytosis pathway. Certain proteins such as dehydrin proteins (DHNs), cold-regulated proteins (CORs) and heat-shock proteins (HSPs) act in conjunction with symplastic and apoplastic soluble osmolytes, such as glucose, sucrose, fructose, trehalose, raffinose, to stabilize both membrane phospholipids and proteins and cytoplasmic proteins (Livingston et al. 2006). These metabolites maintain hydrophobic interactions and ion homeostasis, scavenge reactive oxygen species (ROS) and protect the adhesion of ice to plasma membrane, thus preventing cell disruption (Janska et al. 2010).

4.3 Differential Proteome Profile in Response to Cold Stress

Tian et al. (2015) compared proteome of seedling leaves of cold-tolerant and cold-sensitive soybean varieties and found 57 proteins significantly changed in abundance and they were identified by MALDI-TOF/TOF MS. Proteins identified were found to be involved in 13 metabolic pathways and cellular processes, including 15 differentially expressing proteins involved in photosynthesis, protein folding and assembly, cell rescue and defense, cytoskeletal proteins, transcription and translation regulation, amino acid and nitrogen metabolism, protein degradation, storage proteins, signal transduction, carbohydrate metabolism, lipid metabolism, energy metabolism and unknown. The proteins associated with photosynthesis were implicated in plastid division and heme and chlorophyll biosynthesis: protein coproporphyrinogen III oxidase and cell division protein ftsZ homolog 1, photosystems I (PSI) and II (PSII), ribulose bisphosphate carboxylase/oxygenase (RuBisCO) proteins and interconversion of CO_2 and HCO_3. Differentially expressed proteins identified were coproporphyrinogen III oxidase, cell division protein ftsZ homolog 1, chlorophyll *a*/*b*-binding protein type II, chlorophyll *a*/*b*-binding protein, light-harvesting complex I type II, ferredoxin-NADP reductase, chloroplastic cytochrome b_6/f complex iron-sulfur subunit, photosystem II stability/assembly factor HCF136, NDH-dependent cyclic electron flow, ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, carbonic anhydrase, chloroplastic protein folding and assembly proteins that include protein disulfide isomerase, peptidyl-prolyl *cis–trans* isomerase CYP37, chloroplastic-like 92; protein degradation proteins; cysteine proteinase, cysteine proteinase RD21a-like, cysteine proteinase inhibitor, proteasome

subunit beta type-3-A, proteasome subunit beta type, putative 20S proteasome beta subunit PBC2, ubiquitin fusion degradation 1, aminopeptidase N-like; cell rescue and defense proteins; thiol methyltransferase, nucleotide-binding site-containing resistance-like protein, ferritin; cytoskeletal proteins, such as tubulin/FtsZ family protein, transcription and translation regulation, eukaryotic translation initiation factor, elongation factor 1-delta-like cytidine/deoxycytidylate deaminase-like protein, 60S acidic ribosomal protein; amino acid and nitrogen metabolism proteins; arginase, O-methylthioadenosine/S-adenosylhomocysteine nucleosidase 1, putative pterin-4-alpha-carbinolamine dehydratase 1-like; Storage proteins glycinin A-2-B-1a subunit precursor; carbohydrate metabolism: chloroplast ribose-5-phosphate isomerase, phosphoglycerate kinase; ipid metabolism: beta-hydroxyacyl-ACP dehydratase, Allene oxide cyclase 3; energy metabolism: ATP synthase delta chain, electron transfer flavoprotein subunit alpha, mitochondrial-like stem-specific protein TSJT1. Proteomic analysis of a spring wheat cultivar in response to prolonged cold stress showed relative abundance of proteins involved in ascorbate recycling (dehydroascorbate reductase, ascorbate peroxidase), protein processing (proteasome subunit, cysteine proteinase) and enzyme involved in tetrapyrrole resynthesis (glutamate semialdehyde aminomutase). Downregulation of proteins involved in Krebs cycle; enzymes (isocitrate dehydrogenase, malate dehydrogenase), photosynthesis-related proteins (oxygen-evolving complex proteins, ATP synthase subunits, ferredoxin-NADPH oxidoreductase and some Calvin cycle enzymes) after cold stress was observed (Rinalducci et al. 2011). Zhang et al. (2017) used iTRAQ for comparative protein profiling of cold-tolerant and susceptible wheat varieties and identified 140 proteins that showed decreased protein abundance. These proteins were components of the following main groups: protein metabolism, stress/defense, carbohydrate metabolism, lipid metabolism, sulphur metabolism, nitrogen metabolism, RNA metabolism, energy production, cell wall metabolism, membrane transport and signal transduction. They also identified three novel proteins that play a vital role in conferring cold tolerance in bread wheat; the proteins are Hsp90, BBI and REP14. Comparatively low abundance of two fasciclin-like arabinogalactan proteins (FLAs), fasciclin-like arabinogalactan protein 11-like (M8BBJ1) and fasciclin-like arabinogalactan protein 7-like (A0A0A9EJ37), was reported in bread wheat. These FLAs belong to cell wall glycoprotein family arabinogalactan proteins (AGPs) and are implicated in cell wall biosynthesis, cell wall remodelling and signalling. Earlier FLAs were reported to express differentially in response to salt stress; this study provided first evidence of their degradation in response to salt. Shi et al. (2019) used an isobaric tag for relative and absolute quantification (iTRAQ)-based quantitative proteomic approach to compare the protein profile of self-grafted (SG) and pumpkin rootstock-grafted (RG) watermelon seedlings in response to cold. Root grafting improved cold tolerance in watermelons. A total of 752 proteins were accumulated in grafted watermelon seedling leaves post cold stress; root-grafted watermelon was more cold tolerant than self-grafted watermelon. RG watermelon had improved the scavenging capacity of ROS and arginine biosynthesis, besides producing more energy through photosynthesis, carbon metabolism and oxidative phosphorylation. Activity of the certain antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, ascorbate

peroxidase and catalase also increased in response to cold (Chen and Li 2002). Shi et al. (2014) conducted comparative proteomic analysis to identify proteins involved in calcium-mediated cold response mechanisms in the Bermuda grass. Differentially expressing proteins mainly were involved in redox, tricarboxylic acid cycle, glycolysis, photosystem and amino acid metabolism. Study also confirmed that $CaCl_2$ plays a vital role in ROS detoxification, which might have contributed to enhanced freezing tolerance. Identification of a cold-responsive novel protein 1-FFT, the enzyme involved in inulin synthesis in the roots of biannual crop chicory that has to adapt to freezing temperatures, points towards possibilities of identification of novel proteins involved in freeze tolerance in different organs of different crops. In addition to expected proteins (e.g. related to metabolism, energy, protein synthesis or cell structure), proteins related to folding and stability, proteolysis and stress response were also observed (Degand et al. 2009). Comparative phosphoproteomics in response to cold in banana revealed three unique phosphoproteins MKK2, HY5 and STN7. The findings of the study suggest that phosphorylation happens at the early stage of cold stress as a primary response to cold stress. A conserved MKK2 network associated with the regulation of cellular functions is responsible for the high cold tolerance in the cold-tolerant banana variety. Transcription factor HY5 is a bZIP transcription factor involved in processes such as light signalling and photomorphogenesis and mediates plant responses to UV-B and different hormones, such as ABA, gibberellin, cytokinin and auxin. Serine/threonine-protein kinase STN7, chloroplastic (STN7) plays a role in assembly of the photosynthetic machinery and control of redox balance in the electron transfer chain (Gao et al. 2017).

4.4 LEA Proteins/Dehydrins

These are a group of heat stable, glycine-rich LEA proteins that impart membrane stabilization and protect proteins under cold-induced dehydrating conditions. Some important dehydrins that play an important role in cold acclimation include COR15am (protectant preventing protein aggregation) (Nakayama et al. 2008), ERD10 (early response to dehydration) and ERD14 (chaperones) (Kovacs et al. 2008). COR41an integral chloroplast inner envelope protein. The SFR2 protein outer envelope membrane (Fourrier et al. 2008).

4.5 HSP Proteins

Induced in response to cold, these proteins confer a strong cryoprotective effect by refolding denatured proteins, preventing their aggregation and imparting membrane protection. Expression of HSP90, HSP70, several small HSPs and chaperonins 60 and 20 in response to cold increases fold times in plants (Timperio et al. 2008).

4.6 PR Proteins

Some PR proteins such as PR-2 (β-1,3-glucanase); PR-3, PR-4, PR-5 (thaumatin-like proteins); PR-11 (chitinases) (apoplastic antifreeze proteins), PR-8, PR-10 (Bet v-1 homologues); and PR-14 (lipid transfer proteins) are also responsive to cold (Wisniewski et al. 1999; Griffith and Yaish 2004; Renaut et al. 2006; Janska et al. 2010).

5 Metabolomics in Response to Cold Stress in Plants

5.1 Introduction

Exposure to freezing situations results in genuine harm to the plant cell owing to ice formation and dysfunction of cell membranes. Many plant species increment freezing resilience amid presentation to non-freezing low temperature by a procedure known as cold acclimation (Ghatak et al. 2018). The molecular premise of this procedure has been broadly considered and the commitment of specific metabolites including compatible solutes and the transcriptional regulatory system is elucidated (Zuther et al. 2018). Metabolomics is a generally new methodology for improved comprehension of metabolic systems and the resulting biochemical organization of plants and other biological organisms. It relates to non-biased identification and quantification of all metabolites in an organic framework, and thus the selectivity and affectability of the explanatory strategy must be high (Du et al. 2018). Analytical tools inside metabolomics, including mass spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy, can decide the effect of time, stress, wholesome status and ecological challenges on several metabolites all the while, bringing about enormous, complex informational indexes. The most commonly utilized strategies for plant metabolite examination are gas chromatography coupled to mass spectrometry (GC-MS) and fluid chromatography coupled to mass spectrometry (LC-MS), and further vital expository systems incorporate fluid chromatography (photodiode exhibit discovery) coupled to mass spectrometry (LC-PDA/MS), narrow electrophoresis coupled to mass spectrometry (CE-MS), Fourier change particle cyclotron reverberation mass spectroscopy (FT-ICR/MS) and NMR spectroscopy (Rinschen et al. 2018). Metabolic profiling can likewise be utilized for identification and quantification of a selected number of pre-characterized metabolites, for the most part identified with a particular metabolic pathway(s). Metabolic fingerprinting is additionally utilized for global analysis of samples to give sample order, in which quantification and metabolic identification are usually not utilized; however, such screening allows to segregate between samples of different biological status or origins (VanWallendael et al. 2019).

5.2 Metabolomics: Role in Cold Acclimation

The first metabolomic investigations of cold acclimation were performed by two groups (Le Signor et al. 2018). Cook et al. (2004) investigated metabolomic changes amid cold acclimation in two ecotypes of *Arabidopsis thaliana*, Wassilewskija-2 (Ws-2) and Cape Verde Islands-1 (Cvi-1), which are moderately freezing tolerant and sensitive individually. The metabolome of Ws-2 plants was widely modified because of low temperature. Seventy-five percent of metabolites observed were found to increment in cold-acclimated plants, including metabolites known to increment in *Arabidopsis* plants upon introduction to low temperature, for example, the amino corrosive proline and sugars glucose, fructose, inositol, galactinol, raffinose and sucrose. They likewise discovered novel changes, particularly the expansion of trehalose, ascorbate, putrescine, citrulline and some TCA cycle intermediates. There was extensive overlap in the metabolite changes that happened in the two ecotypes in light of low temperature. Nonetheless, quantitative contrasts were apparent (Nounjan et al. 2018). Kaplan et al. (2007) led metabolome examination of *Arabidopsis* over the time course following the shift to cold and heat conditions. Shockingly, most of heat shock reactions were shared with cold shock, including the expansion of pool sizes of amino acids from pyruvate and oxaloacetate, polyamine precursors and compatible solutes. The after-effects of this study were analysed together with following transcript profiling information by a similar group and it was found that the regulation of gamma-aminobutyric acid (GABA) shunt and proline accumulation under cold conditions are accomplished by transcriptional and post-transcriptional habits separately. Gray and Heath (2005) analysed the impacts of cold acclimation on the *Arabidopsis* metabolome utilizing a non-targeted approach on metabolic fingerprinting. It uncovered global reprogramming of metabolism just as differential responses between the leaves that moved to and those that developed wide open to cold (Bor and Ozdemir 2018). Hannah et al. (2006) exploited the genetic variation of *Arabidopsis* to elucidate the capacity of metabolomics in cold acclimation. In spite of the fact that there is no clear connection between global metabolite changes and contrasts in acclimation capacity or contrasts between the acclimated freezing resistance, the plausible significance of carbohydrate metabolism is shown by the identification of glucose, fructose and sucrose among metabolites emphatically relating to freezing resilience (Schwacke et al. 2019). Kaplan et al. (2007) analysed the impact of diurnal gene/metabolite guideline amid cold acclimation by methods for metabolomics and transcriptomics. Roughly 30% of every single analysed metabolite demonstrated circadian motions in their pool size and low temperature influenced the cyclic pattern of metabolite wealth. These outcomes showed that the collaborations seen among circadian and cold guideline are likely significant components of cold acclimation (Walia et al. 2018).

Metabolomics was likewise used to reveal the functions of some specific genes in cold acclimation. In the above investigation, Cook et al. (2004) additionally

explored plants overexpressing CBF3, which is one of the C-repeat/dehydration element binding factor (CBF) transcriptional activators instigated quickly under low-temperature conditions. The metabolite profiles of non-acclimated CBF3 overexpressing lines were like those of the cold-acclimated Ws-2 ecotype, suggesting a conspicuous role for the CBF cold response pathway in designing the low-temperature metabolome of *Arabidopsis* (Aghcheh and Braus 2018). Maruyama et al. (2009) investigated metabolic and transcript changes in *Arabidopsis* plants overexpressing CBF3/dehydration-responsive element-binding proteins (DREB1A and DREB2A) and observed a minor impact on metabolic profile of CBF3-overexpressing plants. The eskimo1 mutants of Arabidopsis were as such disregarded as freezing tolerant without past acclimation, yet the capacity of this gene was obscure (Tardieu et al. 2018). Lugan et al. (2009) attempted to illustrate the premise of the freezing resilience of esk1 by performing metabolomic analysis under different natural conditions, in particular cold, salinity and dehydration. At that point, the most explicit metabolic response to cold acclimation was not phenocopied by esk1 transformation. Be that as it may, esk1 amassed lower measure of Na in leaves than the wild type and its metabolic profile and osmotic potential were somewhat affected under dehydration stress (D'Amelia et al. 2018). These findings suggest that ESK1 could rather be associated with water homeostasis and all things considered featured the significance of cellular water status in stress resistance.

5.3 Metabolite Profiling in Response to Cold Stress

Global metabolite profiling investigation holds the guarantee to allow synchronous observation of precursors, intermediates and results of metabolic pathways. It is a discovery tool that can recognize and screen unidentified mass spectral tags (MSTs) just as distinguished metabolites that assume critical roles in metabolism and physiology and stress resistance (Zhou et al. 2019). In one investigation, metabolic profiling analysis was performed to decide metabolite temporal dynamics related to the acceptance of procured thermotolerance in light of heat shock and acquired freezing resistance because of cold shock. Low-Mt Polar metabolite investigations were performed utilizing gas chromatography–mass spectrometry. Eighty-one recognized metabolites and 416 unidentified mass spectral tags portrayed by retention time indices and specific mass fragments were checked. Cold shock affected metabolism more significantly than heat shock. The steady-state pool sizes of 143 and 311 metabolites or mass spectral tags were changed because of heat and cold shock, individually. Correlation of heat and cold shock response designs revealed that most of heat shock responses were shared with cold shock responses, a formerly obscure relationship. Arrange increments in the pool sizes of amino acids obtained from pyruvate and oxaloacetate, polyamine precursors and compatible solutes were observed amid both heat and cold shock. Also, a large number of the metabolites

that indicated increment in light of both heat and cold shock in this analysis were earlier not linked to temperature stress (Longo et al. 2018).

Alcohol dehydrogenase (ADH) plays an important role in the metabolism of alcohols and aldehydes, and it is a key catalyst in anaerobic fermentation. ADH1 responds to plant development and natural pressure. Nevertheless, the capacity of ADH1 in response to short-term freezing stress stays obscure (Dar et al. 2017). Utilizing real-time quantitative fluorescence polymerase chain reaction (PCR), the quantitative expression of ADH1 was investigated at low temperature (4 °C). The lethal temperature was determined by the electrolyte spillage tests for both ADH1 deletion mutants (adh1) and wild-type (WT) plants. To additionally examine the connection among ADH1 and cold resistance in plants, low-Mr polar metabolite analysis of *Arabidopsis* adh1 and WT were performed at cold temperatures utilizing gas chromatography–mass spectrometry. This analysis concentrated on freezing medications (cold acclimation group: −6 °C for 2 h with earlier 4 °C for 7 days; cold shock group: −6 °C for 2 h without cold acclimation) and recovery (23 °C for 24 h) for seedling development at an ideal temperature. The exploratory outcomes uncovered a critical increment in ADH1 expression amid low-temperature treatment (4 °C) and at a higher lethal temperature in adh1 contrasted with that in the WT. Retention time indices and explicit mass fragments were utilized to screen 263 factors and elucidate 78 distinguished metabolites. From these investigations, contrasts in the level of metabolite accumulation among adh1 and WT were distinguished, including soluble sugars (e.g. sucrose) and amino acids (e.g. asparagine). Likewise, the correlation-based analysis system featured a few metabolites (e.g. melibiose, fumaric acid, succinic acid, glycolic acid and xylose) that upgraded connectedness in adh1 arrange under cold shock. At the point when considered aggregately, the outcomes demonstrated that adh1 had a metabolic response to freezing stress and ADH1 assumed a critical role in the cold stress response of a plant (Wani et al. 2018).

CB-1 and K326 are closely related tobacco cultivars. However, their cold resistance limits are unique. K326 is significantly more cold tolerant than CB-1 (Masoodi et al. 2016). In an investigation, transcriptomes and metabolomes of CB-1 and K326 leaf samples treated with cold stress uncovered about 14,590 differentially expressed genes (DEGs) in CB-1 and 14,605 DEGs in K326. There were additionally 200 differentially expressed metabolites in CB-1 and 194 in K326. In addition, there were many overlapping genes (around half) that were cold responsive in both plant cultivars in spite of the fact that there were additionally numerous distinctions in the cold-responsive genes between the two cultivars. Significantly, for a large proportion of the covering cold-responsive genes, the degree of the adjustments in expression was regularly considerably more expressed in K326 than in CB-1, which may help elucidate the unrivaled cold resilience of K326 (Zhou et al. 2019). Comparable outcomes were found in the metabolome analysis, especially in the analysis of essential metabolites, including amino acids, organic acids and sugars. A substantial number of specific responsive genes and metabolites feature in the complex regulatory mechanisms related with cold stress in tobacco.

5.4 Glycine Betaine Against Cold Stress in Plants

Both the exogenous application of glycine betaine (GB) and the genetically engineered biosynthesis of GB build the resistance of plants to cold stress and they can upgrade ensuing development and yield (Showkat et al. 2017). Reactive oxygen species (ROS) are delivered persistently as a result of different metabolic pathways even when plants are subjected under non-stress conditions. These ROS are scavenged by an assortment of antioxidant defense frameworks that keep ROS from achieving lethal levels (Rastogi et al. 2019). All types of abiotic stress, including salinity, cold, freezing and drought, cause an oxidative burst in plant cells. The utilization of hydroxyl radicals (OH∗) in *Arabidopsis* roots brought about a massive, dose-dependent efflux of K^+ particles from epidermal cells into the elongation zone. Notwithstanding, the nearness of GB at 5 mM in the incubation medium essentially decreased this efflux of K^+ particles. Besides, in tomato plants, exogenously applied GB altogether diminished the chilling-induced generation of H_2O_2. Since GB does not scavenge ROS specifically, GB must relieve the damaging impacts of oxidative stress in different ways, for instance by enacting or settling ROS searching proteins and additionally stifling the creation of ROS by an obscure system (Tada et al. 2019). Two important factors that impact the resilience of plants to cold stress are concentration and localization of GB in the cell. In numerous investigations of the engineered accumulation of GB in plants, GB-biosynthetic enzymes have been targeted to chloroplasts, while in others the catalysts have been targeted to cytosol or mitochondria or to both the cytosol and the chloroplasts at the same time. Three kinds of transgenic tomato plants were produced by utilizing a *codA* gene that was targeted to chloroplasts (Chl-codA plants), cytosol (Cyt-codA plants), or chloroplasts and cytosol at the same time (ChlCytcodA plants). Cyt-codA and ChlCyt-codA plants accumulated up to 5.0- and 6.6-fold, individually, larger amounts of GB in their leaves than did Chl-codA plants (0.3 mmol g^1 FW). Each one of the three kinds of transgenic plants showed more noteworthy cold resistance than wild-type plants (Dumont and Rivoal 2019). In Chl-codA plants, the stress resistance of photosystem II (PSII) and the recurrence of seed germination were like those in the other two kinds of transgenic plants. In any case, the stress resistance amid the development of seedlings of Chl-codA plants was higher than that of transgenic plants, despite the fact that the dimension of GB was much reduced in the former. Subsequently, the amassing of GB in chloroplasts is more viable than the collection of GB in the cytosol for the protection of plants against cold stress (Schwachtje et al. 2019).

5.5 Differential Metabolic Response to Low Temperature

Zoysia grass local to high latitude may have advanced higher cold resistance than the ones local to low latitude. A study was conducted to explore the cold stress response in *Zoysia* grass local to different latitudes at phenotypic, physiological and

metabolic dimensions (Kudo et al. 2019). Two zoysia grass (*Z. japonica*) genotypes, Latitude-40 (higher scope) and Latitude-22 (lower scope), were exposed to four temperature medications (ideal, 30/25 °C, day/night; suboptimum, 18/12 °C; chilling, 8/2 °C; freezing, 2/−4 °C) dynamically in growth chambers. Low temperature (chilling and freezing) expanded leaf electrolyte spillage (EL) and reduced plant development, turf quality, chlorophyll (Chl) content, photochemical productivity (F_v/F_m) and photosynthesis (P_n, net photosynthetic rate; g_s, stomatal conductance; intercellular CO_2; Tr, transpiration rate) in two genotypes, with increasingly quick changes in Latitude-22. Leaf carbohydrate content (glucose, fructose, sucrose, trehalose, fructan, starch) expanded with the decrease in temperature to an extraordinary reach in Latitude-40. Leaf abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) content expanded, while indole-3-acetic acid (IAA), gibberellic acid (GA_3) and *trans*-zeatin riboside (t-ZR) content diminished with the decrease of temperature, with higher content in Latitude-40 than in Latitude-22. *Zoysia* grass local to higher latitude showing higher freezing resistance might be credited to the higher carbohydrates and stress protectants that balance out cellular membranes (Takahashi et al. 2019).

The impact of short-term cold stress on the metabolism of non-structural carbohydrates in polar grasses has been researched. Flowering plants of the family Poaceae growing in the Arctic and Antarctic were researched (Zhu et al. 2019). Their response to cold stress were analysed under research centre conditions. Samples were collected after 24 and 48 h of cold treatment. Quantitative and qualitative changes of sugars were found among various species; however, they could vary within a genus of the family Poaceae. The estimations of the examined parameters in *Poa annua* contrasted extensively depending to the biogeographic origin of plants. At the start of the trial, plants of the Antarctic were acclimatized in nursery portrayed by essentially higher content of sugars, including storage reserves, sucrose and starch, however lower all out protein content. After 24 h of introduction to cold stress, small changes in the analysed parameters were noted in Antarctic plants than in locally grown samples. Absolute sugar content and sucrose, starch and glucose levels were about steady in *P. annua*; however, they changed fundamentally. These progressions are responsible for the high adaptability of *P. annua* to survive and develop in exceedingly unsupportive environments and colonize new regions (Fàbregas and Fernie 2019).

6 Conclusion

Cold tolerance is a complex trait resulting from multiplicative molecular interaction at genome, transcriptome, proteome and metabolomics levels in an organism. Tolerance to cold is developmental stage specific and can manifest as a mechanism of cell stability in response to stimuli. Genomic loci governing cold stress tolerance in plants share a certain degree of homology across species, yet the relative expression and localization of protein products may vary with systems across, which may

change altogether, the context defining 'cold response'. Eventually, in order to understand cold stress tolerance in plant species, the role of omics technologies is of immense value and of direct solicitation in drawing out new pathways underlying such a mechanism.

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Beneficial Role of Metalloids in Plants: Molecular Understanding and Applicability

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1 Introduction

Metalloids are elements that have chemical properties between metals and non-metals. In the periodic table, metalloids are recognized as boron (B), silicon (Si), arsenic (As), germanium (Ge), antimony (Sb), tellurium (Te) and polonium (Po). These elements are paced diagonally between the metals and non-metals. Along with other elements, these metalloids are widely distributed in earth crust. Like many minerals, metalloids are known to regulate optimum growth and development of all animals and plants. However, it is also commonly known that enhanced metalloid concentration negatively impacts plant health by interfering in various

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biochemical, structural and physiological processes of plant metabolism (Nagajyoti et al. 2010; Adrees et al. 2015; Afshan et al. 2015). These effects may range from substitution of essential functional groups to production of reactive oxygen species (ROS) and cellular damage (Anjum et al. 2015). Additionally, increased concentration of metalloids has been found to adversely affect plant biomass, growth, photosynthesis, accumulation and translocation of essential elements (Wagner 1993; Adrees et al. 2015). Consequently, extensive research into metalloid accumulation and resistance has been taken up across the globe in the last two decades to assess their immediate and long-term impact on environmental, human, livestock and plant health to optimize their bioavailability and uptake by plants to aid plant growth and yield (Nascimento and Xing 2006; Adrees et al. 2015). Compared to other organisms, plants are better equipped with strategies to withstand influx of metalloids and also regulate their metabolism to not affect their survival and reproductive success. Plant genes encode expanded gene families of transporters that regulate uptake and subsequent sequestration of metalloids. These transporters have specific substrate specificities, expression and localization on cellular membranes as they manage translocation of respective metalloids across the whole plant (Hwang et al. 2016). Analysis of these transporters has been conducted in numerous plant systems and their relevance has been assessed using several forward and reverse genetic approaches. Only a few metalloids such as boron, silicon and selenium have been studied extensively and are now well established to play beneficial roles in plant growth and metabolism. These beneficial metalloids serve as essential micronutrients and ensure optimum plant growth, development and productivity (Peterson et al. 1981). At optimum concentrations, metalloids effectively regulate function of many enzymes and metabolic pathways. Furthermore, these metalloids are instrumental in various biosynthetic pathways such as nucleic acid, chloroplast and protein besides governing structural and functional integrity of cellular membranes (Adrees et al. 2015; Oves et al. 2016). Therefore, in this chapter we discuss such incidences of metalloid relevance to plants, mechanisms of their transport and uptake, essential transporters, and various forward and reverse genetic approaches adapted to assay and optimize uptake of metalloids in plants.

2 Sources of Metalloid Ions

Presence of metalloids in soil is a combined result of various anthropogenic and natural processes. Most common source of natural metalloid contribution to the soil in question is rock substratum and geological bedrock (Tchounwou et al. 2012). The amount and composition of metalloids in the parent bedrock and weathering conditions determine concentration of metalloids in the resultant soil (Wuana and Okieimen 2011). Agriculture is another major source of metalloid contamination. Typically, all soils more or less have all known metalloids. However, their concentration varies; while some metalloids are found below the detection limit, some may be present at toxic amounts (Alloway 2013). Accordingly, the concentration of the

metalloids can be categorized as ‘total’ and ‘available’. The ‘available’ metalloids constitute only a part of the ‘total’ concentration of metalloids present in the soil at a given time. Total concentration refers to presence of all forms of element in the soil such as adsorbed to minerals like clay, bound to organic matter, bound in crystal structure of minerals, carbonates, oxides, soluble organic and inorganic complexes in soil solution, and free ions. More often than not, only a fraction of this ‘total’ concentration is available for immediate uptake by plants. This ‘available’ concentration of the element is present in soil as soluble complexes, free ions or readily amenable forms. Further, this availability of the element to the plant is governed by many soil factors such as redox status, pH, temperature, macronutrients and water content. Additionally, plants are known to produce root exudates that can also significantly affect the availability of the metalloids. Assessing total concentration of a given metalloid is not a good indicator of the bioavailability of the element, notwithstanding these measures do indicate presence of anomalously increased or decreased concentrations of the metalloids. Such a measure is instrumental in assessing the effect on soil flora and fauna. Low levels of an element indicate that either the soil is derived from a bedrock that was deficient in the said element or the soil has become depleted over the years. In either case, such soils need to be supplemented with essential metalloids to ensure their bioavailability to growing plants.

3 Molecular Interactions During Plant Elemental Uptake

Essential metalloids required for plant growth and development are taken up primarily from the soil. To ensure regulated uptake of required metalloids (B, Si and Se), specific transporters and signalling mechanisms are in place. Such metalloids that are beneficial to several biochemical and physiological processes in plant growth are components of various cellular enzymes and regulate various oxidation–reduction reactions (Adrees et al. 2015; Emamverdian et al. 2015). Boron (B) is avital element to numerous processes in plant development such as protein and amino acid (AA) biosynthesis, seed germination, nucleic acid metabolism, carbohydrate transportation, cell division and elongation, cell membrane integrity, sugar translocation, biosynthesis and transport of plant hormones, phenolic metabolism, gas exchange and water uptake (Kouchi and Kumazawa 1976; Camacho-Cristóbal et al. 2008; Han et al. 2008; Jehangir et al. 2017; Lu et al. 2015). High-affinity transport systems ensure boron uptake, accumulation and absorption in plants occur via specific boron transporters and chelators (Jehangir et al. 2017; Lu et al. 2015). Selenium (Se) is an essential element for plant growth owing to its antioxidant capacities. It has been established as a component of selenoenzymes such as thioredoxin reductases (TR) and glutathione peroxidase (GSH-Px). In addition, its availability is cardinal to optimum functioning of the enzymes that maintain the redox potential of the cell (Rayman 2000). Silicon (Si) plays a central role in important physiological processes of the plants such as transpiration and photosynthesis. Si is also central in conferring plants with adaptive capacities to tide over drought

conditions (Tubana et al. 2016). These essential and/or non-essential metalloids are absorbed from the soil in accordance to a concentration gradient that favours selective uptake of a certain ion over the other at a given time (Peralta-Videa et al. 2009). First step towards uptake is exploration of soil by root system for availability of the micronutrients. Root responses to explore macronutrients such as nitrogen (N) and phosphorus (P) are well established. However, root responses to metalloid deficiencies do not remain well characterized. In response to nutrient deficiency, plant tends to increase the surface area of roots by promoting branching of roots. Such incidences in root development have been noted in response to Fe deficiency in both monocots and dicots plants (Moog et al. 1995; Schmidt 2002). Second parameter that determines metalloid availability by roots is their bioavailability (Palmgren et al. 2008). Metalloids are present adsorbed to soil particles or in an insoluble form. Plant roots ensure to increase their bioavailability by uptake of specific transporters by changing them into appropriate forms by interacting with the surrounding rhizosphere (Marschner 1995; Palmer and Guerinot 2009). Plant roots exude acidification of the rhizosphere to generate a high membrane potential that drives cation uptake (Palmgren 2001). Additionally, protons that are released participate in cation exchange to release divalent metal ions that are bound to soil particles and consequently the acidification of rhizosphere releases metals from hydroxides (Palmer and Guerinot 2009; Palmgren 2001). Post mineral uptake, minerals migrate to apoplastic spaces from where metals are actively transported across plasma membrane into symplastic pathways.

Uptake of boron from soil and its subsequent transport to shoots is now explained using a model that has been developed based on available data from various different plant systems so far (Takano et al. 2008; Miwa and Fujiwara 2010; Durbak et al. 2014; Baxter and Dilkes 2012). According to this model, boron diffuses from the soil to the apoplast of root epidermis. The influx proteins present on the plasma membrane of the epidermis, endodermis and cortex transport boron to the cytosol. Boron then reaches the pericycle through the symplastic pathways, and efflux proteins subsequently load it into xylem vessels that ensure its availability across the plant system (Kohl and Oertli 1961). Uptake of silicon from soil occurs in the form of silicic acid (Takahashi and Hino 1978; Mitani et al. 2005). Three distinct models from Si uptake have been proposed in plants having varying concentration of Si accumulation and uptake (Takahashi et al. 1990). These models primarily profess active, passive and rejective mode of Si uptake. During passive uptake of Si the rate is similar to that of water uptake resulting decreased concentration of Si in the uptake solution. However, during rejective mode of Si uptake the Si concentration in uptake solution increases. These models have been found valid in a few plant systems. However, these mechanisms remain largely uncharacterized in plant systems so far. Selenium (Se) uptake in plants also varies between different plant species. Additionally, Se uptake is dependent on phases of plant development, soil conditions (salinity and pH) and concentration of Se (Renkema et al. 2012; Gupta and Gupta 2016). Se is present in two forms: selenate in alkaline soils and selenite in acidic soils (Gupta and Gupta 2016). Both of these forms have differential

absorption and mobility (Li et al. 2008). Selanate (SeO_4^{2-}) is the most prevalent and available form of Se (Missana et al. 2009; Gupta and Gupta 2016). In order of preference selenate is absorbed first in plants followed by selenomethionine (SeMet) and selenite. In plants, transporters in root cell membrane play an important role. While selenite is transported by phosphate transport mechanism (Zhang et al. 2014a), selenate uptake is mediated by sulphate transporters and channels (Feist and Parker 2008; Li et al. 2008; Zhang et al. 2003). Although high-affinity transport systems are cardinal for regulated plant growth and development, during the period of excess metal concentration in soil unspecific uptake of metal is unavoidable. For example, arsenic (As) is a metalloid with no established biological function in higher plants; therefore, no specific uptake mechanisms for these metals are in place. However, in soils with high As concentration, uptake of As occurs via phosphate transporters as As(V) that is reduced in plants to As(III) (Meharg Andrew and Hartley-Whitaker 2002). In reducing environments, aquaporin nodulin-26-like intrinsic proteins bind to As (III) (Bienert et al. 2008; Isayenkov and Maathuis 2008).

4 Beneficial Role of Metalloids in Plants

4.1 Boron

Boron (B) a trace element, a non-metal, is one of the eight essential micronutrients that are required by plant for their optimum growth and development. Maze (1919) was the first to recognize boron as an essential element for optimum plant growth of maize (*Zea mays* L.) plants. Warington (1923) illuminated relevance of boron in the development of broad bean (*Vicia faba* L.). Following this, Brenchley and Waeikngton (1927) displayed significance of boron in plant growth in different plant species. By the 1930s, boron was well recognized as an essential micronutrient for plants. Plants growing in soil that are deficient in boron concentration were found to have reduced crop yields and compromised crop quality. Boron requirement varies significantly across all plant species. For example, while corn requires increased boron concentrations, gramineae requires much lower amount. Decreased boron availability causes some of the most noted disorders in plants such as cracked stems of celery *Apium graveolens* L., brown heart of *Brassica napobrassica* Mill. and *Raphanus sativus* L. roots, brown heart of *Brassica oleracea* var. *botrytis* L., internal brown spots of *Ipomoea batatas* Lam. and heart rot of *Beta vulgaris* L. (Gupta and Gupta 2016). Boron is essential in regulating cell elongation and division, as a result of which it directly impacts root growth (Shelp 1993). Boron deficiency was found to have an adverse effect on root length elongation. It was found that root elongation in seedlings of *Cucurbita pepo* L. reduced within 3 h of removing boron supply and completely stopped within 24 h. However, restoring boron supply within 12 h restored root length elongation within 12–18 h (Bohnsack and Albert 1977). In *Helianthus*

annus L. presence of boron in soil resulted in development of adventitious roots (Josten and Kutschera 1999). Further it was found that in contaminated soils that have increased aluminium content and are acidic, application of boron prevented aluminium-mediated inhibition of root growth (Lenoble et al. 1996).

In boron-deficient soils, protein synthesis has been reported severely affected (Carpena Artes and Carpena Ruiz 1983). However, in such studies various parameters such as age of the plant, stage of organ development, localization and remobilization of proteins have not been taken into consideration (Shelp 1988). For example, growing bean (*Phaseolus vulgaris* L.) cotyledons without boron for 5 days increased protein concentration in comparison to control plants suggesting hindrance of nitrogen remobilization due to boron deficiency (Dave and Kannan 1981). However, protein concentrations in actively growing regions was found to slow down in incidences of boron deficiency (Duggar 1983; Shelp 1993). Partitioning of nitrogen into soluble components such as ammonium, nitrate and amino acids was found boron dependent in *Brassica oleracea* var. *botrytis* L. This, in turn, was found dependent on plant organ under study and concentration and duration of boron supply (Shelp 1993). Relative amino acid composition was not found affected due to boron deficiency. Inorganic nitrogen in plant tissues and translocation fluids was substantially increased. Boron deficiency increased nitrate reductase activity in *Beta vulgaris* L., *Lycopersicon esculentum* Mill., sunflower and corn (Bonilla et al. 1997; Kastori and Petrović 1989). In *Nicotiana tabacum* L., boron deficiency resulted in decreased leaf nitrogen and also resulted in decreased nitrate reductase activity (Camacho-Cristóbal et al. 2008). Boron-deficient plants *Glycine max* Merr. showed low acetyls reduction activities and damaged root nodules (Yamagishi and Yamamoto 1994). In *Vigna unguiculata* Walp, acute boron deficiency resulted in increased amounts of reducing and non-reducing sugar concentrations and at the same time decreased starch phosphorylase activity (Chatterjee et al. 1990). Boron deficiency has been documented to result in increased accumulation of phenolic substances as a result of upregulation of genes responsible for pentose phosphate shunt (Hajiboland and Farhanghi 2010). Foliar spray of boron to sunflower displayed increased accumulation of non-reducing sugars and starch concentrations (Shehzad et al. 2016). Such findings suggest a specific role of boron in the production and accumulation of starch and sugar reserves in sunflower seeds. Similar instances of increased accumulation of non-reducing sugars and starch were also found in *Brassica nigra* Koch (Sinha et al. 2000) and *Nicotiana tabacum* (Camacho-Cristóbal and González-Fontes 1999), respectively. In leaves of boron-deficient plants of *Pisum sativum* L., concentration of both starch and sugars increased. However, a marked decrease in their accumulation was noted in seeds that severely affected seed quality (Sinha et al. 2000).

In addition, several studies have reported beneficial role of boron in plant growth and metabolism by regulating processes such as auxin and phenol metabolism (Camacho-Cristóbal et al. 2018), formation of flowers and subsequent seed production (Zohaib et al. 2018) and membrane function (González-Fontes et al. 2014).

4.2 Silicon

In plant growth and metabolism several essential macro- and micronutrients play a central role. Many plant scientists, do not consider silicon (Si) as an essential plant nutrient. However, a plethora of evidence has been generated in recent years to suggest a cardinal role of Si in determining plant growth and quality. In addition to being central in governing important biological processes of plants such as transpiration and photosynthesis, Si adapts a plant to grow in adverse conditions of nutrient deficiency, drought, temperature etc. Si supplementation has seen positive effects on plant growth and development in many plants species. This effect was seen more pronounced in plants where plants growing in soils with limited Si concentration were supplemented with optimum Si. In the same study, Si accumulated in a different plant tissue was found to vary between different species, suggesting that the affinity for Si uptake and localization varies between different plant species and tissues, respectively. Plants growing in soils with increased cadmium concentration when supplemented with Si displayed lower ROS species compared to control plants (Hasanuzzaman et al. 2017), suggesting role of Si in antioxidant defence mechanisms. In addition to improving antioxidant defence mechanisms against Cd stress, Si has been established to augment glyoxalase pathways, increase activity of AsA-GSH and production of antioxidant components. Varying dosage of Si supplementation was found to revive Si-deficient plants significantly. In *Zinnia* and *Helianthus* robust stem structures found associated with Si supplementation. The flower size was found increased in *Gerbera* following Si foliar sprays. In all these species, the flower quality increased and flowering time was found substantially reduced with silicon treatments compared to control plants.

Deciphering role of Si in disease resistance and flower size has intrigued a lot of scientists globally. Si supplementation reduces water loss by plants making this research relevant in present times. Si regulates this aspect by regulating development of a waxy layer on plant that significantly reduces rate of transpiration by the plant. Reduction in transpiration rates has proven benefits to plants. Si supplementation was found to regulate functioning of the stomatal valves, thereby affecting stomatal conductance. While growing in greenhouses, the leaves transpired less with increased Si supplementation. While this study implicates role of Si in regulating stomatal conductance, an active role of Si in the process remains to be established with certainty. In addition, Si has also been implicated to alleviate heat stress in plants as it imparts thermal stability to lipids in cell membranes. However, the mechanism remains uncharacterized thus far.

A role of Si has been reported in preventing incidences of powdery mildew disease. Kanto et al. (2009) found that with increase of Si content in leaves, the incidences of powdery milder disease substantially decreased. Similarly, in wheat and barley Si deficiency was found associated with susceptibility to powdery mildew and poor growth (Zeyen et al. 2002). Use of Si foliar sprays was found to prevent powdery mildew disease in grape, muskmelon and cucumber (Bowen et al. 1992).

Datnoff (2005) reports prevention from several disease in turf grass with application of Si foliar sprays. Si supplementation has also been reported to alleviate various chemical stresses such as metal toxicity, nutrient imbalance and salinity. In rice and barley Si supplementation was found to benefit the plant phosphorus deficiency. Beneficial effects of Si include improved structural cell strength, improved absorption of nutrients and reduced salt stress.

One of the first Si sprays to be extensively used is 13 Essentials. It is the first foliar nanoscale (particle size of 1–30 nm) fertilizers to be commercialized in the US market. It boasts an optimum mix of primary nutrients (P and K), secondary nutrients (Ca, Mg and S) and micronutrients (Fe, Cu, Mn, B, Zn, Co and Mo) adsorbed on a nano-silica base. In this foliar spray, Si serves as both a carrier for other nutrients and a nutrient, thereby reducing the possibility of complexing and making the nutrients efficiently available to the plants.

4.3 Selenium

Selenium is an important trace chalcogen metalloid which exists in very low concentration in the earth (Hawrylak-Nowak et al. 2014; Pilon-Smits et al. 2009). Selenium essentiality for the optimal development in higher plants has been well debated (Terry et al. 2000). However, the consistent efforts in this field of research have confirmed the role of selenium in plant's growth (Hartikainen and Xue 1999; Hawrylak-Nowak et al. 2014), reproduction (Cao et al. 2018; Hladun et al. 2013), metabolism (Schiavon and Pilon-Smits 2016; Ning et al. 2013) and in delaying senescence (Rahmat et al. 2017; Pukacka et al. 2011). In addition, it has been established to play a significant role in tolerance against various stresses such as oxidative stress (Mroczek-Zdyrska and Wójcik 2012), biotic stress (Hanson et al. 2004) and abiotic stress (Malerba and Cerana 2018; Nawaz et al. 2015). Table 1 shows some of important studies on the role of selenium in plants.

Table 1 Significant studies highlighting the role of selenium in plants

Sl no	Plants	Effects	References
1	*Triticum aestivum*	Drought stress	Nawaz et al. (2015)
2	*Acer saccharinum*	Recalcitrant	Pukacka et al. (2011)
3	Oryza sativa	Biofortification	Boldrin et al. (2013)
4	*Cucumis sativus*	Salt stress	Hawrylak-Nowak (2009)
5	*Brassica rapa*	Increases seed production	Lyons et al. (2009)
6	*Spirulina platensis*	Decreases Cr uptake	Belokobylsky et al. (2004)
7	*Brassica juncea*	Aphids resistance	Hanson et al. (2004)
8	*Lactuca sativa*	Antioxidative and growth promoting	Xue et al. (2001)

5 Metalloid Distribution in Plants

Many metalloids are considered an essential micronutrient for higher plants because of assigned important roles in various processes. These metalloids' concentrations in plants are directly related to phyto-availability of these elements in the soil due to natural presence, anthropogenic contamination or foliar application of fertilizers (White 2015; Camacho-Cristóbal et al. 2018). In addition, these beneficial elements are taken in different forms by many transporters (Ding et al. 2008; Archana and Verma 2017; Kumarathilaka et al. 2018). In addition, there are few articles in the literature that have tabulated distribution of various metalloids in various genera and species (Pilon-Smits et al. 2017; White 2015; Camacho-Cristóbal et al. 2018). Table 2 shows the distribution and accumulation of metalloids.

6 Uptake Mechanism and Transporters Involved in Metalloid Uptake in Plants

6.1 Boron Transporter

Boron (B) is an essential metalloid required for the development and growth of the plant. B is an important component of the cell wall that cross-links rhamnogalacturonan-II, a pectic polysaccharide to it, and maintains the integrity of cell wall and growth of the plant (Kato et al. 2009). B deficiency causes a severe impact on the organ expansion including abnormal cell wall, altered cytoskeletal polymerization, defects in the leaf expansion, root elongation, flower and fruit development (Marschner 2012). In plants, B is prevalent in leaves; however, its excess causes the retarded growth, peculiarity of shoots, and chlorosis of leaf tips and margins

Table 2 Accumulation of different metalloids in plant species, parts and regions

Elements	Genera	Regions	Plant parts	References
B	*Lycopersicum esculentum, Hordeum vulgare, Brassica napus*	Turkey, USA, Asia	Stem, leaves	Pommerrenig et al. (2015), Camacho-Cristóbal et al. (2018)
Si	*Oryza sativa, Glycine max, Helianthus annus*	China, Southeast Asia, Africa	Stem, leaves, straw, flag leaf, husk and grains	Ma and Yamaji (2015)
As	*Oryza sativa, Pteris vittata*	China, Southeast Asia	Stem, leaves, straw, husk, germ, and grains	Kumarathilaka et al. (2018)
Se	*Astragalus praelongus, Brassica oleracea, Stanleya pinnata, Lecythis ollaria*	USA, Australia, China, Mexico, Europe	Fruits, stem, leaves, Cladodes	Pilon-Smits et al. (2017), Lindblom et al. (2018)

(Reid and Fitzpatrick 2009). Toxicity mediated by B is prevalent throughout the world, including Turkey, South Australia, Mediterranean countries, Chile and California (Miwa and Fujiwara 2010). In plants, B transportation is mainly done from root to shoot through the symplastic and apoplastic movements. Studies using sunflower suggested the passive mode (gradient) of transportation of B (Dannel et al. 2000). Boron is generally present in soil solution as boric acid and is taken up by the plants in the same form. Boron is transported in the plants through two transporters BOR1 and NIP5;1, which are involved in efficient translocation of boron under its deficit. NIP5;1, boron importer, is a member of nodulin-26-like intrinsic protein (NIP) subfamily of the aquaporins (Maurel et al. 2015). High boron concentrations lead to reduction in the expression of level of NIP5;1 root elongation and root hair zone. It is established as an essential transporter. A T-DNA insertion mutant of nip5;1 was found to have reduced biomass and plant growth under boron-limited conditions (Tanaka et al. 2011, 2016). In maize, TASSEL-LESS1 (TLS1) gene encodes NIP3;1, an aquaporin family member and orthologue of *Arabidopsis* NIP5;1 which is involved in boron transport under boron deficiency (Durbak et al. 2014). In rice, Os NIP3;1 (homolog of AtNIP5;1), is induced during plant growth and development under B-deficient conditions (Hanaoka et al. 2014). In contrast to nip5;1 which displays decreased root and shoot growth under boron deficiency, bor1-1 mutant encoding boron transporter 1 shows reduction only in shoot tissue under B deficit. Promoter studies for BOR1 identified it to be expressed chiefly in the root pericycle cells, and BOR1–green fluorescent protein (GFP) fusion protein was localized to the plasma membrane. Further, experiment done on BOR1 mutant yeast complemented with *Arabidopsis* BOR1 led to a threefold reduction of boron in yeast cells implying BOR1 is an exporter of B. It was also the first B transporter to be identified in the biological system (Tanaka et al. 2008). Rice has four copies of Bor1-like gene which is less compared to the *Arabidopsis* which has seven copies of the same gene. Of the four genes, Os*BOR1* has the maximum similarity to At*BOR1*. OsBOR1 plays an important role in B acquisition by roots and translocation into shoots (Nakagawa et al. 2007).

6.2 Silicon Uptake and Transportation in Plants

Silicon (Si) is the second most copious element after oxygen in soil. Silicon dioxide comprises 50–70% of the soil mass. Abundance of Si in the soil and no visible effects of deficiency led to the consideration that Si is not important for plant growth and yield. Although Si is still not known as essential for plant growth and development, the beneficial effects of this element on the growth, development, yield and disease resistance have been observed in a wide variety of plant species like rice and sugarcane. For example, Si provides resistance against various biotic (Fauteux et al. 2006; Marschner 2012) and abiotic stresses, including drought stress, salt stress, water logging, metal toxicity, nutrient inequity, radiation exposure, freezing and heat (Ma 2004; Coskun et al. 2016), especially for crops like rice and sugarcane.

Plants take up Si as silicic acid [$Si(OH)_4$] from the soil. Si content in plants is equal to or greater than the macro-nutrients N, P, and K, which are supplied through fertilizers (Meena et al. 2014). There are two types of Si transporters in plants: (a) channel type transporters and (b) efflux transporters. Low Silicon1 (Lsi1) and Lsi6 belong to the influx- or channel-type transporters of silicic acid. Lsi1 is a member of nodulin-26-like major intrinsic protein III (NIP III) subgroup of aquaporins and acts as a Si-permeable channel. It is localized in different tissues in different plants, for example, rice Lsi1 is localized in the lateral side of root exodermis and endodermis (Ma et al. 2006). Lsi1 in maize and barley is present in the epidermis and cortex (Mitani et al. 2009; Chiba et al. 2009). Si is transported bidirectionally by Lsi1. However, Si taken up into the root cells by *Lsi1* is instantly effluxed out of the cells by another transporter Lsi2 in rice, generating a concentration gradient from the external solution to the root cells; thus, Lsi1 only functions as an influx transporter in rice roots (Mitani et al. 2008; Ma et al. 2006). Maize Si influx transporters (ZmLsi1 and ZmLsi6) are homologues of OsLsi1 and OsLsi6, respectively, but unlike OsLsi1 their expression is not affected by Si availability (Mitani et al. 2009). *OsLsi2* was first identified in rice; further, its homologues were reported in other plant species. Although *OsLsi2* works in conjunction with *OsLsi1*, they do not bear any structural similarity with *OsLsi1* transporters (Ma et al. 2007). In *Arabidopsis*, the *AtLsi2*-like transporters are prevalent compared to the *AtLsi1* (NIPIII), emphasizing dominant role of *OsLsi2*-like transporters. In tomatoes, the NIPIII and *Lsi2*-like transporters are not involved in the Si accumulation (Mitani et al. 2005) that confirms the role of other factors like gene expression, localization, polarity of the transporters, and others in the Si transportation and accumulation.

6.3 Selenium Transporter

Selenium (Se) is an essential trace element for humans and animals. Selenium in soil varies from 0.01 to 2 mg kg^{-1}, and in selenium-rich areas, Se content <1200 mg kg^{-1} has been reported (Fordyce 2005; Stroud et al. 2010). Elevated Se concentration is toxic to the living organisms since it bears chemical similarity to S that might cause the replacement of S by Se in the proteins (Terry et al. 2000). It also affects the enzymatic activity of peroxidases, which catalyses the oxidation of thiols leading to reactive oxygen species (ROS) production harmful to the plants (Groppa et al. 2007). Se from the soil is acquired in the form of selenate (SeO_4^{2-}), selenite (SeO_3^{2-}; $HSeO^{3-}$; H_2SeO_3) or organoselenium compounds, such as selenocysteine (SeCys) and selenomethionine (SeMet) (White and Broadley 2009). Selenate is the major water-soluble form of Se in aerobic soils, while selenite mostly occurs in anaerobic soils such as paddy soils (Pilbeam et al. 2015). Selenate is taken up by the root cells through high-affinity sulphate transporters (HASTs), homologous to the *Arabidopsis thaliana* sulphate transporters (AtSULTR1;1 and AtSULTR1;2) (Gigolashvili and Kopriva 2014). Similarly, enhanced uptake of selenate in S-starved wheat plants validates the positive relation of sulphate transporter with selenate uptake since plants upregulate the expression of sulphate transporter genes in roots

under sulphur starvation (Buchner et al. 2004; Li et al. 2008). Unlike selenate, sulphur starvation did not have significant effect on selenite uptake in major crops like wheat and rice (Li et al. 2008; Zhang et al. 2006). However, in wheat, selenite uptake was found to be enhanced under the phosphate starvation, which expectedly increases the expression of the phosphate transporter genes (Li et al. 2008). In rice, phosphate transporter, OsPT2, mediates selenate uptake (Zhang et al. 2014a), whereas selenite is transported via OsNIP2;1 transporter encoding aquaporin channel (Zhao et al. 2010b). Selenite assimilated through roots is readily converted to organic forms such as selenomethionine (SeMet) and selenomethionine Se-oxide hydrate (SeOMet) (Li et al. 2008), and only slight selenite was transported into xylem. Faulty incorporation of the products such as SeMet or SeCys in proteins distorts structure as well as function of protein and poses toxicity in plants (Fig. 1) (Gupta and Gupta 2016).

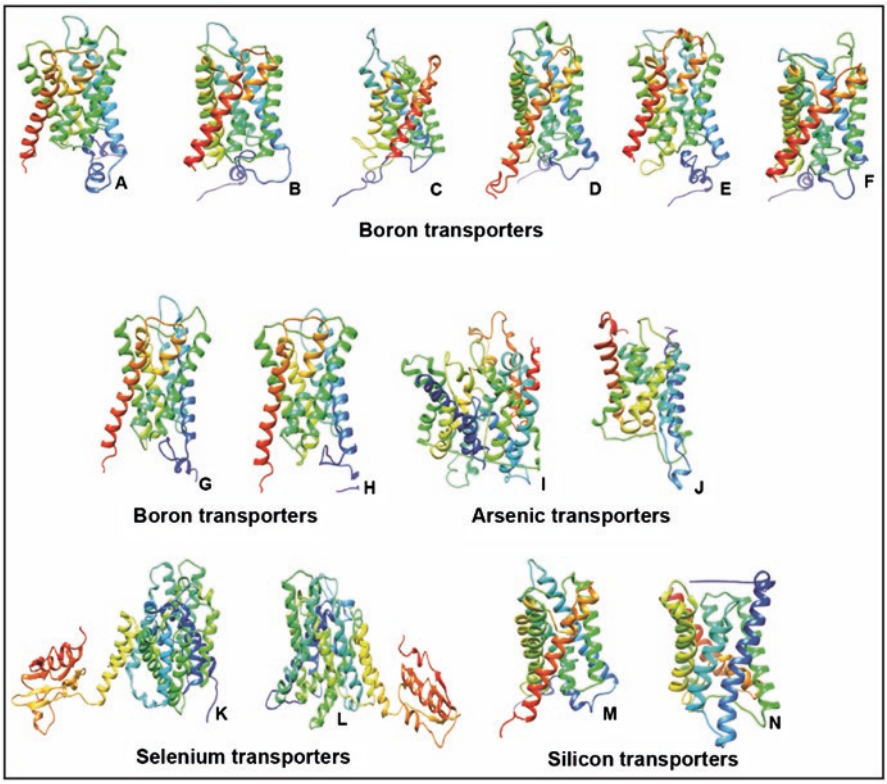

Fig. 1 Model structures of various metalloid transporters: (**a**) OsNIP3.1, (**b**) OsNIP3;2, (**c**) OsPIP2;4, (**d**) OsPIP2;6, (**e**) OsPIP2;7, (**f**) AtNIP3;1, (**g**) AtNIP5;1, (**h**) AtNIP7;1, (**i**) AtBOR4, (**j**) AtTIP4;1, (**k**) AtSULTR1;1, (**l**) OsSULTR1;2, (**m**) SbLsi1 and (**n**) OsLsi2 generated using PHYRE2 web portal (http://www.sbg.bio.ic.ac.uk/~phyre2) (Kelley et al. 2015). Image coloured by rainbow N → C terminus

7 Co-Transporters Like Arsenate and Citric Acid Transporters

7.1 Arsenate Transporters in Plants

Arsenic (As) is a toxic metalloid with an estimated concentration of 1.5–3 mg kg^{-1} in soil (Farooq et al. 2016). Several natural processes like the weathering of rocks, volcanic emissions, hot spring releases, mining, smelting and others are the major sources of As pollution. Among the major crops, rice readily takes up As and translocate it to the grains, making it unfit for the population dependent on it (Zhao et al. 2010a). Arsenic exists in the form of several inorganic and organic forms. Arsenate [As(V)] is the major inorganic form in aerobic soils while arsenite [As(III)] predominates in anaerobic soil environment. Organic forms of As include methylated species such as monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) (Mendoza-Cózatl et al. 2011). As(V) is transported in the plants via phosphate transporters due to the structural similarity with phosphate (Pi). PHOSPHATE TRANSPORTER1 (*Pht1*) proteins are characterized by 12 membrane spanning domains bearing similarity to the yeast Pho84p high-affinity Pi transporter (Rausch and Bucher 2002). Among the phosphate transporter family, *Arabidopsis thaliana PHT1;1* and *PHT1;4* are the two high-affinity transporters involved in As uptake. Further, *pht1;1pht1;4* double mutant in *Arabidopsis* was reported to have tolerance against As(V) stress, suggesting major role of these transporters towards As(V) uptake (Shin et al. 2004). Later a study by González et al. (2005) reported the role of *Arabidopsis* mutant defective in phosphate transporter traffic facilitator 1 (*PHF1*) (involved in trafficking of PHT1;1 from endoplasmic reticulum to plasma membrane) in arsenate metabolism. *Atphf1* mutant was found to have greater tolerance towards arsenate stress compared to the wild type emphasizing importance of Pht1;1 in arsenate uptake. The mutants of the PHS family like the *AtPht1;1* have slow As uptake, but it accumulates twice compared to the wild type (Catarecha et al. 2007). Another study by DiTusa et al. (2015) reported *PvPHT1;3*, a novel PHT1 member cloned in the As hyper-accumulating fern *Pteris vittata*, to have comparable and a higher affinity for Pi and As(V), respectively, compared to *Arabidopsis thaliana AtPHT1;5*. In rice, *OsPht1;8*, transporter which is expressed in both the root and shoot tissue independent of Pi supply, possesses high affinity for both Pi and As (V). Plants overexpressing *OsPht1;8* in rice show increased As(V) uptake and translocation (Wu et al. 2011). Transcript abundance of *OsPht1;8* is known to be regulated by the transcription factor OsPHR2 (Pi starvation response 2) (Wu et al. 2011). Similarly, high-affinity phosphate transporter, OsPht1;1, located in the plasma membrane participates in the As transportation in rice (Kamiya et al. 2013). The regulatory mechanisms governing Pht transporters are still not well defined, but WRKY transcription factors like the WRKY6 and WRKY45 are found involved in the As influx (Castrillo et al. 2013; Wang et al. 2014). WRKY6 is reported to regulate As(V) uptake by repressing expression of the As(V)/Pi transporter PHT1;1 (Castrillo et al. 2013).

7.2 Citric Acid Transporters

Carboxylates like the malate, fumarate and citrate are known to be major constituents of the living system. These metalloids are involved as a precursor or intermediates in the energy, metabolism, biomolecule synthesis, chelators for metallic nutrients and the heavy metals (Ovecka and Takac 2014). Citric acid is a major metalloid that is reported as an iron chelator and is transported by the multidrug and the toxin extrusion (MATE) class of transporter family (Wu et al. 2014). This transporter family is induced during the Fe deficiency.

Citrate transporters like Ferric Reductase Defective 3 (FRD3), OsFRDL1, MtMATE66 and MtMATE69 are prevalent in the leaves, roots and stem that required for the root–shoot translocation of metal ions (Durrett et al. 2007; Yokosho et al. 2009; Pineau et al. 2012). Some transporters like HvAACT1 in barley (Furukawa et al. 2007) and SbMATE in sorghum (Doshi et al. 2017) are well studied. Some of the transporters for the citric acid transportation are FRD3, OsFRDL1 and MATE (multidrug and toxic compound extrusion or multi-antimicrobial extrusion).

FRD3 protein belongs to the multidrug and toxin efflux family that participates in transportation of the chelators like the citrate for efficient distribution of iron throughout the plant (Durrett et al. 2007). Its effluxes citrate into the xylem to form a ferric–citrate complex. The other well-known transporter for the citrate transporter in rice is the OsFRDL1 that shares homology to the HvAACT1 (barley citrate transporter) (Furukawa et al. 2007). This transporter is localized in the pericycle of the cell and transports Fe–citrate complex to the shoot. MATE class of transporters is another well-known citrate transporter which is 400–550 amino acid long comprising 12 transmembrane domains (TMDs). They are involved in the transportation of the secondary metabolite out of the cytosol due to the electrochemical gradient of membrane. They also referred to as a DETOXIFICATION (DTX) protein that participates in the detoxification of the heavy metal contaminants, disease resistance and other biological processes. MATEs are primarily involved in the Al^{3+} detoxification and the Fe uptake by forming complex with the citrate.

7.3 Transporters Unanimously Transporting Beneficial and Harmful Metalloids: Aquaporins

Water transporters that were purified forming the red blood cells were first reported by Peter Agre (Agre et al. 1993). This water channel was later characterized using the *Xenopus* oocyte and named aquaporins1 (AQP-1) that belongs to the major intrinsic protein (MIP) family (Deshmukh et al. 2013). The MIP family comprises aquaporin (water and ion transportation), the glycerol facilitators (glycerol transportation) and the aqua glyceroproteins (water and small uncharged molecules like polyols, urea and arsenite). This family comprises the six transmembrane domains

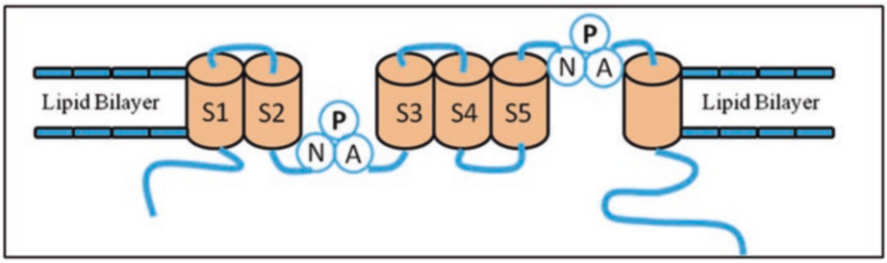

Fig. 2 A typical structure of aquaporin channel in plants

with five loops, and an N-terminal and C-terminal domain (Frick et al. 2014). At the centre, it has an NPA (Asn–Pro–Ala) motif that provides the substrate selectivity (Fig. 2) (Kitchen and Conner 2015).

7.3.1 Aquaporins in Plants

The tonoplast protein γ-TIP (AtTIP1;1) of *Arabidopsis* is a first characterized aquaporin (Rivera-Serrano et al. 2012). The aquaporin transporters are involved in different physiological processes like the cell elongation, seed germination and osmoregulation. A total of 35 MIPs are known in *Arabidopsis* (Deshmukh et al. 2013), 36 in maize (Bansal and Sankararamakrishnan 2007) and 33 in rice (Sakurai et al. 2005) compared to the mammals (Borgnia et al. 1999), *Escherichia coli* (Agre and Kozono 2003) and *Saccharomyces cerevisiae* (Pettersson et al. 2012). The higher number of aquaporins is due to continuous water absorption, flux and evaporation during growth and development (Li et al. 2014).

7.3.2 Aquaporin Mode of Activity

Aquaporin has Ar/R (aromatic–arginine) pore region that is located near to the NPA–NPA region and provides selectivity to the transporter (Deshmukh et al. 2013). In NIPs, the Ar/R provides an additional advantage by allowing neutral metalloids, undissociated acids and small solutes like glycerol across the plasma membrane.

8 Transgenic Plants with Improved Uptake of Beneficial Metalloids

From the last two decades, the researchers have expedited the field of transgenic biotechnology several folds mainly with advances in high-throughput transformation protocols, donor genes isolation from diverse organisms and available

databases (Mitani et al. 2011; Deshmukh et al. 2015; Song et al. 2017). As a result, the scientists have pushed their research prospects in improving the photosynthetic capacity, yield, nutrition content, aroma, biotic stress tolerance, heavy metal toxicity and metalloid uptake (Helliwell et al. 2013; Quilis et al. 2014; Kudo et al. 2017; Bardor et al. 2018). This is also evident from the fact that the term 'transgenic plants' fetched around 517,000 publications in the Google scholar (https://scholar.google.co.in/). It is very challenging to include all the transgenic plants in this book chapter; therefore, we will only list about the main transgenic plants with improved uptake of beneficial metalloids (Wang et al. 2017, 2018; Sun et al. 2017; Song et al. 2017; Mitani et al. 2011; Pérez-Castro et al. 2012; Tanaka et al. 2013; Chen et al. 2017).

8.1 Enhancement of Boron Uptake

Besides being rare element, B is considered as an essential element for normal growth, development, metabolism, signalling and reproduction in plants (Camacho-Cristóbal et al. 2018). Earlier it was a notion that the plant cells maintain the levels of boron at optimum concentration through unregulated simple diffusion (Dannel et al. 2000). However, Takano and co-workers (2002, 2008) discovered the involvement of specific transporter proteins in boron trafficking across the roots cell membrane and xylem loading. Their finding was also supported by the work of Miwa et al. (2006) and other groups (Uraguchi and Fujiwara 2011; Marschner 2012). Therefore, the understanding is necessary to manipulate and develop plants that are resistant to B deficiency and toxicity. Due to this, the researchers have aimed their research prospects in understanding the (1) molecular aspects of the transporters of these metalloid elements; (2) role in uptake, distribution and utilization; (3) effects of deficiency or toxicity of metalloids; and (4) development of plants with increased metalloid use efficiency (Camacho-Cristóbal et al. 2018). As a result, the scientists have made multiple transgenic plants over the last decades (Pang et al. 2010; Pérez-Castro et al. 2012; Tanaka et al. 2013; Wang et al. 2017).

Miwa et al. (2006) developed the *Arabidopsis* transgenic lines by overexpressing *AtBor1* and reported the enhanced tolerance to B-deficient conditions and increase in the seed content. Kato and group (2009) overexpressed the *AtNIP5;1* in *A thaliana* plants and observed an increase in the tolerance to B-deficient conditions. They speculated the enhanced tolerance was probably due to enhanced B initial uptake.

Pang et al. (2010) overexpressed the *AtTIP5;1* in *Arabidopsis* and found a significant increase in tolerance under high boron conditions. Tanaka et al. (2013) examined the OsBOR4 function in relation to the accumulation of boron in the leaves and flowers by using the knockout mutant lines in rice. Their results established that OsBOR4 is cardinal to the reproductive process in rice. Similarly, the overexpression of *AtBOR1* in *Solanum lycopersicum* showed a better survival under the B-deficient conditions (Uraguchi et al. 2014). Takada and group developed *AtBOR2* overexpression lines in

Arabidopsis and found the enhancement in root growth and seed setting under B-deficient conditions (Takada et al. 2014). Additionally, the AtBOR1 orthologoues genes have been overexpressed in plants, including citrus, grape, maize and rice (Nakagawa et al. 2007; Pérez-Castro et al. 2012; Cañon et al. 2013; Chatterjee et al. 2014) growing under B-deficient conditions.

Kumar et al. (2014) characterized the role of *OsPIP2;7* and *OsPIP2;4* genes in B permeability. They found that the high B conditions elevated the *OsPIP2;7* and *OsPIP2;4* expressions in rice roots using transcriptome analysis. Furthermore, they heterologously overexpressed *OsPIP2;7* and *OsPIP2;4* in the *A. thaliana*. The overexpression lines displayed a significant enhancement in higher biomass, root length and shoot length. Furthermore, there was an increase in B accumulation in transgenic plants in comparison to the control plants. Hanaoka et al. (2014) characterized the *OsNIP3;1* in rice (*Oryza sativa*). They expressed the *OsNIP3;1* gene in yeast cells and found the enhancement in the uptake of boric acid compared to control cells. They even heterologously expressed GFP-tagged OsNIP3;1 in the tobacco plant and reported the OsNIP3;1 localized to the plasma membrane of exodermal and pericycle cells. Furthermore, they revealed the OsNIP3;1 transcript accumulation increased up to fivefold in roots under low B conditions only. Even, using RNA-interference (RNAi) technology, the effect of OsNIP3;1 knockout was seen on growth under different B supply. In another instance, Liu and group characterized a dwarf and tiller-enhancing 1 (dte1) mutant of rice, which exhibited many defects such as impaired pollen fertility, retarded growth and more numbers of tillers under low B conditions. Using RNA-interference, transgenic complementation and map-based cloning, they revealed the *DTE1* gene encodes an *AtNIP5;1* orthologoue. In addition, they found the subcellular localization using β-glucuronidase (GUS) staining and studied the DTE1 transcript accumulation profile in vegetative organs under B starvation. The RNAi mutant lines showed a steep decline in the total B content under B-deficient conditions.

Similarly, Wakuta et al. (2015) studied the polar localization and evolutionary divergence in borate exporter family. Additionally, they generated both *AtBOR1* overexpression and RNAi mutant lines to study boron-dependent vacuolar sorting. In another study, Mosa et al. (2016) provided the experimental evidence about the bidirectional transport of boron by OsPIP1;3 and OsPIP2;6 in rice. Heterologous overexpression of OsPIP2;6 and OsPIP1;3 in *A. thaliana* led to enhancement of tolerance to B toxicity. Interestingly, the *10B* was effluxed from the roots in the transgenic plants. More recently, Wang and co-workers (2017) studied the role of clathrin-mediated endocytosis in *NIP5;1* polar localization of epidermal and endodermal cells in the roots. Additionally, they found the role of arrangement is mediated by the phosphorylation of Thr residues of the N-terminal region. Lv et al. (2017) reported a *shb1* (sensitive to high level of boron 1) mutant which exhibited hypersensitivity under high boron conditions. They found that *SHB1* gene upregulates in roots under excessive boron treatments. Additionally, it upregulates the transcription of the *BOR4* gene and alters the boron uptake in root cells. More recently, Porcel et al. (2018) screened complementary DNA (cDNA) library of *Beta vulgaris* and identified a BvCOLD1 gene which codes for a protein with a role in the

transport of several molecules, including boron. The heterologous overexpression of *BvCOLD1* in *A. thaliana* led to enhancement in tolerance against many abiotic stresses as well as boron uptake.

8.2 Silicon Uptake and Transgenic Plants: A Short Story

In the earth's crust, silicon is the second most abundant element; however, it is considered important for plant defence. In the literature, the articles about the transgenic plants with higher uptake of silicon are very less. For the first time, Ma and group (2006) described the low silicon rice 1 (Lsi1) gene in the *O. sativa* cultivar Oochikara, which plays a role in silicon accumulation. They studied the cellular and subcellular localization; in addition, they reported the Lsi1 RNAi plants that showed a decline in silicon uptake. Similarly, Chiba et al. (2009) described the cellular and subcellular localization of Lsi1 gene in *Hordeum vulgare*. They heterologously expressed the Lsi1 gene in mutant rice with defects in Si uptake. Surprisingly, the *HvLsi1* expression enhanced the Si uptake and radial transport in rice. In another instance, Montpetit and co-workers (2012) functionally characterized Lsi1 gene in wheat. In addition, they heterologously expressed *TaLsi1* and *OsLsi1* orthologoues in *A. thaliana*. The heterologous expression significantly increased the uptake by fivefold in overexpression Lsi1 lines in comparison to the wild-type control plants. Similarly, Dallagnol et al. (2013) evaluated the effect of soluble silicon on the wild-type and mutant rice plants with defects in the Lsi1 transporter. In addition, they evaluated the effect of *Bipolaris oryzae* on biomass accumulation, photosynthesis and soluble sugar levels. In another study, Mitani and co-workers (2011) identified a Si influx transporter in *Cucurbita moschata* cultivars Super-unryu and Shintosa. They isolated this transporter and expressed in a rice mutant with a defect in Si uptake. The transgenic lines showed the heterologous expression led to the influx of Si. In addition, the amino acid change of proline to a leucine at the position 242 by site-directed mutagenesis leads to Si transport activity loss. In another study, Fang and workers (2011) evaluated the role of the Lsi1 transporter in the defence against Ultraviolet B (UV-B) stress in rice. In order to elucidate, they generated both overexpression and knock-out Lsi1 lines and subjected to UV-B stress. They found a correlation between the Lsi1 transcript levels with Si uptake in roots. In addition, they reported the Lsi1 upregulated expression of genes related to resistance and photosynthesis, including phenylalanine ammonia-lyase using suppression subtractive hybridization. In another study, Deshmukh et al. (2015) characterized the NIP-III aquaporins which play a role in Si permeability. They performed the comparative analysis of more than 100 aquaporins in many species and predicted about 30 Si transporters with a GSGR filter and significant asparagine–proline–alanine (NPA) domain. In addition, they assessed the effect of 108 amino acids spacing on Si permeability on poplar and tomato mutants. Recently, Sun et al. (2017) studied the cellular localization and functionally characterized the *CsLsi1* gene in *Cucumis sativus* cultivar Mch-4. The *CsLsi1* gene heterologous expression in a mutant rice significantly enhanced the silicon uptake.

8.3 Transgenic Plants with Enhanced Selenium Uptake

Compared to the other metalloids, the publications for the transgenic plants with higher uptake are very few (Ellis et al. 2004; LeDuc et al. 2004; Zhao et al. 2010b; Zhang et al. 2014b; Song et al. 2017). The trial of adenosine triphosphate (ATP) sulphurylase (APS) transgenic lines in a greenhouse pot experiment displayed accumulation of Se more than threefold levels compared to wild-type *Brassica juncea* (Huysen et al. 2004). Ellis et al. (2004) isolated the SeCys methyltransferase (SMT) gene from the donor *Astragalus bisulcatus*, and the heterologous overexpression in *A. thaliana* led to a slight increase in the overall uptake of selenate. Similarly, LeDuc and colleagues overexpressed the SMT gene in the plant *B. juncea* and found the changes in the profile of Se volatilization, uptake, transport and accumulation (LeDuc et al. 2004). Later, the same group developed ATP sulphurylase (APS), SMT transgenic and double transgenic APSxSMT lines and compared the accumulation efficiency of all transgenics to the control plants (LeDuc et al. 2006). Sors et al. (2005) overexpressed the Adenosine 50-phosphosulphate reductase (PaAPR) in *A. thaliana*. The transgenic lines showed an increase in selenite uptake in comparison to the control plants. Banuelos and co-workers (2005, 2007) heterologously expressed the AtATPS1 and SMT in the plant *B. juncea* and observed an increase in selenate uptake. Additionally, they performed the field trials of these transgenic Indian mustard lines and reported no effect on the Se tolerance in the rhizosphere. El Kassis and group (2007) characterized the SULTR1;2 and SULTR1 transporters from *A. thaliana* mutant and confirmed their role in selenite uptake using a gain of function approach. Additionally, they confirmed the SULTR1;2 play a predominant role in selenate uptake. In another study, Zhang and co-workers (2014a) confirmed that OsPT2 is a negative regulator of selenite (HSeO3) uptake using OsPT2 overexpression and RNAi plants. In another instance, Zhao and co-workers (2010b) evaluated the role of OsNIP2;1 in the uptake of H_2SeO_3 in rice plant using mutant analysis and expression studies. Their work was also supported by the Pommerrenig and group (2015).

Recently, Song et al. (2017) reported the role of OsPT8 in Se uptake in tobacco. The OsPT8 overexpression in the tobacco plant led to a significant increase in the biomass, total P concentration and Se accumulation in comparison to the control plants. More recently, Wang et al. (2018) studied the differences in transcriptome profiles of shoot and root from *Stanleya pinnata* and *S. elata* when grown with or without selenite supply. They reported that genes related to selenate cycle, defence-related, oxidative stress resistance and antioxidant activity were found highly upregulated in the *S. pinnata* compared to the non-accumulator species. They reported the Se hyper-accumulation as well as hyper-tolerance were due to upregulation of SA, ethylene and JA pathway genes. They highlighted that these upregulated genes will be the targets of biofortification mediated by genetic engineering in the future publications.

8.4 Manipulating the Arsenic (As)

In the environment, arsenic is present as a well-known metalloid which exists in two variable forms, arsenite (As(III)) or arsenate (As(V)). In the recent past, the biotechnological approaches have been used to identify the genes and develop transgenic plants to increase the As uptake (Isayenkov and Maathuis 2008; Remy et al. 2012; LeBlanc et al. 2013; Xu et al. 2015; Chen et al. 2017).

Dhankher et al. (2002) pyramided two bacterial genes, γ-glutamylcysteine synthetase and As(V) reductase, in *A. thaliana* and found enhancement in the accumulation of As in shoots as compared to the control plants. Navaza et al. (2006) overexpressed glutathione synthetase and gamma-glutamyl cysteine synthetase in *Brassica juncea* and observed higher As accumulation and uptake. In a series of publications, Ma et al. (2007, 2006) studied the cellular localization and role of low silicon rice family members (Lsi1 and Lsi2) in As(III) influx and efflux. They knock out the expression of both transporters and studied the effect on As uptake, trafficking and concentrations in the grain and straw. Grispen et al. (2009) heterologously expressed the *Arabidopsis* metallothionein gene in the tobacco and reported the significant change in As accumulation and uptake. Isayenkov and Maathuis (2008) confirmed the role of *AtNIP7;1* in arsenite uptake using overexpression and RNAi. Wu et al. (2011) overexpressed two genes Phosphate transporter (*Pht1;8*) and Phosphate Starvation Response 2 (*PHR2*) in the susceptible cultivar of *O. sativa* and increase in uptake of As(V) and phosphate. The heterologous co-expression of many members of OsPIP family in *A. thaliana* led to enhancement in the plant's tolerance towards *H2AsO3* rather than the enhancement in the As uptake as well as higher biomass accumulation (Mosa et al. 2012). In another instance, the heterologous expression of phytochelatin synthase gene from *Ceratophyllum demersum* in plants such as *Arabidopsis* and tobacco (Shukla et al. 2012, 2013) led to significant enhancement in As uptake and accumulation. However, there was no effect on the plant growth. LeBlanc et al. (2013) overexpressed the *AtPht1;7* gene in *A. thaliana* and observed the significant accumulation of As(V) in transgenic lines.

In another case, it was reported that WRKY45 and WRKY6 regulate the *AtPht1;1* expression of and, hence, modulate As(V) uptake from the soil (Castrillo et al. 2013; Wang et al. 2014). Xu et al. (2015) identified the NIP subfamily to be involved in arsenite uptake. Furthermore, they found AtNIP3;1 play a role in the arsenic uptake as well as root-to-shoot distribution under different arsenite conditions using reverse genetic strategies. The single *nip3;1* mutant accumulated less arsenic in shoots in comparison to the control plants, whereas the double mutant displayed improved growth in shoots and roots under arsenic stress conditions. They also found the *NIP3;1* gene was expressed exclusively in roots using GUS analysis. Recently, He et al. (2016) characterized a *PvTIP4;1* gene from *Pteris vittata*, which mediates the uptake of As(III) using functional complement cDNA library. Further, they analysed the effect of arsenic accumulation in *A. thaliana*; subcellular localization and the tissue expression profile of *PvTIP4;1*. The transgenic lines showed an increase in

arsenic uptake and accumulation. In another study, Wang et al. (2016) knocked out the *OsPht1;8* gene in rice in order to evaluate the effect on the uptake; contrastingly, they found that As(V) uptake significantly decreased by about 55% in transgenic lines in comparison to the control. Shi et al. (2016) identified the differential expression of OsHAC1;2 and OsHAC1;1 genes in different cells of rice roots under different arsenate treatments. The knockout mutant of both genes displayed a decrease in arsenate reduction and increase in As accumulation in the mutant plants. In the latest study, Chen et al. (2017) reported that OsNIP3;2 plays a significant role in arsenite uptake in rice lateral roots using mutant analysis and overexpression studies. More recently, Wang et al. (2018) investigated the effects of arsenate reductase, γ-glutamylcysteine synthetase and phosphate effluxer knockout on As tolerance and uptake in *A. thaliana* plants. In addition, their group overexpressed *PvACR3* from the plant *Pteris vittata*. They observed a slight change in As uptake as well as shoot-to-root translocation. All knockout mutants showed higher root-to-shoot translocation of arsenic.

9 Challenges to Improve Solute Specificity

These metalloids are elements with chemical properties between metals and non-metals. They comprise many physiologically important elements with roles in growth, development, reproduction, flowering, stress tolerance, desiccation and ultimately yield. In addition, some are toxic to plants such as arsenic, germanium and antimony as their exposure seriously downgrades the plant's metabolism. However, most of metalloids are considered beneficial for plants, and their uptake, translocation and homeostasis are mediated by various membrane transporters. The large data about these various types of transporters have already been gathered majorly by application of techniques such as the gain of function or loss of function approaches, GUS assays, functional complementation assays and promoter analysis. However, there are still many questions associated with the metalloid transport and transporters which need to be addressed.

In the future, many questions associated with metalloid transport in plants will be addressed, including (1) how metalloid-permeable transporters are regulated on metalloid exposure? (2) how do the various plant species orchestrate the transport of a given metalloid? (3) which motif of transporters determines the metalloid selectivity? (4) at which point in the evolution time scale, the nature abled the transporters to channel two types of metalloids? (5) how do the uncharged forms of metalloids are transported *in planta*? and (6) what are the potential transporters of rare elements such as Po, Te and At? In addition, the researchers will generate plants, especially major crops with higher uptake as well as higher tolerance using both breeding and transgenic approaches.

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