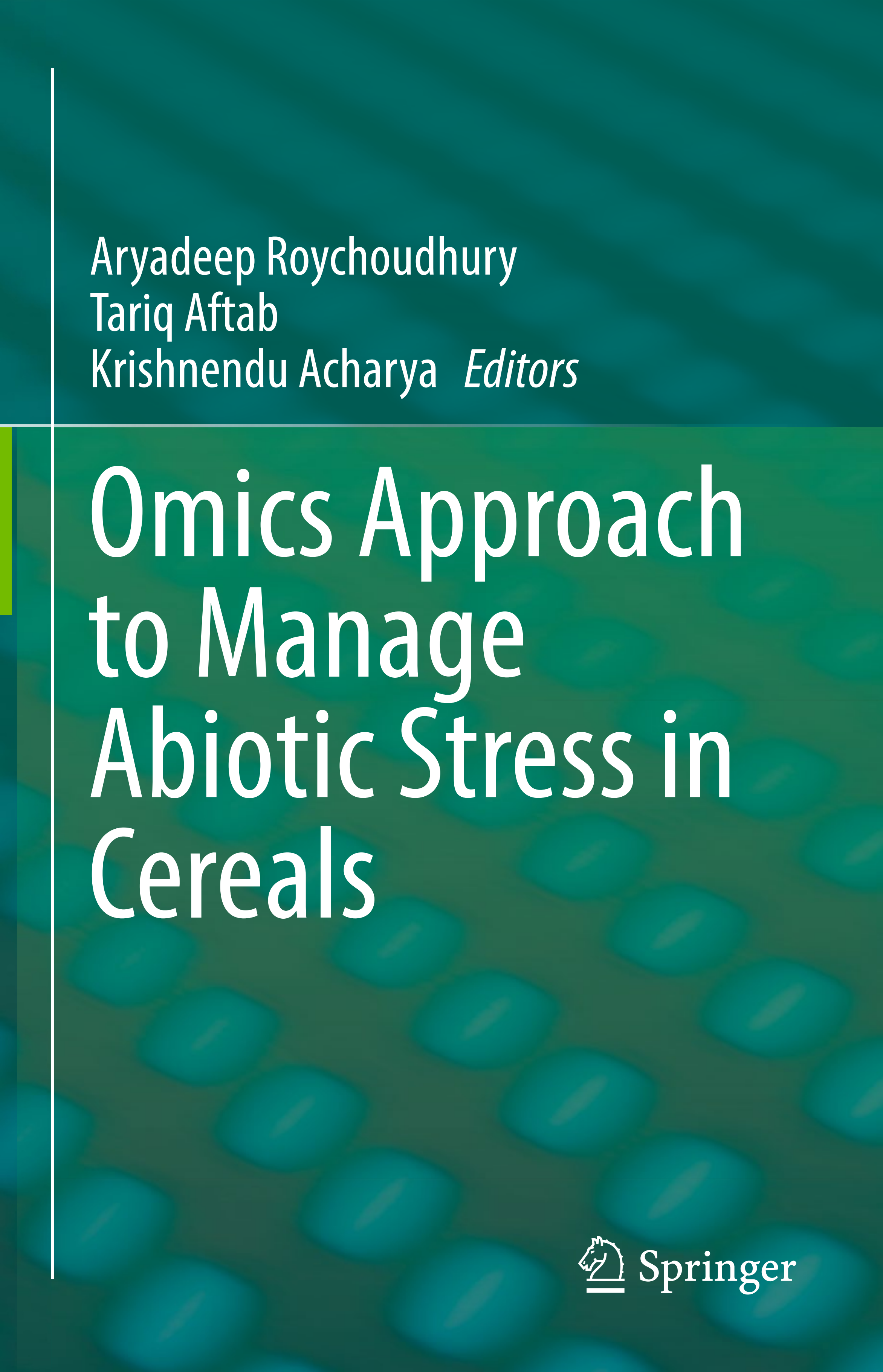

Aryadeep Roychoudhury
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Omics Approach to Manage Abiotic Stress in Cereals

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ISBN 978-981-19-0139-3 ISBN 978-981-19-0140-9 (eBook)
https://doi.org/10.1007/978-981-19-0140-9

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1

Morphological, Architectural and Biochemical Modifications of Cereal Crops During Abiotic Stress

Swarnavo Chakraborty and Aryadeep Roychoudhury

Abstract

The myriads of abiotic stressors like salinity, drought, temperature extremes, etc. can severely affect growth, yield and development of cereal crops across the world. Moreover, the devastating rise in human population needs enhancement of productivity of cereal crops. Hence, there has been an increased demand of cereal crops harbouring adaptive strategies against various abiotic stressors, in order to sustain crop productivity during stress exposure. A series of alterations at the morphological, physiological, anatomical, molecular and biochemical levels lead to the acclimation of major cereal crops under stressful environment. In addition, important plant metabolic circuitry and signalling molecules and enzymes get triggered during abiotic stress, which also forms a part of plant adaptive responses. The commonly observed mechanism associated with stress acclimation in most cereal crops involves changes in root depth and architecture, improved osmoregulation, accumulation of osmolytes and antioxidants and enhanced water use efficiency. This chapter focuses on the various adaptive modifications associated with important cereal crops across the globe, viz., rice, maize, wheat, millets, barley, rye and oats.

Keywords

Abiotic stressors · Stress acclimation · Root depth · Osmoregulation · Osmolytes · Antioxidants · Water use efficiency

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_1

1.1 Introduction

There is a devastating rise in the world population each year, which demands increased productivity of staple food crops across the globe. However, crop productivity is progressively declining due to the adverse effects of various abiotic stresses like salinity, drought, heavy metal toxicity, etc. imposed due to rapid changes in the world climate (Takeda and Matsuoka 2008; Newton et al. 2011). Moreover, insufficient and changing patterns of rainfall, rise in salinity of cultivable lands and indiscriminate application of chemical pesticides and fertilisers have resulted in conversion of cultivable crop fields into barren unfertile lands. This in turn has resulted in global food insecurity (Maiti and Satya 2014).

Cereal crops serve as the most important source of food and nutritive material for humans, as well as livestock throughout the globe. Cereals belong to the Poaceae family of monocots. These are mostly grasses, widely cultivated for their edible grain components, including the endosperm, bran and germ. Cereals have displayed great evolutionary power to different stressed environments (Giordano et al. 2021; Kumari et al. 2021). These crops constitute the most widely utilised plants in agriculture all over the world, with rice, maize and wheat forming the three most leading cereal crops, constituting almost 75% of the total grain production in the world. Cereals are the major energy and nutritional sources for both developing and developed nations and contain carbohydrates, proteins, amino acids, minerals, fibre, vitamins and micronutrients (O'Neil et al. 2010; Papanikolaou and Fulgoni 2017). Cereals like rice, millet and sorghum are maximally cultivated in the Asia; sorghum and corn are chiefly produced in Europe while oats, rye and barley in the European continent. However, the pattern of utilisation of these cereals varies amongst different countries. In developed countries, the maximum proportion of the cereals produced are channelised for feeding animals, while developing and under developed nations utilise the cereals produced for human consumption (Olugbire et al. 2021). Due to rise in global population, the pressure on the production of cereal crops has been increasing day by day. However, the progressive increase in impact of various forms of abiotic stresses has rather endangered the cereal crop productivity, leading to food crisis, starvation and even death all across the globe. The adverse effects of abiotic stresses in plants range from morphological to molecular alterations. Any abiotic stressor is associated with a resultant oxidative stress within the plant system. Stress acclimatisation in cereals is characterised by the maintenance of cellular turgor pressure and water potential; overproduction of protective osmolytes and antioxidants; accumulation of soluble sugars, amino acids and total proteins; and restoration of normal photosynthetic and transpiration rates and retarded senescence under stressful environments. In addition, a series of morphological adaptations at the cellular and tissue level, associated with the root and shoot system of such stress-tolerant plants, ultimately promotes restoration of optimal vegetative and reproductive growth and productivity during stress (Zenda et al. 2020).

1.2 Stress-Induced Modifications of Cereal Crops at the Morphological and Architectural Level

1.2.1 Rice (*Oryza sativa*)

Rice, often referred to as the “grain of life”, serves as the second most extensively consumed cereal crop across the globe. It forms the staple food crop of around half of the world population and almost 90% of the inhabitants of the Asian continent, with China, India, Pakistan and Bangladesh being the leading rice producing countries (Banerjee et al. 2020, 2021). Rice constitutes around 80% carbohydrate, 3% fibre and fat and 7–8% protein (Chaudhari et al. 2018). Owing to its dominance as a food crop, rice has been widely exploited for the improvement of health of millions of consumers in the world. Moreover, this cereal crop has also aided in the prevention of several lifestyle-related diseases, including diabetes, high blood pressure and heart ailments, cancer, Alzheimer’s disease, skin-related health issues, etc. (Muraki et al. 2015; Tan and Norhaizan 2017; Okuda et al. 2018). Unfortunately, rice plants have been reported to be highly affected by different abiotic stressors, for instance, salinity, drought, extremes of temperature, etc. (Almeida et al. 2016). Rice plants have been categorised as a salt-sensitive crop, which displays sensitivity at earlier stages of development and growth, and this in turn leads to limitation of production efficiency at maturation (Todaka et al. 2012; Das et al. 2015; Alam et al. 2020). Lee et al. (2003) established an interesting observation, pointing at the higher ability of *indica* rice varieties to tolerate and adapt against different forms of abiotic stresses, as compared to *japonica* varieties. Moreover, the type of rice species and the stage of growth also govern the ability of this cereal to tolerate different stressors (Hasanuzzaman et al. 2013).

Salinity stress is the most common form of abiotic stress experienced by rice crops. Increased levels of toxic salts result in osmotic stress and subsequent inhibition of plant growth and development due to variable alterations in the cellular metabolism of the plant system (Roychoudhury et al. 2008). Heavy metal stresses including arsenic, copper, cadmium and even fluoride salts adversely affect the growth and yield of different rice cultivars (Roychoudhury et al. 2012). Maiti et al. (2006) studied salinity-induced genetic variability and tolerance threshold of a number of rice hybrids, along with their parents, which aided in the genetic improvement of the sensitive rice varieties by breeders, in order to generate salinity tolerance. The salt-sensitive genotypes exhibited fall in the levels of chlorophyll a and b, and overall reduction in total chlorophyll and carotenoids, as compared to the tolerant ones (Maiti et al. 2009a; Cha-um et al. 2009). Moreover, salinity stress also resulted in decreased photosynthesis, due to detrimental effects on the stomatal conductance, leaf relative water content, osmotic potential, rate of transpiration, availability of CO_2, biological macromolecules like soluble carbohydrates, total amino acids and proteins and photosynthetic pigments (Sultana et al. 1999). Salt-sensitive aromatic Basmati rice varieties exhibited reduction in starch synthase activity and viability of the pollens upon exposure to excess salts (Khan and Abdullah 2003). Salt stress can enhance the senescence process in plants. Sensitive rice cultivars exhibited an acceleration of the deteriorative process associated with senescence, which has been found to be more pronounced in the older leaves, as compared to the younger ones (Roychoudhury et al. 2008). Salt-induced modifications in rice plants included rise in basal non-variable chlorophyll fluorescence (F_0) and total soluble protein concentration (Lutts and Bouharmont 1996). Salt-tolerant rice varieties exhibited upregulated activity of antioxidative enzymes like ascorbate peroxidase (Salekdeh et al. 2002) in order to combat ill effects of toxic reactive oxygen species (ROS) generated (Paul and Roychoudhury 2017). Usually, a negative correlation prevails between the extent of NaCl accumulation by respective plant parts and the plant survival under such conditions (Flowers and Yeo 1981). Tolerance in plants against salinity arises due to certain genes encoding for protective proteins like aquaporins and transporters, which tend to limit the rate of uptake and subsequent transport of salts from the soil to the different parts of the plant. This in turn helps in the maintenance of osmotic and ionic homeostasis of the plant cells and aid in the regulation of leaf development and slowing down of senescence. Most tolerant rice species tend to accumulate higher loads of salts within their roots and also compartmentalise the sodium ions within their vacuoles, in order to generate tolerance against salinity (Roychoudhury and Chakraborty 2013). Interestingly, brassinosteroids have been found to lower the impact of toxic salts on the overall growth and yield of many rice cultivars. In addition, brassinosteroids have also resulted in the restoration of the levels of important plant pigments and also elevated the nitrate reductase activity (Anuradha and Rao 2003). Overexpression of certain bacterial genes associated with trehalose production in rice led to increased salinity tolerance and improvement in overall growth and yield parameters (Garg et al. 2002).

Extremes of temperature, i.e. heat and cold stress, have serious effects on the growth, productivity, phenology, morphology and physiology of rice plants. High temperature exposure alters several plant metabolic processes including photosynthesis. At temperatures higher than 35 °C, rate of carbon dioxide assimilation declined, mainly during daytime. As a part of protection of the Photosystem II from photo-inhibition at higher temperature, the xanthophyll cycle de-epoxidation status improved and chloroplast ascorbate level increased at high temperature (Yin et al. 2010). Certain sensitive rice genotypes, upon exposure to higher temperature-induced water deficit, revealed reduction in anther dehiscence, fertility of pollens and spikelets, overall grain yield and subsequently sterility, unlike the tolerant genotypes where such effects were rather minimum (Levitt 1980; Cao and Zhao 2008; Paul and Roychoudhury 2019). Flag leaf mesophyll tissues undergo adaptations in response to heat stress. Flag leaf mesophylls of the tolerant rice cultivars tend to exhibit more tightly arranged mesophyll cells, well and completely developed vascular bundle and associated tissues and more number of closed stomata, as compared to the sensitive rice plants. However, heat-tolerant rice plants also demonstrated the capacity to maintain an intact mesophyll ultrastructure under normal temperature conditions (Zhang et al. 2009). In addition, plants having tolerance properties against high temperature stress indicated the crucial role of brassinosteroids in generating heat stress tolerance via upregulation of certain protective enzymes and proteins in leaf tissues (Cao and Zhao 2008). On the other hand, freezing-induced dehydration stress occurs as a result of intercellular accumulation of ice crystals, thereby leading to disruption and damage of cells and tissues, denaturation and precipitation of proteins and other cellular macromolecules and extensive damage at the membrane level due to the formation of multiple membrane-associated lesions and intercellular adhesions involving cell membrane and cell wall (Levitt 1980; Steponkus et al. 1993; Zhang et al. 2009). However, as a protective strategy, cold-induced ethanolic fermentation in the shoots and roots of rice plants helps in the adaptation and alteration of the membrane lipids under low temperatures of 5–10 °C (Kato-Noguchi and Yasuda 2007). Many rice cultivars exhibit low temperature sensitivity mostly during the early microspore developmental stage. Grain yield, growth rate and spikelet fertility were the maximally affected parameters under cold stress (Shimono et al. 2002). Most of the cold-tolerant rice varieties displayed better chlorophyll levels, higher root and shoot growth and vigour, lesser extent of electrolyte leakage and malondialdehyde content, higher accumulation of total amino acids like proline and soluble sugars and improved expression of antioxidative enzymes like catalase, peroxidase, superoxide dismutase, etc., unlike the sensitive ones. Induction of a gene named *OsRLK1* encoding for a putative receptor like protein kinase associated with leucine-rich repeats has been reported to play an important role in the molecular basis of both chilling and salt stresses in rice plants (Lee et al. 2004).

In addition to salinity, drought stress serves as the major constraint to yield and production of rice crops across the world, which induces a series of molecular, morphological, biochemical and physiological mechanism within the cells. During drought, many rice varieties tend to develop higher root length density and deeper roots, in order to allow extraction of more water, develop drought tolerance and combat water shortage (Lilley and Fukai 1994). Moreover, these plants also exhibited lower initial leaf area, transpiration rate and specific leaf weight, while carbon isotope discrimination in the leaves increased, with more degradation of starch as compared to sugar and increased accumulation of such carbohydrates in the leaves (Cabuslay et al. 2002). Just like any other abiotic stress, drought also resulted in reduction of grain number and size, with increased formation of unfilled grains in rice (Lilley and Fukai 1994; Rabara et al. 2021). The aromatic rice variety Pusa Basmati showed high susceptibility to polyethylene glycol-mediated dehydration stress (Basu et al. 2010). Plants recruit robust antioxidative machinery in order to counteract the toxic effects of the ROS generated during stress (Banerjee and Roychoudhury 2019a, b, c). Some important antioxidative enzymes like manganese-containing superoxide dismutase (MnSOD) have crucial role in the ROS-scavenging system associated with drought stress tolerance in rice plants (Wang et al. 2005). Such tolerant varieties exhibit lower levels of toxic hydrogen peroxide, malondialdehyde, etc. due to efficient detoxification of ROS generated due to stress (Nguyen et al. 1997). Moreover, certain protective osmolytes like glycine-betaine and proline also have marked implications in combating drought stress (Wang et al. 2010). Rice crops adapt specific phonological features in order to escape drought and dehydration conditions, to recover from water scarcity and also to generate tolerance against water shortage conditions. The most common features are the deep root system and retention of green leaves, which aid in the extraction of water from great depths and sustenance under prolonged drought conditions, respectively (Fukai and Cooper 1995).

Rice is a type of cereal crop that usually demands prolonged submergence and anaerobic conditions for its development. Rice crops require huge quantities of water for their optimal growth and yield (Banerjee and Roychoudhury 2019b). Therefore, rice plants tend to embrace certain adaptive strategies in order to sustain during flooding. These adaptive features include greater rate of elongation of submerged shoot organs and development of aerenchyma for the efficient transport of oxygen to the submerged organs. Germination under anoxic conditions is governed by the coleoptile and not via emergence of roots in rice. Rice plants initially tend to maintain higher carbohydrate reserves in order to survive submergence stress and quickly revive following de-submergence (Singh et al. 2001). However, excessive utilisation of carbohydrates like aldehydes during submergence rather leads to depleted energy reserves and poor plant survival. Some varieties often exhibit reduction in the number of adventitious roots during flooding, in order to maintain an optimal water uptake by roots and also transpiration rate (Kato and Okami 2010). Flooding-tolerant rice varieties demonstrated an induction in the expression of cell cycle regulatory cyclin proteins and certain enzymes like pyruvate decarboxylase and alcohol dehydrogenase during excess water stress, serving as a strategy for quick recovery after de-submergence and oxygen deprivation (Dubey and Grover 2003). The phytohormone ethylene has been found to play an important role during submergence stress, as this hormone modulates the fine balance between two other important plant hormones, i.e. gibberellin and abscisic acid, in order to regulate shoot elongation and adventitious root formation during flooding (Fukao and Bailey-Serres 2008).

1.2.2 Maize (*Zea mays*)

Corn or maize is another important cereal crop, which is grown in diverse agro-ecological regions across the world, since several decades. Maize serves as the third most important cereal crop in the world, after rice and wheat. It is commonly referred to as the “Queen of cereals” due to its huge production potential amongst other cereal crops (Sandhu et al. 2007). Maize is a chief source of a range of antioxidative phytochemicals including phenolic compounds, carotenoids, plant sterols, etc. having immense human health benefits and also aids in the prevention of different chronic human diseases like diabetes, cancers, cardiovascular disorders, nutritional deficiency disorders, etc. (Liu 2004; Shah et al. 2016; Díaz-Gómez et al. 2017). Moreover, maize plants have been found to have anti-HIV (Human immunodeficiency virus) activity, owing to the presence of *Galanthus nivalis* agglutinin (GNA) lectin in maize (Shah et al. 2016). It has been reported that maize productivity can be reduced by a series of different abiotic stresses like drought, extreme temperatures, waterlogging, nitrogen-induced stress, etc. In order to acclimatise to such stressors, maize plants develop several physiological and morphological traits, including alterations in plant architecture, root depth and morphology, thickness of cuticle, antioxidant capacity, osmotic adjustment, hormonal regulation, stomatal and photosynthetic maintenance, stability of proteins and membrane lipids (generating desiccation tolerance) and reproductive events (Bohnert et al. 1995; Shinozaki and Yamaguchi-Shinozaki 1996; Bray 1997).

Exposure to higher concentration of salts is manifested in the form of water deficit in maize plants. Such plants exhibit reduced stomatal conductance, transpiration rate, leaf water potential and root-shoot growth. Moreover, salinity stress in maize also induces a series of biochemical changes including disruption of ionic balance, cellular homeostasis and metabolic activity of several enzymes associated with photosynthesis and other plant metabolic events. Root radial reflection coefficient served as a significant parameter in the regulation of the NaCl assimilation by roots of maize seedlings under salinity stress (Zhu et al. 2010). Salt-resistant maize genotypes displayed an array of adaptive features like increased root growth and development with the formation of profuse lateral roots and enhanced activities of protective antioxidative enzymes like catalase, superoxide dismutase and a range of peroxidases, thereby generating osmotic adjustment and efficient scavenging of toxic radicals and metabolites produced in response to salt stress (Maiti et al. 1996, 2009b; Neto et al. 2006).

Drought stress in maize particularly disrupts transpiration and photosynthetic efficiency with accelerated leaf senescence (Banziger et al. 2000), marked by reduction in the levels of photosynthetic pigments, leaf fall as well as reduced plant size. Tolerant varieties have been shown to possess greater adaptive power due to increased leaf transpiration and photosynthetic efficiency, owing to improved reserves of protective minerals, plant proteins and enzymatic metabolites like pigments, osmolytes and antioxidants. Cultivars exhibiting drought tolerance tend to have a robust root system, possessing faster growth rate and deeper penetration power for efficient water uptake under conditions of water deficit. These cultivars also demonstrate higher accumulation of abscisic acid, thereby rendering them with better survival potential during water shortage, via osmotic adjustment and protection of cellular membranes and macromolecules like enzymes (Lemcoff et al. 1998). Similar manifestations like drought stress have been reported in maize crops during waterlogging, i.e. severely depleted growth and yield due to oxidative damage-induced alterations of stress-responsive biochemical parameters within the system. Tolerant varieties display enormous adventitious root formation and development of aerenchyma during stress imposition. Aerenchyma acts as a saviour under anoxic conditions, because it provides a low-resistance diffusion path for oxygen from aerial plant parts to submerged plant organs (Laan et al. 1989). Moreover, aerenchyma also allows easy diffusion of important plant volatiles like ethylene, ethanol, methane, acetaldehyde, carbon dioxide, etc. (Visser et al. 1997).

Maize plants have a rather higher potential to survive when exposed to a wide range of temperature, i.e. ranging from 0 to 44 °C. However, prolonged exposure to temperature extremes can have serious effects on the vegetative and reproductive growth and yield of the crops, with effects being more pronounced in case of chilling stress. As a protective strategy, cold-tolerant maize genotypes exhibited upregulation of the activity of important enzymes associated with the antioxidative machinery, i.e. ascorbate peroxidase, glutathione reductase, superoxide dismutase, etc. (Aroca et al. 2001). Moreover, such stress-adapted plants depict alterations in their early stages of shoot morphogenesis, which renders maintenance of the vegetative growth under chilling temperature. It has been observed that the photosynthetic apparatus forms the major target of cold stress in maize plants, but the molecular mechanism associated is rather poorly deciphered. Hence, alteration in the expression of a series of chloroplast-related genes and other photosynthesis-related genes served as a hallmark of adaptation to cold stress in the tolerant maize varieties (Trzcinska-Danielewicz and Bilska 2009). Such varieties also exhibited a fall in leaf conductance and rise in water flow via roots upon stress imposition.

1.2.3 Wheat (*Triticum aestivum*)

Wheat also belongs to the grass family and serves as the major staple food crop of many countries (Jeyasri et al. 2021). The Asia-Pacific region has been found to be the leading wheat consumer worldwide. This cereal crop happens to be grown in countless number of varieties across the world. With progressive population growth and changing socio-economic status and food preferences, consumption of wheat has steadily increased during the last few years (Shewry and Hey 2015; Hura 2020). Apart from carbohydrates, wheat is a source of many essential nutrients like proteins, lipids, fibre, minerals, vitamins and certain important phytochemicals having crucial role in the human diet (Luthria et al. 2015; Barros et al. 2020) and also imparts protection against dreaded human diseases like colorectal cancer, type II diabetes and heart-related ailments (Aune et al. 2016; Della Pepa et al. 2018). Like other cereal crops, wheat has also been reported to be severely affected due to various abiotic stressors like drought, salinity and high and low temperature exposures. So, breeders tend to look for cultivars having greater stress tolerance capabilities and enhanced health benefits, to ensure better crop yield under unfavourable conditions.

Wheat cultivars exhibit a wide range of genotypic variability, as an adaptive response to salinity-induced osmotic stress (Maiti et al. 2007). Salinity stress in wheat plants primarily affects seed vigour, apart from negative consequences on overall growth, yield and biochemical parameters. Tolerant wheat cultivars displayed accumulation of various amino acids like proline and both free and bound forms of the stress hormone abscisic acid, resulting in reduction of transpiration rate during salt stress. Moreover, these plants also exhibited higher accumulation of certain cations like sodium, magnesium and calcium ions, but levels of potassium ions within the system were reduced in response to stress (Hossain et al. 2006). Certain salt-tolerant varieties of winter wheat have been shown to possess immense power of delaying plant senescence during reproductive phases (Zheng et al. 2008), even after detrimental effects of high salt on the photosynthetic and transpiration rates, growth and yield parameters and the levels of important plant pigments, ions and macromolecules. Interestingly, salinity-tolerant wheat plants displayed greater production of ATP as an adaptive response to stress, unlike the sensitive ones (Kong et al. 2001). These plants have been reported to overproduce certain protective antioxidative enzymes like peroxidases and superoxide dismutase (Sharma et al. 2005). In addition, for the restoration of photosynthetic efficiency during salinity stress, wheat plants upregulate the expression of certain osmolytes like glycine-betaine to ensure osmotic adjustment within the system (Raza et al. 2006). Certain important proteins involved in salt tolerance, i.e. H^+-ATPases, triose-phosphate isomerase, ferritin and glutathione-S-transferase, were overproduced in tolerant wheat cultivars (Gao et al. 2011). Salt tolerance has also been rendered via interaction of sodium and chloride ions and also due to rise in proline and soluble sugar accumulation (Almansouri et al. 1999).

Drought stress in wheat results in serious morphological, physiological, molecular and biochemical alterations, which in turn adversely affected quality and quantity of grain yield. The most common manifestation of drought stress in any plant including wheat is associated with change in root traits (Adda et al. 2005). The most prominent factors associated with drought tolerance in wheat are limited sensitivity to the rate of exchange of carbon dioxide, stomatal resistance, ratio between water loss and net uptake of carbon dioxide, relative water content and higher potential of osmotic adjustment upon stress imposition (Hafid et al. 1998). The side chains associated with rhamnogalacturonan 1 and 2, commonly called xyloglucans, showed an increase in drought-tolerant wheat cultivars, indicating their protective role against drought stress (Leucci et al. 2008). Tolerant cultivars also showed an accumulation of soluble sugars, amino acids like proline and potassium ions, to ensure efficient osmotic adjustment against drought stress. Apart from proline, another important osmolyte overproduced in these cultivars is glycine-betaine, both of which are responsible for imparting protection against oxidative damages. Moreover, glycine-betaine also renders protection to the photosystem II complex, via increased turnover of D1 protein and maintenance of expression of antioxidative enzymes during water shortage. Similarly, water excess conditions also trigger activities of antioxidative enzymes in the tolerant wheat varieties. Moreover, such plants tend to enhance their potential to tolerate waterlogging by increasing the rates of transpiration and photosynthesis, altering the stomatal conductance and increasing the use efficiency of light energy absorbed, to ensure proper growth and development (Li et al. 2011).

Exposure to increasing temperature conditions results in disruption of grain developmental stages in wheat. Flag leaves exhibit adaptation against heat stress-induced photo-oxidative damages, to sustain higher yield. In addition, the expression of a series of different heat shock proteins serves as the hallmark of heat stress tolerance in wheat plants. Ethylene hormone production is also triggered upon heat stress imposition in certain varieties, which renders optimal maturation even under stressed conditions (Hays et al. 2007). Sometimes, salicylic acid can even relieve damages imposed due to heat and higher light intensity exposures on wheat crops, via restoration of functioning of D1 protein and PSII system in leaves, and in turn normalise photosynthetic functions (Zhao et al. 2011).

It is well-known that ice tends to accumulate in intercellular spaces, leading to adhesion in between the intercellular layers of ice and the cellular walls and membranes (Levitt 1980). Such ice accumulation can result in cellular and membrane damages due to protein denaturation and cellular macromolecular precipitation. Acclimatisation to freezing stress in wheat has been associated with reversal of freezing-induced protein denaturation, reducing molecular precipitation and preventing direct physical injury due to intercellular ice accumulation via stabilisation of cellular components and membranes against chilling stress (Zhang et al. 2009). Cold-tolerant cultivars exhibit higher membrane freezing tolerance owing to alterations in the composition of membrane lipids (Steponkus et al. 1993). Generally, wheat plants exhibiting low canopy temperature have been found to be more superior as compared to the other conventional wheat crops, in terms of some crucial biological characteristics, cellular structures and greater potential of ecological adaptability. Hence, these cultivars have shown great prospects in terms of biological research. In addition, cold-tolerant wheat cultivars have improved rate of grain filling and higher weight of grain per ear due to adaptation to extremes of temperatures (Mashiringwani and Schweppenhauser 1992). Sometimes, in certain varieties of winter wheat, lower temperature exposure leads to higher accumulation of soluble proteins in tissues of callus and root meristems (Karimzadeh et al. 2000). Therefore, several biochemical, physiological and morphological mechanisms contribute to the acclimatisation of wheat against different abiotic stressors. These factors include root depth, water use efficiency, osmoregulation, accumulation of osmolytes like proline, etc.

1.2.4 Millets

Millets comprise of a group of crops having high nutritive value and are often referred to as “Nutri-cereals”. This group constitutes sorghum or jowar; finger millet or ragi; pearl millet or bajra; and small millets including little millet or kutki, barnyard millet or sawa/jhangora, kodo millet, porso millet or cheena, foxtail millet or kakun/kangni. These cereals are well-known for their drought-tolerant properties, low photo-sensitivity, wide range of adaptability to climate changes and nutri-rich contents. Millets are commonly grown in semi-arid tropical continents like Asia, Africa and parts of the American continents (Jeyasri et al. 2021). They are rich in proteins, starch, important vitamins, minerals and unsaturated fatty acids (Ramatoulaye et al. 2016). Moreover, certain millets serve as source of some crucial plant secondary metabolites like phenolics, flavonoids, tannins, etc. having role in restoration of intestinal microbiota and prevention of human disorders like hypertension, dyslipidaemia, inflammation, cancer, diabetes, obesity and oxidative damages (Rao et al. 2008; Ba et al. 2010; Xiong et al. 2019).

Millets are well adapted to grow in arid and semi-arid conditions, associated with combination of various abiotic stressors like drought, high temperature and salinity. Vegetative and reproductive growth stages displayed increased expression of antioxidative enzymes like catalase and glutathione peroxidase, in order to combat stress-induced injuries. Desai et al. (2006) demonstrated the structure and functional implications of a pearl millet gene that encodes for a voltage-dependent anion channel (VDAC), which depicted differential upregulation during salinity stress. It has been reported that certain protective molecules like proline, glycine-betaine, salicylic acid and certain plant growth regulators have crucial role in the alleviation of salt stress in millet species (Ashraf et al. 2008). Drought-tolerant varieties have improved resource allocation patterns to ensure optimal grain yield under stressful conditions. Heat and drought stress-adapted millet crops exhibited better survival percentage, root and shoot dry mass, total root length and depth, osmotic adjustment and rate of leaf elongation even under combination of abiotic stresses (Joshi et al. 2005; Kusak et al. 2005). Moreover, stress-induced upregulation of different antioxidative enzymes, viz. glutathione-S-transferase, glutathione reductase and superoxide dismutase, aided in the control of toxic ROS and maintenance of photosynthetic pigment stability during water shortage (D'souza et al. 2009). In addition, high temperature conditions resulted in alteration in uptake of different elements and ions like N, K^+ and P along with the enhancement of membrane thermo-stability for acclimatisation to heat stress (Howarth et al. 1997; Ashraf and Hafeez 2004).

1.2.5 Rye (*Secale cereale*)

Rye is considered as a secondary cereal crop and is grown in European countries since many decades. By virtue of its tolerance capacity to wide range of temperature and ability to grow in poor quality soil, rye serves as one of the leading cereal crops and suppliers of carbohydrate in the human diet in Europe (Persson et al. 2006). This cereal crop belongs to the wheat tribe, shows resemblance with both wheat and barley and contains beta-gluten, which helps in lowering cholesterol levels in blood, thereby reducing risks of cardiovascular disorders.

Salinity stress and presence of higher loads of different sodium salts in the soil are one of the most serious limiting factors in rye productivity. Moreover, certain salts like carbonates and bicarbonates of sodium lead to increased alkalinity of the soil, which has even greater injurious effects on the plants. Acclimation to such stressors in rye involved higher root cell viability; alteration in the levels of different cations like Na^+, K^+, Ca^{2+} and Mg^{2+}; higher ability of water uptake; and enhanced antioxidative power associated with upregulated expression of different antioxidative enzymes like catalase, glutathione reductase, etc. (Liu and Saneoka 2019). Similarly, overwintering and drought stress tolerance are also related to changes in the metabolome, which results in subsequent morphological and genetic adaptations in certain varieties of rye. Cold adaptation has been associated with higher accumulation of total proteins, free amino acids, soluble sugars, osmolytes and antioxidative enzymes. In addition, brassinosteroids have been found responsible for winter survival and acclimation of certain cultivars of winter rye (Pociecha et al. 2016). Rye crops are typically well adapted to grow in sandy soil marked by poor water holding capacity. Hence, most varieties of rye are usually tolerant to water shortage and demonstrate optimal yield potential.

1.2.6 Barley (*Hordeum vulgare*)

Barley is the fourth most abundantly cultivated cereal crop in the world. This cereal can be grown in regions of low moisture like arctic, sub-arctic and sub-tropical regions, where other conventional cereals like rice, wheat and maize will fail to adapt. The Mediterranean region is well-known for growing barley, which is tolerant to a combination of abiotic stressors like drought, salinity and high temperature (Zhou 2009). Currently, a number of developing countries including India, Bangladesh, Sri Lanka and Nepal have resorted to barley cultivation, due to the acclimation power of barley against stress imposed due to water shortage (Ceccarelli et al. 2007). Barley is a high source of fibre, minerals and vitamins. It is also responsible for maintenance of healthy blood sugar and cholesterol levels. Therefore, barley is a versatile cereal crop, but the severity of a particular abiotic stress is a rather non-uniform event and can severely affect development and yield.

Drought and salinity stresses serve as the major constraints for the production of barley crops. It has been found that this cereal crop adapts to various physiological and agronomic traits, in order to acclimatise to these stressors. Stress-tolerant and sensitive barley cultivars can be differentiated on the basis of a series of factors, viz. alterations in the assimilation rates, levels of photosynthetic pigments, stomatal conductance, generation of ROS, rate of gaseous exchange, etc., which modulates the overall growth and productivity of these crops, represented in terms of number of grains per spike, grain yield per spike and productive tillering. Tolerant cultivars display higher levels of growth regulators, osmolytes and antioxidants, thereby mediating improved osmotic adjustment and stomatal performance under stressful environment (Sabagh et al. 2019). Moreover, these cultivars have improved nutrient translocation and water reserves, altered photosynthetic and transpiration rate, slower leaf senescence, better cellular growth and enlargement and lesser disruption of cellular enzymes, membranes, pigments and other macromolecules, thereby demonstrating lesser oxidative damages as compared to the sensitive varieties. During drought stress, the ear and flag leaves of barley are the sole photosynthetic organs adapted to cater for the grain filling assimilates. Barley plants are capable of multiple adaptive responses against stressors, due to accumulation of an important signalling molecule, proline (Maggio et al. 2002). Moreover, several structural and osmotin-like protein molecules, along with other soluble proteins, tend to accumulate within the system during salt stress, which might have a crucial role in modification of cell wall and cell membrane against stress (Ashraf 2004). It is evident that the combined effects of drought and salinity on barley have a rather disastrous effect, than that imposed due to a single stress alone (Yousfi et al. 2010). In addition, both of these stresses result in higher electron leakage towards oxygen in respiratory and photosynthetic events, due to oxidative damage-induced ROS production (Asada 2006). However, adaptation of a robust antioxidative system aids in combating abiotic stress-related injuries in the tolerant barley cultivars. Similarly, upon exposure to extremes of temperature (heat and cold stress), most barley cultivars represented morphological and physiological alterations, owing to the alterations of the proteome. Several leaf proteins, including certain proteases and chaperones, were found to be differentially regulated, in order to ensure acclimation to heat stress and maintenance of optimal growth and metabolism in barley (Rollins et al. 2013).

1.2.7 Oats (*Avena sativa*)

Oat is grown mainly in the temperate zones as a spring cereal crop and is adapted to wet and cold summer, prevailing in the regions of Canada and North-western Europe. Oats are considered as a “health food”, particularly due to their ability to lower blood cholesterol levels. Some oat genotypes depict tolerance against chilling stress, due to adaptation of an efficient osmoprotective and antioxidative defensive machinery. Root and shoot tissues exhibited accumulation of soluble proteins, free amino acids like proline, soluble sugars, osmolytes and antioxidant enzymes ((viz., catalase, superoxide dismutase and a range of peroxidases). In addition, levels of protective molecules like glutathione and ascorbate were also upregulated during cold stress in oats, as a part of stress acclimation (Goyal and Kaur 2018). During salt stress, certain tolerant oat crops demonstrated optimal root and shoot growth and development, better regulation of osmotic potential and ionic homeostasis, as compared to the sensitive crops (Liu et al. 2020). Salt-acclimatised roots of oat seedlings demonstrated protective and interceptive role for mediation of stronger salt tolerance, via alterations in the Na^+ distribution patterns, thereby posing lesser damages to the shoots. Similar protective mechanism was observed in case of water deficit stress in oats. In addition, certain important proteins including membrane-localised H^+-ATPase have been reported to be activated and responsible for acclimation to drought stress in oats (Gong et al. 2010).

1.3 Conclusion and Future Perspectives

Various types of abiotic stresses have serious and complex effects on the plant dynamics including changes at the cellular, architectural, physiological and transcriptome level, affecting different plant developmental stages, flowering, maturation, grain filling, etc. (Atkinson and Urwin 2012; Maiti and Satya 2014). It is known that agriculture consumes the highest toll of quality water. So, all agricultural operations and activities will be rather hampered by contaminated, poor-quality water and water scarcity. There has to be an increased focus on the reuse of waste and ground water, reclaiming degrading agricultural lands, sustainable utilisation of inputs from cultivation, identification of stress-tolerant genotypes of cereal crops, stressing upon micro-irrigation procedures, etc. in order to promote sustainable use. It has been the focus of researchers and plant breeders to exploit the yield resilience power of various stress-tolerant cereal crop species and promote their improvement

(Halford et al. 2014). Under this current scenario, it is necessary to carry out multidisciplinary research in order to promote selection and breeding of cereal cultivars displaying higher yields and tolerance to different stressors, which in turn will sustain the productivity of crops in agricultural fields. High yielding cereal crops can be evaluated using simple yet effective screening techniques, for tolerance against a range of abiotic stresses like heat, cold, drought, salinity, flooding, heavy metal toxicity, etc. (Maiti 2010). Therefore, it is necessary to understand the molecular cross-talk of plant stress responses, in order to generate broad spectrum abiotic stress-tolerant cereal crops.

Acknowledgements Financial assistance from Science and Engineering Research Board, Government of India, through the grant [EMR/2016/004799] and Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal, through the grant [264(Sanc.)/ST/P/S&T/1G-80/2017] to Dr. Aryadeep Roychoudhury is gratefully acknowledged.

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2

Cereal Physiology, Flowering, and Grain Yield Under Salinity and Drought Stress

Savita Bhardwaj, Tunisha Verma, Bharat Kapoor, and Dhriti Kapoor

Abstract

Cereal crops are the vital source of food and nutrients for human health and nearly cultivated in all parts of the world. Demand for cereal yield has been increasing extensively nowadays due to their high nutritional value and production of first-generation biofuel from cereal starches. However, cereal crops are frequently exposed to several abiotic stresses such as drought, salinity, extreme temperature, heavy metal stress, etc. which dramatically impact their physiological and developmental processes and grain yield. Among these abiotic stresses, drought and salinity stress are one of the major environmental constraints which pose significant threat to cereal crops with subsequent economic impacts. Loss in cereal productivity was primarily determined by the severity and duration of the stress. Declined net photosynthetic activity, damage to the chloroplast and stomatal closure, alterations in various plant developmental processes like flowering, and oxidative stress due to the over-accumulation of reactive oxygen species (ROS) are the major reasons for poor grain set and development in cereals under drought and salinity stress. Although these stresses impede cereal performance at all developmental phases, cereals are highly sensitive during their flowering and grain-filling phases which consequently cause severe yield losses. Therefore, there is an immense demand of efficient approaches to mitigate these stresses to ensure food security and nutrition. Various transgenic and molecular breeding

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_2

strategies are in practice to improve cereal tolerance against drought and salinity stress.

Keywords

Cereals · Drought stress · Salinity · Yield · Flowering

2.1 Introduction

Major portion of the world's food grain production is contributed by cereals such as rice, wheat, maize, and barley. However, abiotic factors like drought, salinity, temperature, heavy metal stress, etc. adversely impact world food grain production. Moreover, these abiotic factors often communicate with each other and cause significant effects on plants in comparison to individual stress exposure (Kadam et al. 2014). Alterations in climatic conditions affect crop productivity via influencing plant growth and physiological and biochemical aspects, ultimately resulting in declined cereal yield (Lesk et al. 2016; Zhang et al. 2018). Cereals are the vital components of food material and nutritional constituents for human health. Food demand is increasing day by day with the rapid increase in population; however, crops are facing high risk due to the occurrence of abiotic stresses. In addition to this, two-thirds of arable land is saline, and one-fifth of it is arid and semiarid which are severely impacting cereal yield. To deal with this situation, the practice of raising drought- and salinity-tolerant crop genotypes is of utmost importance and recognized as a beneficial approach to regulate crop yield (Maiti and Satya 2014).

Around 20% of irrigated land is highly exposed to salinity stress, ultimately decreasing crop productivity (Qadir et al. 2014). However, plant physiological responses to salt stress are generally diverse, which makes research quite complex. Plant response to salinity can be exhibited in two crucial phases. The first phase is the shoot ion-independent response, which occurs within minutes to days, and is considered to be associated with Na^+ sensing and signaling (Gilroy et al. 2014; Roy et al. 2014). During this phase, salinity influence on water status can be significant, resulting in closing of stomata and the suppression of leaf expansion (Munns and Termaat 1986). Ion-dependent response is the second phase to salinity, which occurs from days to weeks and associates with the accretion of ions in the stem to noxious amounts, mainly in the older leaves. During this phase, premature senescence of leaves, yield decline, or even plant death can occur (Munns and Tester 2008). Decreased shoot length, leaf area, CO_2 assimilation, reduced nutrient mobilization to growing parts, transpiration, and low relative water content are some of the adverse effects of drought stress in crops (Lipiec et al. 2013; Aslam et al. 2013). Drought stress decreased grain number in rice and wheat by damaging the pollen and spikelet, during the early reproductive stage (Dolferus et al. 2011; Kato et al. 2008).

The aim of the present chapter is to summarize the effect of salinity and drought stress on physiological aspects, flowering, and yield of cereals.

2.2 Effect of Salinity Stress on Cereals

2.2.1 Physiological Aspects

Salinity stress alters morphological, physiological, and biochemical features of cereals which ultimately results in declined plant biomass and crop yield.

2.2.2 Plant Growth

Decreased growth rate is one of the primary impacts of salinity stress. Salinity-mediated inhibited plant growth is due to two reasons. First is osmotic stress or limited water availability. Plant growth is reduced due to decline in the ability of the plant to take up water. Second reason for the reduction in plant growth is the damage to cells in the transpiring leaves. Salinity stress exhibited a significant decrease in plant height, tiller number, and leaf area index in rice plants (Hasanuzzaman et al. 2009; Paul and Roychoudhury 2019). Decreased shoot and root weight, total biomass, plant height, and leaf number was observed in *Glycine max* under salt stress conditions (Dolatabadian et al. 2011). Similarly, reduced plant height, root length, and dry weight of stems and roots was found in winter wheat under salinity stress (Zheng et al. 2008). Dry weight of leaf, stem, and root was reduced in two wheat genotypes, namely, “Sakha 95” and “Misr 2” against salinity stress (Yassin et al. 2019).

Salinity stress-mediated reduction in the total biomass was due to the decrease in cell division, elongation, and inhibition of growth hormone (auxin) production. Osmotic stress conditions are induced by salinity stress which further causes loss of turgor. Salinity stress reduced the relative water content (RWC) in *Triticum aestivum* genotypes as compared to the control plants (Singh et al. 2020). Plant response to osmotic stress starts to display its effects instantly after the salinity level around the roots elevates to a threshold level (Munns and Tester 2008), due to which water uptake is influenced and plant growth and development is inhibited (Chinnusamy et al. 2005). A gradual reduction in fresh and dry weight of stem was observed with increasing salt concentration in *Triticum aestivum* (Daoud et al. 2018). This might be attributed to the elevation in salt uptake, which further causes production of reactive oxygen species (ROS), resulting in disturbance of the plasma membrane and ionic imbalance to inhibit various plant metabolic aspects and growth (Mittler et al. 2011).

2.2.3 Photosynthetic System

During the process of photosynthesis, solar energy is converted into chemical energy by plants. Salinity-induced decrease in water potential is the major reason for the decline in photosynthetic rates in plants. Chlorophyll is the major component of photosynthesis which is directly responsible for plant vigor; however, the process of photosynthesis is suppressed when high amounts of Na^+ and Cl^- are transported to chloroplasts (Zhang et al. 2005). Salinity stress-mediated reduction in chlorophyll content is a well-known phenomenon, Moreover, chlorophyll content has been recognized as a sensitive indicator of the cellular metabolic state in several studies (Chutipaijit et al. 2011). Decrease in content of chlorophyll *a* and *b* in leaves of rice was found by treatment with NaCl (Roychoudhury et al. 2008; Amirjani 2011). A decrease of 30%, 45%, and 36% was observed in the contents of chlorophyll *a*, *b*, and carotenoids, respectively, in rice with the treatment of 100 mM NaCl (Chutipaijit et al. 2011). Salinity stress reduced the content of photosynthetic pigments, i.e., total chlorophyll, chlorophyll *a* and *b*, carotenoids, and xanthophylls, by 31%, 22%, 45%, 14%, and 19%, respectively, and also declined the intensity of chlorophyll fluorescence in *Vigna radiata* (Saha et al. 2010).

Munns and Tester (2008) observed that salinity stress caused significant reduction in stomatal aperture, but sometimes photosynthetic rate per unit leaf area remains unaffected. Decrease in stomatal conductance was observed in two contrasting durum wheat genotypes under salinity stress (James et al. 2002). James et al. (2002) found that PSII efficiency remained unchanged in the tolerant wheat genotype, whereas in the sensitive genotype, quantum yield of PSII was decreased along with leaf ageing, higher Na^+ and Cl^- level in the leaf, and chlorophyll degradation. Internal CO_2 level was decreased due to declined stomatal closure which further declined the functioning of various photosynthetic enzymes such as RuBisCo (Chaves et al. 2009). Hence, carboxylation and net photosynthetic rate were reduced.

Salinity stress decreased the stomatal conductance and intercellular CO_2 content in sorghum, and inhibition of net photosynthetic rate was mainly due to lower stomatal conductance (Netondo et al. 2004). Salinity stress is also known to interrupt the biochemistry of photosynthetic system, which results in alterations of chloroplast integrity to decrease efficiency of two photosystem (Ibrahim et al. 2015; Basu and Roychoudhury 2021). Salinity stress caused changes in leaf chloroplast ultrastructure by disrupting the thylakoid membrane and chloroplast envelope and also decreased the leaf chlorophyll content in wheat. Ionic toxicity or imbalance, greater amount of lipids in chloroplasts, and osmotic imbalance between chloroplast and stroma are the major reasons for the disruption of thylakoids. In addition to this, photosynthetic efficiency and electron transport activity of chloroplasts were also reduced (Zeeshan et al. 2020).

2.2.4 Antioxidant Defense System

Oxidative stress is the most general consequence of salinity stress in plants due to the generation of reactive oxygen species (ROS) such as singlet oxygen, superoxide radicals, and hydrogen peroxide which dramatically disrupt plant biomolecules such as proteins, lipids, and nucleic acids (Das and Roychoudhury 2014; Ozfidan-Konakci et al. 2015). Activity of antioxidative enzymes, i.e., peroxidase (POD) and superoxide dismutase (SOD), was enhanced in barley and wheat cultivars under salinity stress (Izadi et al. 2014). Salinity stress increased the activity of antioxidative enzymes, i.e., SOD, catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and guaiacol peroxidase (GPX), in a concentration-dependent manner and also improved the content of non-enzymatic antioxidants, i.e., ascorbate (AsA) and glutathione (GSH) in *Triticum aestivum* (Ahanger and Agarwal 2017). Salinity stress also improved the activity of SOD in *Oryza sativa* cultivars (Vighi et al. 2017). Content of oxidative stress markers, i.e., malondialdehyde (MDA) and H_2O_2, was increased under salinity stress, while activities of SOD, POD, CAT, and APX enzymes were improved to diminish salinity-induced oxidative stress in *Zea mays* (Hussain et al. 2014). Babaei et al. (2017) observed that salinity stress upregulated the functioning of CAT, POD and polyphenol oxidase (PPO) enzymes in *Triticum aestivum*. Salinity stress caused oxidative stress in two genotypes of *Triticum aestivum*, i.e., salt sensitive (Darab 2) and salt tolerant (Arta), by generating high level of ROS and via increasing lipid peroxidation. Salt-tolerant cv. “Arta” exhibited greater ROS scavenging activity by improving the functioning of APX and POD as compared to “Darab 2.” Moreover, functioning of SOD and GR remained unchanged in sensitive cv. “Darab 2” under salinity stress (Esfandiari and Gohari 2017). Salinity stress increased the activity of CAT and SOD enzymes in rice genotypes (Jan et al. 2019), while Wang et al. (2005) found decreased activity of SOD and CAT enzymes under saline conditions.

2.2.5 Mineral Nutrients

Crop yield may be harmfully impacted by nutritional imbalance caused by salinity stress, and the relation between nutrients and salinity is very complex (Grattan and Grieve 1998). Altered nutrient availability, competitive accumulation, passage, and distribution within the plant are the major reasons for salinity-induced nutritional imbalance. Salinity stress-triggered decrease in the availability of phosphorous in soil is mainly due to the following reasons: (1) activity of PO_4^{3-} reduced by ionic strength, (2) phosphate content highly regulated by sorption mechanism in soil solution, and (3) little solubility of calcium-phosphorus minerals. With the increase in the salinity level, phosphate content in agricultural crops declines (Qadir and Schubert 2002). Salinity stress significantly increased the Na^+ levels in the stem and roots, while K^+ amount was observed to decrease in stem and roots of *Pistacia vera* (Rahneshan et al. 2018). Ca^{2+} content was increased in the shoot of *Pistacia vera* plants under salinity stress (Karimi and Maleki Kuhbanani 2015).

2.2.6 Flowering

Flowering is the major and sensitive growth phase of the life cycle that is responsible for production of seed. Salinity stress is considered to adversely affect flowering stage in various crop plants (Singh et al. 2004). Grain yield is determined by the flowering phase. Pollen viability is impacted by salinity stress at booting stage due to which fertilization and percentage of filled grains were reduced, ultimately resulting in the declined total plant yield. Mohammadi-Nejad et al. (2010) studied the effect of salt stress on the viability of pollen and grain yield in various rice cultivars and found decreased pollen viability under salt stress in most of the rice genotypes. In addition to this, cultivars in which acute decrease in the pollen viability and crop yield were observed were categorized as salinity-sensitive cultivars for flowering stage (Khatun and Flowers 1995). Characteristics like number of flowers per plant coupled with stamen length, pollen viability, in vitro pollen germination, and pollen tube growth were decreased under salinity stress in cluster bean (Deepika and Dhingra 2014). Salinity-induced decline in pollen quality, i.e., viability, germination, and tube growth in *Zea mays*, was mainly due to the accretion of non-nutrient ions like Na^+, Cl^-, and declined starch and protein content (Dhingra and Varghese 1985).

2.2.7 Grain Yield

Almost 30–50% yield losses are mainly due to salinity stress which can restrict crop growth and yield by three reasons: (1) ionic toxicity, (2) reducing osmotic potential, and (3) altered enzyme activity due to disarrangement and imbalance of ion uptake (Joseph and Mohanan 2013). Salinity stress induced changes in plant growth parameters ultimately resulting in declined crop yield. Nahar and Hasanuzzaman (2009) found that salinity stress adversely affected different yield components by decreasing the numbers of pods per plant, seeds per pod, and seed weight of *Vigna radiata*. The reproductive growth was also found to be reduced under salinity stress. A decrease of 77%, 73%, and 66% in yield of different cultivars of *V. radiata*, i.e., cv. BARI mung-2, BARI mung-5, and BARI mung-6, respectively, was observed as compared to control plants (Nahar and Hasanuzzaman 2009). Salinity stress highly influenced the grain yield of rice varieties. Grain yield was reduced by 50%, 38%, 44%, and 36% in cultivars BR11, BRRI dhan41, BRRI dhan44, and BRRI dhan46, respectively, at 150 mM NaCl, as compared to control (Hasanuzzaman et al. 2009). The main reason behind salinity-induced inhibition of fertility may be due to the competition in carbohydrate supply between vegetative growth and suppressed transport of these carbohydrates to developing panicles (Murty and Murty 1982).

2.3 Effect of Drought Stress on Cereals

2.3.1 Physiological Aspects

Scarcity of water has a detrimental impact on crop growth and production. As a result, understanding the physiological, biochemical, and ecological processes associated with drought stress is critical for improved management of crops (Rabara et al. 2021).

2.3.2 Plant Growth

Drought stress is widely recognized as a constraint factor that influences numerous aspects of plant growth and development, although the ranges of stress imposed are highly variable based on timing and intensity of stress imposed. Plant advancement is primarily determined by seed germination, seed vigor, and coleoptile length which are foremost for plant development (Sourour et al. 2017). The major element of development that is susceptible to drought stress is seed germination. Drought stress caused considerable alterations in seed germination in a variety of plant species, including some of the most commonly farmed crops such as maize, wheat, and sorghum (Queiroz et al. 2019; Patanè et al. 2013). Plant height, leaf size, and stem girth were significantly reduced under water-limiting conditions in maize (Khan et al. 2015). Similarly, reduced germination potential, early seedling growth, root and shoot dry weight, hypocotyl length, and vegetative growth have been reported in important field crops including pea (*Pisum sativum* L.), alfalfa (*Medicago sativa* L.), and rice (Basu et al. 2010) under drought stress. Several studies have found that drought stress has detrimental influence on germination and seedling development (Kaya et al. 2006; Farooq et al. 2009).

Drought conditions reduce plant nutrient absorption owing to low soil moisture, resulting in shorter stem length. Likewise, shoot length decreased in *Lathyrus sativus* L. under water scarcity. In response to water deficit, plants try to collect water from deeper layers of the soil by strengthening the root architecture. Drought inhibits cell development primarily through loss of turgor (Taiz and Zeiger 2006) and impairs elongation of cell mostly due to insufficient flow of water from xylem to adjacent cells (Nonami 1993). Plant height, leaf area, stem diameter, and plant biomass in different field crops were significantly reduced under drought stress (Apel and Hirt 2004; Farooq et al. 2009; Zhang et al. 2012). Reduced number of branches and total seed yield decreased significantly via drought stress in *Glycine max*. Kamara et al. (2003) observed in another investigation that biomass build-up in maize was considerably decreased under drought conditions induced at various stages of growth.

2.3.3 Photosynthetic System

Photosynthesis is one of the major physiological processes in plants that is affected by drought stress (Farooq et al. 2009). Among the key limitations for photosynthesis are reduced leaf area, high rate of stomatal closure, and subsequent lower leaf cooling by evapotranspiration as well as increased osmotic stress, which leads to damage of photosynthetic machinery. Closure of stomata under water-deficit condition lowers the availability of CO_2, making the plant more vulnerable to photodamage (Lawlor and Cornic 2002). Reduced moisture availability causes unfavorable changes in photosynthetic pigments, damages the photosynthetic machinery (Fu and Huang 2001), and affects the operation of key enzyme resulting in significant damage to plant growth and yield. Non-stomatal limitations can be defined as an inhibition of RuBisCo, lower ATP production, and a decrease in photosynthetic pigment content. Drought wreaks havoc on photosynthetic pigments and thylakoid membranes (Anjum et al. 2011) and affects chlorophyll content in plants. Decrease in the content of chlorophyll was reported in cereals under conditions of drought stress. Drought stress increased chlorophyll content in some cultivars of *Vigna mungo* L., while in some others, they were decreased under moisture stress. The difference in this response was linked to changes in the activity of enzymes involved in the production of chlorophyll. It has been observed that the concentration of chlorophyll a in drought-stressed plants was greater than chlorophyll b (Jain and Pennacchiotti 2010).

Drought stress shortens the length of photosynthesis of wheat during the blooming stage. A lack of water decreases the absorption rate during the flag leaf stage and hastens the breakdown of absorption during senescence. As a result, drought exerts impacts on wheat growth differently at different phases of development. It reduces the height, biomass, and production of winter wheat by limiting photosynthesis. Chlorophyll concentration is critical for photosynthesis and is another characteristic of utmost importance for photosynthesis that is highly impacted by water shortage and has been identified as a unique indicator of photooxidation and chlorophyll degradation (Chowdhury et al. 2017). Drought stress, for example, alters leaf chlorophyll production and chlorophyll a/b proportion.

2.3.4 Antioxidant Defense System

The majority of the plant defense mechanism is dedicated to counteracting the negative effects of drought-induced ROS (Hussain et al. 2019). In order to cope with oxidative damage, plants have evolved excellent machinery to lessen and repair the harmful effects caused by production of ROS. Increased levels of several enzymatic (SOD, POD, CAT, GST, GPX, etc.) and non-enzymatic antioxidants (reduced and oxidized glutathione, α-tocopherol, and carotenoids) in plants are reported to maintain the cellular homeostasis and mitigate the oxidative damages (Gill and Tuteja 2010). Content of non-enzymatic antioxidants and antioxidant enzyme (SOD, CAT, and APX) activities increased in *Coleus plectranthus* under drought stress conditions. Likewise, it was observed that SOD, POD, CAT, and APX activities were changed which played an important role in protecting peony plants from severe water shortage. Antioxidant substrates found in cell organelles and cytoplasm play an important role in detoxification of ROS (Shao et al. 2008). Methionine sulfoxide reductases are another type of antioxidant enzyme that serve to protect proteins from damages induced by production of ROS in plastids (Rouhier et al. 2006).

Thioredoxin is another enzyme that is used to decrease methionine sulfoxide residues in proteins caused by oxidative stress. Moreover, several authors have demonstrated the function of several enzymatic and non-enzymatic antioxidant activities in drought tolerance of maize (Anjum et al. 2017), rice (Sharma and Dubey 2005), and wheat (Sairam and Saxena 2000; Keleş and Öncel 2002). Drought stress boosted the levels of SOD and POD in *Vigna mungo*, and the authors concluded that increased levels of enzymes stimulated tolerance against drought stress (Gurumurthy et al. 2019). Conditions of water scarcity increased the level of CAT, POX, and SOD levels in leaves of *Glycyrrhiza glabra* L. which sought to fight against the spread of H_2O_2 (Hosseini et al. 2018).

2.3.5 Mineral Nutrients

Micronutrient uptake of plants under dry conditions is typically jeopardized (Ekiz et al. 1998; Karim and Rahman 2015; Pant et al. 2014). Drought stress has different consequences depending on the timing, length, and degree of the deficiencies (Pandey et al. 2001). Water scarcity has a substantial impact on soil nutrient accessibility, translocation, root mineral translocation, and ion concentration in different tissues. Condition of water scarcity reduced absorption of potassium in plant. This decrease in K is caused by decrease in the mobility of potassium that further declined the transpiration rate and weakened the action of root membrane transporters. Similar results were observed in *Malus hupehensis* when the level of potassium decreased in drought-stressed plants. The quantity of P in the leaves of *Ocimum gratissimum* and level of K in *Thymus daenensis* were decreased under water-deficit conditions. Drought stress decreased K level in *Ocimum basilicum* and *Ocimum americanum* when subjected to limited water availability. Recent findings have shown that drought stress in wheat became more pronounced in plants suffering from Zn deficiency. It was suggested that these results are due to the reduced activity of enzymes scavenging O_2 and H_2O_2 in tissues (Cakmak 2000). Current data on barley revealed that adequate mineral nutrition can enhance drought tolerance (King 1994), but in wheat, the loss in grain due to drought stress in wheat was shown to be more marked when plants were Zn deficient (Bagci et al. 2007; Ekiz et al. 1998).

2.3.6 Flowering

Drought stress has been shown to alter a variety of vegetative and reproductive characteristics of plants. It may have direct impact on plant reproduction through physiological changes, as well as indirect impacts on pollination. Water scarcity causes plant to spend less in reproduction, resulting in fewer flowers, fruit abortion rates, and non-viable pollen and seeds. Stress disrupts germination, vegetative growth, tiller production, dry matter partitioning, and reproductive organ development (Boyer and Westgate 2004). Apical morphogenesis is vulnerable to scarcity (Mahalakshmi and Bidinger 1985; Wopereis et al. 1996; Winkel et al. 1997). Craufurd and Peacock (1993) observed a delay in flower initiation caused by water-limited conditions in cereal. Recent study on maize reported delay in female organ development, whereas male inflorescence was less affected (Damptey et al. 1978). Likewise, grain crops showed sensitivity to drought during floral initiation and pre-meiotic floral component differentiation (Barlow et al. 2001). Effects of drought on floral meristem are one of the least known elements of crop reproductive development under water scarcity (Saini and Aspinall 1981). Drought significantly affected flower and grain development under stress conditions in wheat (Blum 2005).

2.3.7 Grain Yield

Drought stress significantly reduces both grain output and quality (Bagci et al. 2007; Kamara et al. 2003). As a result, any drought-tolerant mechanism would be welcome in ongoing attempts to address the problem of worldwide water shortages in agricultural production. Dry conditions reduced the yield and yield components of maize (Kamara et al. 2003) and wheat (Barnabás et al. 2008). Drought-induced stress had a significant impact on grain yield, pod output, pod abortion, and seed size. In recent study, it was found that drought stress significantly reduced global production by 33% (Kashiwagi et al. 2015). The number of branches and total seed yield was reduced in *Glycine max* due to drought stress (Frederick and Ogden 2001). When compared to control or non-irrigated seedlings, medium-sized seeds generated under drought stress had lower germination rates and seed vigor in chickpea (Samarah et al. 2009). The reproductive stage of growth is more sensitive to drought than the vegetative stage, resulting in fewer flowers and poor pod or fruit set, which decreases seed numbers (Seghatoleslami et al. 2008). Water deprivation caused flower abscission, ovule abortion, pollen sterility, pollen tube distortion, poor fruit set, and eventually poorer final yields in grain crops throughout the reproductive development (Thakur et al. 2010).

Drought stress reduced the proportion of progeny germination in soybean at the early stages of seed filling as compared to control plants. Water-deficit conditions reduced the proportion of progeny germination in soybean at the early stages of seed filling as compared to control plants (Smiciklas et al. 1992). Dornbos and Mullen (1992) observed a decline in seed germination, decline in seedling vigor, and increase in electrical conductivity of seed leachate in soybean seeds derived from drought-stressed crop. Conversely, dry circumstances decreased seed development and seed germination in peanut (*Arachis hypogaea* L.), but had no effect on seedling vigor (Ketring 1991).

2.4 Conclusion

Alterations in rainfall pattern occurs due to change in climatic conditions to regulate crop production in rain-fed areas, due to which food crops are facing drought stress globally. Moreover, soil salinity conditions arise in those areas where cultivation is majorly done by irrigation. Cereal crops are the major staple crops of the world, but these crops are facing high risk of various abiotic stresses that adversely affect crop productivity. Abiotic stresses like drought and salinity inhibit cereal growth via inhibiting their morpho-anatomical, physicochemical, and molecular aspects. Significant progress has been made in knowing the plant mechanisms to tolerate these stresses, but there is still limited information for increasing productivity under stress conditions. Plant growth parameters like plant height, number of leaves, leaf area, etc., photosynthetic parameters, antioxidant defense system, and osmolyte and mineral nutrition level are declined under both drought and salinity stress in cereals. However, due to the rapid increase in world population, there will be critical food shortages to fulfill their food demands in the nearby future. Hence, integrated approach from agronomists, plant scientists, and plant breeders is needed to develop cultivars with high yield and improved tolerance to drought and salinity stress.

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Cereal Physiology, Flowering, and Grain Yield Under Abiotic Stress Imposed by Different Heavy Metals

3

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Abstract

Cereals are the most critical contributor to the global food supply. It is a staple in the human diet because it avoids cancer, constipation, bowel problems, and high blood sugar. It covers more than half of the world's total calorie needs. The availability of high-quality cereal grains on a long-term basis is crucial in addressing the high-priority challenge of food security. Heavy metal

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_3

concentrations in the soil have been shown to negatively impact the biochemical and physiological processes that regulate grain quality in cereals. Several metabolic processes such as the electron transport chain, cell membrane stability index, connective PSII compound, mineral metabolism, oxygen-forming complex, and enzyme activity can be disrupted by excessive heavy metal concentrations in plant systems. In this chapter, we discuss the following topics related to the effects of heavy metals on (1) plant productivity, (2) accumulation and remobilization of seed reserves, (3) plant growth, (4) photosynthesis, and (5) nutrient uptake.

Keywords

Cereals · Heavy metals · Abiotic stress · Oxidative stress · Plant defense

3.1 Introduction

Rapid growth and massive economic expansion in agriculture and manufacturing resulted in a polluted environment. This polluted environment is a result of entry of hazardous compounds from various sources, including anthropogenic and natural activities. Abiotic elements and biotic communities are threatened by man-made synthetic industries, coal conversion, and waste incineration. The environmental toxicants are typically comprised of heavy metals and pesticides, and they pose a hazard to the entire ecosystem, causing significant damage to its function and structure.

Heavy metals such as copper (Cu), zinc (Zn), lead (Pb), and cadmium (Cd) are producing an alarming combination of environmental and health issues. The chemical qualities of heavy metals are more practically responsible than their physical characteristics. Increasing attention has been paid to environmental toxicity that exceeds established maximum residue limits (MRL) by think tanks worldwide. Heavy metals can be found in various places such as housing industries, mining sites, and agricultural fields. The risk of heavy metal contamination in the environment has increased dramatically, causing havoc across society and mainly in the agricultural sector, where they accumulate in the soil and are further absorbed by plants. The problem of heavy metal contamination has had a serious impact, and it requires creative and realistic solutions to decrease the risks. The plants need heavy

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metals to develop numerous organs, but they become poisonous when the concentration exceeds a particular limit. Agriculture, mining, agrochemicals, and industry are the principal sources of heavy metals.

Under traditional agriculture, the soil is a fertile environment for the accumulation of heavy metals and their subsequent release to plants through absorption of water by the roots, which is then carried over into the plant's vascular system. Heavy metals are classified by scientists into two primary categories: natural (sources varying from sedimentary rocks, volcanic eruptions, soil formation, and rock weathering) and anthropogenic (sources varying from agriculture, mining and household waste).

As a source of great nutritional value for humans, cereal products play a critical part in economic development (Güleç et al. 2010). Cereals have high protein content and are used as animal feed, raw material in bread production, and primary ingredients in a wide range of industrial products, among other applications. It is produced on a large scale to meet the nutritional requirement of humans (Karaoğlu and Kotancılar 2001).

Wheat is one of the significant cultivated plants around the globe and is considered as an essential food product. About 70% of all cereal production consists of wheat (Yavuz 2005). There is a constant exchange of information between plants and the surrounding environment in which they grow. There are a variety of environmental challenges that these plants must deal with (Abu Obaid et al. 2018; Al-Tawaha et al. 2018a, b, c; AL-Issa et al. 2020). Heavy metals are the major part of chemicals that lead to large destruction of the ecosystem. The sudden increase in environmental pollution due to excessive use of these harmful chemicals, industrialization, and technological development ultimately leads to contamination of the food chain. The result of contaminated food products in turn poses a threat to human health. Some pollutants can be accumulated in living organisms by passing through the food chain (Nagajyoti et al. 2010). As a result of biological accumulation, the concentration of these pollutants dramatically increases in plants. Plants accumulate these pollutants—most importantly heavy metals—through the application of agrochemicals and contaminated water (MacFarlane et al. 2003). Unlike other chemicals, heavy metals fail to decompose. Hence, they accumulate in plants quickly, thereby leading to destruction of soil structure, which further leads to compromising soil quality and plant production (Wu et al. 2010). Plant phytochemicals, e.g., phenolic compounds, help neutralize and eliminate free radicals (Šiukšta et al. 2019). Copper (Cu) plays a significant role in enzyme activation and is directly involved in lignification (Hall and Williams 2003). It is also known as a micronutrient and is regarded to be needed for proper plant growth and development (Hall and Williams 2003). Zinc (Zn) plays an essential catalytic and structural role in enzymes, and proteins used in energy metabolism depend on this particular metal (Hall and Williams 2003). The cultivation of cereal crops on highly contaminated soils with waste and heavy metals will lead to a food security threat because these heavy metals can accumulate in cereals and incorporate into the food chain (Munzuroglu and Geckil 2002). Such conditions also lead to the complete failure of crops. In this chapter, we will study how accumulation of heavy metals in cereal crops can affect seed germination, growth, and development of the plant.

3.2 Effect of Heavy Metals on Crop Productivity

Heavy metals and pesticides are on top of the list of environmental toxins that threaten the ecosystem. Agricultural methods produce a wide range of pollutants, categorized into biotic and abiotic by-products of agricultural practices. Various pollutants, including agricultural pollutants, often affect agro-ecosystems. These pollutants are typically linked to the pollution and degradation of the agro-ecosystem in which they occur. Fertilizers, insecticides, and sewage sludge are the most common agricultural sources of heavy metals. However, the nature of poisonous heavy metals and how they accumulate, whether in soil or plants, differ. Fertilizers provide a variety of essential nutrients to plants to promote growth and productivity while also increasing the organic matter of the soil. As a result, fertilizers increase the fertility of the soil by adding organic fertilizers and inorganic fertilizers.

Heavy metals are trace elements that the plant requires in order to grow and develop, although they can also cause severe damage to plants. The rhizosphere, where metalliferous minerals and chemicals interact with root exudates, is where heavy metals influence plants. Heavy metals are responsible for inducing many morphological, physiological, and biochemical abnormal functions that lead to reduced crop productivity. Heavy metal toxicity induces illnesses depending on heavy metal content, length of time spent exposed, and stage of plant growth.

3.3 Effect of Heavy Metals on Germination

Abiotic and biotic stress, environmental changes, and hormonal disturbances can all impact seed germination, making it one of the most susceptible physiological processes in plants (Moosavi et al. 2012). The extent of heavy metal impact on seed germination depends on their ability to pass through its seed coat and enter embryonic tissues (Ko et al. 2012). Moreover, Munzuroglu and Geckil (2002) reported that when the same amount of heavy metal is applied to different seeds, it shows a different effect based on the seed coat's anatomy and structure. Copper and cadmium stop seed germination by reducing water uptake (Kranner and Colville 2011). Various experimentations also proved that all heavy metals reduced seed germination in different plants (Márquez-García et al. 2013). The reduction in germination is also linked with the limited oxygen supply and physiological malfunctioning which further leads to failure in providing food reserves. Plants can uptake heavy metals via roots or dust and rain (Jozic et al. 2009). Four times more heavy metals are found in plants near the road than those that are not. Seeds are susceptible to heavy metals, especially to metallic traffic pollutants. There are abnormal embryos and seeds with very low viability that have been reported in heavily trafficked highways (Stvolinskaya 2000). Seeds collected from these areas are not germinated even after pregermination treatments (Ganatsas et al. 2011).

3.4 Effect on Accumulation and Remobilization of Seed Reserves

Seed biology is one of the crucial topics in plant physiology (Bewley 1997). Therefore, different plant species have been studied to examine how heavy metals are assimilated, concentrated, and distributed in seeds (Wang et al. 2014). Reestablishing transcriptional and translational setups is the key reason on which the failure and success of a seedling development depend, especially during early ontogeny (Finkelstein 2010). Many other essential factors are also involved, like remobilization of stored seed reserves (Nadeem et al. 2013); growth of seedling root; soil, temperature, and water conditions; and concentrations of heavy metals (Wang et al. 2014). Germination of seed is vulnerable to heavy metals, most importantly when Cu, Cd, Cr, Zn, Ni, and Mn are present in the environment (Márquez-García et al. 2013). Nickel can help in the remobilization of macronutrients when present in higher concentrations. The higher concentrations of copper and cadmium reduce the remobilization of reserves of phosphorous because of a reduction in the acid phosphatase, alkaline phosphatase, and α-amylase activities in the endosperm (Kalai et al. 2014). Cd and Cu can also enhance soluble sugar and protein content, which causes lipid peroxidation and accumulation of proline in the form of radicals (Kalai et al. 2014). The reduction in the germination of seeds is not the result of a decrease in uptake of water but maybe because of disturbed remobilization of food reserves from the endosperm (Kalai et al. 2014). An increase in the concentrations of Cr, Cu, Co, Zn, Ni, Pb, and Mn can reduce the rate of germination in various crops (Wang et al. 2014). This is due to the lack of oxygen and physiological alterations in the supply of stored food.

3.5 Effect of Heavy Metals on Plant Growth

A major constraint to agricultural production around the world is the effects of environmental stress (Al-Tawaha et al. 2018a, b, c). Heavy metal ions can enter plant tissues with the uptake of other nutrients from the soil (Hossain and Komatsu 2013). Exposure to heavy metals is one of the key reasons to decrease of plant growth (Hu et al. 2013). Other physiological disorders include changes in the structures of leaves and other physiological changes and a decrease in photosynthesis and respiration rate. All of these changes affect metabolism and decrease energy production. Transpiration and material exchange between different organs and the efficiency of roots to uptake water and mineral salts from the soil are also disturbed (Ying et al. 2010). Heavy metals can also cause phytotoxicity in plants, leading to chlorosis, decreased yield and plant growth, limited uptake of nutrients, and decreased legume plants' ability to fix nitrogen (Guala et al. 2010). When entering into the food chain, heavy metals can cause a reduction in agricultural yield and severe health issues. Cadmium is one of the most hazardous heavy metals for plants, ranking second only to mercury because of its inhibiting effect on photosynthesis and the growth of root and shoot. Cadmium also has the additional effect of inhibiting the function of numerous enzymes by developing an affinity with the thiol group of the enzyme (Mendoza-Cozatl et al. 2005). Higher copper concentration can reduce biomass by causing chlorosis and restrict the function of the electron transport chain of photosynthesis (Patsikka et al. 2002). Uptake of nickel at higher concentration can cause physiological disorders, most importantly chlorosis and necrosis (Pandey and Sharma 2002). Plants have decreased nutrient balance when growing on soil highly contaminated with nickel, because it disturbs the function of the cell membrane. Ni can also disturb the activity of H-ATPase and lipid composition of the plasma membranes (Yadav 2010).

3.6 Effect of Heavy Metals on Photosynthesis

Environmental contamination and ecological destruction have become increasingly serious in recent years, owing to the rapid rise of industrialization and the consequent increase in population. One of the most serious reasons of these difficulties is soil heavy metal pollution, which has risen to become one of the world’s most serious environmental problems. Heavy metals have a negative impact on the development and metabolic processes of plants. Photosynthetic fixation is significantly impacted by heavy metal toxicity, both in vivo and in vitro. Heavy metals have an adverse effect on both in vivo and in vitro photosynthesis. Heavy metal toxicity can cause serious issues in photosynthesis, respiration, and growth in the algal model system (Volland et al. 2012). Other than toxic metals, there are some metals like Fe, Cu, and Zn which are an essential part of many cofactors and important nutrients for plants because of their requirement in photosynthesis (Kovacik et al. 2010). Photosynthesis is the main process to produce energy which is based on the continuous working of many important molecular and cellular organelles and structures. The functions of these structures are highly susceptible to metal toxicity. Interaction with heavy metals can decrease the content of light-harvesting pigments and will cause an interruption in transferring energy to light-harvesting antennae (Kuzminov et al. 2013). The absorption and build-up of Cd in plants can have an impact on their normal growth. Cadmium stress results in a decrease in the biomass and photosynthetic rate of plants. It also causes oxidative damage, a shift in the balance of nutrients taken up by plants, decreases photosynthetic efficiency, and also interferes with the development of photosynthetic pigments. Cd stress can also increase the concentration of active oxygen in cells and promote peroxidation. By producing and synthesizing a wide range of enzymatic antioxidants, non-enzymatic antioxidants, osmolytes, and chelating agents, plants can alleviate the stress induced by heavy metals. Achieving an effective way of remediating Cd-contaminated soils is critical because Cd can accumulate in plants and then enter the human body through the food chain, resulting in chronic poisoning and putting human health at risk.

On the other hand, heavy metal pollution can also disturb the normal function of photosynthetic proteins like Rubisco and reaction center proteins (Franco et al. 1999). Thus, heavy metal pollution causes disturbance in both light and dark reaction of photosynthesis. Heavy metals when present in higher concentrations can disturb the electron transport of photosystem II. It can also inhibit the transport of electrons between both photosystems due to heavy metal toxicity in membrane lipids. Despite the fact that heavy metals have a significant impact on photosynthesis, there is still a lack of precise information about the physiological modifications caused by heavy metals (Appenroth 2010).

3.7 Effect of Heavy Metals on Nutrient Uptake

It is reported through various studies that exposure to lead can decrease the nutrient uptake in plants (Chatterjee et al. 2004). It can also decrease the concentration of important cations like Mg^{2+}, Mn^{2+}, Ca^{2+}, Zn^{2+}, and Fe^{2+} in leaves of *O. sativa*, *B. oleracea*, *M. sativa*, *Z. mays*, *R. sativus*, and *V. unguiculata*. Lead exposure to plants can also considerably reduce the content of nitrate in shoots, free amino acid, and nitrate reductase in *B. pekinensis*. The presence of Cd in the soil disturbs the fraction and functions of minerals, hence affecting the biochemical, structural, and physiological processes in plants (Feng et al. 2010). Cd is not essential for plant growth, but it is also absorbed by plants with other nutrients and transported to other parts of the plant. Uptake of Cd can reduce the yield and quality of the crop (Hassan et al. 2005) by inhibiting (1) the uptake and translocation of nutrients in plants and (2) photosynthesis. Moreover, Cd inhibits the openings of stomata, therefore, decreasing the uptake and transportation of water (Vassilev et al. 1997). Cd uptake also disturbs the function of enzymes involved in the Calvin cycle which leads to a decrease in carbohydrate metabolism (Khan et al. 2009).

3.8 Conclusion

Contaminated soil with heavy metals is a major concern for human health and the environment. Heavy metals are already present in the crust of the earth, but these heavy metals are redistributed to different environmental compartments through anthropogenic activities. When released into the soil, heavy metals significantly affect the productivity of the crop and the quality of the environment. Heavy metals absorbed by roots of the plants from the soil enter the food chain, through food. Most of the metals absorbed by roots are also stored in roots in the form of insoluble metal salts. They can bind with pectin, get immobilized within the cell wall, and can further make their way by accumulating in the vacuoles and precipitating within intracellular spaces. Accumulation of heavy metals in higher concentrations in plants can cause a decrease in the biomass, decrease seed germination, inhibit the biosynthesis of chlorophyll, and reduce root elongation. Heavy metal toxicity also affects many other physiological processes like photosynthesis, mineral nutrition, enzymatic reactions, and respiration. Heavy metal accumulation is also the reason for the maximum production of ROS by interacting with electron transport. To cope with up high production of ROS, plants have adapted many detoxification mechanisms. It involves reducing heavy metal uptake by complexation with pectin molecules of the cell wall. This mechanism is regarded to be the most significant resistance of plants against heavy metal toxicity.

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Priming Effect in Developing Abiotic Stress Tolerance in Cereals Through Metabolome Reprograming

4

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Abstract

Cereals are often subjected to a diverse array of biotic and abiotic stresses. Abiotic stresses, which are inevitable, have a significant negative effect on cereal production potential around the planet. These environmental constraints, which include insufficient and irregular rainfall, alkalinity, salinity, and high temperatures, among others, not only restrict cereal yield but also appear to be gradually worsening. Given the current scenario, it is critical to adopt certain more advanced approaches that can effectively address environmental problems and increase crop yield. Among these, seed priming is a frequently used technology for increasing seed vigor and resistance to stress. Seed priming is the process of achieving a desired physiological condition by the use of natural or synthetic chemicals. Crop plants grown from primed seeds demonstrate an instantaneous cellular reaction to abiotic stresses. Resistance is acquired by primed seeds through a variety of cellular and metabolic pathways that include a series of signaling networks. Primed seeds have many benefits over conventional approaches, including standardized germination, decreased emergence and germination times, and a wide spectrum of disease and environmental stress resistance. Seed priming is a commonly used new technique to improve plant tolerance under abiotic stress conditions.

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_4

Keywords

Oat · Barley · Maize · Rice · Wheat · Drought · Oxidative injury · Salinity · Oxidative defense · Nutrient acquisition

4.1 Introduction

Crop productivity is being hindered in many areas as a result of the effects of global warming. Various environmental limitations, including heavy metals, cold, heat, salt, and drought, can have a significant impact on plant growth and development (Rhaman et al. 2021; Hasanuzzaman et al. 2013). These environmental limitations have a negative impact on the early phases of plant development, such as seed germination and establishment of seedlings (Vishal and Kumar 2018; Yadav et al. 2020). In this era, significant emphasis has been placed on finding ways to alleviate abiotic stress in crop plants. There are several physiological and non-physiological strategies for increasing plant tolerance to abiotic stresses (Rhaman et al. 2021). Seed priming is a cost-effective and efficient technique that promotes seed germination, improves plant growth and development, and promotes morphological characteristics (Jisha et al. 2013; Banerjee and Roychoudhury 2018; Muhei 2018; Rhaman et al. 2020). Priming induces a certain physiological state in plants, making them more tolerant and responsive to future stress exposure (Marthandan et al. 2020). Water absorption in seeds during the imbibition stage promotes protein biosynthesis and respiratory activity. During the second stage, important processes such as protein synthesis, mitochondrial reorganization, and changes in soluble sugar concentration occur (Ali et al. 2017a). The efficacy of seed priming is affected by factors such as priming duration, priming concentration, medium, type of plant species, and temperature. Seed priming is classified into two types based on the priming agent: biotic and abiotic priming (Sher et al. 2019). Seed priming is the controlled hydration of seeds in water or low osmotic potential solution to start the germination process without the emergence of radical. Several reports found that seed priming increases the stand establishment and yield production potential of crop plants (Jisha and Puthur 2016a, b; Thomas and Puthur 2019; Paul and Roychoudhury 2017). Seed priming can be done in a variety of ways, including (1) phytohormone priming, (2) nutripriming, (3) biopriming, (4) solid matrix priming, (5) osmopriming, (6) halopriming, and (7) hydropriming.

4.2 Phytohormone Priming

Phytohomones or plant growth regulators (PGRs) are chemical compounds generated by plants that play significant functions in controlling plant growth and development. Phytohormones, including ethylene, salicylic acid, abscisic acid, gibberellins, cytokinin, and auxins, are essential for plant growth and development (Rhaman et al. 2021). Phytohormones act as essential chemical messengers in plants, modulating numerous cellular processes. Further, phytohormones possess the potential to interact with multiple signaling pathways in plants under abiotic stress (Voß et al. 2014). Studies reported that interaction among phytohormones plays a critical function in controlling plant physiology under different biotic and abiotic stresses (Nishiyama et al. 2011; Colebrook et al. 2014). Hormonal priming, viz., priming seeds with hormone solutions, has become an essential mechanism in mediating seed metabolism (Rhaman et al. 2020). Hormonal priming is currently a widely utilized approach for improving germination, seedling development, and agricultural production in unfavorable conditions (Masood et al. 2012). Abiotic stresses impede plant growth and development, and seed priming with indole acetic acid has been described as an effective method for mitigating the impacts of these challenges (Akbari et al. 2007). Hormonal seed priming involves pre-soaking seeds in an optimum level of phytohormone, which improves seedling growth, germination, and yield production by promoting nutrient absorption via improved physiological processes and root formation (Rhaman et al. 2021). Seed priming using phytohormones has been researched in a variety of plant species and has been shown to influence numerous physiological events, including transpiration, respiration, and growth (Sneideris et al. 2015). Plant hormones play an important part in plant defense, biochemical indices, and signaling processes (Sytar et al. 2019). Phytohormone seed priming can alter molecular and biochemical processes that allow plants to withstand environmental constraints, and these approaches are currently showing great potential.

4.3 Nutripriming

The use of various micronutrient solutions to prime seeds has recently gained much attention so as to increase the availability of micronutrients to plants and their assimilation in seeds to minimize malnutrition. Several research investigations have found that seed priming with pre-determined ratio of magnesium (Mg), boron (B) and zinc (Zn) significantly enhanced productivity of several crops by improving yield characteristics, germination, and growth. Micronutrients also participate in a variety of biochemical and physiological processes during seedling establishment and germination. Micronutrients are needed especially during germination because B, Zn, and manganese (Mn) play important roles in nutrient acquisition, secondary metabolism, free radical scavenging, and membrane integrity particularly under stress conditions. Plant researchers are studying different seed augmentation methods for better germination, uniform emergence, and seedling establishment in different fields and horticultural crops (Mondal and Bose 2019). Roots absorb Zn as the divalent cation (Zn^{2+}). Zn activates several enzymes, including alcohol dehydrogenase that catalyzes the conversion of carbonic acid and acetaldehyde. Carbonic acid catalyzes the hydration of carbon dioxide to bicarbonates. Besides, Zn deficiency in soil indicates an altered metabolism of the phytohormone auxin (Sharma 2006). Seed priming with micronutrients (Fe and Zn) significantly increased chlorophyll content and plant biomass in maize (Sharifi et al.

2016). Likewise, priming with ZnO nanoparticles significantly enhanced salinity tolerance in lupine plants by improving chlorophyll and oxidative defense (Abdel Latef et al. 2017). Similarly, Zn administration promoted salinity tolerance of maize plants reflected as better nutrient acquisition under salinity (Imran et al. 2018).

4.4 Biopriming

It is a novel seed treatment approach that combines biological (inoculation of seeds with microorganisms) and physiological (seed hydration) processes (Reddy 2013). Biopriming has lately been utilized as an alternative technique of controlling seed- and soil-borne diseases. The process of biopriming was first introduced by Callan et al. (1990). Biopriming, like other seed priming methods, does not allow radical protrusion. During biopriming, the exudates produced from the seed may provide energy and nutrition to the biocontrol agents (El-Mougy and Abdel-Kader 2008). Therefore, water/nutrient absorption is facilitated by the growth and colonization of these biocontrol agents on the surface of seeds. Biopriming with beneficial microorganisms provides a new crop protection method by enhancing the seedling vigor, seed quality, and plant ability to endure adverse environmental conditions, therefore assuring long-term crop production (Ma 2019). Biopriming plays an essential part in plant growth promotion activities (Mahmood et al. 2016). Biopriming has been shown to improve plant tolerance against drought (Kasim et al. 2013). Further, biopriming has shown promising results in improving plant salinity tolerance (Mahmood et al. 2016). Kaymak et al. (2009) reported that biopriming with PGPR enhanced germination in radish under saline conditions.

4.5 Solid Matrix Priming

Matrix priming (solid matric conditioning) is achieved by regulated and restricted hydration, similar to osmopriming and hydropriming. However, in solid matrix priming, seeds are primed with solid medium that supplies nutrients and water to seeds before radical emergence. The solid matrix may include diatomaceous polymer, water-absorbent polymer, and vermiculite (Taylor and Harman 1990). The materials used in solid matrix priming are low in osmotic potential and bulk density, while these materials hold higher water retaining capacity. Solid matrix priming is recommended due to its higher water retaining capacity, and seeds can be easily separated from such materials (Khan et al. 2009). During the hydration process from solid matrix priming, oxygen supply to the seed is much better. In a study, solid matrix priming enhanced metabolism during seed germination in *Helichrysum bracteatum* L. plants (Grzesik and Nowak 1998). Likewise, fruit quality and protein contents were higher in plants from seeds primed with matrix priming in hot pepper plants (Ilyas et al. 2002). Similarly, solid matrix priming enhanced germination in pepper and tomato plants. Solid matrix priming has the potential to improve germination in various plant species, particularly horticultural crops (Sher et al. 2019).

4.6 Halopriming

Soaking seeds in aerated solution of different levels of inorganic salts such as calcium chloride, calcium sulfate, sodium chloride, and potassium nitrate is referred to as halopriming. Halopriming improves seedling establishment, germination, seedling development, and yield production capacity in different plant species under stress and non-stress environments. Nakaune et al. (2012) reported that NaCl priming significantly enhanced seedling vigor and tolerance to salinity stress. Khan et al. (2009) also reported better germination, seedling vigor, and salinity tolerance in hot pepper plants. İşeri et al. (2014) found higher salinity tolerance in tomato plants as a result of NaCl priming. Srivastava et al. (2010) reported that priming seed with $CaCl_2$ enhanced tolerance to drought and salinity stress in mustard plants. Yadav et al. (2011) reported that seed priming with KNO_3 significantly enhanced germination and tolerance to salinity in pepper. Mahmoudi et al. (2012) found significantly improved salinity tolerance in lettuce plants as a result of KNO_3 priming. Entesari et al. (2012) reported that seed priming with salicylic acid and KNO_3 resulted in a significant improvement in seed germination and salinity tolerance in mung bean plants. Lara et al. (2014) also found improvement in nitrate reductase activity and oxidative defense in tomato plants.

4.7 Osmopriming

The term osmopriming was first introduced by Heydecker and Coolbear (1977). It is a common commercial procedure in which seeds are hydrated to a regulated level in order to facilitate pre-germination metabolic events. In this process, seeds are subjected to a predetermined amount of imbibition due to excess water entry into the seed, leading to the build-up of reactive oxygen species and oxidative damage injury to essential cell components, including nucleic acid, proteins, and lipids. Osmopriming by delaying water entry into the seed lowers ROS generation and so prevents the cell from oxidative damage. To reduce water absorption, osmotica, including sorbitol, mannitol, sugar, and polyethylene glycol (PEG), is supplemented to the solution. Furthermore, other salts such as KNO_3, NaCl, $MgCl_2$, and $NaNO_3$ are utilized in osmopriming. Di Stasio et al. (2020) reported higher yield in salinity-stressed tomato as a result of osmopriming with seaweed extracts. Abid et al. (2018) found higher drought tolerance in wheat due to osmopriming with PEG. Mouradi et al. (2016) reported that osmopriming with PEG substantially enhanced growth, chlorophyll fluorescence, nutrient uptake, and nodulation in alfalfa under drought. PEG osmopriming also improved tolerance to water-deficit conditions in sunflower (Bourioug et al. 2020). Osmopriming is more financially and technically viable than hydropriming as seeds with osmopriming germinate faster with greater water conservation, providing farmers with a promising option (Kumar and Rajalekshmi 2021).

4.8 Hydropriming

Hydropriming involves soaking of seeds in water before sowing for a set amount of time determined by the radical emergence time in plant species. This is a low-cost and simple technology where water is utilized to prime seeds. Following hydropriming, seeds are surface-dried or re-dried to their initial weight. Several studies found that hydropriming improves stand establishment, yield production, and seedling vigor in plants under stress or non-stress environments. For instance, seed hydropriming significantly enhanced seed germination in wheat under salinity (Roy and Srivastava 1999). Similarly, hydropriming improved germination and growth in maize at the vegetative and reproductive stages (Nagar et al. 1998). Several studies reported better seed germination and seedling growth as a result of hydropriming in rice (Basra et al. 2005), maize (Dezfuli et al. 2008), and chickpea (Kamithi et al. 2016). In a nutshell, hydropriming significantly enhanced germination and yield production potential of plants.

4.9 Effect of Seed Priming on Cereals Under Abiotic Stress

In conventional breeding methods to improve the tolerance to abiotic stress, the selection for better grain yield is expensive and slow, needing several years of field trials. The production of grain depends primarily on the features and functions of the flag leaf and is linked to the plant capacity to manage its water content, and synthesize, conserve, and move the carbohydrates from leaves to grains. The *Poaceae* family, which includes bamboos, sugarcane, pasture grass, and cereal grains, generated the most significant bioenergy, feed, and food crops. Grasses provided more than 70% of the essential calories to humans. It is difficult to determine the economic worth for grasses, although wheat production alone in 2014 was estimated at about USD 200 billion worldwide. Abiotic stress is one of the major causes that limit agricultural production in the globe (Shahinnia et al. 2019). Seed priming contributes significantly in mitigating the effects of abiotic stress on crop plants, particularly cereals (Mickky 2021). The mechanism by which seed priming improves tolerance to abiotic stress in cereals is given in Fig. 4.1.

4.10 Wheat

Wheat is an important cereal crop and widely cultivated in different parts of the globe. However, growth and productivity of wheat are severely diminished due to abiotic stress (Mickky 2021). Jafar et al. (2012) reported that seed priming significantly enhanced salinity tolerance in wheat. Seeds were hydroprimed (water priming), primed with $CaCl_2$ (50 mg L^{-1}), kinetin (50 mg L^{-1}), salicylic acid (50 mg L^{-1}), and ascorbate (50 mg L^{-1}). The stand established was maximal in plants from seeds primed with different priming treatments, and this was particularly significant in osmopriming ($CaCl_2$ priming). Different yield-related attributes such

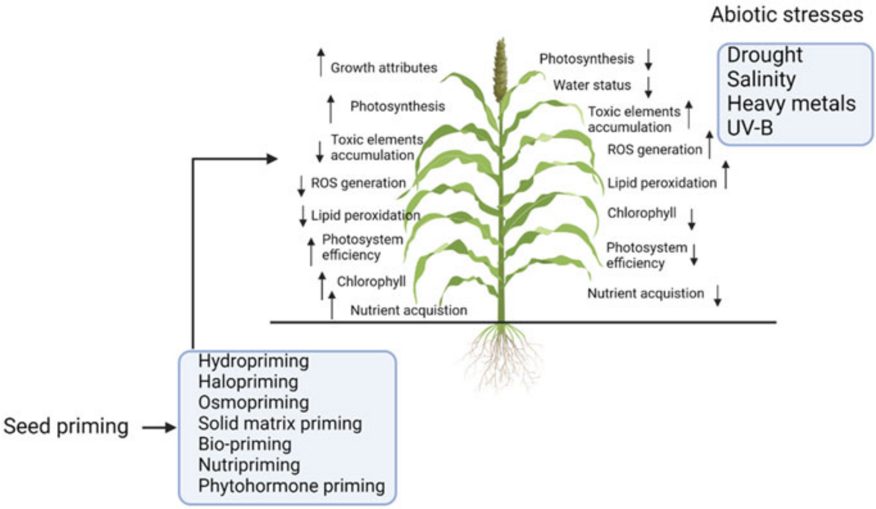

Fig. 4.1 Role of seed priming in mediating plant defense response in cereals under environmental constraints. ↓ Represents decrease, and ↑ represents increase

as harvest index, grain yield, grain weight, 1000 grain weight, grains per spike, and fertile tillers were manyfold higher in plants with osmopriming followed by seed priming with ascorbate. Seed priming, particularly osmopriming, significantly impeded with the uptake of Na^+ alongside better K^+ uptake. Further, priming also resulted in a substantial rise in protease activity, α-amylase activity, total soluble proteins, and total phenolics in wheat under salinity. Seed priming significantly subsided the negative effects of salinity, especially osmopriming. Iqbal et al. (2006) reported improvement in salinity tolerance in wheat due to seed priming with NaCl, $CaCl_2$, and KCl (100 mM). Seed priming with $CaCl_2$ significantly enhanced plant tolerance to salinity compared with other priming treatments. Priming treatments notably enhanced plant fresh biomass and grain yield. Further, hormonal imbalance created by salinity was also regulated by $CaCl_2$ priming. Likewise, Feghhenabi et al. (2020) found greater salinity tolerance in wheat plants primed with ascorbic acid, potassium silicate, spermidine, and saline water. Among different priming treatments, seed priming with potassium silicate was the most effective in terms of seed germination and seedling establishment under salinity. Ali et al. (2017b) reported that seed priming with sodium nitroprusside significantly improved physiological and biochemical processes in wheat under saline conditions. Four wheat cultivars, namely, Galaxy-2013, Millat-2011, Punjab-2011, and Sehar-2006, were used in the study. The wheat cultivars displayed a substantial difference in their relative salinity tolerance potential in the form of grain yield and oxidative defense mechanism. Wheat seeds were primed with different sodium nitroprusside levels (0.1 and 0.2 mM). The authors reported a significant reduction in plant biomass and grain yield alongside a significant rise in proline, lipid peroxidation, and reactive oxygen generation (ROS) in wheat under salinity. Seed priming with sodium nitroprusside (SNP) showed manyfold decline in lipid peroxidation that increased grain yield and plant biomass in wheat cultivars under salinity.

Iqbal and Ashraf (2006) studied the effects of seed priming with different levels (100, 150, and 200 mg L^{-1}) of cytokinins (benzyl aminopurine and kinetin) on plant growth, yield, endogenous levels of polyamines and salicylic acid in wheat cultivars under salinity. In priming treatments, kinetin priming proved efficient in increasing growth and yield in wheat plants under salinity. Salicylic acid concentration increased in wheat plants in response to priming with mild kinetin dose (100 mg L^{-1}) and moderate benzyl aminopurine level (150 mg L^{-1}) under salinity. The levels of polyamines increased substantially in wheat plants primed with benzyl aminopurine under salinity. The authors reported better salinity tolerance in wheat as a result of seed priming with kinetin and benzyl aminopurine. Similarly, seed priming with 30 mM NaCl significantly enhanced growth and biochemical attributes in six wheat cultivars under saline conditions. Seed priming significantly enhanced growth, chlorophyll contents, and seed germination in plants under salinity (Jamal et al. 2011). Wheat seed collected from drought-stressed plants showed significant tolerance to salinity when primed with $CaCl_2$ (1.5%). Seed priming notably enhanced plant water relations, osmolyte accumulation (proline and glycine betaine), leaf area, and grain yield under salinity. Besides, seed priming also dropped lipid peroxidation and protected plants from specific ion toxicity under salinity (Tabassum et al. 2017).

Bajwa et al. (2018) reported better salinity tolerance in wheat as a result of seed priming with 5 mg L^{-1} benzyl aminopurine (BAP) and sorghum water extract (5% v/v). Seed priming treatments improved growth, chlorophyll, total phenolics, proteins, soluble sugars, α-amylase activity, and tissue K^+ contents in wheat under salinity. Further, seed priming treatments also impeded the tissue Na^+ levels in wheat plants under saline conditions. Similarly, seed priming with $CaCl_2$ (50 mM) significantly improved growth in wheat under salinity (Khan et al. 2019). Likewise, Mohamed et al. (2017) reported improvement in salinity tolerance in wheat due to seed priming with silver nanoparticles. Seed priming significantly improved growth, chlorophyll, proline, and activities of antioxidant enzymes in wheat under salinity. Hussain et al. (2018b) reported increase in salinity tolerance in wheat as a result of seed priming with aspirin. The priming treatments regulated the starch metabolism and strengthened the activities of antioxidant enzymes in wheat under salinity. Islam et al. (2015) studied the effects of hydropriming and halopriming ($CaCl_2$ and KCl) on salinity tolerance potential of wheat plants. Salinity caused a substantial depression in germination, yield, and growth. Further, there was a remarkable rise in lipid peroxidation and ROS production in wheat under salinity. Halopriming significantly protected plants from salinity-mediated oxidative injury due to strengthened antioxidant enzyme activities. K^+/Na^+ was also better as a result of halopriming in wheat plants under salinity. The role of seed priming in mediating defense responses in wheat under abiotic stresses is enlisted in Table 4.1.

Table 4.1 Effect of seed priming on wheat (*Triticum aestivum* L.) under abiotic stresses

Seed priming	Abiotic stress	Plant response	References
Hydropriming and ascorbic acid (2 mM)	Drought	Drought resulted in a significant reduction in plant growth, water potential, chlorophyll contents, and membrane integrity due to oxidative stress. Hydropriming induced a notable improvement in stand establishment and seedling growth. Further, osmopriming with ascorbic acid was better in improving early growth and stand establishment in wheat under drought. Ascorbic acid priming resulted in substantial increase in leaf phenolics, proline, ascorbic acid, and chlorophyll contents. Also, ascorbic acid priming subsided the oxidative injury due to drought. Ascorbic acid priming improved drought owing to enhanced proline levels and antioxidant function of phenolics and ascorbic acid that, in turn, enhanced water potential, membrane integrity, and seedling growth	Farooq et al. (2013)
Hydropriming and silica gel, silicic acid, and sodium silicate (0.5%, 1.0%, and 1.5%)	Drought	Silicon plays a significant role in establishing plant tolerance to drought conditions. Silicon uptake was assessed at three-leaf, anthesis and maturity stages. The uptake of silicon was dose dependent with maximal silicon levels at 1.5% silicon priming. Further, silicon priming increased membrane integrity, proline, relative water content, and epicuticular wax under drought. Seed priming with silica gel resulted in a notable rise in grain yield compared to seed priming with other silicon sources. Silica gel came up as an excellent seed priming agent to improve plant drought tolerance	Ahmed et al. (2016)

(continued)

Table 4.1 (continued)

Seed priming	Abiotic stress	Plant response	References
Hydropriming and osmopriming with $CaCl_2$ (1.5%)	Drought	Seeds collected from field-grown wheat plants exposed to normal irrigation and drought at the reproductive stage were primed with hydropriming and osmopriming ($CaCl_2$ 1.5%). The primed seeds were grown in pots filled with soil. Following germination, 50% water holding capacity (drought) and 100% water holding capacity (well-watered) were maintained. The authors found a significant reduction in plant growth, water potential, and yield alongside a remarkable increase in lipid peroxidation and osmolyte accumulation. It was noticed that plants from seeds collected form terminal drought plants were better in terms of growth and osmolyte accumulation. Further, lipid peroxidation was also minimal in these plants. Osmopriming was superior to hydropriming as it significantly increased leaf area, water potential, grain yield, and osmolyte accumulation	Tabassum et al. (2018)
Hydropriming and priming with salicylic acid and gibberellic acid (10^{-4} M)	Drought	Drought resulted in a significant decline in grain yield. Seed priming with hormones induced a noteworthy improvement in yield-related attributes in wheat under drought conditions	Ulfat et al. (2017)
Osmopriming with $CaCl_2$ ($\Psi s = -1.25$ Mpa)	Drought	There was a remarkable reduction in grain yield due to drought-induced drop in leaf area index and crop growth rate. Osmopriming resulted in a uniform germination and stand establishment alongside better allometric attributes under drought conditions	Hussain et al. (2018a, b)

(continued)

Table 4.1 (continued)

Seed priming	Abiotic stress	Plant response	References
Hydropriming and priming with sodium selenite (25, 50, 75 and 100 μM)	Drought	The priming treatments produced a significant improvement in dry matter stress tolerance index, root length stress tolerance index, and seedlings biomass. Selenium priming resulted in a considerable increase in total free amino acids and total soluble sugars in plants under drought	Nawaz et al. (2013)
Silicon nanoparticle (0, 300, 600, 900, 1200 mg L^{-1})	Cadmium (Cd)	Seed priming with silicon nanoparticles significantly improved plant growth and photosynthetic pigments compared with control plants under cadmium toxicity. Priming substantially diminished oxidative injury by strengthening the antioxidant enzyme activities. Further, seed priming with silicon nanoparticles abridged Cd accumulation in leaf and grains alongside a considerable rise in Si accumulation. It was suggested that silicon nanoparticle priming is an efficient tool to improve tolerance in wheat against Cd toxicity	Hussain et al. (2019)
Zinc (0, 25, 50, 75, and 100 mg L^{-1}) and iron nanoparticles (0, 5, 10, 15, and 20 mg L^{-1})	Cd	Priming with higher doses of nanoparticles significantly improved plant growth characteristics and photosynthesis. Further, priming treatments diminished electrolyte leakage and peroxidase and superoxide dismutase activities under Cd toxicity. Also, the accumulation of Cd in grains were manyfold greater in plants with nanoparticle priming in roots, shoots, and grains; however, Cd accumulation was far below the threshold limit for Cd in grains	Rizwan et al. (2019)

(continued)

Table 4.1 (continued)

Seed priming	Abiotic stress	Plant response	References
Salicylic acid (SA) priming (0.25 and 0.5 mM)	Cd	Cd toxicity resulted in a considerable depression in plant growth and photosynthetic pigments. The uptake of potassium, calcium, and sodium was diminished in plants under Cd stress. However, the uptake of toxic Cd was several fold greater in plants under stress. Seed priming with SA manifested a significant improvement in growth attributes, photosynthetic pigments, and nutrient acquisition alongside limited aerial translocation of Cd in plants	Gul et al. (2020)
Mercapto-triazoles (10, 20, 30, and 40 mg L^{-1})	Cd	Seed priming with mercapto-triazoles manifested a significant improvement in plant potential to tolerate Cd toxicity. Priming treatments substantially increased soluble proteins, esterase, and antioxidant enzyme activities with a conspicuous reduction in lipid peroxidation	Hameed et al. (2020)
Salicylic acid (100 μM)	Chromium (Cr), zinc (Zn)	Wheat plants under when exposed to Cr and Zn toxicity in the growth medium depicted a significant depression in growth with enhanced production of ROS, leading to oxidative injury. Further, Zn and Cr stress resulted in inactivation of antioxidant enzyme activities. SA priming significantly diminished oxidative damage and ROS accumulation. SA priming did not affect SOD and CAT activities, while the levels of antioxidant compounds, namely, glutathione and ascorbic acid, were manyfold greater in plants primed with SA under stress	Mazumder et al. (2021)

(continued)

Table 4.1 (continued)

Seed priming	Abiotic stress	Plant response	References
Melatonin (0, 5, 10, 50, 100, and 150 μM)	Cr	Cr toxicity resulted in a significant drop in seed germination and seedling growth. However, melatonin administration markedly subsided Cr effects on germination and seedling growth in a concentration-dependent manner. Cr accumulation was also minimal in plants primed with melatonin. Further, priming treatments remarkably enhanced α-amylase activity and soluble sugar and soluble amino acid contents. The production of ROS was minimal alongside better antioxidant enzyme activities in plants primed with different levels of melatonin	Lei et al. (2021)

4.11 Maize

Abiotic stresses induced substantial reductions in growth and productivity of maize plants (Abdel Latef and Tran 2016). Ashraf and Rauf (2001) reported the effect of seed priming with 200 meq L^{-1} NaCl, $CaCl_2$, and KCl in maize under salinity. The authors reported better tolerance in maize plants in response to all the priming treatments at the seedling stage. Plants primed with $CaCl_2$ manifested greater germination percentage and plant biomass. Seed priming with different priming agents produced a substantial rise in Ca^{2+} and K^+ in maize under salinity. Imran et al. (2018) reported hydropriming and $ZnSO_4 \cdot 7H_2O$ priming in maize plants under salinity. The priming treatments resulted in a significant increase in plant biomass and minimal shoot Na^+ contents. Seed priming with Zn significantly enhanced salinity tolerance of maize by improving nutrient acquisition in maize. Abraha and Yohannes (2013) also reported enhanced salinity tolerance in maize plants as a result of seed priming with NaCl. Priming treatment conspicuously enhanced plant fresh biomass under stress conditions. Seed priming with titanium dioxide significantly enhanced seed vigor, antioxidant enzyme activities, and leaf water status in maize plants under salinity (Shah et al. 2021). Seed priming with different doses of melatonin (0.4, 0.8, and 1.6 mM) led to a significant increase in seed germination and seedling growth in maize under salinity. Melatonin priming resulted in a remarkable increase in relative water contents (RWC); total phenolics, proline, and K^+ contents; and activities of superoxide dismutase, catalase, and phenylalanine ammonia. Further, priming treatments significantly diminished electrolyte leakage, lipid peroxidation, and Na^+ contents in maize under salinity. Melatonin priming showed an efficient approach to enhance salinity tolerance of maize plants (Jiang et al. 2016). In another study, maize seeds were primed with NaCl. It was observed that plants from seed primed with NaCl depicted a significant improvement in plant biomass, germination, and seedling growth in maize under salinity (Yohannes and Abraha 2013). Likewise, salinity stress resulted in a conspicuous drop in leaf area, leaf number, shoot length, shoot dry weight, electrolyte leakage, and chlorophyll. However, seed priming with NaCl substantially enhanced the potential of maize plants to tolerate salinity, reflected as higher growth characteristics and photosynthetic pigments (Farahbakhsh and Saiid 2011). In a study, hydropriming and biopriming with *Artemisia* L. and *Rosmarinus officinalis* L. leaf extract significantly mitigated the negative impacts of salinity on maize plants, reflected in the form of better germination percentage and strengthened antioxidant system (Panuccio et al. 2018). The effect of seed priming on mediating defense responses in maize under abiotic stresses is given in Table 4.2.

4.12 Barley, Rice, and Oat

Seed priming with salicylic acid SA (0.25 and 0.5 mM) and hydropriming significantly improved Cr tolerance in rice plants. Cr resulted in a remarkable decline in germination percentage, growth, seedling vigor, and dry biomass. In contrast, seed priming with SA led to improved germination and plant biomass production under Cr toxicity. The uptake of nutrients (N, K^+, Na^+, and Ca^{2+}) was several fold lower under Cr stress. The damage to membrane was also higher in Cr-stressed plants (Shinwari et al. 2015). The priming of rice seed with 0.01 μM brassinosteroids significantly alleviated the negative effects of Cr toxicity on germination attributes, plant growth, and photosynthetic ability. Cr toxicity resulted in a significant rise in the endogenous levels of malondialdehyde (MDA) and H_2O_2 in rice plants. Brassinosteroid priming resulted in a significantly lower oxidative damage due to strengthened antioxidant enzyme activities. The uptake of Cr was also minimal in plants primed with brassinosteroids. Further, subcellular damage was also lower in plants primed with brassinosteroids. Sodium selenite priming improved salinity tolerance in rice. Seeds were primed with 6 mg L^{-1} Na_2SeO_4. Total biomass increased in rice plants under saline conditions. The activities of antioxidant enzymes were also higher in plants treated with Na_2SeO_4 as seed priming. The authors concluded that higher salinity tolerance in rice was due to better oxidative defense and enhanced *osNHX1* transcript levels in response to Na_2SeO_4 administration (Subramanyam et al. 2019). Seed priming with spermine (2.5 mM) and spermidine (5 mM) significantly improved salinity tolerance in rice. The spermine and spermidine priming produced a remarkable rise in the expression of genes for antioxidant enzymes with significantly greater expression in plants primed with spermine. The expression of genes involved in the biosynthesis of osmolytes was also higher in plants with spermine and spermidine priming. The expression of genes

Table 4.2 Effect of seed priming on mitigating the adverse effects of abiotic stress in maize

Seed priming	Abiotic stress	Plant responses	References
Multi-walled carbon nanotubes (0, 100, and 200 mg L^{-1})	Cadmium (Cd)	Cd toxicity caused a significant reduction in seed germination and plant growth in maize plants. Various priming treatments resulted in a remarkable improvement in germination and plant biomass alongside the strengthened activities of antioxidant enzymes under Cd stress. Plants primed with multi-walled carbon nanotubes manifested a noticeable abridge in lipid peroxidation	Chen et al. (2021)
Biopriming (*Spirulina platensis* and salep gum)	Cd	Biopriming resulted in a conspicuous improvement in seed germination in both stress and non-stress environments. There was a substantial increase in plant biomass due to biopriming under Cd toxicity. The aerial transport of Cd was manyfold lower in plants primed with biopriming agents under Cd stress. Under Cd stress, plants primed with biopriming agents showed better non-photochemical quenching and enhanced photosynthetic electron flow. In a nutshell, biopriming agents significantly enhanced growth and photosynthetic potential of maize plants by restricting the aerial transport of Cd under Cd stress conditions	Seifikalhor et al. (2020)
Hydropriming and proline (1 mg L^{-1})	Cd	Cd stress resulted in a significant reduction in plant growth attributes alongside the significant accumulation of Cd in the aerial parts. However, seed priming with proline led to a noteworthy improvement in growth characteristics and antioxidant activities. Further, the accumulation of Cd in the aerial parts was also minimal in plants primed with proline. The biosynthesis of sugars and proline was several fold greater in plants from seed primed with proline under Cd toxicity	Karalija and Selović (2018)

(continued)

Table 4.2 (continued)

Seed priming	Abiotic stress	Plant responses	References
Salicylic acid (SA) 500 µM	Chromium (Cr) and UV-B	Stress conditions significantly dropped plant growth characteristics and chlorophyll fluorescence attributes, including qP, Fm/Fo, Fv/Fo, and Fm alongside a remarkable rise in values of NPQ and Cr accumulation. Seed priming with SA resulted in a noteworthy improvement in growth attributes and a significant abridge in Cr accumulation. The stress conditions led to enhanced production of ROS, which were significantly lowered by SA priming of maize plants. Stress conditions resulted in a significant damage to membrane in the form of lipid peroxidation. The activities of antioxidant enzymes were also better in response to SA priming in maize plants under stress conditions. SA might have functioned as a signaling molecule that reduced the ROS generation and Cr accumulation. Further, SA signal also strengthened the activities of antioxidant enzymes	Singh et al. (2016)
Silicon (Si) 2 mM	Drought	Drought significantly reduced the grain yield in maize plants. Exogenous Si notably enhanced grain yield, 100-kernel weight, and cob length under stress	Sirisuntornlak et al. (2019)
SA (100 µM) and plant growth-promoting bacteria (T2Cr and CrP450)	Cr	Cr resulted in a significant increase in cellular levels of Cr, electrolyte leakage MDA (malondialdehyde), and H_2O_2 alongside a remarkable decline in plant growth, carbohydrate metabolism, and chlorophyll in maize plants. However, SA administration and inoculation with plant growth-promoting bacteria (PGPB) improved plant growth by decreasing lipid peroxidation, ROS generation, and Cr accumulation. SA and PGPB subsided the oxidative injury by strengthening the antioxidant	Islam et al. (2016)

(continued)

Table 4.2 (continued)

Seed priming	Abiotic stress	Plant responses	References
		enzyme activities. Further, the levels of antioxidant compounds were also higher in plants under Cr stress	
H_2O_2 (80 μM)	Cr	Cr produced a significant drop in germination percentage, seedling growth, and chlorophyll contents in maize. There was significant rise in proline and sugar contents in plants under Cr toxicity. Seeds pretreatment with H_2O_2 remarkably improved germination and chlorophyll contents that clearly indicated the involvement of H_2O_2 priming in mitigating the negative effects of Cr on plant growth and development	Ayesha et al. (2014)
Hydropriming and ZnO nanoparticles	Cobalt (Co)	Seed priming with nanoparticles resulted in a significant improvement in growth, photosynthesis machinery, and biomass in maize under Co stress. Zn nanoparticles priming protected plants from oxidative injury reflected as minimal production of MDA and H_2O_2. The uptake of Co was also significantly lower in plants treated with Zn nanoparticles. Further, oxidative defense and nutrient acquisition were better in plants primed with ZnO nanoparticles under Co toxicity	Salam et al. (2022)

mediating the biosynthesis of abscisic acid (ABA) was also higher in plants with polyamine priming. The expression of ion transporter, namely, *NHX1*, was also better in plants with spermidine priming. The results proposed an intricate association of polyamines with key stress-related mechanisms (Paul and Roychoudhury 2017). In another study, seed priming with spermine (2.5 mM) significantly enhanced germination and oxidative defense that counteracted the oxidative injury reflected in the form of lower lipid peroxidation and ROS generation (Paul and Roychoudhury 2016). Similarly, the exposure of rice to salinity resulted in a significant drop in growth, chlorophyll, and anthocyanins, while an increase in proline, Na^+/K^+, and H_2O_2 production was prominent (Roychoudhury et al. 2008). Priming treatments noticeably counteracted salinity effects on growth and photosynthetic pigments by reducing ROS generation and Na^+/K^+ ratio in rice plants

(Chunthaburee et al. 2014). In another study, seed priming with $CaCl_2$ (-0.75, -1.00, -1.25, and -1.50 MPa) resulted in a significant improvement in salinity tolerance potential of rice as reflected in the form of better germination rate, speed of germination index, germination energy, germination coefficient, seedling growth, and water uptake (Yousof 2013). Jisha and Puthur (2016b) studied the effect of beta-amino butyric acid (BABA) priming on the biochemical, physiological, and growth attributes in rice seedlings under NaCl and PEG-6000 stress conditions. BABA priming resulted in a significant improvement in seedling growth, photosynthetic pigments, chlorophyll a fluorescence-related attributes, and better activities of photosystems. Mitochondrial activities were also better in plants with BABA priming. Proline accumulation, nitrate reductase activity, and activities of antioxidant enzymes were higher in plants with BABA priming. The peroxidation of membrane lipids was also minimal in plants with BABA priming. Similarly, seed priming with ethephon and 1-methylcyclopropene improved germination and biomass of rice under salinity. Further, 1-methylcyclopropene priming showed lower ROS levels compared with ethephon seed priming under salinity (Hussain et al. 2020). Similarly, seed priming with 30% PEG improved chlorophyll contents and activities of antioxidant enzymes and reduced lipid peroxidation of membrane lipids under nano-ZnO stress (Salah et al. 2015). Farooq et al. (2008) reported seed priming with 10 and 20 ppm spermine, spermidine, and putrescine solutions improved salinity tolerance in rice as reflected in the form of better growth characteristics. Similarly, seed priming in 0.3 and 0.5 mM solutions of calcium compounds, namely, $Ca(OH)_2$, $CaCl_2$, and $Ca(NO_3)_2$, notably mitigated fluoride toxicity in rice plants. Plants stressed with fluoride toxicity manifested greater fluoride accumulation, significant reduction in growth attributes, and prominent cellular injuries. Seed priming with calcium compounds improved germination, seedling biomass, chlorophyll contents and decreased lipid peroxidation, ROS generation, electrolyte leakage, and fluoride accumulation (Singh et al. 2020). Likewise, Banerjee and Roychoudhury (2020) observed that seed priming with gibberellic acid improved fluoride tolerance in rice by regulating antioxidant and phytohormone homeostasis. Barley plants showed a significant reduction in growth characteristic under aluminum toxicity. There was a remarkable increase in lipid peroxidation and ROS production that produced oxidative injury. The degree of oxidative damage was manyfold lower in plants primed with salicylic acid and ascorbic acid. Further, priming treatments strengthened the activities of antioxidant enzymes (Shahnawaz and Sanadhya 2017). Sazegari et al. (2020) reported improvement in drought tolerance in barley plants with $CaCl_2$ seed priming. Likewise, seed priming with acetylsalicylic acid promoted growth, physiological parameters, and yield components in barley plants under drought conditions (Kabiri and Naghizadeh 2015). Kiran et al. (2021) reported improvement in salinity tolerance of oat plants in response to seed priming with $ZnSO_4$ and $CuSO_4$ (100 and 200 ppm). Salinity stress caused a significant reduction in germination percentage and growth attributes in oat plants. Priming treatments resulted in a noticeable rise in growth attributes and germination percentage in oat plants under salinity.

4.13 Conclusion

The aforementioned literature clearly indicated the sensitivity of cereals to various abiotic stresses. Seed priming treatments with various methods could be an essential approach to mitigate the effects of abiotic stress on plant growth and productivity. The priming treatment manifested tremendous potential to regulate defense responses in cereals exposed to different environmental constraints. Germination percentage, seedling growth, photosynthesis capacity, nutrient acquisition, and photosystems efficiency are regulated by seed priming treatments of cereals under abiotic stress. The activities of antioxidant enzymes and concentrations of antioxidant compounds also increased significantly with concomitant decline in oxidative injury due to lipid peroxidation and ROS production in cereals under abiotic stress conditions.

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Understanding Abiotic Stress Tolerance in Cereals Through Genomics and Proteomics Approaches

5

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Abstract

Abiotic stress severely decreases agricultural productivity worldwide. Understanding the molecular mechanism of plant defense responses using conventional methods has been a challenging task. Cereals and grain-legumes, as a primary source of vegetarian food, are quite essential in satisfying the expanding nutritional demands. The prevailing low yield of major cereals (rice, wheat, barley, etc.) has made researchers switch their focus upon enhanced abiotic stress tolerance of plants. This stands out to be rather one of the most sustainable solutions owing to the increasing nutritional demands in context to changing climate. Omics like genomics, proteomics, and metabolomics are important for better understanding, uncovering the underlying biological pathways and mechanisms in response to stress. By a rational combination of the high-throughput large-scale data of the omic approaches and bioinformatic tools, a crucial role toward the holistic understanding of the biological architecture has been established. Stress perception, signal transduction, and molecular mechanisms of defense responses are regulated by gene transcription level to cellular protein complements and metabolite profile level of stressed tissues. In this book chapter, we discuss the integration of physiological trait-based approaches with ever-evolving "omics" technology and its existing tools. These will be critical in further understanding the genetically complicated biological process of abiotic stress that could be accepted by the global omics research community. This deep understanding will thereby provide a novel insight for a great impetus to the development of crop breeding.

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_5

Keywords

Abiotic stress · Omics · Cereals · Genomics · Proteomics

5.1 Introduction

Plants are the primary producers providing approximately 80% of food for human consumption. About 50% of the global production is dominated by cereals that include rice, wheat, maize, and sorghum. The plants are exposed to harsh environmental stress conditions that impede growth and agricultural production. Biotic and abiotic stresses have an adverse effect on plant yield. Plants are frequently subjected to abiotic stresses that include water deficit, salinity stress, flooding, temperature extremities, nutrient deficiencies, and metal toxicity, considered as primary reasons to reduce crop productivity (Yadav et al. 2020). Such plants are considered more prone to weeds, insects, and pests which enhance concomitant loss (Reddy et al. 2012). Earlier, it was predicted that abiotic stress factors are involved in the loss of 70% crop yield, but later in the twenty-first century, it aggravated to 96.5%. The alarming population growth with climatic change exacerbated global food security altogether (Lesk et al. 2016). The sessile nature of plants modulates the development of various strategies. It includes transient rearrangement in the molecular level like transcription factors (TFs), heat shock protein (HSP), late embryogenesis abundant (LEA) proteins, biochemical pathways like reactive oxygen species (ROS) production and photosynthesis, physiological responses like flowering time, gene to protein level expression pattern, and modification of transport channel activities, as survival mechanisms (Soda et al. 2015).

Various omics tools and techniques have been adopted to identify the survival mechanism of plants. The hallmark of omics technology lies in its holistic approach that aims to find out novel candidate genes, proteins, and underlying pathways (Roychoudhury et al. 2011; Soda et al. 2015). The stress tolerance mechanism of plants is still not unveiled, while advancement over the technologies of genomics and proteomics has rapidly assembled data about multiple abiotic stress (Molina et al. 2008). The concept of omics has been developed to aid us with a better understanding of the genetic make-up of plants and their adaptive capacity under stressful circumstances (Soda et al. 2015).

Recent advances in research have demonstrated the idea of plant stress responses utilizing genomics and proteomics. It requires quantitative data at every stage of gene expression. Studies have deciphered the importance of post-transcriptional modifications and regulation in the translational system in plant adaptation to various abiotic challenges (Liu et al. 2012). Genomics has been tremendously advanced in the twenty-first century, with QTL (quantitative trait loci) mapping (Li et al. 2013), NGS (next-generation sequencing), WGAS (whole-genome associated sequencing), etc. Proteomics is an excellent technique for demonstrating the key proteins which recognize major pathways through 2DE (two-dimensional gel electrophoresis), SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), etc.

contributing to stress (Gong et al. 2015). These approaches have been intensely discussed in the chapter. The wealth of knowledge obtained from advanced genomics and proteomics is used to explore the underpinning molecular mechanisms that make the plant stress-resilient. However, a highly synchronized perspective of systems biology with bioinformatics data is required for a complete understanding of the complex regulatory structure of plants. Substantial study on various abiotic stresses has been done to understand the regulatory processes and unravel the different stress-tolerant genes and traits in different species. The chapter outlines the proteomics and genomics approaches toward abiotic stress of crop plants and understanding the molecular mechanisms for stress tolerance. The chapter also highlights several omics techniques that have been used in unraveling notable genes and proteins of cereal crops altered under specific abiotic stress conditions. The identified novel genes, allelic variants, and traits would bring an improved degree of adaptive competence and plasticity (Gürel et al. 2016). This "upregulated" stress-tolerant variety can be further used for breeding on a large scale in field conditions.

5.2 Genomics and Proteomics for Understanding Stress Tolerance in Plants

Omics is a branch of applied biology which includes genomics, proteomics, metabolomics, transcriptomics, epigenomics, nutrigenomics, and metagenomics (Yuan et al. 2008). The "omics" approaches bring a paradigm shift for researchers toward understanding, exploring, and comprehending the behavior of plants toward biotic and abiotic stress. Omics approaches are often misinterpreted and considered relevant solely for plant adaptation associated with the signaling responses of a plant. However, it is a novel approach for paving scopes for research toward crop improvement (Bagati et al. 2018). Omics demonstrate the changes in gene expression level, causing a complex interplay with the composition of the plant proteome and the overall metabolic pathways. "Omics" study is not just limited to the terms "genomics" and "proteomics" but rather extends to both qualitative and quantitative parametrical approaches of the high-throughput techniques for detection of the identified genes, proteins, transcripts, or metabolites related to multifarious signaling pathways. Forward genetics includes observing the variation (natural or induced mutagenesis) and correlating it with genetic loci and phenotypic positional cloning of the allele. Reverse genetics comprises targeting a gene of interest and characterizing its function by studying the genetic manipulation through an experimental approach on the gene (Bowne et al. 2011).

The study of genomics is widely classified into structural, functional, mutational, and comparative genomics. Structural genomics deals with the structure of each and every protein encoded by a genome. Functional genomics, an important branch of omics, facilitates the gene function and interaction of gene products at the cellular level. The enormous information about genes, when analyzed with high-throughput genomic technologies gives data of a genome on a wide scale and validates the genomic difference between different species through “comparative genomics.” The genome sequences help in deciphering the stress-specific gene, protein profiles, and their dynamic regulation changes during stress screening. The genomic approaches enable the identification, improvement, and development of desirable genes by their over- or under-expression. Any addition or lack of information in plant genomes due to mutation is studied under “mutational genomics.” The lack of genomic information is further remunerated with the availability of expressed sequence tags (ESTs), cDNA sequence libraries, microarray, and serial analysis of gene expression (SAGE). Molecular markers enabled identification of the genes since the 1980s. Still, on a large scale, functional analysis was more promising with gene tagging. QTL mapping and designed marker-assisted selection advanced further. It further paved an effective response toward unraveling biological pathways and inheritance of the traits which might be controlled by single or multiple genes called the QTL (Micheletto et al. 2007). QTL analysis for screening can be classified under four categories: biochemical, phenological, morphological, and physiological. Recent breakthroughs implemented positional cloning that enabled multiple potential candidate genes responsive to stress conditions within cereal germplasms (Sutton et al. 2007). Resources of genome sequences of cereals (rice, maize, etc.) are invaluable for exploiting syntenic alignment and fine mapping with many other unsequenced genomes of graminaceous species. The advent of NGS is considered as a hassle-free, cost-effective, and speedy approach for annotation of the genome sequences (including introns and exons), which earlier were challenging to differentiate. NGS techniques, along with the GWAS (genome-wide association studies), have just accentuated the pace and opportunities of comparative genome sequencing of stress-related genes within and across the diversity of species (Ma et al. 2012). High-throughput NGS platforms include Roche 454, pyrosequencing, Illumina, polony sequencing, ABI SOLiD technology, Helicos, nanoball sequencing, Ion Torrent, and Oxford Nanopore. An already sequenced plant genome serves as a reference genome for genome annotation and identifies genetic discrepancies across a large number of sequences (Akpinar et al. 2013). The significant role of the MAPK (mitogen-activated protein kinase) signaling pathway in the abiotic stress has been marked. TILLING (targeting induced local lesions in genomes) is yet another recent high-throughput technology predominantly used to identify and analyze the allelic variations (mutants and SNPs) and primary phenotyping (Cordeiro et al. 2006). With the advancement of genome editing technologies, it is now feasible to introduce alterations at specific locations in the genome with genome editing techniques such as ZFNs (zinc-finger nucleases), TALENs (transcriptional activator-like effector nucleases), and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas 9 (CRISPR-associated nuclease 9). It has enabled the integration of specific mutations, insertions, or deletions and precise sequence alteration using tailored nucleases in various organisms (Kumar and Jain 2015). Transcriptional level changes always do not get translated into proteins. Hence, the study of post-translation changes is significant for investigating plant mechanisms toward abiotic stress.

Proteomics is another interesting branch of omics that further validates the data disparity between mRNA abundance and accumulation of protein in adverse conditions. It also evaluates the gene expression level resulting in alteration of the transcriptomic, proteomic, and metabolomic make-up. The proteomic study is classified into a wide array of applications. It includes structural proteomics, functional proteomics, protein-protein interaction, proteome mining, protein expression profiling, and post-translational modification. Proteomics do not always deal with structural identification of the protein complexes, organelle composition, or sub-proteome isolation but also creates (3-D) maps for localization, signal transduction, and underlying molecular mechanisms through protein expression profiling. The presence of two proteins further elucidates the study of protein-protein interaction through yeast-two hybrid, co-precipitation, or phage display techniques. Several studies have elucidated that alteration in gene expression studies is always not proportional to protein level change. Hence, the functional aspect of proteomics is analyzed through yeast genomics, knockout systems, affinity-purified protein complexes, etc. Proteomes are direct effector molecules for plant stress response catalyzing several changes in the machinery of transcriptional and translational signaling pathways. Few modifications also occur through post-translational modification which includes the study of glycosylation, proteolysis, and phosphorylation. PTM (post-translational modification) is crucial for the investigation of proteomes by proteome mining through differential display, identification or validation, etc. Hence, proteomics deals with a wider perspective for improving crop productivity that has been opened with the advancement in omics. The aeon of "proteomic" research approach commenced through the 2D gel and DIGE (difference gel electrophoresis) (Subudhi 2011). The alternative technique of protein electrophoresis is isotope-coded affinity tags, multidimensional protein identification, and chromatographic techniques. ICAT (isotope-coded affinity tag), cation exchange, and reverse and liquid chromatography approaches convert the whole protein into peptides by enzymatic digestion. The digested peptide is identified by MS (mass spectrophotometer). MS further enables the structural information of the peptide and amino acid sequence, against the protein database (MASCOT, Expasy, PepSea, PepFrag, etc.), to predict the protein. Integration of several omics platforms includes the enormous omics data that has been generated due to computational software resources; such data are stored, analyzed, and made easy access through "databases." These databases reveal an interactome system between molecular interactions and the genotypic-phenotypic relationship network of a plant system for improving and developing novel approaches and paving scopes toward crop improvement (Bagati et al. 2018).

5.3 Plant Response Toward Abiotic Stress and Understanding Through Omics Approach

Plants have been serving as a primary renewable source of energy for the higher tropic levels since evolution. Humans have been utilizing roughly 3000 plant species as food, and 90% of our food energy intake has been served by "cereals." It provides approximately 75% calories and 67% protein intake, which makes cereals more demanding. Stress is defined as any stimulus that exceeds the normal range of homeostatic control in any living organism (Fraire-Velázquez et al. 2011). With an exponential increase in the human growth curve, there are concomitant challenges of abiotic stress. Abiotic stress includes global warming (extreme temperature conditions); a steep decline in the availability of freshwater (drought); hypersaline conditions of the soil (salinity stress); flood and leaching of the soil (nutritional deficiency); overuse of fertilizers, pesticides, insecticides, and herbicides (heavy metals (HMs) increasing soil toxicity); and high photon irradiance (UV, blue light, high-intensity light, etc.).

Additionally, biotic stress reduces crop yield by 4.1% and 2.6% for being infected with pests and weeds. Around 69.1% of loss is attributed to abiotic stress conditions with a lack of management practices, economic hurdles, and training (Godfray et al. 2010). 70 million hectares and 100 million hectares of cereal crop-growing land have been affected solely by drought and salt stress worldwide, respectively, rendering the land uncultivable and retarding its nutritional benefits (Ahmad et al. 2014). With such substantial loss of annual productivity due to abiotic stress, food security is one of the critical issues, particularly in rural areas. With the concomitant loss of agricultural land to urbanization, industrialization has tightened the availability of crop land owing to the ever-increasing population. Crop breeding with abiotic resilience through traditional approaches has met limited success. The population explosion challenges to fulfill the enormous demand toward maintaining quality and supply of the agricultural productivity to satiate hunger parallelly (Bowne et al. 2011). When a plant suffers from abiotic stress, the response of the plant toward abiotic stress ranges from the molecular level to whole-plant physiological level depending upon the duration of exposure, age, developmental stage, species, and genotype of the plant. Plants change their "omics" profiles to adapt to changing environments. Hence, integrating the phenotypic, genotypic, and omics approaches will aid in identifying genes and their underlying mechanisms for the selection and development of stress-resilient and high-yielding cereal plants (Takeda and Matsuoka 2008). Abiotic stress targets the vegetative phase, yet it initiates many responses in the various level of cell types. Plants, over a period of time, generate responses to acclimatize to the prevailing stress conditions. Throughout evolution, this acclimatization leads to adaptation, which is generally established by acquiring mechanisms and metabolic alterations.

Plants have been divided into two categories based on their sensitivity to stress: (a) glycophytes (stress-susceptible) and halophytes (stress-tolerant). The majority of plants come under glycophytes for their tolerance, avoidance, and resistance mechanisms from damage. Various physiological processes by which plants function at the cellular level to adapt to abiotic stress are an accumulation of osmoprotectants (glycerol, sorbitol, polyols), amino acids and its compounds (proline, phenylpropanoid from phenylalanine and tyrosine), terpenes (squalene, lycopene), sulfur-containing compounds (dimethylsulphoniopropionate, defensins, glutathione), phenolic compounds (coumarins, flavonoids, isoflavonoids, lignin, ferulic acid), and ammonium compounds (glycine betaine, alkaloids, cyanogenic glycosides). The production of secondary metabolites confers protection of the protein structure from getting disaggregated and maintains osmoregulation and membrane integrity during environmental stress conditions. Accumulation of proline is a marked response defense mechanism (Roychoudhury et al. 2015). It regulates cellular potential, membrane stabilization, protein solvation, cell-proliferative mechanisms, and autophagy, altering the mitochondrial activity during adverse conditions (Ashraf and Foolad 2007). ROS is detrimental to the plants. Hence, plants produce ROS-scavenging antioxidant enzymes like ascorbate peroxidase (APX), superoxide dismutase (SOD), and peroxidase and catalase (CAT), conferring tolerance and protection to stress conditions by maintaining homeostasis (Horváth et al. 2007; Das and Roychoudhury 2014). Abscisic acid (ABA) signaling shows an evident molecular cross-talk in pathways that induce both abiotic stress resilience and pathogen resistance (Roychoudhury et al. 2013). Plants trigger multiple signal transduction cascades, activating the stress-responsive genes (Ge et al. 2009). Therefore, screening and selecting well-adapted germplasm with high-throughput omics technologies is a challenging job. “Omics” approaches have been quite persuasive for elucidating the molecular mechanisms controlling stress response in plants (Fraire-Velázquez et al. 2011). Generating improved lines of cereal crops for abiotic stress conditions has followed a continuous application of molecular breeding.

5.3.1 Drought Stress

Drought is one of the most devastating factors affecting world agriculture among all the abiotic stress conditions. One-third of the total agricultural land suffers from inadequate water supply triggering dehydration stress conditions impacting the overall growth and yield of a plant (Araus et al. 2003). Irrigation utilizes 80% of the usable water resource. The plant prevails in drought conditions by improving its water usage capacity. The first noticeable impact is the hardening of the soil due to dryness. Physiologically, it impacts plant growth and yield by harnessing mechanical stress on the roots, accompanied with removal of extracellular water content, the decline in photosynthetic activity, and loss of pigment. It leads to the accumulation of compatible solutes, amino acids, polyols, amines, ions, and organic acids. Osmotic adjustment is an inherited trait to counter ROS that stimulates oxidative stress and activates chaperonins and signaling pathways for dehydration−/drought-responsive genes in plants. The development of stress tolerance in crops by traditional approach is a very time-consuming and labor-intensive process (Manavalan et al. 2009). Hence, the focus has been shifted on improving the genetic make-up of the high-demanded cereal crops through modern transcriptomic techniques associated with in vitro genomic studies for verifying the response of the stress and efficacy of the construct of the identified potential gene (Roychoudhury and Banerjee 2015). Abiotic stress induces the transcription factors in rice (*Oryza sativa)* like DREBs (dehydration responsive element-binding protein), CBFs (C-repeat-binding factor), NACs (NAM, ATAF, and CUC), members of gene family 14.3.3 (GF14b and GF14c), and mitogen-activated protein kinase (MAPK) signaling pathway (Pitzschke et al. 2009). G14 genes possess cis-elements in their promoter responsible for tolerance toward abiotic stress and pathogen attack in rice.

Various stress-related and developmental pathways have been marked for expanding understanding of the interactome processes which play a crucial role in selecting beneficial candidates for molecular breeding (Budak et al. 2015). The genomic approaches include molecular breeding techniques, which dissect quantitative traits of a genetic compartment through techniques like QTLs. Progeny lines of extreme phenotypic characters are crossed for segregation and screened with molecular marker-assisted techniques like RAPD, AFLP, and RFLP for verifying genetic polymorphism. Molecular markers associated with drought-tolerant sub-traits are further used for segregation maps analyzed by computer software like ArcMap, MadMapper, RECORD, and JoinMap (Cheema and Dicks 2009). Transfer of the drought-resilient traits into cereal crops is only possible if there is a linkage of the sub-traits of drought-tolerance to the QTLs of the molecular markers. Drought-resilient QTLs associated with the cloning of gene/DNA sequences have marked a vital approach in molecular breeding.

5.3.2 Salinity Stress

About 20% of the available irrigated land suffers from salinity. It affects key metabolic processes, leading to genomic instability, cell wall destruction, and cytoplasmic lysis. Salinity stress alters membrane trafficking, photosynthesis, transcription level, energy metabolism, signal transduction, and protein biosynthesis pathway (Roychoudhury and Chakraborty 2013). Salt-tolerant genes are categorized based on salt uptake, transport, homeostasis, and osmoprotectants. Ion imbalance leads to the accumulation of Na^+ over a long period having a two-phase response to salinity. Osmotic stresses affect the root and shoot elongation and Na^+ toxicity of the plant (Roychoudhury et al. 2008). Salt stress is highly regulated by ion homeostasis (Witcombe et al. 2008). Sulfates and chlorides also contribute to enhancing the toxicity levels of saline soil. Salt stress results in calcium deficiency because the calcium uptake is reduced due to enhanced toxicity levels of other minerals. Hyposalinity (concentration of 80 mM NaCl or below) can also exacerbate stress. It suppresses vegetative growth, seed germination, and root weight causes decline in leaf area and reduces yield (Kanayama and Kochetov 2015). The salt tolerance of plants has been improved by metabolic readjustment (Na^+ exclusion or regulation).

Traditional breeding approaches to enhance salt tolerance included hybridization, wide hybridization, and ideotype breeding. The success rate is not ample for the development of stress-resilient varieties. Modern genomic approaches like QTLs have marked an improvement in the screening efficiency for selecting polygenic traits in the hypersaline environment. Plant transformation methods that include callus induction and tissue regeneration of the explants served crucially for developing salt-tolerant transgenics of model cereal crops. Genetic engineering by introducing cloned genes has also proved highly promising (Verulkar and Verma 2014). Expression profiling of a single gene under varying salt concentrations with differential sensitivity has been explored (Basu and Roychoudhury 2014) with the roles of miRNA. Thorough proteome analysis of rice anthers, wheat root seedlings, and rice plasma membranes has been conducted as part of tissue-specific proteomic research of salinity stress. The wheat chloroplast proteins were linked to a variety of physiological factors responsive to salinity. However, in most of these investigations, only a few salt-responsive proteins were discovered (Sarhadi et al. 2012). Omics analysis reported that sorghum undergoes moderate tolerance under salinity stress, and the most affected cereal includes maize. Rice, barley, and wheat (glycophytes) are the most salt-sensitive cereal, yet rice serves as the best model crop for studying salt tolerance. A few limitations to salinity stress include screening complications due to the expression of salt-tolerant polygenes in several cereal crops (Ashraf et al. 2008).

5.3.3 High-Temperature Stress

Climate change and global warming have been accompanied by an unprecedented rise in temperature by 2–4 °C. High temperature hampers nutrient security by escalating the loss in agricultural productivity in the tropical and subtropical regions. The natural tolerance mechanism of plants includes activating a cascade of events that activates stress-responsive genes, TFs and proteins (HSP), etc. Heat-tolerant genes (HT) are vital for the breeding program (Cossani and Reynolds 2012). High temperature interferes with a variety of important physiological activities in plants, including photosynthesis, respiration, and transpiration, by limiting carbon absorption and obstructing overall reproductive processes, resulting in significant yield loss (Zinn et al. 2010).

Temperature above 40 °C has detrimental impacts on the development of the warm season crops (rice, sorghum) (Akman 2009). It includes impairment in plant height, dry weight, tillers, reproductive traits, fertility, kernel development, seed viability, and fruit formation (Manigbas et al. 2014). Cool-season crops (wheat) are more vulnerable to damage by high temperatures. A temperature difference of 2 °C causes 50% retardation in grain growth and number (Rane et al. 2007). Morphological abnormalities of the damage include unusual ovary, tapetum degradation, and ultimate sterility (Zinn et al. 2010).

The productivity of major cereal crops (wheat, rice, maize, soybean, barley, etc.) is lost by almost 0.45 tons/ha by just 1 °C rise of temperature. The morphological and physiological characteristics for assessing crop diversity via the traditional breeding approaches could not cope with the loss. Hence, researchers shifted their focus to modern omics techniques (You et al. 2009) that included the screening of heat-tolerant genes or QTLs. The molecular study integrates the signaling pathway as a response generated by the activated receptors on the plasma membrane of cells undergoing heat stress. The pathway activates secondary messengers that include calcium sensors like calmodulins (Wu and Jinn 2010), calcium ions, CNL (calcium neurin like), CDPKs (calcium-dependent protein kinases), etc. It further activates the MAPK, TFs, and the concerned *HSP* genes. Stress-responsive genes are regulated via the TFs (Mizoi et al. 2012). *HSP* genes confer survival to the plant under heat stress conditions by maintaining the conformation of the protein structure (Saidi et al. 2010). The identified QTLs gave impressive genetic variation data between the wild type and the HT species. Heat-tolerant wheat genotypes “WH1021 and WH730” (Dhanda and Munjal 2012), soybean genotype “DG 5630RR,” and maize genotype “AZ100” were developed. Apart from creating hybrids, researchers have also exploited certain defense traits (heat escape) to create genetic variability and stabilize yield. Genotypes of heat escape developed included “Waha-1” and “Omrabi-5” of wheat (Al-Karaki 2012). These varieties aimed for enhanced photosynthetic efficiency, harvest index, reproductive traits, a decline in respiratory rate, delayed senescence, etc. (Cossani and Reynolds 2012). The intervention of molecular markers for trait mapping of the identified QTLs has been deployed extensively for studying *HT* genes and their mechanisms which assisted in a better and faster breeding strategy (Shirasawa et al. 2013). Proteomic and functional genomic analyses have made progress by elucidating the role of the *HT* genes. The differential expression analysis of tolerance and regulation has been studied in HT plants (Urano et al. 2010). Several up/downregulated proteins were observed and identified at varying temperatures with their role in plant metabolism. To combat heat stress, several omics techniques have been refined for developing “transgenics.” Omics technology, paired with systems biology approaches, might substantially boost traditional breeding to reduce HS problems and simplify the future of sustainable agriculture (Ahuja et al. 2010).

5.3.4 Cold Stress

The human population explosion has doubled the need for agricultural yield. However, low temperature is an intimidating stress factor affecting plant growth with unpredictable duration and intensity (Sinha et al. 2015). Cold-/low-temperature stress, disrupting metabolic homeostasis, can be classified based on its intensity into chilling (0 to 20 °C) and freezing (<0 °C). Freezing results in ice formation in plant tissues (inter and intracellular spaces) causing cellular dehydration that restricts plant metabolism and hampers growth and development with concomitant loss to global agricultural productivity (Chinnusamy et al. 2007). The severity of cold stress damage shows detrimental symptoms affecting both vegetative and reproductive phase that includes poor germination rate, arrested growth of seedlings, surface lesions on the plant (below −10 °C), waterlogged condition, dehydration, discoloration, osmotic changes (−2 to −4 °C), lamellar phase transitions (−4 to −10 °C), tissue disintegration, protein denaturation, hastened senescence with abscission, floral sterility, and deformation in the pollen tube and ovule growth with decreased fruit yield. Tropical and subtropical belt has chilling-sensitive cereals including rice and maize. Temperate belt grows chilling-tolerant cereals (Chinnusamy et al. 2007).

The cold acclimation of a plant is related to the metabolic pathway in both ABA-dependent and ABA-independent signaling. It induces a change in numerous gene expressions, membrane fluidity, lipid composition, and proteins at molecular levels (Lin et al. 2016). In cold stress circumstances, cytosolic calcium, which is maintained by chelators and channel blockers, plays a crucial role during signal transduction by altering conformations of the Ca^{2+} sensor proteins, including CaM (calmodulins), CDPK, etc. (Tuteja and Mahajan 2007). The alteration in expression levels is associated with the induction of several antifreeze proteins (AFPs). It includes LEA, DMSO (dimethyl sulfoxide)-induced COR (cold-regulated) proteins, HSPs, CSDPs (cold shock domain proteins), chaperones, PR (pathogenesis-related) proteins, etc. (Heidarvand et al. 2010). Apart from this, there is also an accumulation of several amino acids, antioxidants, sugars, pigments, and secondary metabolites that act as a protective shield from membrane desiccation, cell disruption, injury, and unbalanced hydrophobic interaction, promoting membrane rigidity and homeostasis (Janská et al. 2010). Enhanced membrane lipid unsaturation and bilayer fluidity are the significant adaptations for successful cold acclimation, as evident from knockout mutation studies in *Arabidopsis* (model plant) toward sensitivity to low temperature (Chen and Thelen 2013).

The stress-responsive gene expression has been studied enormously (through QTL identification, GWAS, marker-assisted breeding) in various species of cereals (Fig. 5.1). Protein expression level and interaction network are directly affected by stress and were hence investigated with advanced proteomic approaches (such as 2 DE, LC-MS, MALDI-TOF/MS, etc.), providing a complete overview during stress responses (Agrawal and Rakwal 2011) (Table 5.1). The differentially expressed cold-responsive proteins are involved in the regulatory and functional network. Few of them include photosynthetic and photorespiratory proteins, which are very susceptible to damage by proteases induced by ROS.

Genomic and proteomic analysis aided in the deciphering of freezing-inducible/freezing-tolerant genes and proteins and understanding of molecular networks. It generated a shift toward a metabolomic approach to link and uncover the physiological and adaptive stress responses in cereal crops. To understand the phenotype of plants under cold stress, a collaborative approach toward understanding physiological and metabolic components of cold stress is required. Nonetheless, integrating these “Omic”-based methods in diverse cereal crop plants through a holistic approach to address the problem of cold- and freezing-related plant damage remains a major challenge.

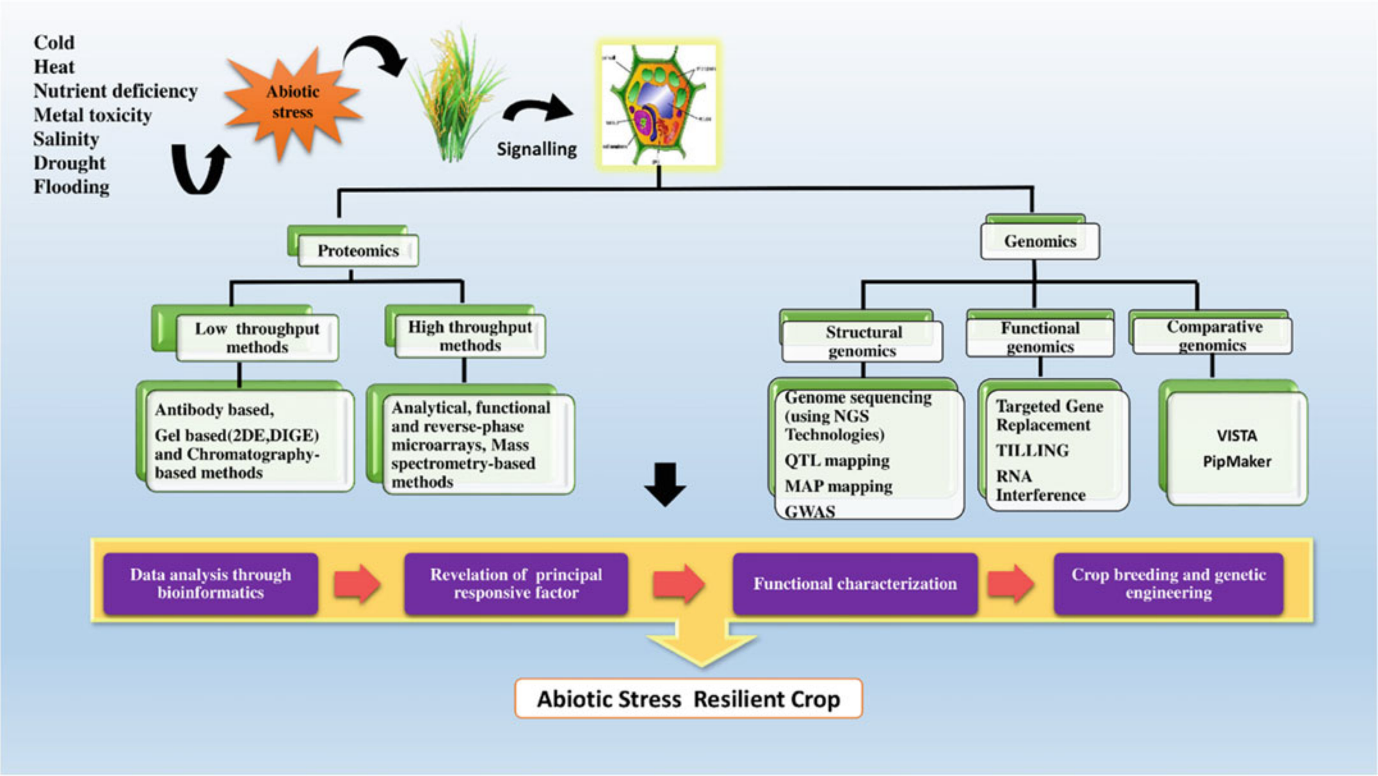

Fig. 5.1 Integrated omics approaches for the development of abiotic stress-resilient crop

Table 5.1 Application of omics tools in various cereal crops under abiotic stress

Crops	Genomic/proteomic tools	Stress conditions	Genes/proteins identified	References
Rice	QTL identified	Anoxia submergence and drought	*QTL9* and *Sub1A*, *Sub1B* and *Sub1C* on chromosome 9, *QTL2,11*, *12.1*- on chromosome 2, 11, 12	Ahmad et al. (2014)
	Genes identified	Drought	OsDREB1A, OsDREB2A, SNAC1, OsERD1, OsNAC6	Ahmad et al. (2014) and Ohnishi et al. (2005)
	QTL identified	Salinity	OsDREB2A, OsERD1, OsNAC6upregulated	Ohnishi et al. (2005)
	GWAS, QTL	Cold	42 QTLs in seedling, 29 QTLs in response to metabolism pathway	Shakiba et al. (2017) and Borjas et al. (2016)
	2-DE, MS, MALDI-TOF MS, label-free and iTRAQ	Cold	Several up- and downregulated unique protein spots identified	Neilson et al. (2011)
	2DE, MS, and MALDI TOF-MS analysis	Heavy metal (Cd, As)	Upregulated proteins and DEP found in roots and leaves	Lee et al. (2010) and Liu et al. (2013)
	GWAS, QTL, Genetic mapping	Heavy metal (As, Cd, Cu, Zn, Fe, and Hg)	QTL identified on Chr 2, 3, 5, 6, 7, 8 DEP, candidate genes for Fe and Zn identified	Gautam et al. (2012), Wang J et al. (2018), Zhu et al. (2020) and Zhang et al. (2017a, b)
	2-DE-MS, QTL GWAS. Linkage mapping	Nutrient deficiency (P, K, N, S, Fe)	Protein spot, OTL (*qRST9.14*), gene and QTL were identified related to survival in stress	Torabi et al. (2009), Kim et al. (2011), Fang et al. (2015), Pariasca-Tanaka et al. (2020), Jewel et al. (2019), Chen et al. (2015) and Hakeem et al. (2013)
	QTL, genotyping, RAPD, and RFLP linkage	Flooding	QTL on Chr. 1,2,5,7,10,11 SNP-mapped on Chr. 9,d	Toojinda et al. (2003) and Singh et al. (2017)
Wheat	2 DE, LC-MS/MS	Drought	Proteasomal factors and protease inhibitors, LEA proteins	Alvarez et al. (2014), Wang et al. (2016a, b), Zhang et al. (2014) and Li et al. (2014, 2018)
	QTL identified	Flooding, drought	–	Shukla et al. (2015), Barakat et al. (2015) and Merchuk-Ovnat et al. (2016)

(continued)

Table 5.1 (continued)

Crops	Genomic/proteomic tools	Stress conditions	Genes/proteins identified	References
	Nano-LC (LC)-MS/MS, Shotgun tandem MS approach	Waterlogging, drought	TaBWPR-1.2 genes	Haque et al. (2014) and Bernardo et al. (2017)
	2 DE, LC-MS/MS	Salinity	Metabolic enzymes, enzymes involved in ETC and ATP synthesis	Singh et al. (2017)
	QTL identified	Salinity	–	Xu et al. (2012a, 2013)
	2-DE, 2D-DIGE, MALDI-TOF	–	Unique protein spots, DEP identified	Rinalducci et al. (2011) and Kosová et al. (2013)
	MS, 2 DE, iTRAQ, and LC-MS analysis	Heavy metal (Cd, Cu, Zn, Hg)	Proteins and DEP identified	Li et al. (2013), Wang et al. (2016d) and Kang et al. (2015)
	GWAS, QTL	Heavy metal (Cr, Al, Cu, P)	71 loci for plant development, ALMT1 mapped on Chr 4DL (Al tolerance) as transporter for malate efflux against stress	Almas et al. (2018) and Raman et al. (2005)
	GWAS	Cold	63 loci and 76 SNP identified	Zhao et al. (2020)
	LC-MS/MS, 2 DE	High temperature	–	Lu et al. (2017)
	Label-free, MS	Nutrient deficiency (N)	Protein spot detected and QTL identified	Karim et al. (2020), Ren et al. (2017) and Chandna and Ahmad (2015)
	Gel-based and LC-MS/MS	Flooding	Protein identified between susceptible and tolerance	Kong et al. (2010) and Pan et al. (2019)
Sorghum	NILs and gene labelling studies	Drought	BADH1, BADH15	Chen and Murata (2008)
	GWAS	Cold	QTLs, SNP 44,515, marker traits on chr SBI-1, 2, 3,6, 9, and 10	Shakoor et al. (2019) and Parra-Londono et al. (2018)
	2-DE, MS, chromosome loci mapping, WGAS, QTL identified	Heavy metals (Cd, Cu, Al)	DEP identified and Alt_{SB} on Chr 3 identified as aluminum-activated citrate transporter	Roy et al. (2016, 2017)
	iTRAQ, LC–ESI-MS/MS	Cold stress	Stress-responsive proteins identified	Wang et al. (2016c)

	QTL identified	Cold stress tolerance	QTLs on Chr 2,4,8	Rodríguez et al. (2014)
	2DE, iTRAQ, and LC-MS/MS	Heavy metal (Cr, Cd, Pb)	Proteins and DEP found after stress treatment	Terzi and Yıldız (2021), Wen et al. (2019) and Li et al. (2016)
	QTL, GWAS, linkage mapping	Heavy metal (Hg, Cd, Pb, Zn, and As)	SNPs identified with seeds and shoots (Hg) QTL (*qKHC9a*, *qKHC9b*, and *qBHC9*) identified (As, Cd, Pb), QTL gene GRMZM2G137161 and GRMZM2G132995 identified (Cd-Zn transporting)	Zhao et al. (2017, 2018) and Hou et al. (2021)
	2DE, MS	Nutrient deficiency (P)	DEP identified	Li et al. (2007) and Zhang et al. (2014)
	MAS, single-molecule real-time sequencing and optical mapping, GWAS, 2D-DIGE, iTRAQ LC-MS/MS	Flooding	DEP on Chr 4, 8 related to ARF, 184 AP2/ERF stress-responsive genes present on Chr 1–10	Mano et al. (2005), Jiao et al. (2017), Du et al. (2014), Chen et al. (2014) and Yu et al. (2015)
	2DE, LC- MS/MS, MALDI-TOF	Cold	Several up- and downregulated protein spots with DEP and unique proteins identified	Mohammadi (2021) and Longo et al. (2017)
	QTL, GWAS	Frost	Chr 5HL	Visioni et al. (2013)
	2D, MS TMT/LC-MS/MS	Nutrient deficiency (N, K)	DEP spot related to root 67, shoot 49, and 288 tolerance found	Møller et al. (2011) and Zeng et al. (2015)
	QTL and GWAS Tandem mass spectrometry analysis	Flooding	QTL on Chr. 2H and 4H, DEP detected and three potential genes (*PDC*, *ACO* and *GST*) upregulated	Gill et al. (2019), Broughton et al. (2015) and Luan et al. (2018)
Rye and oat	Shotgun proteomics and DRM	Cold	DRM and HSP70 proteins identified with P-type ATPase, aquaporins	Takahashi et al. (2013)

5.3.5 Flooding Stress

The decade of scientific advancement has artificially separated nature from mankind. The revolutions of industrial progress and urbanization in the twenty-first century have enhanced the rate of global warming, deforestation, cyclone, and climatic discrepancy. The emission of greenhouse gases has not just depleted the ozone layer but also exerted a profound impact on the precipitation patterns. These varying conditions have affected the whole ecosystem (Eigenbrod et al. 2015).

Flooding is one of the major abiotic stress factors that have a significant impact on crop growth, eventually resulting in a lower yield of cereal crops (Normile 2008). Unpredictable heavy or sometimes irregular rainfall and cyclones induce waterlogging stress in the soil due to poor drainage. It causes a detrimental impact by reducing the gaseous exchange between the soil and the water (Bailey-Serres and Voesenek 2010). Flooding ruins about 13% of the world's total area and nearly 10% of agricultural land (ten million hectares in India alone). The overall yield loss in different cereal crops affected by the aftermaths of flooding ranges from 15% to 80%, depending on the kind of plant, soil, and severity of the stress. It has a profound impact on rice and wheat farming, causing a global yield loss of 18% and 39–40%, respectively.

The incompetency of the crops to tolerate stress is generated due to prevailing anoxic conditions by reducing the level of oxygen diffusion to 104-fold as compared to air (Armstrong and Drew 2002). It alters the respiration and nutrient uptake pathways due to submergence or accumulation of water around, affecting root-shoot development, overall plant yield, and sustainability. The hypoxic conditions (reduced level of oxygen) further transmute to anoxia (absence of oxygen), which facilitates the anaerobes to participate conveniently for the partial respiration and other anticipated activities.

Floods affect several crops, including maize, wheat, rice, and soybeans. Intolerant plants are barred from growing in flood-prone areas, whereas tolerant plants grow, based on escape and quiescence strategy (Colmer and Voesenek 2009). The escape strategy allows reaeration of flood-damaged tissues by inducing anatomical and morphological alterations. At the same time, the quiescence strategy follows the conservation of carbohydrates for a prolonged supply of energy underwater (Bailey-Serres and Voesenek 2008). Flooding alters several morphological, physiological, and anatomical changes in plants that include reduction of dry mass, growth, pH, nutrient deficiency, and microelement toxicity (Voesenek and Bailey-Serres 2015). Developmental adaptation involves changes in organ anatomy and cellular composition to improve oxygen accessibility. It includes the growth of adventitious roots (Zhang et al. 2017a, b), aerenchyma tissue formation (for the ease of oxygen passage from root-shoot), and hypertrophied lenticels. Flooding leads to the transport of 1-aminocyclopropane-1-carboxylate (ACC, precursor of ethylene) from roots to shoots. It triggers the activation of ethylene for regulating systemic responses such as tissue chlorosis, leaf petiole elongation to reach water surface, nastic movements, etc. (Sasidharan and Voesenek 2015). The activation of auxin, abscisic acid, gibberellic acid, and polyamines also directs the stress signal transduction cascade. Ethylene response factors (ERFs), specifically ERF VII, are a low-oxygen detecting group (Licausi et al. 2011). ERFs govern the adaptation mechanism of plants in flooding stress by changing the course of biochemical pathways to preserve the viability of the cell. It deviates from OXPHOS (oxidative phosphorylation) to less energy-efficient glycolysis, fermentation, and anaerobic respiration. The primary limitation of the pathways is the rate of NADH oxidation. Ethanolic fermentation uses alcohol dehydrogenase (ADH), and lactic acid fermentation requires LDH (lactic dehydrogenase) (Bailey-Serres and Voesenek 2008). There is also an upregulation in the production and accumulation of antioxidants, osmoprotectants, and HSF (heat shock proteins). It occurs in response to oxidative damage by ROS via the NADPH-oxidase pathway during anoxia. Antioxidants include catalase, glutathione reductase, ascorbic acid, glutathione, etc. (Lekshmy et al. 2015). HSF act as sensors of the hydrogen peroxide generated during stress. Activation of genes and TFs encoding HSF for proteolysis promotes flood amelioration.

The unpredictable onset of floods due to climatic variations has drawn a significant concern for researchers. The transformation of the traditional knowledge into contemporary has only been possible with the approach of omics. The notable flood-tolerant genes and proteins that have been identified and characterized with genomics and proteomics have been described in Table 5.1. Appreciable conclusions have been embarked with the identification, screening, and characterization of the genes and proteins. It has given an impetus for further advancement of genomics and proteomics (like validation of the biomarkers, marker-assisted breeding, GWAS, LC-MS, etc.). The identified stress-tolerant genes and proteins have been used for genetic engineering to increase agricultural production and subdue the impact of the flood. Metabolomics is yet in a nascent stage of advancement. An amalgamation of metabolomics with genomics and proteomics through a consolidative phytochemical pathway would give a complete picture of flood-tolerant cereal crops (Tewari and Mishra 2018).

5.3.6 Heavy Metal Stress

Among the various abiotic stresses, heavy metal toxicity has a detrimental effect on plants. The bioavailability and leaching of heavy metals (HMs) contaminate the soil. Along with that, the anthropogenic invasions for urbanization and food production result in several contemporary man-made activities. It includes mining, sewage sludge flow, and wide-scale usage of artificial fertilizers and chemicals (Gupta et al. 2010). Natural causes include weathering of soil and climatic change that leaches the minerals, altering the eco-physiological properties of the elements into toxic HMs (Rajkumar et al. 2013). The toxic heavy metals, which are potential carcinogens (transition elements at mild concentration), mix with the environment on a higher scale, negatively impacting soil, water, and air quality, worldwide. Based on the physicochemical properties, atomic number (greater than 20), and specific gravity (greater than 5), the elements have been recognized under non-essential and essential HMs. Zinc, copper, molybdenum, nickel, cadmium, arsenic, beryllium, chromium, aluminum, lead, etc. are very critical for biological growth and developmental process (Tiwari and Lata 2018). The detailed role of every element is mentioned in Table 5.1. Exceeded concentrations above supra-optimal levels cause a detrimental impact on the ecosystem by entering the plant metabolism through ion carriers and channels (Pierart et al. 2015). Due to soil contamination, a low concentration of the HMs accumulated by the plants results in high toxicity levels as it passes on to the subsequent trophic levels of the inverted pyramid in the ecosystem. HMs alter and deteriorate the quantitative and qualitative crop yield. To safeguard from the toxic effects of HMs, plants have evolved intricate defense and escape mechanisms. Escape mechanisms include compartmentalization and sequestration of the HM inside the cellular organelles (mostly vacuoles) or reduction in the passive absorption of HM into the plant cell. Defense mechanisms include activation of the antioxidant armory (SOD, APX, betaine, proline), binding to phytochelatins, deactivation of organic compounds, and elimination of the HM via transporters, ion channels, TFs, signaling molecules, etc. (AbdElgawad et al. 2020; Jamla et al. 2021). The impacts of copper (Cu), zinc (Zn), lead (Pb), magnesium (Mg), and sodium (Na) were investigated on cereal crops grown on contaminated soil. It was concluded that these metals have detrimental effects on the cellular mechanism, gene expression levels, seed germination, and plant physiology. Phytotoxicity results from the accumulation and interaction of toxic heavy metals with ROS. It interrupts the lipid peroxidation (Branco-Neves et al. 2017) cellular homeostasis and causes oxidative damage (Huihui et al. 2020) whose impacts are chlorosis, reduced nutrient uptake, protein biosynthesis, photosynthesis, biomass production, and loss in plant growth and development at all stages (germination, vegetative, reproductive) (Chandra and Kang 2016).

Omics methods have advanced considerably because of the evidence of biochemical and molecular alterations in the plant due to HM stress. The resources and updates on plant genome, transcriptome, and proteome plasticity against the HM resilience have been developed with the accession of the HM stress response on the cereal crops. Apart from the elemental assessments via omics, significant upregulation of the epigenetic regulators (like metal detoxification transporters) was identified (Shafiq et al. 2019). The massive sequencing and omics data are further developed through several in silico tools for proper annotation and ultimately generates improved resilience in the plant systems for HM stress research. Comprehensive and detailed investigations can lead to unraveling novel candidate genes for phytoremediation, hyperaccumulation of HMs, transporter or carrier system of the retrograde signaling, and metal co-uptake in HM resilient plant species.

5.3.7 Nutrient Deficiency

Plants cannot complete their life cycle unless they receive the necessary nutrients. Plants require approximately 14 elements, which are classified as micro- and macronutrients, based on the levels of such elements in plant dry matter. The occurrence of both micro- and macroelements throughout plant growth and development is fundamental to the overall physiological condition. Micronutrients required in small concentrations include boron, chloride, copper, iron, manganese, molybdenum, nickel, and zinc. These elements are essential for enzyme function and protein stability (Hänsch and Mendel 2009). Macronutrients needed in relatively significant quantities with more than 0.1% of dry mass include calcium, magnesium, nitrogen, phosphorous, potassium, and sulfur (Maathuis 2009).

The mineral elements like carbon, nitrogen, hydrogen, and sulfur are absorbed from the soil or environment in ionic form from carbon dioxide, oxygen, bicarbonate, water, nitrate, ammonium, nitrogen, sulfate, and sulfur dioxide. These are components in the basic cellular structure like amino acids, proteins, enzymes, and nucleic acids and play crucial roles in enzymatic or oxidation-reduction reactions. Potassium, sodium, calcium, magnesium, manganese, and chlorine are nutrients that contribute to maintaining osmotic potential, enzyme conformation (enzymatic activity), regulating anions, membrane permeability, and electrical and chemical potentials. Inorganic compounds containing lead, boron, and silicon are frequently generated from the soil and contribute to the maintenance of electrochemical potential, electrostatic interactions, and power transmission reactions (Mengel et al. 2001).

The poor and unpredictable presence of many of these essential nutrients, particularly macronutrients, significantly inhibits crop development and yield in most soil samples (Gojon et al. 2009). Plants exhibit extensive morpho-physiological responses to mineral nutrient deprivation, including lower productivity and seed yield, significant changes in leaf color through alteration in pigmentation, as well as modification in the root system. During the early emergency response phase, the plant strives to compensate for mineral deficits by employing bidirectional adaptation mechanisms such as increased root zone absorption, mobilizing existing resources, and avoiding biosynthetic activities. Lateral root growth is decelerated in support of main root exploration expansion (Gruber et al. 2013). Furthermore, any kind of macronutrient shortage has an instantaneous deleterious effect on photosynthesis, reducing carbon availability (Wulff-Zottele et al. 2010). The continuous depletion of one or more mineral elements causes an instantaneous reaction phase. The developmental program of the plant is irrevocably turned to maturation and senescence (Watanabe et al. 2010). Plants alter their root development in response to nutritional requirements, and these variations can serve as a nutrient status indicator. Transporters in the root plasma membrane are the entrance point for nutrients into the plant, and their selectivity is significant in determining the toxicity of specific elements (Miller 2014).

Nutrient scarcity has a severe negative impact on crop productivity worldwide, resulting in decreased yield and poor-quality food and feed. At the same time, improper fertilizer use can contaminate both terrestrial and aquatic habitats and a significant amount of energy required in fertilizer production, which leads to climate change. Mineral nutrient efficiency in crops is usually characterized as the ability of the crop to absorb mineral nutrients and produce biomass or yield using the mineral nutrients obtained. As a result, due to inadequate nutrient efficiency, more than half of the chemical fertilizers applied are lost. So, creating crops with high mineral nutrient efficiency is crucial for agricultural sustainability, which needs a better understanding of crop responses to mineral nutrient insufficiency (Xu et al. 2012a, b).

5.4 Integrated Omics for the Development of Abiotic Stress Resilience in Cereals

According to recent research, among all the abiotic stresses, drought has been the most severe one affecting cereal crop productivity. Traditional breeding programs including screening were more laborious, yield-oriented, and focused on disease resistance rather than ameliorating the underlying mechanism of abiotic stress. The decline in the rate of major cereal crops prompted scientists to build a molecular breeding approach related to the constitutive (stress avoidance) and intrinsic traits (stress tolerance) of plants. The cumulative effect of several stresses at the same time just exacerbated the situation (Dolferus et al. 2011). Hence, to reduce the gap between studies and challenges, multidimensional modern omics approaches reduced the technical difficulties to a large extent and substantially expanded the depth of research in physiology and systems biology (Shelden and Roessner 2013). The advancement in MAS (marker-assisted selection) (Akpinar et al. 2013), WGS, RNA sequencing, etc. removed several limitations of QTL studies, EST, and microarray for generating genomic data. It was validated by both organellar and tissue-specific proteomic studies which provided insight into stress-acclimatized proteins. Proteomic research resulted in the identification of several abiotic stress-sensitive proteins. The most common technique for separating proteins is through two-dimensional electrophoresis (2-DE). The emerging proteomic advancement has enhanced the accuracy for screening the developmental phase of the stress, analyzing the resistivity of cereal crops, and monitoring their high-throughput stress-responsive proteins by MS.

The discovery of abiotic stress-resilient mechanisms with omics has provided enormous opportunities in cereal research including (1) genome similarity and conserved genome order on the chromosome (synteny) between several cereal species for positional cloning, (2) development of the mutant line of populations with single base pair mismatches in cereals and transposon-tagged lines with Ac/Ds, (3) extensive mapping of the stress-resilient traits and map-based cloning by merging of the QTL identified on different chromosome maps of cereals for analysis, and (4) exploiting alternative efficient screening methods and designing a plant model system (*Arabidopsis*) to compare, identify, and transform stress-resilient germplasm by the omics approach. Interactome studies with traditional approaches proved complex due to the massive genome sequences, products, and related defense responses. Therefore, a shift toward transgenic methods through omics approaches offered a lot of potential for transferring stress-tolerant genes across species (Jha et al. 2014). Omics approaches are being seen as a prominent and promising tool for venturing into the profiles of stressed tissues.

5.5 Future Perspectives

A targeted research point of view is desirable to combat environmental stress and reuse natural resources. The impact of stress during the reproductive stages in the field are critical for rain-fed, drought-prone environments. The current omics studies dominate more with the comparative analysis of the differential expression of the stressed plant with respect to wild-type crops. However, focused strategies on proper integration and interpolation of the genomic and proteomic accessible data are emerging exponentially. Specific omics tools will play an important role to widen the window of molecular breeding, genetic-phenotypic mapping, QTLs, hybridization, single-cell genome sequencing, positional cloning, alternatives to gene silencing, transgenic approaches, etc. Future studies would be more specific on tissue, cell line, or growth stages, rather than the whole plant organ with improved dissection and isolation techniques. PTM, protein-protein interactions, protein isoforms, GWAS, and stress markers are the emerging fields of studies, revealing novel candidate genes in response to abiotic stress (Fig. 5.1). This advancement not only would open up new avenues for co-expression and network analysis but are highly recommended to produce high-yielding and stress-resilient crops.

Omics is an essential step toward accelerating such breeding initiatives throughout the world, which may provide massive insight into stress physiology and metabolism. Identification of novel genes controlling stress tolerance can increase crop yield. It has been reported that drought and salinity hit crop productivity more severely. Hence, in-depth attention is required for the characterization of the traits, compatibility evaluation, and development of stress-resilient crops under various environmental conditions. Both environmental stress and population growth has put alarming pressure on plant breeders. It is becoming increasingly evident that crop development must rely on omics tools for gene identification and high-yielding germplasms to ensure future food security across the world.

Acknowledgments This work was supported by the Department of Biochemistry, Central University of Rajasthan, Ajmer-305817, Rajasthan. DG gratefully acknowledges SERB-DST grant (SRG/2020/001024). SD and RM would like to gratefully acknowledge financial support from DST-SERB and CSIR.

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Metabolome Analyses in Response to Diverse Abiotic Stress

6

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Abstract

Advanced technologies, particularly "omics" approaches, include the most immediate and promising options for enhancing crop abiotic stress resistance. Omics approaches help discover the molecular mechanism to stress response and tolerance. The application of metabolite profiling techniques in the detection, unambiguous identification, quantification, and quick analysis of even minute quantities of cellular metabolites has risen dramatically in recent years. Metabolomics is essential for understanding plant chemical imprints at various stages of growth and development. The major issues that world agriculture faces today are feeding an ever-increasing population with finite inputs and in a rapidly changing environment. In this light, metabolic engineering technology appears to be a feasible choice, with enormous promise for delivering future crops. Proteomics and metabolomics investigations in cereals, in reaction to abiotic stress, are still negotiable. Gene manipulation involving regulatory genes for improved abiotic stress tolerance has proven to be more effective than employing single or multiple stress responsive genes. Recent breakthroughs in "omics" technologies and access to genomic sequences have a significant potential to

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_6

improve stress tolerance in cereals. This chapter describes several methodologies to detect metabolites in response to abiotic stress in significant cereal crops.

Keywords

Cereals · Abiotic stress · Omics · Metabolomics

6.1 Introduction

Plants that are sessile in nature are driven to survive in harsh environments resulting from either physical stress (abiotic stress) or through other living species (biotic stress). Drought, salt, waterlogging, severe temperatures, and heavy metals are all abiotic stressors that limit plant growth and development, resulting in yield reductions of greater than 50% for important agricultural plants (Bray et al. 2000). Non-stress agricultural land accounts for only 10% of total arable area worldwide, suggesting that crops grown in the rest cultivable lands are prone to abiotic stresses (Dita et al. 2006). Furthermore, crops that are subjected to abiotic stresses are more susceptible to weeds, insects, and pathogen infections, resulting in significant losses (Reddy et al. 2004). The advancement of omics technology and functional analysis of individual genes has revealed that environmental acclimation, which is critical for plant survival, is tightly regulated (Kissoudis et al. 2014; Lopez et al. 2008). Abiotic stress factors like soil salinity and high temperatures, in addition to drought, have a huge impact on crop output. They cause yield losses that are variable in nature related to stress timing, intensity, and time duration. As a result, improving abiotic stress tolerance in crop plants is critical for increased yield stability.

6.2 Omics and Abiotic Stress Tolerance in Cereals

A thorough understanding of both the target crop species and the underlying stress tolerance processes, which includes measurements from the whole plant to the molecular level, is required for successful application of biotechnological techniques to overcome abiotic disturbances. The kind, duration, and severity of stress have a major impact on plant stress response (Bray 1997). Given that plant responses are dictated by the genome, current efforts have focused on deciphering the molecular response to abiotic stressors. Until recently, model plants like *Arabidopsis* and rice were the focus of plant stress response research, while orphan crops like millets languished far behind.

Omics is a term that refers to all biotechnological applications that require knowledge of stress response at the molecular level, such as genomics, functional genomics, genetic engineering, gene expression, protein or metabolite profile(s), and their overall phenotypic effects in response to environmental perturbations, which are usually accompanied by significant changes in the plant transcribing system (Ahuja et al. 2010). Cereal crops have recently benefited from "omics" techniques to better understand the molecular and genetic underpinnings of abiotic stress perception, response, and tolerance. Transcriptome and expression profiling investigations have been performed on a regular basis in the past. Abiotic stress research on major cereals has made significant progress so far, as detailed in the following sections.

6.3 Different Platforms to Gather Metabolomics Data

Because most research projects focus on understanding or improving a particular target feature, targeted metabolomics is by far the most prevalent method. There is a wealth of information available to explain phenotypic variance, but it may not be readily available. Small molecules have a big impact. Tomatoes, for example, can taste bitter, sweet, insipid, or wonderful depending on the ratio of sweetness to acidity (Baldwin et al. 2000). Large-scale, low-cost assays that can analyze hundreds of samples in a short amount of time are required to accelerate advancements through breeding programs.

Combining numerous datasets from complementary analytical platforms, on the other hand, provides a powerful technique for metabolome analysis. Color and aroma are two further areas where tomatoes can be improved. Isoprenoids, such as carotenoids, make up the majority of tomato pigments, while polyphenolics (such as flavonoids) make up the rest (Grotewold 2006). For carotenoid profiling, liquid chromatography (LC) with commercial standards has been employed in the past (Enissi et al. 2005). However, LC-MS is employed to provide more complete metabolome estimation, particularly for isoprenoids.

The quality (i.e., isomerization) and identity (i.e., heat degradation) of carotenoids can change throughout food preparation and processing. For a comprehensive description of isoprenoids, examination of both raw and cooked samples is required (Dewanto et al. 2002; Re et al. 2002). Carotenoids provide a variety of benefits in addition to color. Carotenoids, as well as fatty acid and amino acid derivatives, contribute to fruit aroma in addition to color (Tieman et al. 2006). All three are volatile chemicals, and they are separated and identified by GC and GC-MS (Tikunov et al. 2005).

One of the common issues encountered while examining MS or NMR data in order to better comprehend the spectra formed during an experiment is the need for a well-curated database. Recent advances in tomato metabolomics have resulted in the emergence of such community-oriented tools. Several software and analysis tools have been developed in recent years to process and evaluate metabolite data, but none of them is self-sufficient enough to meet user expectations.

6.4 Metabolomics

The term “metabolome” refers to the entire set of low-molecular-weight chemicals found in a sample that may be by-products or substrates of enzymatic activities. Furthermore, changes in the cell metabolome are not always related to changes in the proteome or transcriptome, underscoring the importance of metabolomics. Target analysis, fingerprinting, and metabolite profiling are some of the conceptual techniques in metabolomics that may be utilized for large-scale applications, such as phenotyping transgenics, gene function identification, significant equivalence testing, and stress response monitoring. As a result, metabolomics bridge the gap between genotype and phenotype. Most of the metabolomics studies used model plants and cereals, with no or very little studies on main millet crops. Only one study used gas chromatography, to investigate variation among primary metabolites and phenoli acids in three kinds of proso millet.

6.5 Metabolomics Intervention Toward Better Understanding of Plant Traits

The above techniques enabled the collection of metabolome data from samples taken for primary and secondary metabolite analysis, as well as lipidomics under varied growth conditions. The metabolomics research looked into biotic stress (Kumar et al. 2017; Uchida et al. 2020), abiotic stress (Feng et al. 2020), improving the quality of legumes and cereals (Tripathi et al. 2015; Okazaki and Saito 2016), biofuel production and lipid profiling (Hou et al. 2016; Tenenboim et al. 2016), the impact of climate change and high CO_2 levels (Pal et al. 2014; Reich et al. 2016), hormone profiling (Kumar et al. 2018), and improving fruit quality (Toubiana et al. 2012; Ainalidou et al. 2015; Upadhyaya et al. 2017). These efforts allowed researchers to examine metabolic pathways in order to build stress-tolerant and nutrient-dense crop plants (Kumar et al. 2017). Several review publications have previously focused on providing thorough methodology and the availability of advanced tools utilized in omics studies, including metabolomics (Kumar et al. 2017; Raja et al. 2017).

Metabolomics is a broad term that refers to the study, characterization, identification, detection, and quantification of metabolites from cells, tissues, and living organisms under specific environmental conditions (Parida et al. 2018; Kumar et al. 2016). The metabolome is made up of a large number of tiny molecules (lower than 2000 Da) with a wide range of chemical structure and makeup. In their analyses of endogenous metabolites and metabolites from external sources, the researchers use both non-targeted and targeted techniques (Li et al. 2014). In comparison to proteomics and transcriptomics, this method generates more detailed data (Dos Santos et al. 2017). The development of high-performance liquid chromatography (HPLC), mass spectrometry (LC-MS and GC-MS), NMR, direct injection mass spectrometry (DIMS), and other techniques has aided in the understanding of metabolite profiling and stress tolerance mechanisms in plants (Parida et al. 2018). Metabolomics studies have gained significance in plant science research due to their accuracy, sensitivity, and precision in mitigating agricultural losses (Genga et al. 2011) and revealing various facts about plant signaling and regulatory pathways (Shen et al. 2016). The overall metabolite content in plants is estimated to be around 250,000 (Kim et al. 2010). The overall quantity, concentration, and kinds of metabolites in plants are greatly increased under stress situations. Plant metabolite profiles show a clear correlation with changes in gene expression. It is critical to learn about the crucial metabolites that play a role in growth, development, and survival, as well as how they are modulated when diverse abiotic stresses occur. Various researchers (Freund and Hegeman 2017; Kumar et al. 2016) identified potential metabolomics markers that are critical for plant abiotic stress tolerance. Several researchers have employed the metabolomics approach to investigate metabolic patterns in stressed plants (Yan et al. 2014; Muthuramalingam et al. 2018; Shen et al. 2016; Srivastava et al. 2013; Witt et al. 2012). As a result, it has become a crucial tool for deciphering the molecular pathways that underpin stress responses. Drought stress caused the increase of many metabolites in *Arabidopsis thaliana* plants, including proline, raffinose like oligosaccharides, GABA, and several tricarboxylic acid (TCA) cycle metabolites (Urano et al. 2009). The function of various subunits of the mitochondrial alternative electron transport route was changed under dark stress (Araujo et al. 2011). Abiotic stress, such as salt, drought, and other factors, also increased the amounts of branched-chain amino acids (BCAAs). The amount of amino acids accumulated is determined by the degree of desiccation, as evident from amino acid profiling of maize and wheat during water desiccation (Witt et al. 2012). Colmsee et al. (2012), for example, created the OPTIMAS-DW data resource platform to solve various concerns about *Zea mays*. It can be used to integrate metabolomics, transcriptomics, proteomics, and ionomics data as well as handle different data domains. Amiour et al. (2012) identified important regulatory processes in nitrogen metabolism control by combining metabolomics, proteomics, and transcriptomics research.

Srivastava et al. (2013) reported on a study involving a transgenic plant that included the *superoxide dismutase* (*SOD*) gene. They used a data processing platform to generate information on ROS metabolism at the system level. Yan et al. (2014) concentrated on the use of omics techniques to better understand secondary metabolism. Exposing plants to salt stress, AbdElgawad et al. (2015) found that tocopherol levels in maize shoots increased, but ascorbic acid levels plummeted. Wang et al. (2015) also found that when *Kosteletzkya virginica* seedlings were exposed to salt conditions, their proline levels increased. Under salt stress, Shen et al. (2016) found a quick decline in the levels of sugars associated with glycolysis pathway in barley. When the peach plant was subjected to higher temperature, it was found to accumulate proline. Sun et al. (2016) examined how the metabolome of maize changed after being exposed to diverse stresses such as drought, heat, and salinity. Based on the metabolomics data, they found that the individual stresses affect differently from that arising from a mixture of stresses. Khan et al. (2018) used untargeted metabolic profiling techniques to examine the influence of drought on the metabolome of chickpea genotypes. Muthuramalingam et al. (2018) investigated threonine profiling using genome-wide computational metabolomics. Using in silico expression investigations, they discovered roughly 16 genes that regulate threonine levels in abiotically stressed rice plants.

Plants create an ample amount of metabolites, having a variety of structures and essential for plant growth and development. These metabolites lay the chemical foundation of agricultural output and quality, as well as an important source of nutrition and energy for humans and livestock (Hall et al. 2008). These metabolites could be primary and secondary; primary metabolites are requisite for plant growth, while secondary metabolites do not have a role in growth, but required for a plant to survive in stressful situations by maintaining a balance with the environment. Exploration of the underlying biochemical nature of plant metabolites and their function in complex regulatory mechanisms is necessitated by the diversity of plant metabolites and their role in intricate regulatory mechanisms (Hall et al. 2008). Because of the complexity of the varied metabolic properties and abundances of chemicals, studying the metabolome of plants will be extremely difficult. Various abiotic stressors disrupt plant metabolism. Under stress, plant metabolic networks must be reconfigured to allow for maintaining the metabolic homeostasis as well as the generation of chemicals that alleviate the stress. Plant development and productivity are reduced when they are exposed to unfavorable growing conditions, such as abiotic stress. Plant metabolism is disrupted for a variety of causes, including inhibition of metabolic enzymes, a lack of substrate, an excess demand for certain chemicals, or a combination of these and other variables. As a result, the metabolic network must be reorganized so that essential metabolism is preserved and a new state for adaptation to the current stress conditions is adopted.

Another issue is the generation of ROS, causing oxidation that disturbs the function of biological components and, in the worst-case scenario, cell death. To reduce ROS formation, the reducing power to scavenge ROS is provided via organellar electron transport chain and must be well maintained (Das and Roychoudhury 2014). Despite the importance of metabolic control in stressful situations, our knowledge of the process is still fragmented and incomplete. As a result, metabolomics is used to gather a huge number of useful information for gene discovery and pathways (Tohge et al. 2014). Plants appear to have a complex regulatory network covering these small molecules, and metabolomics research contributes greatly to understand the relationship between genotype and metabolic outputs by targeting important network components and finding interactions among these metabolites (Toubiana et al. 2013). The domains of transcriptomics, genomics, proteomics, and metabolomics have all seen significant advancements during the "Omics" era. The data collected by these "Omics" methodologies has improved the precision and speed with which breeding programs are generating nutrition-rich and climate-smart germplasm to ensure food security (Parry and Hawkesford 2012). In recent years, the importance of phenomics-based breeding has become clearer.

Based on the technique, metabolomics can be divided into two categories: untargeted metabolomics and targeted metabolomics. Untargeted metabolomics refer to comparing the metabolomes of control and test groups in order to uncover changes in metabolite profiles that may be related to specific biological circumstances. Targeted metabolomics acts as a quantitative method for identifying and analyzing specific metabolic components in living organisms. It provides details on the amount and composition of metabolites, which are directly linked to biological functions and can vary substantially depending on physiological conditions. As a result, metabolomics techniques are critical for understanding physiological functions of various organisms. Metabolomics is a strong method for gaining a thorough understanding of how metabolic networks are regulated, and it has been used in a number of studies in recent years.

Plant plasticity can also be better understood for future biotechnological engineering of stress-tolerant plants. In spite of the fact that it is currently impossible to perform an accurate and thorough full metabolome analysis of a sample, plant metabolomics approaches and technology are quickly evolving (Hegeman 2010). A variety of integrated technologies, such as non-destructive NMR, mass spectrometry (GC-MS and LC-MS), and FI-ICR-MS (Fourier transform ion cyclotography-MS), have made possible large-scale analysis. Metabolomics could be carried out at the subcellular level with the help of various sample technologies (Misra et al. 2014). A number of common plant species, like tomato, wheat, rice, and maize, have proved their potential in plant metabolomics investigations for many reasons (Francki et al. 2016). However, because each analytical platform has inherent limitations, combination techniques are increasingly being used in metabolomics investigation.

6.5.1 GC-MS

The most widely utilized technology for plant metabolomics research is gas chromatography-mass spectrometry (GC-MS). Before separating them through GC, polar metabolites are being derivatized to make them volatile. The fact that this technology has been used for metabolite profiling for a long time means that there are established methods for chromatogram evaluation and interpretation (Lisec et al. 2006). The short run time and cheap running cost of GC-MS make it more significant, but the technique can only be applied for thermally stable volatile chemicals, making analysis of high-molecular-weight molecules (more than 1 kDa) challenging.

6.5.2 LC-MS

In this technique, a prior sample treatment is not required, and separation of the components occurs in a liquid phase, thus not limited by compound volatilization. Reversed phase, hydrophobic interaction, and ion exchange columns are among the columns that can be used to separate metabolites depending on their chemical features, making the technique applicable to a wide range of plant metabolites. The recent development of ultra-performance liquid chromatography (UPLC), with higher resolution and sensitivity, makes the approach more potent. For ionization to connect LC and MS, electrospray ionization (ESI) is extensively utilized.

6.5.3 NMR

Nuclear magnetic resonance spectroscopy is a completely different type of analytical technique than MS, which is based on atomic interaction. Atoms with nonzero magnetic moments, such as 1H, 13C, 14N, and 15N absorb and reflect electromagnetic radiation in a strong magnetic field. The intensity, frequency, and magnetic relaxation qualities of the radiation are all characteristics that reveal the particular environment of the identified nucleus. As a result, the atoms of a molecule produce a unique spectrum that can be used to identify and quantify metabolites in a complicated biological sample. NMR can also create in vivo kinetic data and investigate metabolic reactions in a single plant, rather than a group of similar plants (Terskikh et al. 2005). The magnetic characteristics of nuclei in a magnetic field are used to detect metabolites via NMR. NMR is a nondestructive approach for identifying metabolites with a molecular weight of less than 50 kDa for a variety of applications, including metabolite fingerprinting, metabolic flux, profiling, and extracting the atomic structure information of compounds in biological materials (Winning et al. 2009). MS has improved its accuracy because of the advancements in ionization technologies like electrospray ionization (ESI), air pressure chemical ionization (APCI), and MALDI-TOF (Issaq et al. 2009). Metabolomics is becoming more prevalent in plant sciences and biochemistry, and it has been used in a huge number of researches to date. In this chapter, we shall look at how metabolomics profiling can be used to figure out why plants are so resilient to abiotic stress.

6.6 Abiotic Stresses

6.6.1 Water Stress

Water scarcity is one of the most serious risks to crop output, and it is expected to worsen significantly in the future years (Cominelli et al. 2009). As a result, a lot of research has gone into understanding how people react to this important and prevalent stress. These investigations have indicated that metabolic regulation, particularly photosynthetic regulation and osmolyte buildup, plays a vital role in drought stress response (Verslues and Juenger 2011; Rabara et al. 2021). Plants respond to drought stress by accumulating several metabolites (Urano et al. 2010). The principal metabolites that changed in abundance in response to water deficiency in wheat were amino acids, organic acids, and sugars. Polyamine production is linked to tryptophan build-up via the shikimate pathway and glutamate reduction. Non-targeted metabolomics data analysis revealed that the biomolecules and secondary metabolites were differentially accumulated in the leaves in response to drought and heat stress. Excess water, such as flooding or rhizosphere waterlogging, creates issues due to a reduction in oxygen supply (hypoxia/anoxia). Fermentation in anoxic conditions is required to create ATP, resulting in cytosolic acidification and the build-up of hazardous chemicals. There is a large-scale metabolism reprogramming under oxygen-reduced conditions, which was supported by transcriptome data indicating a widespread downregulation of energy-consuming activities (van Dongen et al. 2009).

6.6.2 Temperature

Cells are severely affected due to ice formation and cellular membrane malfunction (Guy 1990) due to freezing. The molecular foundation of the cold acclimation process has been thoroughly investigated, as has the role of certain metabolites, such as compatible solutes (Wanner and Junttila 1999), as well as the regulatory network (Medina et al. 2011). Cook et al. (2004) and Kaplan et al. (2004) observed that the bulk of heat shock reactions, such as increased pool sizes of amino acids, polyamine precursors, and suitable solutes, were shared between heat stress and cold shock. The findings of this work were compared to transcript profiling data (Kaplan et al. 2007), which demonstrated that transcriptional and posttranscriptional mechanisms regulate GABA shunt and proline buildup under cold circumstances, respectively.

6.6.3 Light

Light drives photosynthesis and also causes secondary damaging processes during excess irradiance. Wulff-Zottele et al. (2010) measured metabolites in plant leaves for 6 days after switching to high light. The level of most glycolysis, Kreb cycle, and pentose phosphate pathway metabolites was altered, showing that the plants exposed to high light undergo a metabolic shift. In addition, an increase in glycine suggested that photorespiratory pathway was activated. Metabolites like glycine and glycolate, which are photorespiratory intermediates, were observed to be accumulated in the early period (5–60 min after changeover). Kusano et al. (2011) observed that the metabolic effect of UV light stress on plants during the mid-phase (80–360 min) embraces the buildup of phenylalanine, shikimate, and fructose, plus the decrease of succinate.

6.6.4 Ion Stress

High salinity levels in the soil impede crop growth, posing severe issues for global food production (Munns 2005; Roychoudhury et al. 2008). High NaCl concentration generates both hyperionic and hyperosmotic stress in plant cells, resulting in turgor loss, disrupted metabolism, and suppression of critical ion absorption, as well as other issues (Kim et al. 2007; Roychoudhury et al. 2007). The metabolite concentrations were frequently many times greater in *Thellungiella*, and stress aggravated the condition. Some metabolites differ from one another. The metabolic differences between *Arabidopsis* and other plants and *Thellungiella* were tested for a wider spectrum of salt and osmotic stressors (Lugan et al. 2010). The examination of global physicochemical parameters of both the species revealed several metabolites, but the shift in *Thellungiella* was far more dramatic. Heavy metals, in general, cause cellular oxidation, enzyme inhibition, and metabolic disturbance, which results in retarded growth and, in extreme cases, mortality (Sharma and Dietz 2009). Dubey et al. (2010) performed a transcriptomics and metabolomics investigation of rice roots treated with Cr. Proline, as well as ornithine, accumulated at three times the rate of the control under these conditions. Following Cr stress, the concentration of various additional metabolites such as lactate, fructose, uracil, and alanine increased. The findings showed that control of sucrose degradation was acting as a rescue mechanism when respiration was stopped.

6.6.5 Combined Stress

In nature, adverse environmental conditions are frequently the result of a combination of variables, with one stress usually being followed by another (Králová et al. 2012). Plants are frequently exposed to multiple stresses, each of which is followed by another. The single stress level is most convenient for experimentation and discussion; however, the plants are actually exposed to a variety of abiotic stress in their habitat (Paul and Roychoudhury 2019). When maize plants are subjected to both drought and salinity stress, levels of citrate, fumarate, valine, phenylalanine, isoleucine, and leucine levels in leaves alter dramatically, indicating a crosstalk effect in manifold stresses (Sun et al. 2015). Combined cold and water stressors in rice resulted in overexpression of carbohydrate metabolism-related genes, which is consistent with the accumulation of glucose, sucrose, and fructose in the aerial portions (Maruyama et al. 2014). When plants are subjected to combined stressors, sugars typically accumulate in plant cells, possibly buffering plants from oxidative damage via osmotic adjustment (Wulff-Zottele et al. 2010).

6.7 Conclusion

Molecular biology and biotechnology have emerged as a viable method for combating stressors in plants in recent years. However, knowledge of stress response at the molecular level is required for efficient application of omics for abiotic stress tolerance. High-throughput sequencing technologies are capable of providing researchers with a wide range of sequencing applications, including the detection of short RNAs and SNPs and the finding of molecular markers. NGS techniques, in combination with genome-wide expression profiling studies, will overcome the challenges posed by large genomes, particularly those found in cereal grains. Proteomics and metabolomics investigations in cereal crops are still lagging behind genomic and transcriptomics studies. However, advances in high-throughput proteomics and metabolomics tools like time-of-flight mass spectrometry and flow injection will simplify the study on complex biological systems like cereals. It not only will help in identifying genes, proteins, and metabolites responsible for many molecular and signaling pathways but will facilitate our fundamental understanding of the molecular basis of abiotic stress tolerance in such crops. As a result, it is critical to carry out considerable research in cereals to overcome abiotic stress. A collaborative effort involving all omics will be critical in unraveling abiotic stress tolerance mechanisms, which may then be used for MAS or traditional breeding. QTLs in many cereals are yet undetected, with no indications of gene pyramiding. In addition, rigorous and meaningful phenotyping is necessary for understanding stress adaptation mechanisms before locating and tagging genes. Nonetheless, current improvements in omics technologies, as well as advances in transgenic technology and MAS, will help improve the existing situation, and it will be possible to genetically modify all cereals in the future.

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Metabolomic Profiling of Different Cereals During Biotic and Abiotic Stresses

7

Bushra Hafeez Kiani

Abstract

The major part of the world's food supply depends on the production of cereal crops such as rice, maize, wheat, barley, sorghum, oat, and millet. These crops are constantly exposed to different environmental stresses, i.e., biotic (pathogens and pests) and abiotic (drought, salinity, flooding, cold, heat, UV radiation, heavy metals, nutrient deficiency) stresses, resulting in huge loss in production and consequently eminent economic problems. As with all organisms, plants grow under suitable environmental conditions, which are required for their optimal growth and development. Plants respond through different defense mechanisms to any deviations from these environmental conditions such as biotic and abiotic stresses. Environmental stresses are often responsible for loss in the production of cereals which usually link with limitations in the cultivations of these crops due to change in growing season under these stresses. Plants have vast reservoir of more than 200,000 secondary metabolites with many diverse and important functions. Secondary metabolites are the major response factors under different environmental stresses through signaling, physiological regulation, and defense responses. Metabolic activation is very important for the acclimatization and survival of plants in response to different stressors by restoring chemical and energetic imbalances of the cells which ultimately helps plant cope with these stresses. Metabolomic profiling of cereal crops is a very important and systematic approach to analyze and monitor the metabolic networks produced and modified in response to stress and tolerance processes. In this chapter, the progress and

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_7

perspective of metabolic patterns of cereals during diverse environmental stresses are described.

Keywords

Metabolomics · Cereals · Environmental stress · Flavonoids · Alkaloids · Secondary metabolites

7.1 Introduction

Plants are sessile, so they have developed different methods to adapt to the varying growth preconditions so that plants can perform their functions without affecting their cellular and developmental processes (Yang et al. 2018; Arnold et al. 2019). This is done by producing a list of secondary metabolites which are involved in various roles in growth and development (Kroymann 2011; Berini et al. 2018).

The production of metabolites in plants is considered a coping mechanism in harsh and dynamic environments, especially those relating to growth. These changes may produce different complex chemicals and induce different structural and functional changes by signaling pathways. These changes or stabilizations in chemical structures lead to different interactions within the plant (Edreva et al. 2008). Numerous secondary molecules that are present in plant cells are synthesized from primary metabolites. Treating plant cell cultures with signaling and eliciting molecules both living and non-living can promote the production of secondary metabolites (Ramakrishna and Ravishankar 2011; Rejeb et al. 2014; Caretto et al. 2015; Narayani and Srivastava 2018) (Fig. 7.1).

Plants have different responses to biotic and abiotic stressors, exclusive for every species. These stressors negatively impact the development and production of plants. Due to these stressors, the world is suffering agriculturally (Barnabas et al. 2008; Athar and Ashraf 2009). It has been calculated that crop yields of major plants have been reduced to half due to these environmental stressors. However, the severity of this yield loss depends on the early stages of plant development when the stress occurs, how strongly it affects, and how long it lasts (Bray et al. 2000; Ashraf et al. 2008; Atteya 2003; Monneveux et al. 2006; Lafitte et al. 2007).

Plants have acquired diverse metabolites throughout continuous evolution, with over 200,000 known secondary metabolites with a vast array of functions. The chemicals involved in the defense mechanism in kingdom Plantae arise from different catabolic and anabolic pathways and can be divided into alkaloids, isoprenoids, and shikimates (Großkinsky et al. 2012) (Fig. 7.2).

Isoprenoids, alkaloids, and phenylpropanoids are produced through the tricarboxylic acid cycle (TCA cycle) or the shikimic acid pathway, acetate-mevalonate or methylerythritol phosphate pathway, and shikimic acid pathway, respectively (Großkinsky et al. 2012). These metabolites formed through different pathways are collectively known as the metabolome of the plant. The plant metabolome is collectively represented as the physical traits of the plant. Biochemical phenotypes

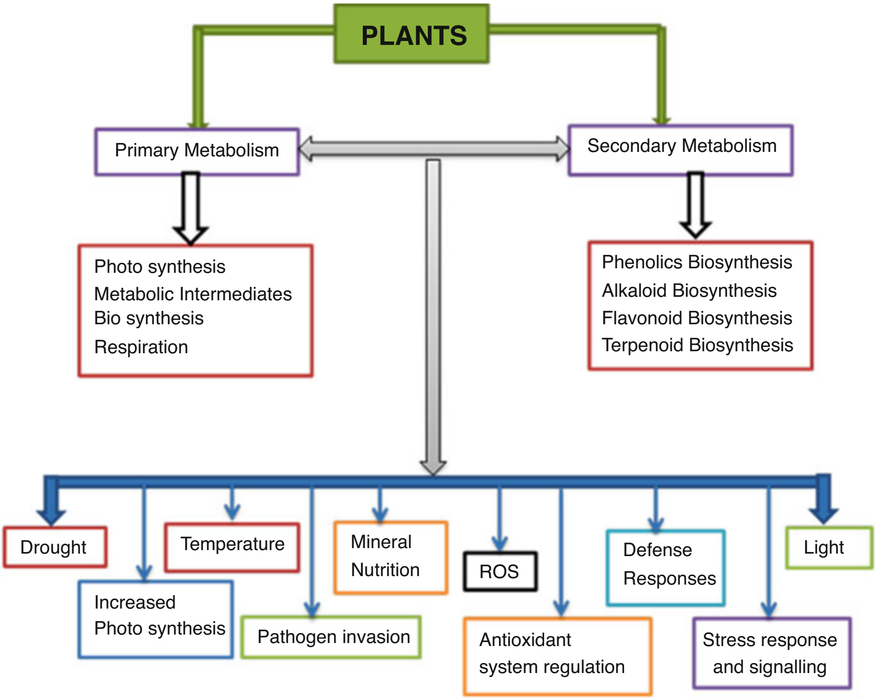

Fig. 7.1 A flow chart of different primary and secondary metabolite production as a response to different environmental stressors in plants

as such can be profiled on a large scale based on their quality and quantity during metabolic analysis. Recently, profiling of metabolites has become a basic tool in plant sciences, for example, examining the species phenotype, analyzing resistant genes, or determining plant responses to different toxicants (Schauer and Fernie 2006).

Metabolomics has been used as a tool to have a comprehensive outlook of different metabolic pathways and their regulation. This has been applied in many pieces of research and can also be used to explore genes as a tool in systems biology and functional genomics. As defined by Fiehn, a complete and quantified analysis of all the molecules present in a living system is called metabolomics (Fiehn 2001). Kingdom Plantae may contain metabolites ranging from 200,000 to 1,000,000, while a single species like *Arabidopsis* may only be limited to only a few thousand (De Luca and St Pierre 2000; D'Auria and Gershenzon 2005; Saito and Matsuda 2010; Davies et al. 2010). The KNApSAck Database contains information on around 50,000 plant metabolites as described through published research (Afendi et al. 2011).

Metabolomics has evolved into a vital tool for screening germline cells during plant breeding (Langridge and Fleury 2011). Plant metabolites are involved in

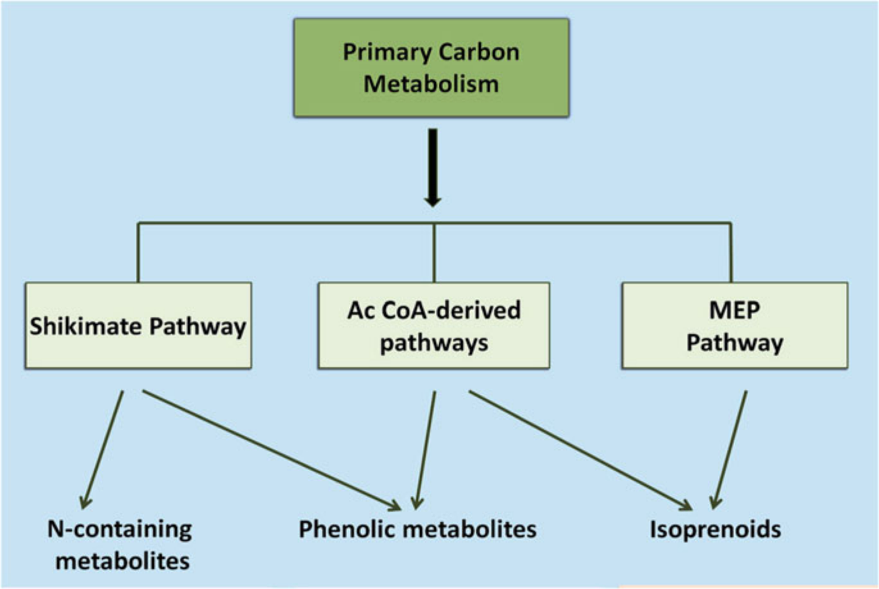

Fig. 7.2 Major metabolites and their pathways involved in cereal defense mechanism. (Adapted from Balmer et al. 2013)

various physiological functions, and current studies on plants have benefited greatly from new metabolome profiling techniques (Großkinsky et al. 2012). Except for a few studies, not much research has taken place on the relationship between the plant, specifically the cereal metabolome, and stress caused by biotic factors (Allwood et al. 2010; DuFall and Solomon 2011).

In this chapter, metabolites, their response to environmental stressors, and their role in the plant's defense arsenal have been discussed. In addition, breakthroughs in metabolome analysis and profiling methods, with their detailed overview in cereal metabolome, are summed up.

7.2 Metabolomic Profiling of Cereals to Biotic Stresses

Metabolomic profiling of plants, especially cereals in response to their interactions with biotic stressors, has not been fully studied, and there are only a few studies reported to support this. In this section, we shed light on the role and response of metabolites to pathogens along with a comprehensive overview of the analysis of cereal metabolites.

7.2.1 Cereal Response to Necrotrophic Pathogens from a Metabolomic Perspective

The metabolite profiling of plants in response to fungal disease must be done by taking the exact determination of compounds or the presumptive determination of metabolites, statistical importance for the studied variables, a strong conversion in the concentration of unsusceptible or vulnerable pathogen-plant metabolites, and, if applicable, reallocation to a plant defense mechanism on record.

One of the most destructive diseases that affect monocotyledonous grains like barley, maize, wheat, and triticale is *Fusarium* head blight (FHB) (Choo 2006). *Fusarium* uses mycotoxins to disrupt host cells before feeding on them, making them necrotrophs. More than 100 quantitative trait loci (QTLs) have been linked with FHB resistance, and they have been reported in seven wheat and barley chromosomes. Only Qfhs-ndsu-3BS QTL plays a role in resistance according to different reports. This is due to its involvement in the formation of a less toxic DON-3-*O*-glucoside from a highly toxic deoxynivalenol (DON) (Lemmens et al. 2005). In such circumstances, research on resistance controlled by polygenes is time-consuming and not practical because of how their low heritability is affected by the environment, time, and location. Metabolites linked with resistance in complex landscapes can easily be detected through metabolomic resources. In 2010, 496 metabolites were identified in barley by using liquid chromatography-mass spectroscopy (LC-MS) (Bollina et al. 2010). These metabolites were overexpressed in the resistant cultivar in comparison to a susceptible cultivar. A peculiar thing to note is that majority of the resistance cluster metabolites were secondary products of both catabolic and anabolic pathways of terpenoid, flavonoids, and fatty acids (Fig. 7.2). The presumptive role of these metabolites in resistance was verified through in vitro assessment for antifungal activity. Several kaempferol precursors found in the resistance cluster were involved in enhancing the defense mechanism of resistant cultivars (Bollina et al. 2010).

The same genetic history should be used to examine the role of metabolites in resistance, as metabolite variations may arise by discrepancies in the genetic makeup of the plant. Moreover, attaining metabolites derived from pathogen-plant interactions is possible. Nevertheless, resistance is expected to be also linked with low fungal growth levels which is why the criteria for selecting fungal compounds as resistant metabolites based on their abundance in the resistant plant is improbable.

Strains of *F. graminearum* are favorable for studying metabolic processes that monitor fungal disease resistance in barley (Kumaraswamy et al. 2011). This study reported the presence of induced resistance-related (RRI) and constitutive resistance-related (RRC) metabolites. Coniferyl aldehyde, vitexin, 8E-heptadecenoic acid, and pelargonidin 3-*O*-rutinoside are examples of RRC compounds present in high levels in resistant barely (Kumaraswamy et al. 2011). It was reported that picolinic acid, IAA (indole acetic acid), and a feruloyl alcohol glucoside were present in increased levels in resistant barely strains that produced trichothecene.

Wheat spikes have developed the resistance to the onset of *F. graminearum* through *Fhb1* (*Fusarium* head blight 1 resistance locus) QTL. This is chiefly due to the activated metabolic pathways of terpenoids, phenylpropanoids, and fatty acids (Fig. 7.2) (Gunnaiah et al. 2012). *Fhb1* contributes to resistance by controlling and regulating the phenylpropanoid pathway. This shows that metabolomic researches extend further from the genetic research of QTLs (Gunnaiah et al. 2012). These studies also showed resistant wheat cultivars had high concentrations of jasmonic acid isoleucine (JA-Ile) hydroxycinnamic acid amide (HCAAs), feruloyl putrescine/agmatine, and coumaroyl putrescine/agmatine (Fig. 7.2) (Gunnaiah et al. 2012).

Maize is another model organism that has been studied in relation to the plant-necrotrophic interaction in maize. Benzoxazinones (BX) have been reported to play a new role in necrotrophic resistance against the fungus *Setosphaeria turcica* (Ahmad et al. 2011). Aglucones are considered toxic when plastid-targeted β-glucosidase enzymes break down BX-glucosides (Morant et al. 2008). QTOF MS combined with ultrahigh-pressure liquid chromatography (UHPLC) is a powerful technique for determining the presence of these chemicals under different research parameters (Ahmad et al. 2011; Glauser et al. 2011).

7.2.2 Cereal Response to Biotrophic Pathogens from a Metabolomic Perspective

Magnaporthe oryzae is characterized by its unique hemibiotrophic lifestyle in which its host cells can undergo plasmolysis (Koga et al. 2004). On the contrary, rice cells that have incompatible interactions with this fungus lose their ability to plasmolyze and show a loss of cell membrane rigidity, along with other symptoms like granulation that are usually signs of infection by fungal necrotrophs. Hence, the lifestyle of *Magnaporthe oryzae* is conditioned by the degree of incompatibility, as it shows a pure biotrophic lifestyle in interactions that are completely compatibilized. *Magnaporthe oryzae* enters the host through the developing conidia present on the epidermis of the leaf. The germ tube produces an appressorium through which the penetration peg grows into the host cell. Multiple invasive biotrophic hyphae originating through the penetration peg are separated from the plant's intracellular fluid by a membrane generated by the plant host. The fungal infection is likely spread through the plasmodesmata in the neighboring cells as the veracity of the plasma membrane of the plant is undisturbed (Kankanala et al. 2007). Additionally, if it infects the epidermal or consequent layers of cells, the biotrophic invading strategy of rice will be different. The interconnected metabolic pathways are difficult to study in such a finely regulated process.

Two important findings have been reported in the detailed analysis of the *Magnaporthe grisea* and rice metabolites. The diseased leaf tissues showing injury were concentrated with amino acids including proline (Pro), alanine (Ala), cysteine (Cys), tryptophan (Trp), and histidine (His). Concentrated accumulation of glucose, sucrose, malate, and fructose were also reported (Jones et al. 2011; Parker et al. 2009). These accumulations were observed in the genetic makeup of susceptible rice, implying that metabolic photosynthetic sinks are formed by noticeable lesions (Parker et al. 2009). The biotrophic mode of living observed in *Magnaporthe grisea* fits in alignment with this information. Nevertheless, a higher concentration of phenylpropanoid and phenolic compounds has a similarity to the necrotroph and plant response explained above. It is assumed that the rice is reinforcing its cell walls, but these reinforcements are not evident because of the reduction of hydrogen peroxide in susceptible phenotypes. This results in reduced phenolic cross-linking in the cell compared to resistant plants. In the later phase of infection, dead cells discharge minerals that may serve as a fuel source for fungus to further sporulate (Parker et al. 2009).

The combination of several compatible and incompatible *Magnaporthe grisea* strains can be used to study metabolic reprogramming in rice's defense arsenal. Jones et al. (2011) evaluated the plant interaction to the fungus at various points in time by using metabolomics based on nuclear magnetic resonance (NMR) and mass spectroscopy (MS). Major changes in the interaction between sugars and amino acids like malate, glutamine, alanine, proline, and cinnamate were reported. It was proposed that the high levels of alanine triggered by then fungal invasion facilitated cell death and promoted further *Magnaporthe grisea* invasion. This information suggests that bringing such responses to a halt may cause an incompatibility in this interaction and limit the infection. Regardless, further studies are needed to be conducted to exhibit the formation of compatibility and the function of alanine (Jones et al. 2011).

7.2.3 Cereal Response to Herbivores and Nematodes

Metabolites derived from primary metabolites are also used by plants to protect themselves against harmful organisms like herbivores, pests, and nematodes. Primary and secondary metabolic pathways are regulated by a great portion of the genome. For example, one-fourth of the *japonica* subspecies of the *Oryza sativa* genome is dedicated to metabolic activities (Goff et al. 2002).

There is very limited data on the metabolomics of cereals and the interaction of cereal herbivores. Even if many quantitative trait loci that are linked with insect herbivore resistance are being described, the cause for these traits at a genetic level in most of the cases is still unknown. Some of the cereal metabolites are being derived from classes that exhibit antimicrobial properties and have the power to stop the growth of pathogens due to their insecticidal and nematicidal properties.

7.2.3.1 Benzoxazinoids

The best known secondary cereal metabolite that could be used against herbivores is benzoxazinoids. These molecules are made up of 2-hydroxy-2*H*-1, 4-benzoxazin-3 (4*H*)-one skeleton, hydroxamic acids being the most effective among them (Niemeyer 2009). These plant compounds have undergone glycosylation, and their effectiveness increases after they are hydrolyzed to aglycone. The biochemical process that leads to their production is well understood (Niemeyer 1988; Sicker and Schulz 2002).

The insect *Spodoptera littoralis* was used to assess the reaction of maize (*Zea mays*) to *Diabrotica virgifera*, as well as the defense capability of the plant parts above the ground. The concentration of metabolites on the affected leaves produced as a defense was also observed. The metabolites found in these affected leaves were quantified using high-performance liquid chromatography with diode-array detection (HPLC-DAD). After the plant was attacked by the western corn rootworm, an increased level of DIMBOA (2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one) was observed in the plant (Erb et al. 2009a, b).

Phenolic chemicals that were assessed through UPLC-MS (ultra-performance liquid chromatography-tandem mass spectrometer) showed that caffeic acid production was inhibited after either of the insects infected the plant. Interestingly, the initial root infestation of *Diabrotica* prepped the leaf tissues to increase the production of chlorogenic acid in the presence of *Spodoptera*. Through the application of abscisic acid (ABA) to the corn roots, we can mimic the accumulation of chlorogenic acid and increase the production of DIMBOA. However, it is important to note that other metabolites are also involved, as the root treatment with abscisic acid alone did not induce *Spodoptera littoralis* resistance (Erb et al. 2009a, b). Recently, the dual purpose of benzoxazinones in inducing herbivore resistance has been reported (Glauser et al. 2011). DIMBOA, which was released from its correspondent glucoside in the main response to herbivores, could be detoxified by *littoralis* and *frugiperda*, both species of *Spodoptera.* On the contrary, the insect stomach degrades HDMBOA (2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one), an unstable chemical generated in the next stage of the herbivore attack, which acts as an obstacle to *Spodoptera* species *littoralis* and *S. frugiperda.*

Another role for BX that is proposed for the defense for cereal is protection from nematodes. Rye (*Secale cereale*) is a crop that is planted in winter annually, and BX can decrease insects and nematode number in rye (Zasada et al. 2005). Other possible implicated metabolites were examined when aliphatic organic acids with low molecular weight from rye plants having biocidal action against *Meloidogyne incognita* was ruled out (McBride et al. 2000). In rye, 2,4-dihydroxy-1,4-benzoxazin-3-one (BX DIBOA) and its by-product benzoxazolin-2(3H)-one (BOA) were reported to have allelopathic effects along with DIMBOA and its by-product MBOA (6-methoxy-BOA) (Barnes and Putnam 1987; Rice et al. 2005). These compounds were also checked for their effect on nematodes (Zasada et al. 2005).

DIMBOA is the primary metabolite in wheat and corn, while DIBOA is predominantly present in rye; this makes it a possible choice for controlling nematodes (Friebe 2001; Rice et al. 2005). It was reported that in plant interaction with nematodes *Xiphinema americanum* and *Meloidogyne incognita*, DIMBOA induced a lower death rate than DIBOA. However, the adults and juveniles were more harmed than the nematode eggs (Zasada et al. 2005). These in vitro toxicity researches have to be viewed with caution, as it was later reported by the same research group that the concentration of DIBOA may be too low to play a substantial role to control nematodes, based on the agricultural soil reports.

7.2.3.2 Flavonoids

The growth of *Helicoverpa zea* larvae, more commonly known as corn earworm, can be stopped through corn silk flavonoids like C-glycosyl flavones apimaysin and maysin (Lee et al. 1998). As per quantitative trait locus studies, approximately half of the phenotypic deviations against *Helicoverpa zea* could be because of maysin and apimaysin. Something peculiar to note is that the two quantitative trait loci were independent of one another, meaning that the synthesis of these related chemicals was not inter-linked (Lee et al. 1998). Isoorientin is another flavonoid that works against *Helicoverpa zea.* Due to a recessive trait, inbred corn has more isoorientin in its silk (Widstrom and Snook 1998).

Avena sativa produces flavone-C-glycosides when attacked by nematodes as shown in mass spectroscopy. O-methyl-apigenin-C-deoxyhexose-O-hexoside is one of these chemicals that are efficient against the *Pratylenchus* and *Heterodera* genus of the cereal-attacking nematodes (Soriano et al. 2004).

7.2.3.3 Alkaloids

Hordenine (*N, N*-dimethyltyramine) and gramine (*N, N*-dimethyl indole methylamine) are the most well-studied grass alkaloids. Cereal plants like barley, sorghum, and millet have been found to contain hordenine (Smith 1977). Both hordenine and gramine function as eating obstacles for grasshoppers (Hinks and Olfert 1992). The feeding tests for *Heliothis subflexa* and *Heliothis virescens*, which are specialist and generalist caterpillars, respectively, showed that hordenine had obstructive results on their eating style. *Heliothis virescens* was less affected than *Heliothis subflexa* (Bernays et al. 2000). The feeding experiments conducted on barley seedlings and aphids *Schizaphis graminum* and *Rhopalosiphum padi* showed that gramine also influences feeding behaviors, through tissue localization and increased concentrations at attacked sites (Zúñiga et al. 1988).

The above-cited examples have a perspective more focused on these chemicals as a feeding hindrance or deadly substances, rather than a metabolomic perspective. Marti et al. (2013) tried to get an overall idea of changes induced by herbivores in the maize metabolome by an impartial approach. The researchers used UHPLC-TOF-MS to establish local and systemic level changes in *Spodoptera littoralis* infected corn, consequently showing 32 chemicals that were regulated independently. The availability of innovative methods is likely to increase our understanding of changes at a metabolomic level in plant and insect synergies.

7.3 Metabolomic Profiling of Cereals to Abiotic Stresses

Recent studies have been focusing on the genetics of the complex series of occurrences in plants that are exposed to abiotic stresses. Many genes have been associated with environmental defense response and gene regulatory networks with the help of transcriptomics. Changes at the metabolomic level are less understood, but in recent years, worldwide metabolite research of plant response to stress has become an emerging topic of research (Roychoudhury et al. 2011a). In this section, we will dissect the results of recent studies conducted on cereals with a particular focus on abiotic stress factors like varying temperature, water scarcity, reactive oxygen species, and high salt concentration.

7.3.1 Temperature Stress

Plant growth and development are largely influenced by temperature, as changing seasons cause temperature fluctuations. The after effects of this temperature change are largely dependent on whether the changes were within the natural range of the plant or were in extremity. However, the survival of organisms in varying temperatures can be increased by cold acclimation or acquired thermo-tolerance. Extensive changes in the transcriptome, proteome, and metabolome enable plants to use these adaptive mechanisms to increase their temperature tolerance.

7.3.1.1 Cereal Response to Temperature Stress

Morsy et al. (2007) studied two genotypes of rice that had varying cold tolerances, with a particular focus on the plant response toward osmotic stress and salinity. It was found through targeted metabolite analysis that both of these genotypes had varying responses to different stresses. Surprisingly, the chilling-sensitive genotype (CS) was less sensitive toward salinity and water scarcity compared to the chilling-tolerant genotype (CT). This varying stress tolerance may be due to the accumulation of varying metabolites in both plant varieties, as it was reported that raffinose and galactose concentrations decreased in chilling-sensitive genotypes, while these sugars increased in the case of the chilling-tolerant genotypes. Also, chilling-sensitive genotypes had a high accumulation of osmoprotectants in response to different stress conditions. For example, an increase in mannitol concentrations was observed under saline concentrations, and an increase in trehalose concentrations was observed under water scarcity. Measuring endogenic content of oxidative products and observing the enzymatic activity of some antioxidative enzymes, it has been hypothesized that during chilling stress, an effective reactive oxygen species scavenging metabolism exists in chilling-tolerant genotype (CT) (Morsy et al. 2007).

Another study used a constitutive (pCaMV35S) or a stress-inducible (pCOR15a) promoter and performed research on different species in response to stress tolerance in wild-type (wt) and *Osmyb4*-expressing genetically modified plants (Mattana et al. 2005b). The researchers observed that there is a relation between an increase in Myb4-driven cold tolerance in apple, maize, and *Osteospermum* and increased levels of proline and sugars. As the *Osmyb4* gene expression in maize was under the effect caused by the pCOR15a stress-inducible promoter, the genetically modified maize grown under a regulated environment did not show any variance in metabolite accumulation related to the wild type. More concentrations of proline, fructose, sucrose, and glucose were observed by applying a cold treatment for 6 days in both wild-type and genetically modified maize. The concentration of these metabolites was noticeably higher for all of them in *Osmyb4*-genetically modified plants. Increased amounts of sugar and proline were observed in genetically modified *Osteospermum* and apple, pre-stress exposure by utilizing the pCaMV35S constitutive promoter to drive *Osmyb4* expression (Mattana et al. 2005b). Observations on Myb4-driven cold tolerance and metabolic changes were confirmed and enhanced when further studies reported the first metabolic profile of *Osteospermum* species (Laura et al. 2010).

The increase in proline content was a common pattern observed in all Myb4-genetically modified plants, while an increase in the free amino acid pool was observed in the case of wt plants (Laura et al. 2010; Mattana et al. 2005a; Pasquali et al. 2008). These observations highlight the significance of cereal proline concentrations in various roles and under abiotic stress (Verbruggen and Hermans 2008). Many metabolites are accumulated in different species under various growth and stress conditions through metabolite profiling.

7.3.2 Drought Stress

Drought or water scarcity is also a major factor affecting plant efficiency around the globe. The effects of drought depend upon timing and the severity of water scarcity, whether it be due to low rain supply or short periods with no rain. Whatever the circumstances are, plants always have a coping mechanism for it (Basu et al. 2010). The earliest responses observed are a decrease in generative growth, closing of stomata, and a low photosynthetic rate. Decreased osmotic potential due to increased solute concentration in drought is an important mechanism that maintains turgor pressure in plant cells.

The phytohormone abscisic acid (ABA) and ABA-dependent and ABA-independent pathways are triggered by low water levels or drought conditions (Yamaguchi-Shinozaki and Shinozaki 2006; Roychoudhury et al. 2013). So far, many genes have been linked in various species to induce drought, and their gene patterns are also being studied. The overall metabolic variance induced by water scarcity has been studied recently by different researchers.

7.3.2.1 Cereal Response to Drought Stress

It is observed in some of the species that wild types are more tolerant compared with normal cultivated crops, implying that crossing between wild and normal cultivars could improve stress adaptation. Based on this observation, researchers compared the metabolic profile of tomato fruit pericarp grown from irrigated and non-irrigated fields, which could either be *Solanum pennellii* or *Solanum lycopersicum* (cv. M82). Seventy-two metabolites were identified while their differences influenced by genotype and environment were also studied.

When observations were made under irrigated field conditions, it was noticed that there was a great difference between the metabolic conditions of the best cultivar and the F1 hybrid, with a comparatively greater amount of several amino acids in M82 and a higher accumulation of fatty and organic acids observed in the F1 hybrid. Other differences which were observed in these two genotypes were higher concentrations of sugar, sugar alcohols, and sugar-phosphate along with other compounds mainly glucose, fructose, sucrose, maltose, myoinositol, and trehalose in the F1 hybrid. M82 had high concentrations of fructose-6-phosphate and putrescine only (Semel et al. 2007). Stress had strong effects on the metabolite content due to M82 cultivated tomato which shows increased levels of fatty acids, amino acids proline, GABA, β-alanine, glycine and glutamate, TCA cycle intermediates, and organic acids along with some sugars and their derivatives. However, changes occurring in plants due to drought stress are mainly due to some chemical compounds like proline and a few sugars, while the increase in other compounds like branched amino acids, gentiobiose, or TCA cycle intermediates is peculiar. It is important to note that it has been proposed that gentiobiose is a signaling molecule during tomato development. On the other hand, metabolic profiling of F1 hybrids did not show any effect in growth conditions, and this could be due to the consecutive increase in metabolite levels that take part in the drought response mechanisms (Semel et al. 2007).

Researchers also examined the drought-tolerant phenotype while focusing on metabolite concentration in wt and genetically modified tomato plants that possess the rice *Osmyb4* gene either under a stress-inducible (pCOR15a) or constitutive (pCaMV35S) promoter. It was concluded that a higher concentration of glucose, fructose, and sucrose and percentage accumulation of proline was linked with ameliorated tolerance of the genetically modified lines (Vannini et al. 2007).

Systemic studies on metabolite changes induced by water scarcity have also been carried out on the roots of *Salvia miltiorrhiza* and by extraction techniques using various solvents, proton nuclear magnetic resonance, and LC DAD-MS (Dai et al. 2010). Normally phytomedicines are air-dried or sun-dried for ease of transport, storage, and other pharmaceutical needs. The effects of both of these different drying methods have been compared and studied with a freeze-drying method, as a control method. Analysis done by 1H-NMR showed the existence of primary as well as secondary metabolites, while analysis done by LC-DAD-MS identified 44 secondary metabolites, among which polyphenolic acids, genipin, umbelliferone, and tormentic acid have not been discussed previously for this plant.

To find a relation between osmotic adjustment and drought tolerance, we have to consider seed yield and accumulation of specific metabolites. Studies have been carried out on three different *Ricinus communis* L. hybrids and their parent plants which were grown in both irrigated and non-irrigated field conditions (Babita et al. 2010). The studies revealed that genotypes with a higher osmotic adjustment had large amounts of leaf relative water content (RWC) and maintained higher leaf water potential in water scarcity. Also, a positive relationship has been found between seed yield and osmotic adjustment OA under water-scarce conditions. Genotypes with increased OA have higher concentrations of proline, potassium, and total free amino acids and sugars, with sugars contributing the most to osmotic adjustment (Babita et al. 2010).

7.3.2.2 Resurrection Plants

Although the term “drought tolerance” means the capability of a plant to sustain decreased dehydration, the capability of a plant to survive further dehydration to ~0.3 g H_2O g^{-1} dry weight is known as “desiccation tolerance” (Moore et al. 2009). This also means that plants can skillfully recover their normal metabolism and growth by rehydration within a few hours of re-watering the plant. Nevertheless, desiccation is a normal developmental process in seedlings of higher plants, and only some species are having vegetative tissues that are desiccation-tolerant. These species are from different families of angiosperms and are called “resurrection plants” (Moore et al. 2009).

Resurrection plants have been studied for understanding the mechanism that enables them to fight drought conditions so that the gathered information could be utilized to increase drought tolerance in crop species with economic importance. Molecular, biochemical, metabolic, and physiological aspects of this peculiar trait are extensively being studied (Moore et al. 2009). Several upstream-acting genes are an area of interest; genes coding for transcription factors or those involved in RNA regulation were identified from the data collected (Moore et al. 2009).

Antioxidants such as phenolic acids and polyphenols (galloylquinic acid) have great importance as these metabolites are involved in desiccation tolerance. A relationship between the galloylquinic acid concentration and the length of the desiccation period *Myrothamnus flabellifolius* can tolerate has been documented. These molecules can interpret the length of drought period a plant can survive in, before its ability to survive has been permanently affected (Moore et al. 2005). Under the influence of dehydration, an increase in proline and other soluble carbohydrates like trehalose, glucose, sucrose, and raffinose has been observed in resurrection plants. Furthermore, glucose and sucrose localize in plant tissues in connection with their role as cellular protectants during drought conditions (Martinelli 2008).

In spite of all the information gathered about desiccation tolerance through studying metabolic adjustments, gene regulation, and macromolecular stability, the mystery behind resurrection plants still remains unsolved. The comprehensive data on how all these factors relate to one another and the main factor responsible for all this is still undiscovered (Moore et al. 2009).

7.3.3 Salt Stress

The unfortunate consequences for decreased soil fertility in the next 10 years are certain, as both natural processes and agricultural practices increase soil salinity, which will result in a major agricultural land loss by the middle of this century. Most of the species that give us large economic benefits are highly sensitive to high soil salinity. Hyper-ionic stress and hyperosmotic stress can be caused by increased soil salinity (Roychoudhury et al. 2008). The disruption of ionic equilibrium, inhibition of cell division and expansion, reduction in photosynthesis, and reduced growth are the major effects seen in plants due to dehydration, ion toxicity, nutritional deficiencies, and oxidative stress. Ion exclusion, tissue tolerance, osmotic adjustment, and several molecular and biochemical changes are some of the acclimation responses, which may be convergent or divergent depending upon plant species (Sanchez et al. 2008).

7.3.3.1 Cereal Response to Salt Stress

Tomato fruits were one of the first applications for the metabolic analysis of plants for salt stress environments (Johnson et al. 2003). FT-IR spectroscopy and chemometric techniques were used to analyze extracts from two varieties with varied abilities to tolerate salinity. The checking process, discriminant function analysis (DFA), differentiated between both untreated and salt-treated samples of each type, while the unchecked method, principal component analysis (PCA), was not able to differentiate between control and salt-treated fruit of any type. The main regions within the FT-IR spectra needed for differentiation of amino radicals and nitrile-containing compounds were identified by applying genetic algorithms.

Cramer et al. (2007) elaborated a thought-provoking connection between water deficiency and salt stress. It was reported that the changes occurring in the transcript and metabolite profiles due to water scarcity and high saline environments engendered vegetative tissues of *Vitis vinifera*, cv. Cabernet Sauvignon. In order to better mimic field conditions, both of these stresses were moderately applied to the plants. In order to distinguish osmotic effects caused by ion toxicity, the experiment was designed in such a way that equivalent water potentials were kept overtime in two stress conditions. As predicted, the two stress conditions changed the relative accumulation of many metabolites, but salt concentrations had less harsh effects than water scarcity. Proline, glucose, and malate were accumulated in lesser concentrations in salt-treated plants compared to drought-treated plants. Also, water scarcity caused an increase in citrate and tartrate concentrations, while a decrease in sulfate, phosphate, and chloride concentrations was reported in drought stress plants compared to salinity stress (Cramer et al. 2007).

The changes reported at the transcriptional level of genes involved in nitrogen and energy production are associated with changes in metabolite concentration among the two growth environments. The data published thus suggests that photo inhibition, detoxification of reactive oxygen species, and osmotic adjustment need less refinement in salt-treated plants compared to drought-treated plants (Cramer et al. 2007). The previously mentioned study conducted by Morsy et al. (2007) is another illustration of the multiple stress analogy. These researchers used two rice genotypes with varying chilling tolerances and studied the response of both types in high salt concentrations and water scarcity. It was found that the chilling-sensitive genotypes (CS) were less sensitive to both stress conditions compared to the chilling-tolerant genotypes (CT). The chilling-sensitive genotypes had a high accumulation of osmoprotectants such as mannitol observed under saline concentrations and trehalose observed under water scarcity compared to chilling-tolerant genotypes. These metabolites may be the cause of their survivability under stress environments (Morsy et al. 2007).

Fumagalli et al. (2009) conducted an in vitro metabolite analysis on Arborio and Nipponbare rice cultivar under osmotic or salt stress conditions by using 1H-NMR. Arborio was found to be less tolerant to both stress conditions compared to Nipponbare, based on the growth hindrance of root and shoot. The principal component analysis score grouped the samples based on the growth environments into three: untreated, 20% PEG osmotic treated, and NaCl (150 mM) salt-treated plants. Under both stress environments, the shoots of both cultivars had high concentrations of glutamine, glutamate, and glucose, while under high saline environments, increased accumulation of threonine, valine, sucrose, and lactate was observed. Both rice cultivars underwent changes in metabolite concentration under stress, but the ratio of specific metabolites differed, particularly the ratio of sucrose to glucose, glutamate to total amino acids, and glutamine to total amino acids. This result shows that under abiotic stress factors, sugar and glutamine-glutamate metabolic pathways are regulated independently in both rice cultivars.

An analysis of the metabolic responses to high salt concentrations was conducted in barley by Widodo et al. (2009). Barley is a crop of interest as its tissues can withstand high sodium ion concentrations compared to other cereals like rice and wheat. Sahara barley cultivar is more tolerant to salt concentrations, while Clipper is more susceptible to high salinity. Both of these barley cultivars were profiled and compared for metabolites under normal and high saline environments over the course of a given time frame. The leaf metabolites from the two cultivars were analyzed through principal component analysis and were separated based on growth conditions, distance, and time increment between control and treated samples. There was a clear separation in the long-term and short-term responses of both cultivars. Only minute changes in metabolite accumulation were detected in the plant after a day of saline treatment, while exposure of 3 to 5 weeks had a significantly large difference in various metabolite concentrations of both plant cultivars.

It is suggested by researchers that several amino acids apart from proline, which was accumulated in Clipper after 3 to 5 weeks of saline exposure, may be linked to leaf necrosis and/or reduced growth. Therefore, rather than being a part of an adaptive response to high salt concentrations, it is a marker of the overall stress and cell damage caused by high salinity. Meanwhile, the increased concentration of sugars, polyols, organic acids, Krebs cycle intermediates, and other chemicals in Sahara leaves may be key components in building tolerance to high salt concentrations in the tolerant cultivar (Widodo et al. 2009). It has been observed that non-mycorrhizal plants have worse performance than mycorrhizal plants when exposed to osmotic constraints. Plants interacting with arbuscular mycorrhizas (AMs) provide the majority of information on increased stress protection, whereas molecular and physiological processes underpinning the augmentation of stress tolerance in host plants by ectomycorrhizas (EMs) are sparse.

7.3.4 Cereal Response to Oxidative Stress

Reactive oxygen species (ROS) are toxic chemicals that damage macromolecules like proteins, chlorophyll, lipids, DNA, and polysaccharides. Oxidative stress is commonly induced by ROS produced due to abiotic stress factors (Gill and Tuteja 2010a; Das and Roychoudhury 2014). Photosynthesis, respiratory electron transport, and other metabolic processes produce reactive oxygen species as secondary products. The production and desolation of ROS are in equilibrium under normal conditions; however, abiotically induced stress may cause disequilibrium, thereby causing increased amounts of ROS inside the cells. ROS-hunting antioxidants with enzymatic properties are the initial defense response to ROS and have been widely studied (Banerjee and Roychoudhury 2017). On the contrary, not many studies have been conducted on the effects of oxidative stress induced on plant metabolites.

In a study involving *Arabidopsis* cell suspension cultures, researchers used redox-active quinone menadione to induce oxidative stress and identified the metabolic response dynamics through altering 13-carbon labeling kinetics and metabolite abundance (Baxter et al. 2007). Out of the 50 metabolites that were analyzed, only 23 were notably affected. The combine evaluation of changes in the concentrations of sugars hexose, ribose, gluconate, triose phosphates, malate, and some amino acids cause a major hindrance in Krebs cycle which diverts the carbon into the pentose phosphate pathway. The increased concentrations of threonate, a by-product of ascorbate, and the reduction of ascorbate which is a cell antioxidant molecule suggest a long state of severe oxidative stress with an inability to completely recycle the oxidized ascorbate (Baxter et al. 2007).

Similar studies conducted on hydroponic *Arabidopsis* roots show similar metabolite changes, with a notable difference in roots and cultured cells of *Arabidopsis* after long- and short-term oxidative stress induced by menadione responses. 33 of 56 identified metabolites present in roots treated with menadione were affected in the first half-hour, and 39 were modified at two points in time. As observed in cell cultures, the early changes include a decrease in the Krebs cycle metabolites and related amino acids, along with an increase in concentrations of some glycolysis intermediary molecules as well as Ribulose 5-P and Ribose 5-P, both of which are intermediary molecules of the pentose phosphate pathway. With the passage of time, most of the metabolite concentrations normalized and accumulated further in the roots, compared to cultured cells where metabolite concentration decreases over time.

Rice cultured cells showed increased tolerance toward menadione-induced oxidative stress by an increased gene expression of *Arabidopsis Bax Inhibitor-1* (*AtBI-1*) (Ishikawa et al. 2010). Bax Inhibitor-1, located in the ER, functions as a suppression factor in cell death which is most of the time conserved in both animal and higher plant cells. Metabolic responses to cell death-inducible oxidative stress were studied with the help of capillary electrophoresis-mass spectrometry. Based on the data gathered, researchers suggested that due to the possible increase in NADPH demand as a reducing agent, the carbon flow of control rice cells shifts to pentose phosphate pathway from the central pathway, keeping in view the data received by the study of roots and cultured cells of *Arabidopsis* (Baxter et al. 2007; Lehmann et al. 2009).

A considerable amount of amino acids derived from pyruvate, PEP, and oxaloacetate was present in MD-treated rice cells, even though carbon metabolism decreased in the central pathway. However, these results are not analogous to the results gained from MD-treated *Arabidopsis*, which had low amino acid precursor levels; therefore, a decrease in several amino acids was observed (Baxter et al. 2007; Lehmann et al. 2009). The primary metabolism in the non-stressed cell cultures did not have any notable effects by the overexpression of *AtBI-1*, but notable differences in precursors of glutamate and aspartate, redox metabolism, glycolysis, and energy metabolism were observed between cells overexpressing *AtBI-1* and control cells after stress exposure for a day (24 h).These findings suggest that high metabolic acclimation is attributable to AtBI-1 imparted oxidative stress tolerance, with the restoration of metabolites that were reduced earlier.

Ozone can induce both oxidative and programmed cell death in natural and cultivated plants. The increase in tropospheric ozone concentration is becoming a serious environmental stress factor recently, as it has adverse effects on the growth, development, and productivity of plants. Ozone reacts with water in plant leaves and produces reactive oxygen species as it is a photochemically generated air pollutant that has the capability to enter leaves through stomata. Visible chlorosis and necrosis and inhibition of photosynthesis and reduction in yield can occur depending on the effects caused by the plant condition, ozone concentration, and time of exposure. High ozone doses for a short time and a weak ozone dose for a greater time period, exposure of plants to realistic stress environments, are two different aspects that are being studied (Banerjee and Roychoudhury 2018).

By using “omics” tool, research has been conducted to find plant responses to atmospheric pollutants in different species like *Arabidopsis*, birch, and rice (Cho et al. 2008; D’Haese et al. 2006; Kontunen-Soppela et al. 2007; Li et al. 2006; Ludwikow and Sadowski 2008). Using different perspectives from transcriptomics, proteomics, and metabolomics, researchers performed several experiments on rice seedling molecular responses and got systematic results (Cho et al. 2008), thus, providing us with an overall view of how signaling and metabolic pathways are linked with rice response to ozone exposure. Metabolic analysis done on the basis of capillary electrophoresis-mass spectrometry showed that the content of different amino acids, gamma-aminobutyric acid, glutathione, and sakuranetin increased which is one of the major secondary metabolites for rice. All these facts allowed researchers to suggest that glutamate, GABA, and glutamate dehydrogenase could be the possible biomarkers for ozone damage in rice.

Kontunen-Soppela et al. (2007) directed a long-term ozone exposure experiment in realistic open fields to get a comparison between how the ozone-induced leaf metabolome differs in two types of *Betula pendula* Roth (white birch) with different ozone sensitivities. Increased concentrations of phenolic compounds like chlorogenic acid, quercetin glycosides, and lipophilic compounds that cause waxy leaf cuticles (other than 339 low-weight compounds) were reported due to increased ozone concentration. On the other hand, elevated ozone levels caused a decrease in the accumulation of carbohydrates and chlorophyll-related metabolites (Kontunen-Soppela et al. 2007).

7.3.5 Role of Primary Metabolites in Response to Abiotic Stress

Change in metabolism occurring in different directions is one of the strategies embraced by plants in order to survive in hostile stress conditions. Osmotic adjustment is one of the most studied plant responses for water scarcity which involves rise in solute concentration, for example, carbohydrates, amino acids, polyols, tertiary sulfonium, and quaternary ammonium compounds (especially glycine betaine). Some important functions of these molecules include maintenance of cell turgor and stabilization of cell membrane and proteins. According to some hypotheses, their function includes the re-establishment of redox balance by hunting ROS, which could have adverse impacts on cell metabolism and structure. The concentration of cryoprotective molecules like soluble sugars, sugar alcohols, and nitrogen-containing compounds increases in response to cold stress. This prevents ice adhesion to plasma membranes at low temperatures which can otherwise lead to cell disruption (Rontein et al. 2002; Bartels and Sunkar 2005; Valliyodan and Nguyen 2006; Munns and Tester 2008; Janská et al. 2010).

7.3.5.1 Amino Acids

It has been reported that plants exposed to various abiotic stresses show an increased concentration of amino acids. One of the most broadly used osmolytes is proline, and its levels increase in different stress conditions like drought, salinity, and cold stress (Verbruggen and Hermans 2008; Szabados and Savoure 2010). Glutamate is converted into proline through pyrroline-5-carboxylate (P5C) when it undergoes reduction reactions by two enzymes pyrroline-5-carboxylate synthetase (P5CS) and pyrroline-5-carboxylate reductase (P5CR) (Hu et al. 1992; Roychoudhury et al. 2015). An increased expression of P5CS in tobacco caused high proline levels and a smaller decrease in osmotic potentials of transgenic plant leaves in comparison to plants in controlled conditions after a drought environment. Therefore, this confirms the role of proline in transgenic plants in osmotic stress (Kavi Kishor et al. 1995).

Due to the increased concentration of proline in response to abiotic stresses, it was regarded as a stress tolerance trait. The link between stress tolerance and accumulation of osmolytes is under discussion because of its debatable connections to crop yield (Serraj and Sinclair 2002). During research on maize hybrids subjected to drought stress, increased levels of some amino acids along with proline, tryptophan, phenylalanine, and histidine were reported (Witt et al. 2012). In the case of these hybrids, it is interesting to note that only a few metabolites are found to be linked with drought tolerance and drought susceptibility. On the contrary, the metabolomic studies of Andean potatoes and their response to drought showed that plants with drought-resistant genotypes had less proline content compared to higher stress-susceptible phenotypes (Vasquez-Robinet et al. 2008).

Elevated levels of proline and GABA were seen in a cultivar of barley with a sensitive phenotype when studies on barley subjected to salinity stress were conducted. It could be due to more susceptibility of these types of plants toward such stresses. The researchers stated that there can be a relation between leaf necrosis and the slower growth of sensitive genotypes toward the accumulation of amino acids (Widodo et al. 2009). Moreover, proline can be a deadly compound and bad for plant growth during a heat stress environment as shown by research on *Arabidopsis* (Lv et al. 2011).

7.3.5.2 Polyamines

Polyamines have molecular mass and are positively charged which makes it possible for these compounds to interact with negatively charged species like phospholipids, nucleic acids, and proteins (Roychoudhury et al. 2011b). Triamine spermidine, tetraamine spermine, and diamine precursor putrescine are some of the common polyamines. As these compounds are cationic in nature, they are generally linked with heavy metals, water scarcity, UV-B high salinity, and chilling stress (Kaur-Sawhney et al. 2003; Groppa and Benavides 2008; Hussain et al. 2011). Stabilization of membranes and their protection from denaturation under stress conditions (Slocum et al. 1984), scavenging free radicals (Drolet et al. 1986), modulating nucleic acid structures, and also enzyme activities or function (Galston and Sawhney 1990; Paul and Roychoudhury 2017) are some of the roles of polyamines. Furthermore, it could be possible that due to the functional similarity of these compounds with proline and other compatible solutes, they may function as osmolytes (Hussain et al. 2011). Moreover, several researchers concluded that during stress response, polyamines can function as cellular signals (Alcázar et al. 2010; Gill and Tuteja 2010b).

Under environmental stress, levels of polyamines change. These levels can deviate or elevate in different cases. Experiments conducted on wheat show that the concentration increases, as does the concentration of putrescine and spermidine after cold treatment. Higher levels of spermine were reported after wheat was put under osmotic stress induced by polyethylene glycol. In other experiments, a decrease of polyamines was reported in response to stress. A notable decrease in free spermine and putrescine was observed in apple shoots grown in vitro under long-term exposure to saline stress (Liu et al. 2008; Roychoudhury and Das 2014).

7.3.5.3 Carbohydrates

Many researchers have reported that increased concentrations of carbohydrates like sucrose, polyhydric alcohols, and hexose are caused by abiotic stress factors. Osmotic stresses such as water scarcity or saline stress have been linked to carbohydrate accumulation (Bartels and Sunkar 2005). Carbon and energy for plant metabolism are sourced through soluble carbohydrates. The concentration of carbohydrates may be affected in response to stress as carbohydrate content depends on photosynthetic pathways. Barley roots show an increase in raffinose, glucose, sucrose, and fructose sugars, while a decrease in myoinositol is observed under water stress (Sicher et al. 2012). Soluble sugars reduce the deteriorating effects of osmotic stress, maintains turgor pressure, stabilizes plasma membranes, and protects the plant from degrading, therefore serving an osmoprotectants role during water scarcity (Basu et al. 2007).

The breakdown of starch by hydrolytic enzymes causes an increase in sugar concentration (Kaplan and Guy 2004). Soluble sugars (raffinose, sucrose, stachyose, trehalose) and sugar alcohols (ribitol, inositol, sorbitol) protect cell membranes from ice adhesion, therefore functioning as a cryoprotectant (Janská et al. 2010). Carbohydrates may function as signaling molecules and key components in stress adaptation (Ramel et al. 2009). Trehalose accumulates and protects the plant from abiotic stress factors. Its presence has also been related to stress tolerance in fungi (Cao et al. 2008) and bacteria (Purvis et al. 2005).

With the exception of resurrection plants, trehalose is barely detected in crop plants and generally does not accumulate in high concentrations (Fernandez et al. 2010). Grapevine response to chilling stress was studied with respect to trehalose metabolism (Fernandez et al. 2012). It was reported that resistance to abiotic stress factors could be attributed to the increase of trehalose and trehalose-6-phosphate in grapevine tissues. Trehalose-6-phosphate can possibly function as an active compound during stress and may be connected to sucrose metabolism Researches conducted a study on genetically modified rice with overexpression of trehalose biosynthetic genes distinguished by improved tolerance to high salt concentrations and water scarcity (Garg et al. 2002). Sustained plant growth, favorable mineral balance, high concentrations of trehalose, and reduced photo-oxidative damage were all reported in genetically modified rice crops compared to the control group. Rather than being a suitable solute, it is believed that trehalose is involved in sugar sensing and carbohydrate metabolism.

7.3.5.4 Glycine Betaine

Glycine betaine (*N*, *N*, and *N*-trimethylglycine) is a quaternary ammonium compound that is well studied for its role as an osmoprotectant. It stabilizes macromolecules, detoxifies ROS, maintains water balance, and protects photosynthesis (Chen and Murata 2011). It has also been reported that glycine betaine improves tolerance to abiotic stress factors. Nonetheless, barley, wheat, and maize do not accumulate large amounts of glycine betaine as a stress response, while rice does not accumulate any glycine betaine at all (Giri 2011; Roychoudhury and Banerjee 2016). Glycine betaine biosynthesis has been used in attempts to produce genetically modified plants with improved stress tolerance.

Accumulation of higher levels of glycine betaine in genetically modified maize plants as compared to wild type has also been studied. In view of this transformation, chilling stress is sustained in a better way by genetically modified maize in comparison with non-transformants (Quan et al. 2004). Other genetically modified plants like tobacco were also tested against different environmental stresses such as tolerance to high salt concentrations and low temperamental stress (Holmström et al. 2000). Additionally, a greater capacity to germinate under stress conditions and greater growth rate was seen in *Brassica juncea* that was genetically modified to improve tolerance to saline environments as compared to wild type (Prasad et al.

2000). Moreover, tomato plants when compared to wild type had a higher tolerance to chilling stress and showed an increase of 10–30% in fruit yield (Park et al. 2004). Faster recovery rates in genetically modified rice in comparison to wild type were reported after salt stress was eliminated (Sakamoto et al. 1998).

However, concerning osmotic stress that plants face, the amount of glycine betaine synthesized in genetically modified plants is inadequate. Instead, mechanisms of glycine betaine which are involved in plant protection may affect tolerance like protection against stress caused by ROS (Su et al. 2006).

7.3.6 Role of Secondary Metabolites in Response to Abiotic Stresses

Secondary metabolites and their derivatives are key components in various plant biochemical and biophysical pathways. The concentration of these natural compounds is carefully controlled by the developmental stage, ambient circumstances, and adaptation mechanisms, and they are produced by specific plant species. Secondary metabolites are important in defensive response against pathogens; they function as attractants or repellents to herbivores and insects, in addition to protecting from UV damage. Not only this, these secondary metabolites act as protectants under biotic and abiotic stress factors (Ahuja et al. 2010; Fernie and Schauer 2009; Kliebenstein 2004) (Table 7.1).

7.3.6.1 Phenolic Compounds

Phenylpropanoids and their polymers, particularly lignins and tannins, flavonoids, isoflavonoids, anthocyanins, and coumarins, are secondary metabolites that are phenolic compounds in nature. These chemicals are produced in the phenylpropanoid pathway where phenylalanine is a substrate (Boudet 2007). Ultraviolet irradiation, wounding, light, pathogenic infection, herbicide treatment, and lack of nutrients are some of the factors that control the accumulation of these

Table 7.1 Production of various secondary plant metabolites synthesized under stress (Adapted from Ramakrishna and Ravishankar 2011)

S. no	Plant species	Secondary metabolites
1	*Lycopersicon esculentum*	Sorbitol
2	*Sesamum indicum* L.	GABA
3	*Hordeum vulgare*	Flavonoids
4	*Lycopersicon esculentum*	Jasmonic acid
5	*Cakile maritima*	Polyphenol
6	*Datura innoxia*	Tropane alkaloids
7	*Grevillea spec*	Anthocyanins
8	*Glycine max*	Trigonelline
9	*Trifolium repens*	Glycinebetaine
10	*Oryza sativa*	Polyamines
11	*Triticum aestivum*	Glycine betaine
12	*Cenchrus pennisetiformis*	Sucrose and starch

phenolic compounds. The growth and yield of plants are notably affected by environmental factors like water scarcity, soil flood, high salt concentrations, and temperature (Ramakrishna and Ravishankar 2011). Phenylpropanoids are intermediates in lignin biosynthesis, and their concentration changes during water scarcity in different tissues. The concentration of ferulic acid decreases, while the concentration of *p*-coumaric acid and caffeic acid increases in xylem sap in drought-stressed maize. It was proposed that these molecules could be linked with lignifying and strengthening of cell walls (Alvarez et al. 2008).

In another research, it was found that water scarcity was linked with changes in metabolism and the accumulation of wall-linked phenolic compounds in the elongation zone of maize roots. Changes in wall phenolics were revealed to be involved in the gradual restriction of wall extensibility and root development, which may help roots adapt to drying conditions (Fan et al. 2006). Under water deficit, an increase in the polyphenol concentration was observed in leaves of *Gossypium hirsutum.* Decreased levels of these natural compounds were recorded in sensitive genotypes compared to the tolerant genotypes. According to these results, maintaining osmotic potential in cells and scavenging free radicals under water stress conditions are possible through polyphenols (Parida et al. 2007).

It has been concluded by other researchers that changes in gene expression profiles of potato plants under drought stress were linked with varied polyphenolic profiles, but these changes vary for every cultivar type. While developing improved potato varieties, this information can be used (André et al. 2009). An increase in salinity leads to changes in phenolics concentration. Elevated levels of phenolic compounds were reported under high saline concentrations in red pepper fruits (Navarro et al. 2006). Ksouri et al. (2007) observed the production of antioxidative polyphenol compounds in halophytes under saline stress. The production of phenolic compounds and their integration into the cell wall as lignin or suberin are caused by cold stress. Lignins are an important part of a plant's defense mechanism against stress, and stress potency and stages of plant development are two factors that determine the synthesis of lignins (Moura et al. 2010). The resistance against cold stress could be notably increased by lignifying and depositing suberin in the plant cell wall (Janská et al. 2010; Pérez-Ilzarbe et al. 1997).

Different environmental stresses can cause the synthesis of flavonoids, isoflavonoids, and anthocyanins. Due to the antioxidant abilities of flavonoids, they have protective functions in plants that experience water deficiency. Research carried out on tea leaves subjected to drought stress showed an increase in the oxidation products of flavan-3-ols (Hernández et al. 2006). Water stress due to waterlogging or drought showed increased concentration of flavonoids, i.e., quercetin and rutin in the *Hypericum brasiliense*, whereas response toward temperature treatment was varied (de Abreu and Mazzafera 2005). Leave examination of two citrus species led to the identification of 47 flavonoids, and most of their concentrations were altered due to excess water. Quercetin, kaempferol, eriodictyol, and apigenin were compounds that are showing the most changes. Sensitive genotype showed a higher reduction in flavonoids; this may represent that maintenance of more antioxidant activity is due to the stability of flavonoid content in water stress

(Djoukeng et al. 2008). Wu and co-workers elaborated that the use of isoflavonoids to differentiate among closely related soybean genotypes is possible. As genistein is a secondary metabolite that is related to salt tolerance and might be useful as a biomarker in case of traits due to salt tolerance, it could be useful for crop breeding (Wu et al. 2008). In another work, levels of flavonoids were found to change depending on the type of environmental stress faced by two species of hawthorn. Water deficit or cold stress treatment, or when both are applied collectively, causes an increase in flavonoid accumulation levels, whereas a decrease in concentration is seen in the case of flooding and herbivores (Kirakosyan et al. 2004).

Saline, drought, and cold-induced stress caused an increase in anthocyanin concentrations (Christie et al. 1994; Chalker-Scott 1999; Parida and Das 2005). Nevertheless, research carried out on salt-sensitive potato plants reported deviated levels of anthocyanin in high saline environments (Daneshmand et al. 2010). The green cultivar of pepper is less tolerant to water-induced stress than the purple cultivar (Bahler et al. 1991). Elevated levels of anthocyanin were reported in strawberry cell culture subjected to low temperature (Zhang et al. 1997).

7.3.6.2 Terpenoids

Terpenoids are a diverse group of lipophilic secondary metabolites produced from isoprene units, which may be further synthesized and changed in various ways (Langenheim 1994). These chemical compounds positively affect abiotic and biotic stress factors, as they exhibit antibiotic and antioxidant properties. Stabilization of lipid bilayer, defense against herbivores, and improved stress tolerance can be attributed to the involvement of terpenoids (Cheng et al. 2007). Alterations in chlorophyll and carotenoids content have been reported due to water scarcity, and research conducted on cotton reported a decrease in terpenoid levels during water-scarce conditions (Parida et al. 2007; Massacci et al. 2008). Genetically modified tobacco under high saline environments and water-scarce conditions showed higher activity of important carotenogenesis enzymes (Cidade et al. 2012). Furthermore, temperature affects the carotenoid concentration of citrus juice. Mediterranean citrus fruits had a higher carotenoid concentration than tropical citrus fruits (Dhuique-Mayer et al. 2009). The increased concentration of tocopherol is involved in reducing the stress caused by saline solution, heavy metals and osmotic potential as reported by a study on *Brassica juncea* (Yusuf et al. 2010). Saponins were also discovered to be one of the main secondary metabolites linked to saline tolerance in soybeans (Wu et al. 2008).

7.3.6.3 Nitrogen-Containing Secondary Metabolites

Glucosinolates are secondary molecules produced from amino acids and glucose sugar. These compounds contain nitrogen and sulfur and are involved in plant response to abiotic and biotic stress. Increased levels of aliphatic glucosinolate and flavonoid have been reported in *A. thaliana* under water scarcity and waterlogging (Mewis et al. 2012). *Brassica napus* under water scarcity show a similar increase in accumulation of glucosinolate during vegetative growth (Jensen et al. 1996). Alkaloids are another group of nitrogenous secondary metabolites that are produced under abiotic stress factors. They have a bitter taste and are involved in plant response against herbivores and pathogens. High levels of alkaloids have been reported in *Papaver somniferum* under water scarcity (Szabó et al. 2003). Experiments conducted on *Lupinus angustifolius* genotypes during different developmental stages showed that drought altered alkaloid concentrations (Christiansen et al. 1997).

7.4 Conclusion

Metabolomics is the thorough and quantifiable analysis of all the chemicals present in an organism. It can be thought of as the ultimate expression of its genotype in response to environmental changes that are often categorized by multiple abiotic and biotic stresses at the same time. Plant responses to abiotic and biotic stress factors have been studied from a metabolomic perspective revealing information about metabolite composition, including some osmoprotectants that are linked to physiological and biochemical changes that ultimately affect the phenotype of the plant. These researches have also revealed the coordination between metabolic processes, their fluxes, and enzymatic activities in order to meet their cellular energy demands under stressful situations. Metabolomic research methods provide a larger, in-depth, and far more comprehensive picture of metabolic profiles in the adaptive plant response to stress-inducing conditions in a practical setting. The metabolites identified by this data can be potentially applied to economically important crops and can help improve plant adaptability in unfavorable or harsh environments.

Plant metabolism will eventually be viewed as a web of linked biochemicals, instead of a series of processes that contribute to the formation of a final metabolite. This is a difficult task as multiple factors must be kept into account. The plant stress response is intricate as it is dependent on multiple systematic processes, time alterations, and stress dosage. Also, every plant tissue has a different response, as does every plant genus and species, showing a link to a plant genotype. However, the use of more advanced metabolomic tools in the near future will open doors for better understanding, speeding up, and enhancing designs of plant breeding projects. This will result in a new generation of agricultural crops better equipped for coping with abiotic and biotic stress factors.

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Plant Breeding Strategies for Abiotic Stress Tolerance in Cereals

8

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and Samiullah Khan

Abstract

By the year 2050, the global human population is predicted to increase by 2.5 billion reaching 9.6 billion people. To feed the world's 9.6 billion people, the Food and Agriculture Organization estimates that global food production must increase by 70%. Moreover, the productivity of major food crops is affected by environment induced abiotic stressors that further expand the food demand-supply gap. Among the food crops cereals are most important in ensuring food security, yet they are also the most vulnerable to abiotic stresses. Due to various abiotic stressors, cereal productivity is decreasing; thus, mitigating these yield losses is critical for all nations to satisfy rising food demands. Besides abiotic stressors, ongoing climate change are also posing severe obstacles to obtaining the required agricultural production levels to meet the expanding food demands. Among the abiotic stresses drought, temperature and soil salinity are the most severe, resulting in massive crop yield losses. Therefore, tolerance to abiotic stresses has typically been a long-term goal for plant breeders. In this

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_8

chapter, the consequences of abiotic stresses, mechanism of abiotic stress tolerance and the role of various breeding strategies in developing abiotic stress-tolerant cultivars have been discussed.

Keywords

Cereals · Plant breeding · Abiotic stress · Mutation breeding · Quantitative trait loci

8.1 Cereals: An Ideal Crop to Achieve Food Security

Cereal is derived from the Latin word ‘cerealis’, which means ‘grain’, and refers to a type of fruit called a caryopsis, which is made up of endosperm, germ and bran. Cereals, such as wheat, rice, maize, sorghum, millet, barley and rye, are annual grass members of the grass family (a monocot family Poaceae, commonly known as Gramineae), with long, thin stalks and starchy grains used as food. Cereals have evolved to thrive in settings where they are frequently subjected to various stressors, including high temperatures, drought, salt, mineral toxicity and water scarcity (Giordano et al. 2021; Kumari et al. 2021). They are widely used crops in global agriculture, with approximately 2979 million tonnes being harvested worldwide in 2019 (FAOSTAT 2021 http://www.fao.org/faostat/en/). Maize, wheat and rice are the three most significant cereal crops, accounting for at least 85% of global grain output. In 2019, 1148.49, 765.77 and 755.47 million tonnes of rice, wheat and maize, respectively, were harvested (Table 8.1). The cereal statistics for the area harvested and grain yield in the last 10 years showed significant growth and thus

Table 8.1 Worldwide total production of different cereals (2019)

Cereals	Production value (million tonnes)
Maize	1148.49
Wheat	765.77
Rice, paddy	755.47
Barley	158.98
Sorghum	57.89
Millet	28.37
Oats	23.10
Triticale	14.06
Rye	12.80
Cereals nes	7.91
Grain, mixed	3.42
Buckwheat	1.61
Fonio	0.70
Canary seed	0.24
Quinoa	0.16
Cereals, total	2978.98

Source: FAOSTAT 2021, http://www.fao.org/faostat/en/

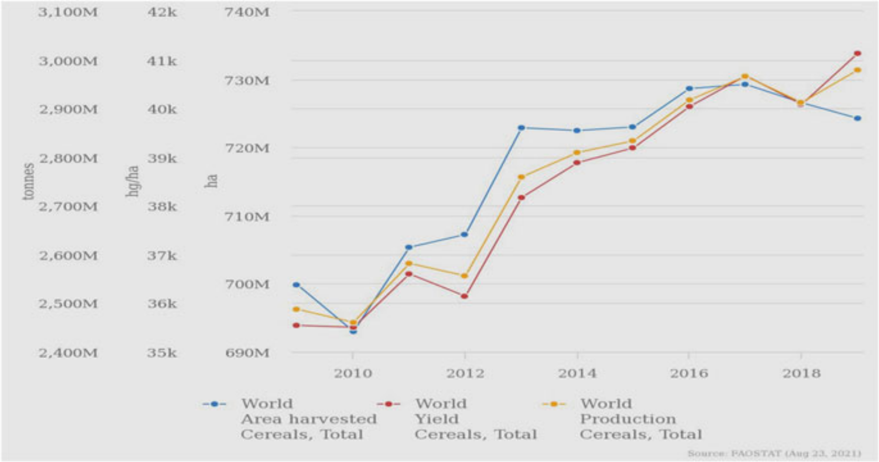
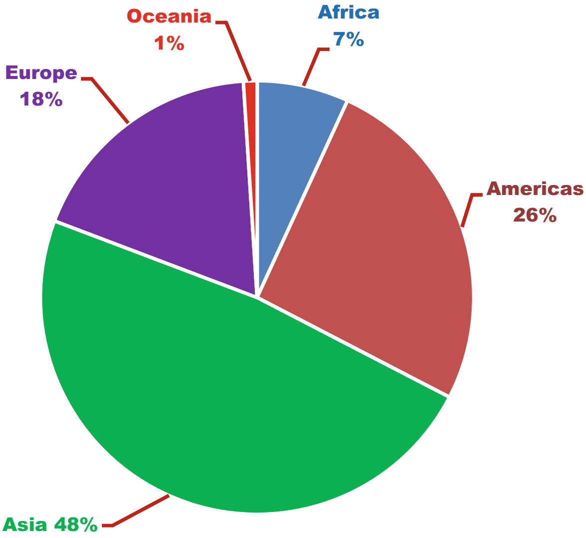


Fig. 8.1 Comparative analysis on area harvested, yield and production of cereals globally (2009–2019)

Fig. 8.2 Region-wise production share of cereals (2019)



positively impacted the production of cereals (Fig. 8.1). Cereals provide essential nutrients including proteins, carbohydrates, minerals, amino acids, fibres and micronutrients, including vitamins, magnesium and zinc (O'Neil et al. 2010; Papanikolaou and Fulgoni 2017).

Globally, 48% of the world's cereal grains are produced in Asia, 26% in America and 18% in Europe (Fig. 8.2). Rice, sorghum, millet and wheat are commonly grown in Asia; corn and sorghum are grown in the United States, while barley, rye and oats are grown in Europe. Cereals are an essential source of nutrients in industrialized and

Table 8.2 Comparative nutritive value of cereal grains

Factor	Wheat	Maize	Rice	Barley	Sorghum
Available CHO (%)	69.7	63.6	64.3	55.8	62.9
Energy (kJ/100 g)	1570	1660	1610	1630	1610
Digestible energy (%)	86.4	87.2	96.3	81.0	79.9
Vitamins (mg/100 g)					
Thiamin	0.45	0.32	0.29	0.10	0.33
Riboflavin	0.10	0.10	0.04	0.04	0.13
Niacin	3.7	1.9	4.0	2.7	3.4
Amino acids (g/16 g N)					
Lysine	2.3	2.5	3.8	3.2	2.7
Threonine	2.8	3.2	3.6	2.9	3.3
Methionine and cysteine	3.6	3.9	3.9	3.9	2.8
Tryptophan	1.0	0.6	1.1	1.7	1.0
Protein quality (%)					
True digestibility	96.0	95.0	99.7	88.0	84.8
Biological value	55.0	61.0	74.0	70.0	59.2
Net protein utilization	53.0	58.0	73.8	62.0	50.0
Utilization protein	5.6	5.7	5.4	6.8	4.2

Source: USDA National Nutrient Database for Standard Reference

developing countries, although their usage patterns differ. More than 70% of total grain output is used as feed for livestock in affluent nations, whereas 68 to 98% of cereal production is used for human consumption in developing countries (Olugbire et al. 2021).

Compared to dietary legumes and oilseeds, cereal grains have a low protein level, with rice having the lowest. In all cereal grains, lysine is the most limited of the necessary amino acids for humans. Cysteine, methionine and sulphur-containing amino acids are abundant in most grain proteins. Among the cereal crops, barley has a higher lysine content. The presence of antinutrients such as metal chelates, antivitamins, goitrogens, cyanogens, protease and amylase inhibitors, toxic phenolic glycosides and amino acid derivatives are known to influence the consumption of legumes (Mohan et al. 2016). As a result, adequate processing of the cereal-legume combination is necessary before ingestion to reduce these antinutrients. Cereal grain products have lower nutritional and sensory characteristics compared to animal meals. Physical, chemical, biological and physiological changes can improve the grain nutritional and visual characteristics (Piltz et al. 2021). Furthermore, natural processes such as fermentation and regulated germination with natural microflora help to improve the quality of cereal-based foods. The nutritional qualities of the most important cereal crops are furnished in Table 8.2.

8.2 Abiotic Stresses: Impact on Cereal Production

It is often difficult to breed a species for more than one robust feature at a time since individual plants react so differently to similar abiotic stress stimuli, but that is precisely what plant breeders are aiming for. Globally, abiotic and biotic stresses reduce average yield by more than 50% (Oerke et al. 1999; Raina and Khan 2020). However, abiotic stresses, especially salinity, drought, and temperature, are the major constraints for cereal production (Acquaah 2007; Martinez-Beltran and Manzur 2005; Munns 2002; Lobell and Field 2007). Abiotic stressors have a negative impact on several growth phases (Raina et al. 2020a). They are highly complicated, affecting crop dynamisms such as blooming, grain filling and maturity at the transcriptome, cellular and physiological levels (Atkinson and Urwin 2012; Maiti and Satya 2014; Paul and Roychoudhury 2019). The primary abiotic stressors impacting contemporary agricultural systems are atmosphere, soil, water and related variables (Sahu et al. 2014). Water is a major factor that induces abiotic stress in cereals, including water scarcity, salinity and waterlogging. Water scarcity, falling rainfall and rising temperature are the major limitations for agriculture, all of which substantially impact agricultural productivity.

8.2.1 Drought Stress

Undeniably, drought is one of the principal abiotic stresses in the world. Drought stress affects various morpho-physiological aspects of the plant from anthesis to maturity and significantly reduces productivity. The need of the hour is to develop stress-resistant genotypes which could thrive well under severe environment (Rabara et al. 2021). In most cases, rainwater produces a flooded condition in the field. Since water replaces almost all air in soil pore space, the oxygen content in flooded conditions decreases to zero within 24 h. Roots require oxygen to maintain vital cellular processes and cell viability. Waterlogging reduces the amount of oxygen available to the roots; if the roots consume any residual oxygen from flooded or waterlogged soils, the biological functions of the roots will get disrupted. As a result, the leaves and stems cannot acquire sufficient minerals and nutrients, and the roots begin to die due to waterlogging (Liliane and Charles 2020).

8.2.2 Temperature Stress

Crop species have been divided into three categories based on their temperature sensitivity: chilling-sensitive, freezing-sensitive and freezing-resistant plants (Kai and Iba 2014). Freezing may affect growth and produce frost-hardening/cold hardening, as well as causing the formation of reactive oxygen species, which could disrupt membrane components and induce protein denaturation (Beck et al. 2004; Baek and Skinner 2012). The crops subjected to a high temperature result in expansion-induced lysis, phase changes, lesions in membranes and physical damage (Tomás et al. 2020).

8.2.3 Salinity Stress

Salinity stress has various consequences in plants, including ionic and osmotic effects, nutritional and hormonal imbalances and the formation of reactive oxygen species (Rao et al. 2019). The buildup of sodium (Na^+) and chloride (Cl^-) ions has a significant impact on plant development and production, resulting in ionic, osmotic and oxidative stress (Yildiz et al. 2020). Multiple metabolic activities, such as protein translation, transcription and enzyme activity, are influenced by Na^+, resulting in osmotic stress. There is a genetic basis for salt responsiveness, as evidenced by salt-tolerant and salt-sensitive crop species (Roychoudhury et al. 2008). Salt tolerance across varieties has been recognized since the 1930s (Epstein 1977, 1983), and intra-specific salt tolerance selection has been documented in rice (Akbar and Yabuno 1977) and barley (Epstein et al. 1980).

8.2.4 Heavy Metal Stress

Abiotic stress arises from soil-related factors including soil properties, pollution and degradation. The injudicious use of certain hazardous pesticide chemicals facilitates their entry in the natural environment in various ways based on their solubility. Abiotic stress can also be caused by a nutrient shortage or the presence of harmful chemicals in the soil, such as heavy metals (Sahu et al. 2014). Heavy metals including manganese, zinc, copper, magnesium, molybdenum, boron and nickel substantially impact plant morphological, metabolic and physiological abnormalities (Roychoudhury et al. 2012). It includes shoot chlorosis, lipid peroxidation and protein breakdown (Emamverdian et al. 2015). Nutrient insufficiency has long been thought to be the root cause of low agricultural yields. Only 3.03 billion hectares (22%) of the world's 13.5 billion hectares is cultivable, while over two billion hectares is not suitable for cultivation. Oil shale disposal, soil heavy metal pollution and crude oil leakage negatively impact the root systems (Shah and Wu 2019).

In addition to the above-mentioned stresses, plant tissues are injured when the weather is hot, humid and foggy with a slight breeze. Plant reproductive development, chlorosis and necrosis are all affected by chilling stress, which includes decreased leaf growth and wilting. Ultraviolet and ionizing radiations have a variety of effects on the growth and development of cereal crops. Radiation affects stomatal function, cell survival, seed development and fertility (Foroughbakhch Pournavab et al. 2019; Metwally et al. 2019). Photon irradiation causes cellular damage in root and leaf tissues of cereals. Fast-flowing winds also reduce the phytohormonal content of cereal crop roots and shoots. The wind direction and velocity have an impact on plant growth and development (Sahu et al. 2014). Rainfall is one of the major abiotic stress variables impacting soil erosion and crop productivity in rain-fed agriculture in semi-arid areas. It regulates the acidity and salinity of the soil. Acid rain occurs when sulphur dioxide (SO_2) and nitrogen oxides (NO_x) combine with water and oxygen in the atmosphere (Gong et al. 2019). Acid rain impairs vital processes of growth and development in cereal crops.

8.3 Origin of Abiotic Stress Tolerance

The origin of abiotic stress tolerance in agricultural plants can be found in a variety of places. Landraces, wild relatives, high-yielding varieties, initial breeding materials and advanced breeding materials may all harbour tolerance. Landraces from arid regions have been effectively employed in breeding to create open-pollinated types or hybrids for water-scarce situations. Abiotic stress providers include wild species and progenitors of our farmed crops (Table 8.3). The likelihood of identifying the desired genes and even the projected challenges and expected success in introgression of these genes into the chosen recurrent cultivar all influence the genetic resource used as a source for abiotic stress resistance. A substantial genetic diversity occurs in the breeding materials and even in some improved cultivars of different crop species for drought and salinity resistance (Basu and Roychoudhury 2021). Because this is the least troublesome of all sources of drought and salinity resistance, an initial aim of the breeder should be to find and use such sources. Drought and salt resistance traits are often found in landraces (old or desi varieties) that have evolved and are adapted to drought and salinity conditions. Efforts in utilizing wild relatives should be concentrated only when the diversity in top breeding materials and landraces has been exhausted.

Table 8.3 Wild sources of resistance to drought and salinity in some cereal crop plants

Crop	Wild species	Resistance
Wheat (*Triticum aestivum* L.)	*Aegilops kotschyi*	Drought tolerance
	Agropyron seirpea	Salinity tolerance
	Triticum urartu	Drought tolerance
Rice (*Oryza sativa* L.)	*Porteresia coarctata (O. coarctata)*	Salinity tolerance
	O. rufipogon	Cold tolerance
	O. glaberrima	Drought and heat tolerance
	O. barthii	Drought and heat tolerance
	O. meridionalis	Drought and heat tolerance
	O. rufipogon	Acid soil and aluminium tolerance
Maize (*Zea mays*)	*Eastern gamagrass*	Drought; acid soil and aluminium; salinity tolerance
	Z. nicaraguensis	Waterlogging tolerance
	Z. luxurians	Waterlogging tolerance

8.4 Response of Cereals Towards Abiotic Stress

Cereals have a diverse set of strategies at the genetic, physiological, biochemical and molecular levels. Nevertheless, new progress in traditional, marker-assisted breeding and genetic engineering has made it possible to develop drought-tolerant crops (Oladosu et al. 2019; Rosero et al. 2020). Many crops are sensitive to high salinity and could not withstand saline conditions; however, certain crops are adapted to thrive in harsher salt environment prevalent in coastal locations like salt marshes. The high rate of evaporation within those areas concentrates salts in the mineral composition of the soil. These crops have evolved morpho-physiological and reproductive adaptations to salty, waterlogged and anaerobic environments. Such crops can withstand salt stress primarily through three main mechanisms: osmotic tolerance, ion exclusion and tissue tolerance. Long-distance signalling waves control osmotic tolerance by reducing cell growth in root tips, leaves and regulate stomatal conductance (Rajendran et al. 2009; Roy et al. 2014). Ion exclusion is primarily concerned with the transfer of sodium (Na^+) and chlorine (Cl^-) into roots, which prevents the buildup of Na^+ in shoots. Tissue tolerance entails exposing tissues to accumulating Na^+ and Cl^- at the cellular and subcellular levels, the build up of suitable solutes and the enzyme that catalyzes the detoxification of reactive oxygen species (Reddy et al. 2017). The capacity of cereal crops to withstand dominating abiotic stress, which includes water deficiency (drought), flood (anoxia), salinity, high/low temperature and other osmotic stressors, is an essential component of yield resilience and has long been a goal for plant breeders (Halford et al. 2014). Due to the ever-increasing population, the fast-changing global environment impacts agricultural production and food supply, resulting in a need for stress-tolerant crop (Takeda and Matsuoka 2008; Newton et al. 2011). Understanding how plant responses to diverse stressors, interact at the molecular level is critical for creating stress-tolerant crops (Paul and Roychoudhury 2019). The adaptive mechanism of abiotic stress in plants is associated with various traits are shown in Fig. 8.3.

8.5 Different Breeding Strategies for Improving Abiotic Stress Tolerance

Genetic modification for effective stress tolerance in plants is difficult due to the complex characteristics of abiotic stress events (Wang et al. 2007). Breeding for abiotic stress is an important strategy to fight yield loss. Even in ancient times, the necessity for stress-tolerant crops was obvious (Jacobsen and Adams 1958). However, efforts to increase crop performance under environmental stressors have mostly failed due to a lack of understanding of the underlying processes of stress tolerance in plants. Information on the genetic basis of abiotic stress tolerance, method of inheritance, size of gene effects, heterosis, combining ability and their mechanism of action are required to plan effective breeding programmes for generating abiotic stress-tolerant cultivars. Breeding methodology for crops should be adopted based on the type of reproduction, i.e. whether the species is self- or cross-pollinating.

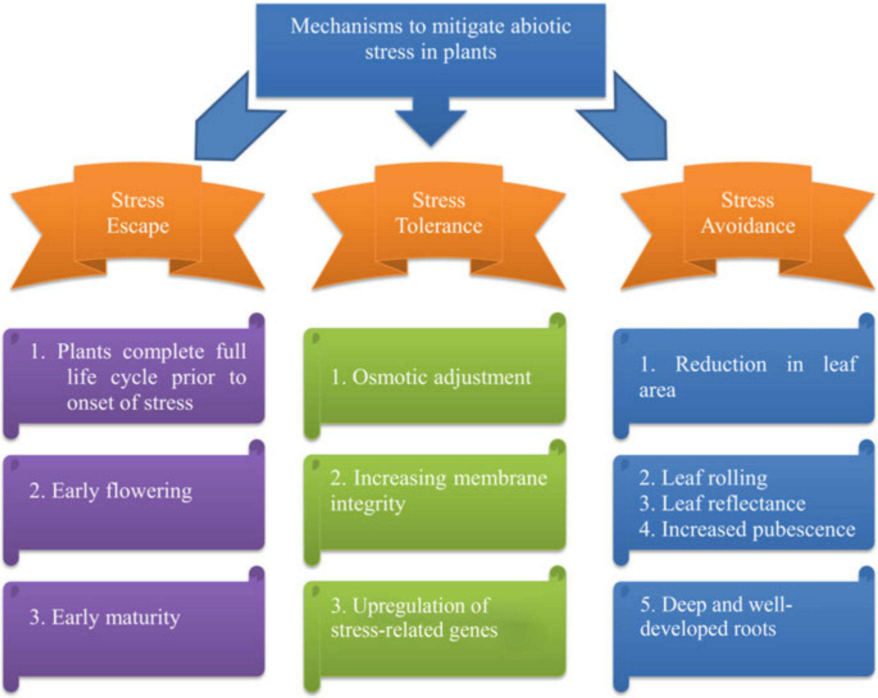


Fig. 8.3 Abiotic stress adaptive mechanism and their associated traits

Pedigree and bulk methods may be employed for self-pollinated crops, whereas recurrent selection could be used for cross-pollinated crops. However, if the goal is to transfer a few stress-resistant characteristics to a high-yielding genotype, backcrossing is an appropriate strategy. Pedigree, modified bulk pedigree and anther culture approaches were used to create salinity-tolerant varieties. On the other hand, biparental mating (half-sib and full-sib) preserves a wide genetic basis while also allowing for the evolution of drought-resistant genotype (Yunus and Paroda 1982).

Nowadays, breeding strategies for improving abiotic stress tolerance include both conventional and non-conventional approaches. Various factors influence selecting an appropriate breeding programme developing stress-tolerant cultivars, including screening methodologies, sources and mechanisms of tolerance, gene action and heredity and their link to agronomic characteristics.

8.5.1 Conventional Approaches

Conventional approaches in plant breeding include selection and introduction, pedigree method, modified bulk pedigree method, shuttle breeding, mutation breeding, diallel selective mating system supplemented by marker-assisted selection (MAS), backcross method and recurrent selection. The timing, duration and severity of the stress are three key aspects for successful stress-resistance breeding. Using traditional breeding procedures, abiotic stress-tolerant lines of crops such as rice, wheat, maize and barley have been developed. However, identifying traits that correlate well with drought tolerance is difficult using this technique. Traditional efforts to develop crop plants resistant to abiotic stress have had mixed results (Richards 1996). This is attributed to various variables, including complexity caused by genotype due to environment (G × E) interactions. Stress-tolerant crop cultivars can be created through various methods, including introduction, selection, hybridization and mutation breeding. However, mutation breeding is considered a coherent and widely accepted tool to enhance abiotic stress tolerance in cereals.

8.5.1.1 Mutation Breeding

Nonetheless, conventional breeding approaches are laborious and time-consuming and do not yield the desired variation (Cassells and Doyel 2003). Therefore, agronomists are putting efforts into searching alternate ways to get the desired variation in crops within a short time. In this regard, mutation breeding is a coherent tool to generate mutants with desirable traits and enhanced genetic variability. Mutations are sudden heritable changes in an organism genome that play an essential role in increasing genetic variability (Hugo de Vries 1901). Mutations occur both spontaneously and are induced using different physical, chemical and combined mutagens. However, the frequency of spontaneous mutations is low and is not enough to meet the needs of crop improvement programmes. Therefore, mutations are induced artificially to enhance the frequency of mutations. In the beginning, Muller (1927), employed X-rays to irradiate *Drosophila* flies to enhance genetic variability. The discovery of the mutagenic potential of X-rays in maize and barley was the pioneering event in the establishment of mutation breeding for crop improvement programmes (Stadler 1928). The induction of mutations within an organism genome has been used in plant breeding since the discovery of the mutagenic effects of X-rays on *Drosophila* flies (Muller 1927). These discoveries on the induction of artificial mutations encouraged plant breeders to use different physical and chemical mutagens for crop improvement programmes. As a result, thousands of mutant varieties with improved yield, quality, stress tolerance and adaptability were developed in various crop species. Mutation breeding is now an established breeding strategy to achieve crop varieties with improved agronomic traits, including abiotic stress tolerance in cereals.

8.5.1.2 Role of Mutation Breeding in Improving Abiotic Stress Tolerance in Cereals

Nowadays, mutation breeding for crop improvement is based on physical and chemical mutagens and variations in in vitro culture, called ‘somaclonal variation’. The use of induced mutation for crop improvement is reflected by the fact that 3364, including 1596 improved mutant varieties of cereals, have been developed and officially released (Raina et al. 2016). These mutant varieties are cultivated on millions of hectares of cultivated land that generate billions of dollars (Laskar et al. 2018a, b; Goyal et al. 2019a, b). This has led to a tremendous economic impact on agriculture and its allied sectors worldwide (Das et al. 2014; Khursheed et al. 2018a, b, c). Ever since the historical discoveries of Muller and Stadler, different mutagens were employed to enhance genetic variability. However, radiations were preferred to achieve the improved genetic variability in food crops (Hassan et al. 2018; Laskar et al. 2019). In the last five decades, various countries such as China, India, Pakistan, Bangladesh, Vietnam, Thailand, Italy, Sweden, the United States of America, Canada and Japan took up extensive crop improvement programmes through induced mutagenesis. This has made remarkable achievements in developing elite mutant varieties in a wide range of food crops, particularly cereals and pulses (Raina et al. 2019; Wani et al. 2021a, b).

Mutation breeding has been successful in developing mutant varieties with improved tolerance to abiotic stresses (Laskar et al. 2015; Khursheed et al. 2019; Goyal et al. 2021). The improved mutant varieties play a vital role in mitigating the chronic hunger and malnutrition issues and achieving global food and nutrition security (Khursheed et al. 2016; Goyal et al. 2020a, b). Mutation breeding offers an opportunity to improve abiotic stress tolerance without altering the genetic constitution (Raina et al. 2017; Tantray et al. 2017). Unlike genetically engineered crops, mutant varieties do not possess any alien genes. Hence, there are no detrimental issues of induced mutations associated with human health, religious and social ethics. In the last two decades, enormous advances have been achieved in this field, and thousands of mutants have been released as new cultivars (Wani et al. 2017; Ansari et al. 2021). In rice, the results have been remarkable. The FAO/IAEA database displays about 853 mutant varieties of rice, 311 mutant varieties of barley, 298 varieties of wheat and 96 mutant varieties of maize developed by the use of induced mutations (https://mvd.iaea.org/ accessed on 10-07-2021). Till now, 160 mutant varieties of cereals have been developed that reflect improved tolerance to a wide range of abiotic stresses such as drought, heat, salinity and cold (Baloch et al. 2002; Saleem et al. 2005; Cassells and Doyel 2003; Parry et al. 2009) (Table 8.4). For instance, rice seeds irradiated with carbon or neon ions have generated a high salt tolerance mutant variety (Hayashi et al. 2007). The gamma radiation mutant rice varieties, Zhefu 802 and Basmati 370, revealed improved cold tolerance (Ahloowalia et al. 2003). Likewise Bastion, Secret and Taran are cold-tolerant barley mutant varieties (Shevtsov et al. 2003). In Finland, a barley mutant, Balder J, had a higher yield and improved drought resistance (Kharkwal and Shu 2009). Luther and Pennrad are high-yielding and lodging-resistant barley mutant varieties (Kharkwal and Shu 2009).

Many such varieties of abiotic stress-tolerant mutant crops have been released in different countries all over the world (Raina and Danish 2018; Raina et al. 2020b). Therefore, induced mutagenesis for resistance to abiotic stresses is a possible breeding approach that creates new desirable genetic variability of agronomic importance (Amin et al. 2016, 2019; Raina et al. 2018a, b). Thus, mutation-assisted plant breeding has a crucial role in developing 'designer crop varieties' to address the qualms and challenges of global climate variability and plant-product insecurity (Raina et al. 2021a, b, c). Both efficiency and efficacy of mutation techniques in crop breeding can significantly be increased through molecular mutation breeding. High-

Table 8.4 Mutant varieties of cereals with improved tolerance to abiotic stresses (Source: MVD 2021)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
Finland	1975	*Secale cereale* L.	Gamma rays (100 Gy)	Low temperature stress
Russian Federation	1993	*Panicum miliaceum* L.	It was developed by hybridization with two chemo mutants	Drought stress
United States	1991	*Avena sativa* L.	It was developed by hybridization with mutant Florida 501	Low temperature stress
Russian Federation	1984	*Sorghum sudanense (Piper) Stapf*	Dimethyl sulphate	Drought and lodging stress
China	1974	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1979	*Oryza sativa* L.	Gamma rays (350 Gy)	Low temperature and lodging stress
China	1977	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1981	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1981	*Oryza sativa* L.	Gamma rays	Low temperature stress
China	1976	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1968	*Oryza sativa* L.	Gamma rays (300 Gy)	Salinity stress
China	1981	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1975	*Oryza sativa* L.	Fast neutrons	Low temperature stress
China	1980	*Oryza sativa* L.	Gamma rays (350 Gy)	Low temperature stress
China	1980	*Oryza sativa* L.	Gamma rays (200 Gy)	Heat stress
China	1973	*Oryza sativa* L.	Gamma rays (200 Gy)	Drought stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
China	1977	*Oryza sativa* L.	Gamma rays (350 Gy)	Low temperature stress
China	1986	*Oryza sativa* L.	Gamma rays (186.9 Gy)	Low temperature stress
India	1980	*Oryza sativa* L.	X-rays (300 Gy)	Drought stress
India	1984	*Oryza sativa* L.	EMS (0.2%)	Lodging stress
India	1976	*Oryza sativa* L.	Gamma rays (220 Gy)	Salinity stress
India	1983	*Oryza sativa* L.	Gamma rays	Salinity stress
India	1988	*Oryza sativa* L.	It was developed by hybridization with mutant Jaya induced by gamma rays	Low temperature stress
Indonesia	1983	*Oryza sativa* L.	Gamma rays (200 Gy)	Salinity stress
Indonesia	1988	*Oryza sativa* L.	Gamma rays (400 Gy)	Drought and low pH stress
Japan	1976	*Oryza sativa* L.	It was developed by hybridization with one mutant variety Reimei obtained by irradiation of seeds with gamma rays (200 Gy)	Low temperature stress
Japan	1966	*Oryza sativa* L.	Gamma rays (200 Gy)	Low temperature and lodging stress
Japan	1985	*Oryza sativa* L.	It was developed by hybridization with mutant variety Mine-asahi obtained by irradiation of seeds with gamma rays (200 Gy)	Low temperature stress
Japan	1988	*Oryza sativa* L.	It was developed by hybridization with mutant variety Mutsukaori obtained by irradiation of seeds with gamma rays (200 Gy)	Low temperature stress
Japan	1989	*Oryza sativa* L.	It was developed by hybridization with mutant variety Reimei obtained by irradiation of seeds with 200 Gy gamma rays	Low temperature and lodging stress
Pakistan	1987	*Oryza sativa* L.	EMS (0.5%)	Salinity stress
Pakistan	1993	*Oryza sativa* L.	Gamma rays	Salinity stress
Vietnam	1990	*Oryza sativa* L.	It was developed by hybridization with two mutants induced by treatment of seeds with 0.015% N-methyl-N′-nitrosourea	Salinity stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
China	1992	*Oryza sativa* L.	Gamma rays	Salinity stress
China	1994	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1992	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1988	*Oryza sativa* L.	Gamma rays (300 Gy)	Low temperature stress
China	1990	*Oryza sativa* L.	Gamma rays	Salinity and alkalinity stress
China	1993	*Oryza sativa* L.	It was developed by hybridization with mutant variety Zhefu 802 obtained by irradiation with gamma rays (300 Gy)	Low temperature stress
China	1998	*Oryza sativa* L.	Gamma rays	Low temperature stress
Pakistan	1999	*Oryza sativa* L.	Fast neutrons (15 Gy)	Salinity stress
Thailand	1978	*Oryza sativa* L.	Gamma rays (150 Gy)	Drought stress
Vietnam	1999	*Oryza sativa* L.	NA	Salinity stress
Philippines	1976	*Oryza sativa* L.	Gamma rays (200 Gy)	Drought stress
China	1981	*Oryza sativa* L.	It was developed by hybridization with mutant Funong 709	Low temperature stress
Vietnam	1999	*Oryza sativa* L.	Gamma rays (200 Gy)	Acid sulphate soil
Vietnam	1999	*Oryza sativa* L.	Gamma rays (200 Gy)	Acid sulphate soil and salinity stress
India	1992	*Oryza sativa* L.	NA	Salinity stress
India	1993	*Oryza sativa* L.	It was developed by hybridization with mutant variety Jagannath (BSS-873) obtained by irradiation of seeds with X-rays (300 Gy)	Low temperature stress
Cuba	2007	*Oryza sativa* L.	Protons (20 Gy)	Salinity stress
Cuba	1995	*Oryza sativa* L.	Fast neutrons (20 Gy)	Low temperature stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
Cuba	NA	*Oryza sativa* L.	NA	Drought and salinity stress
Cuba	NA	*Oryza sativa* L.	NA	Drought and salinity stress
Cuba	NA	*Oryza sativa* L.	NA	Drought stress
Cuba	1995	*Oryza sativa* L.	Fast neutrons (20 Gy)	Drought stress
Malaysia	2015	*Oryza sativa* L.	Gamma rays (300 Gy)	Drought stress
Malaysia	2015	*Oryza sativa* L.	Gamma rays (300 Gy)	Drought stress
Thailand	2017	*Oryza sativa* L.	Gamma ray (20 Kr)	Photoperiod insensitive
Japan	1986	*Oryza sativa* L.	It was developed by hybridization with mutant variety Nadahikari obtained by irradiation with gamma rays	Low temperature stress
Japan	1999	*Oryza sativa* L.	It was developed by hybridization with mutant line derived from mutant variety Reimei induced by irradiation of seeds with gamma rays (200 Gy)	Low temperature stress
Japan	2000	*Oryza sativa* L.	It was developed by hybridization with mutant line derived from mutant variety Reimei and mutant variety Yama-uta induced by irradiation of seeds with gamma rays (200 Gy)	Low temperature stress
Japan	2002	*Oryza sativa* L.	It was developed by hybridization with mutant of mutant variety mine-Asahi obtained by irradiation of seeds with gamma rays (200 Gy)	Low temperature stress
Japan	2004	*Oryza sativa* L.	It was developed by hybridization with mutant variety Dewasansan obtained by irradiation of seeds with gamma rays (300 Gy)	Low temperature stress
Korea	2007	*Oryza sativa* L.	Gamma rays (50 Gy)	Salinity stress
China	1968	*Triticum aestivum* L.	Gamma rays (200 Gy)	Drought stress
China	1968	*Triticum aestivum* L.	Gamma rays (300 Gy)	Low temperature and lodging stress
China	1968	*Triticum aestivum* L.	Gamma rays (300 Gy)	Low temperature and lodging stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
China	1971	*Triticum aestivum* L.	Gamma rays (200 Gy)	Low temperature stress
China	1979	*Triticum aestivum* L.	Gamma rays (350 Gy)	Lodging, drought and salinity stress
China	1979	*Triticum aestivum* L.	Gamma rays (300 Gy)	Drought stress
China	1966	*Triticum aestivum* L.	Gamma rays (300 Gy)	Low temperature stress
China	1983	*Triticum aestivum* L.	Gamma rays (350 Gy)	Low temperature, salinity and drought stress
China	1982	*Triticum aestivum* L.	Beta rays	Drought stress
China	1980	*Triticum aestivum* L.	Gamma rays	Salinity, alkalinity and heat stress
China	1974	*Triticum aestivum* L.	Gamma rays	Salinity, alkalinity and low temperature stress
China	1974	*Triticum aestivum* L.	Gamma rays	Salinity, alkalinity and low temperature stress
China	1971	*Triticum aestivum* L.	It was developed by hybridization with mutant Yuannong 1 irradiated with gamma rays	Drought stress
China	1968	*Triticum aestivum* L.	Gamma rays (100 Gy)	Drought stress
China	1968	*Triticum aestivum* L.	Gamma rays (200 Gy)	Drought stress
China	1973	*Triticum aestivum* L.	Gamma rays	Drought stress
China	1982	*Triticum aestivum* L.	Gamma rays	Drought stress
China	1969	*Triticum aestivum* L.	Gamma rays (200 Gy)	Low temperature stress
China	1975	*Triticum aestivum* L.	Gamma rays (300 Gy)	Low temperature stress
China	1986	*Triticum aestivum* L.	Gamma rays (300 Gy)	Salinity and alkalinity

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
China	1984	*Triticum aestivum* L.	Gamma rays (80 Gy)	Drought and lodging stress
China	1982	*Triticum aestivum* L.	It was developed by hybridization with one mutant M 70A2	Drought stress
China	1980	*Triticum aestivum* L.	It was developed by hybridization with mutant 72 gamma-16 obtained by irradiation with gamma rays (200 Gy)	Drought stress
China	1988	*Triticum aestivum* L.	Gamma rays	Low temperature stress
China	2004	*Triticum aestivum* L.	Gamma rays (1.5 Gy)	Salinity and drought stress
China	2004	*Triticum aestivum* L.	Gamma rays (1.5 Gy)	Salinity and drought stress
Pakistan	1996	*Triticum aestivum* L.	Gamma rays (1400 Gy)	Drought stress
Russian Federation	1984	*Triticum aestivum* L.	Gamma rays (200 Gy)	Heat stress
Russian Federation	1982	*Triticum aestivum* L.	Gamma rays	Low temperature and lodging stress
Russian Federation	1985	*Triticum aestivum* L.	It was developed by hybridization with mutant KK1 induced with treatment of seeds with N-nitroso-N-ethyl urea	Low temperature and lodging stress
Russian Federation	1989	*Triticum aestivum* L.	It was developed by treatment of seeds with water solution of 0.01% ethyl imine	Low temperature and drought stress
Russian Federation	1989	*Triticum aestivum* L.	Water solution of NMU (0.01%)	Low temperature stress
Russian Federation	1991	*Triticum aestivum* L.	It was developed by hybridization with mutant variety Nemchinovskaya 86 induced by treatment of seeds with N-nitroso-N-ethyl urea	Low temperature and lodging stress
Russian Federation	1992	*Triticum aestivum* L.	Water solution of N-nitroso-N-methylurea (0.01%)	Low temperature stress
Russian Federation	1992	*Triticum aestivum* L.	It was developed by treatment of seeds with water solution of 0.01% ethylene imine	Low temperature and lodging stress
Bulgaria	2009	*Triticum aestivum* L.	Gamma rays (50 Gy)	Low temperature and drought stress
China	2007	*Triticum aestivum* L.	NA	Drought stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
China	2010	*Triticum aestivum* L.	NA	Drought stress
Kenya	2001	*Triticum aestivum* L.	Gamma rays	Drought stress
China	2011	*Triticum aestivum* L.	Space mutagenesis and doubled haploid technique	Drought stress
Ukraine	2017	*Triticum aestivum* L.	Gamma rays (100, 150, 200, 250 Gy)	Drought stress
Ukraine	2017	*Triticum aestivum* L.	Nitrosomethylurea (0.0125% and 0.025%)	Drought stress
Ukraine	2017	*Triticum aestivum* L.	Nitrosomethylurea (0.0125% and 0.025%)	Drought stress
Ukraine	2017	*Triticum aestivum* L.	Gamma rays (100, 150, 200, 250 Gy)	Drought stress
Bulgaria	2002	*Triticum turgidum* ssp. *durum Desf.*	Gamma rays (50 Gy)	Low temperature and lodging stress
Bulgaria	1988	*Triticum turgidum* ssp. *durum Desf.*	Gamma rays (20 Gy)	Lodging and low temperature stress
Estonia	1993	*Hordeum vulgare* L.	It was developed by hybridization with mutant variety Liisa obtained by irradiation with X-rays (100 Gy)	Drought and lodging stress
Finland	1960	*Hordeum vulgare* L.	X-rays (60 Gy)	Drought stress
Greece	1969	*Hordeum vulgare* L.	Gamma rays	Low temperature stress
Iraq	1994	*Hordeum vulgare* L.	Gamma rays (200 Gy)	Lodging stress
Turkey	1998	*Hordeum vulgare* L.	Gamma rays (150 Gy)	Low temperature and drought stress
Turkey	1998	*Hordeum vulgare* L.	Gamma rays (150 Gy)	Low temperature and drought stress
United States	1963	*Hordeum vulgare* L.	Thermal neutrons	Low temperature stress
Russian Federation	1982	*Hordeum vulgare* L.	N-Nitroso-N-ethyl urea	Low temperature and lodging stress
Russian Federation	1988	*Hordeum vulgare* L.	Ethylene oxide (0.02%)	Lodging and drought stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
Russian Federation	1988	*Hordeum vulgare* L.	It was developed by hybridization with mutant 52 M1 resistant to winter (induced by NDMU) and with mutant variety Novator ((Zavet × start) × 31 M15) induced by N-nitroso-N-ethyl urea (NEU)	Low temperature and lodging stress
Russian Federation	1995	*Hordeum vulgare* L.	N-Nitroso-N-ethyl urea (0.025%)	Low temperature and lodging stress
Czech Republic	1978	*Hordeum vulgare* L.	It was developed by hybridization with [(/Valticky × Kneifel/ × Diamant) × Arabische G]	Drought stress
Russian Federation	1990	*Hordeum vulgare* L.	N-Nitroso-N-ethyl urea (0.06%)	Low temperature and lodging stress
Germany	1955	*Hordeum vulgare* L.	X-rays (100 Gy)	Low temperature and lodging stress
Syrian Arab Republic	2000	*Hordeum vulgare* L.	Gamma rays (100 Gy)	Lodging and drought stress
Bulgaria	1983	*Hordeum vulgare* L.	Gamma rays (100 Gy)	Low temperature stress
Ukraine	2000	*Hordeum vulgare* L.	It was developed by hybridization with mutant Kharkivskiy 99	Drought stress
Russian Federation	2001	*Hordeum vulgare* L.	N-Nitroso-N-ethyl urea (0.1%)	Low temperature stress
Bulgaria	1982	*Hordeum vulgare* L.	It was developed by hybridization with mutant variety Markeli 5 obtained by irradiation gamma rays (400 Gy)	Low temperature stress
Jordan	NA	*Hordeum vulgare* L.	NA	Drought stress
Bulgaria	1994	*Zea mays* L.	It was developed by hybridization with mutant (from the cross XM-88-113 (female) × Mol17 (male)). The mutant parent was derived from the treatment of seed of B-84 with 0.001% N-nitroso-N-ethyl urea followed by 1% dioxane	Drought stress
Bulgaria	1993	*Zea mays* L.	It was developed by hybridization with mutant (from the cross mutant XM-87-136 (female) and maize cultivar Mol17 (male)). The mutant was derived from the treatment of	Drought stress

(continued)

Table 8.4 (continued)

Country	Year	Latin name	Mode of development, mutagen and dose used	Improved tolerance to
			seed of B-37 with 0.2% dimethyl sulphate followed by 0.05% dimethyl amino azobenzene followed by 1% dioxane	
Bulgaria	1992	*Zea mays* L.	It was developed by hybridization with mutant (from the cross B-579 × B-84 selection (female parent) and the mutant XM-552 (male)	Drought stress
China	1992	*Setaria* sp.	Gamma rays (250 Gy)	Drought stress
China	1999	*Setaria* sp.	Gamma rays (250 Gy)	Drought stress
China	1995	*Setaria* sp.	It was developed by crossing with one mutant	Drought and lodging stress
China	1987	*Setaria* sp.	Fast neutrons	Drought stress
China	1989	*Setaria* sp.	Fast neutrons	Drought stress
China	1985	*Setaria* sp.	NA	Drought and low temperature stress
China	1985	*Setaria* sp.	NA	Drought and low temperature

throughput DNA technologies such as targeting induced limited lesions in genomes, high-resolution melt analysis and Ecotype targeting induced local lesions in genomes are the key techniques and resources in molecular mutation breeding (Das et al. 2014). Unlike biotic stresses, herbicides, pesticides and fertilizers cannot mitigate the harmful effects of abiotic stresses.

8.5.2 Non-Conventional Approaches

The traditional breeding method has been sluggish in creating high-yielding, stress-tolerant cultivars, owing to challenges in accurately defining the target environment, complicated interactions of stress tolerance with surroundings and a lack of adequate screening methods (Cooper et al. 1999; Wade et al. 1999). The necessity for numerous backcrosses to remove undesired characteristics, restriction to loci that provide a readily apparent phenotype and inadequacy if the gene pool lacks sufficient diversity in the trait of interest are all key drawbacks of traditional breeding. As a result, the current focus is on marker-aided breeding, which allows for the 'pyramiding' of desired characteristics for faster crop development with minimal resource input. Non-conventional approaches and discoveries such as the somaclonal approach, F1 anther culture, marker assisted selection and genetically modified crops are the most recent advances in developing abiotic stress tolerance in cereals. The most immediate and future solutions for increasing abiotic stress tolerance in cereal crops might be the recent ground breaking developments in bioinformatics and integrating omics technology. Identifying the genetic basis of stress tolerance and using the required salt stress tolerance-associated genes or QTL (quantitative trait loci) to produce varieties with increased salinity tolerance are prerequisites for improving salt tolerance. Omics techniques, such as genomics, functional genomics, genetic engineering, gene expression, protein or metabolite profile(s) and their overall phenotypic impacts, contribute to a better knowledge of stress tolerance mechanisms at the molecular level. The discovery and characterization of genes and particular genomic areas linked with quantitative and qualitative agronomic characteristics that have been critical in crop breeding approaches. A high-throughput marker-assisted approach has been widely employed in recent breeding projects to improve selection effectiveness and precision. The exploitation of natural genetic variants, either through direct selection in stressful situations or by the identification of QTLs and subsequent marker-assisted selection, and creating transgenic plants to introduce new genes or change the expression levels of existing genes to influence salt stress resistance are two primary techniques now being used to increase stress resistance. Simple genetic models were used to assess the genetic basis of stress tolerance in plants in the beginning. With the advent of molecular markers, inheritance of salt tolerance became more manageable since particular QTLs could be discovered. It is now feasible to establish the genetic basis of a trait and map particular chromosomal segments or QTL and estimate the relative contribution of each QTL to the variation of a trait. Genomic maps have been created in several crops to exploit genetic diversity, tag qualitative and quantitative characteristics (Butruille et al. 1999) and assess the stability of identified QTL across diverse environmental conditions (Hittalmani et al. 2002). Stable and consistent QTLs offer a great way to increase selection efficiency, especially for characteristics that are regulated by several genes and heavily impacted by the environment, such as salinity (Dudley 1993). The effectiveness of marker assisted selection is influenced by several parameters, including the distance between observed QTL and marker loci (Dudley 1993) and the fraction of total additive variation explained by the QTL (Lande and Thompson 1990). New genomic technologies are promising to advance breeding resistance to these stressors due to a better understanding of underlying mechanisms and identifying the implicated genes. Modern biotechnological tools such as genetic engineering have successfully developed transgenic plants resistant to various abiotic stresses (Jewell et al. 2010). However, stress-resistant transgenic plants did not receive public acceptance due to risks concerning human health, social and religious issues and environmental safety (Carpenter 2010; James 2011; Kathage and Qaim 2012; Seralini et al. 2012). Hence, conventional breeding methods seem more appropriate to develop stress-tolerant and environment-friendly crop varieties.

8.6 Conclusions and Future Directions

The majority of crop losses are caused by abiotic stressors, which account for more than half of all harvest losses. According to several research findings, salt and drought stress have a detrimental effect on plant growth, development, physiology and production. During the last century, conventional plant breeding significantly enhanced crop quality and yield and improved abiotic stress resistance, such as drought and salinity tolerance. However, establishing abiotic stress tolerance varieties/hybrids will take longer time. Crop varieties/lines/hybrids with higher tolerance to drought, salinity, high temperature and nutrient deficiency, developed through conventional and molecular breeding methods and genetic engineering, are important for meeting global food demands. Traditional breeding knowledge combined with marker-assisted selection makes it quicker and more effective to generate drought tolerance in crop plants using genotypic data to improve and sustain productivity in drought-prone environmental settings. There is a pressing need to develop strategies to boost food output, particularly in the stressed zones of the world. A breeder must identify the genetic basis of stress tolerance in crop plants to generate improved genotypes using either traditional breeding or biotechnological methods. Scientists from all around the globe are working hard to develop varieties with enhanced heterosis in stress-prone settings. The most promising, less resource-intensive, commercially feasible and socially acceptable strategy is to develop crop varieties with built-in salt, drought and heat tolerance.

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9

Transgenic Strategies to Develop Abiotic Stress Tolerance in Cereals

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Abstract

Cereal grains are the world's dominant contributors of raw food material and dietary supplements for both humans and livestock. Significant yield losses in cereals are recorded due to abiotic stresses such as extreme temperatures, water availability, ion or physiological pH (salinity and alkalinity), UV radiation, and anoxia/hypoxia in terms of quality and quantity. The fact that abiotic stresses are both multigenic and quantitative complicates understanding how plants respond to them. Plants with improved responses to environmental perturbations are being developed worldwide using physiological, biochemical, and molecular genetic approaches. Further, with the advent of next-generation sequencing technologies,

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_9

numerous potential abiotic stress-responsive genes in different crop plants have been identified. As a consequence, biologists are focusing their efforts on elucidating the molecular basis of such genes that confer abiotic stress tolerance, as well as investigating the downstream pathways they are involved in. Using genetic engineering technologies including transgenics, functional validation of various target genes involved in different biological processes such as signalling pathways, transcriptional activation/repression, ion homeostasis, and oxidative defence in multiple model systems has been accomplished. Several of these attempts have been made in cereal crops, viz. rice, corn, barley, wheat, sorghum, and millets. The current chapter summarizes the milestones established in the transgenic research unveiling the role of important genes in different abiotic stress responses as well as the transfer of these genes to other cereal crops to help them thrive under such stressful conditions.

Keywords

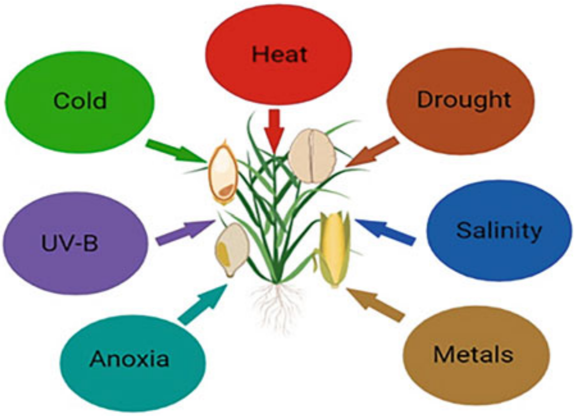
Abiotic stress · Cereals · Transgenic approach · Redox · Ionic balance · Transcription factor · Signalling cascade · Osmotic regulation

9.1 Introduction

The productivity and yield of many agricultural plants, especially grains, are influenced by abiotic stresses. They form the major group of yield-restricting factors for crops (Canter 2018; Zorb et al. 2019). Extreme temperature, drought, salinity, heavy metal stress, anoxia stress, and ultraviolet-B (UV-B) stress, among others, affect the growth of plants and limit crop productivity causing crop failure (Isayenkov and Maathuis 2019; Shikov et al. 2020; Kapoor et al. 2020). Approximately 90% of farmland is subjected to one or more abiotic stress factors (dos Reis et al. 2012). Abiotic stress inflicts 70% of agricultural loss worldwide (Acquaah 2007; Mantri et al. 2012). Studies based on the amalgamation of climate change and crop yield models have anticipated further loss of productivity in major cereals like rice, wheat, and maize causing critical repercussions to total food security (Tigchelaar et al. 2018). Therefore, the development of abiotic stress-resilient crops is important to secure food supply and to establish sustainable agriculture (Fig. 9.1).

Abiotic stresses cause a reduction in biomass production in cereals. For example, high temperature increases the incidence of spikelet sterility and reduces the accumulation of assimilates in rice (Korres et al. 2017). With every 1 °C rise in temperature, there is a 10% loss in wheat yield (Hede et al. 1999). Temperature above 28 °C during anthesis in oat decreases its yield (Hakala et al. 2020). Chilling stress leads to a reduction in germination percentage and emergence efficiency in sorghum, limiting seedling development with stunted root and shoot. It also affects

Fig. 9.1 Potential abiotic factors affecting crop productivity and yield in cereals



photosynthesis, thereby decreasing dry matter accumulation in sorghum (Ercoli et al. 2004; Chinnusamy et al. 2007; Ortiz et al. 2017). In wheat, drought causes loss of leaf area, kernel abortion, obstruction in the movement of food reserves, and a decrease in the number of amyloplast in grains (Saini and Westgate 1999; Shah and Paulsen 2003). Drought stress during the flowering stage in rice strongly influences physiological characters leading to compromised grain yield (Yang et al. 2019). Salinity causes cell damage and retarded growth in finger millets (Satish et al. 2016). Compared to other cereals, rice is highly susceptible to rhizospheric salinity (Maas and Hoffman 1977; Ashraf 2009; Hussain et al. 2017). Salt stress reduces CO_2 fixation, leaf development, leaf cell enlargement, dry mass buildup, and relative growth in rice (Cramer et al. 2001; Khan and Abdullah 2003; Amirjani 2011). Small amounts of heavy metals are required for plant growth and development; however, their excessive amounts can lead to toxicity in plants. For example, arsenic (As) causes a reduction in seed germination, lowering of seedling height, decrease of leaf area, and a reduction of dry matter accumulation in rice (Marin et al. 1993; Abedin et al. 2002). Cadmium (Cd) causes a reduction in seed germination and a decrease in plant nutrient content in wheat (Ahmad et al. 2012; Yourtchi and Bayat 2013). Cd also leads to reduced root and shoot growth in rice and maize (Wang et al. 2007; Ahmad et al. 2012; Roychoudhury et al. 2012; Yourtchi and Bayat 2013). Accumulation of zinc in the leaves of rye causes retardation in growth, decrease in nutrient content, and reduction of photosynthetic energy conversion efficiency (Bonnet et al. 2000). Complete absence of oxygen and UV-B exposure can also affect crop productivity. It was observed that the seeds of barley, wheat, rye, and oat could not synthesize α amylase under anoxia, thus affecting their germination (Perata et al. 1993). Biotechnology provides a plethora of applications in the improvement of cereal crops for abiotic stress tolerance. Although conventional breeding and marker-assisted selection have led to the development of many promising cultivars, transgenic approach has an upper-hand since genes governing important agronomic traits can be sourced from any organism whether plants or microbes and can be introgressed into the plant genome. Conventional breeding of abiotic stress-resistant cereals involves random mixing of thousands of genes together present in both resistant and susceptible varieties, while genetic engineering allows the preferential transfer of desirable genes to susceptible plants and preserve their agronomically important traits. Genetic transformation has been successfully employed to produce many abiotic stress-resilient cereal crops like rice (Sahoo et al. 2012; Ganguly et al. 2020; Shim et al. 2018; El-Esawi and Alayafi 2019), wheat (Zang et al. 2018), and maize (Wei et al. 2011).

9.2 Genetic Engineering Approaches for Plant Abiotic Stress Tolerance

High temperatures, drought, flood, salinity, and heavy metal toxicity are the primary abiotic conditions that hinder growth, culminating in yield declines of up to 70% in vital agricultural crops (Mantri et al. 2012).Traditional breeding, although a strategy of countering such abiotic stress, has several downsides, including the fact that it is a time-consuming procedure that allows for the fertilization of plants of the same or closely related species for hybridization. Genetic engineering technologies to circumvent such barriers are currently in use and one of the significant accomplishments in the generation of biotic/abiotic stress-resilient agricultural crops (Khan et al. 2011). The different genetic transformation techniques are centred on direct and indirect transformation procedures. The gene of interest (GOI) is introduced straightforwardly into the plant genome using direct techniques such as microprojectile bombardment and protoplast transformation or electroporation. In indirect approach, however, either *Agrobacterium tumefaciens* or *Agrobacterium rhizogenes* facilitates the transfer of the binary vector harbouring the GOI into the plant genome.

9.2.1 Direct Gene Transfer into Plant Cells

9.2.1.1 Particle Bombardment

Particle bombardment/biolistics, a direct delivery method in which high-velocity microprojectiles are employed to introduce foreign DNA into plant tissues or cells, was developed by John Sandford and co-workers (Sanford et al. 1987). In this technique, the DNA-coated particles (microprojectiles) are accelerated directly into intact cells or tissues. As the microprojectile enters the cells, the transferred DNA dissociates from the particle and integrates into the host genome. Sandford coined the term “biolistic”, which is taken from the words “biological and ballistic”. The most frequently used terms, however, are particle gun or particle bombardment.

In the original gene gun, described by Sanford et al. (1987), a plastic bullet (macrocarrier) loaded with millions of DNA-coated tungsten particles (microcarriers) was placed in front of the 0.22 calibre barrel. When the gunpowder was fired, microcarriers were accelerated towards a stopping disc containing a tiny hole, through which only the microcarriers can pass and reach the target tissue

(Sanford 1990; Kikkert 1993). The gunpowder model was effectively utilized in the development of transgenics of different plant species in the majority of genetic transformation protocols. However, the uncontrollable impact of the gunpowder caused physical damage to surrounding cells as well as the target cells, posing a barrier to the generation of stable transformants.

Plant genetic transformation procedure could be outlined briefly as follows: The gene of interest for abiotic stress is isolated from the original source and cloned into a functional construct (plasmid) containing promoters and marker genes. This plasmid is then introduced into the target plant cells using particle bombardment. Transgenic plants are then regenerated from the bombarded cells and further tested for gene expression at the laboratory, greenhouse, and field level. The first stable transformation using biolistics was reported in soybean. Later, maize callus tissues were transformed with the selectable marker gene; neomycin phosphotransferase (NptII) (Klein et al. 1988).

First-generation particle bombardment devices used gunpowder charges; however, improved devices have been developed that include the use of helium gas as a particle propellant for the microparticles through target tissues (Biolistic® PCS-100/He particle delivery system), high-voltage electric discharge to accelerate the gold particles coated with DNA (ACCELL™ particle gun), syringe filter to induce a low-pressure helium burst for acceleration of microcarriers (particle inflow gun), and use of plastic tube that directly accelerates the microcarriers to the target tissues (Helios® gene gun). Helios® gene gun is the most advanced design of particle bombardment (Ibrahim and Kuaybe 2020).

9.2.1.1.1 Biolistic® PDS1000/He Particle Delivery System

Biolistic® gene gun has been commercially available in 1991 (Bio-Rad) which symbolizes technical variants over the gunpowder device. It is an efficient and widely utilized gene transfer method. PDS-1000/He system employs a high-pressure helium (He) gas as a particle propellant for the microparticles through target tissue (Fig. 9.2). The bombardment chamber placed at the top of the device is continuously fed by the gas and held by a "rupture disc", which is designed to break as microparticles move and pass inside the target cells. The advantage of using this delivery system is that it regulates different bombardment parameters and uniformly distributes microcarriers throughout the target cell, resulting in higher transformation efficiency (Kikkert 1993). In recent years many agriculturally important crops have been transformed using biolistics. Sarangi et al. (2019) bombarded embryogenic calli of a high-yielding scented indica rice variety, Pusa Basmati 1, with *AmSOD* gene under the control of a ubiquitin promoter against salt stress. Particle bombardment has also been successfully used for plastid transformation, gene silencing, proteolistic, minichromosome delivery, fluorescent dye delivery, and precision genome engineering (Ibrahim and Kuaybe 2020).

9.2.1.2 Protoplast Transformation

Protoplast transformation is based on the use of plant protoplasts (plant cells without a cell wall) in which the naked DNA is treated with polyethylene glycol (PEG) in the

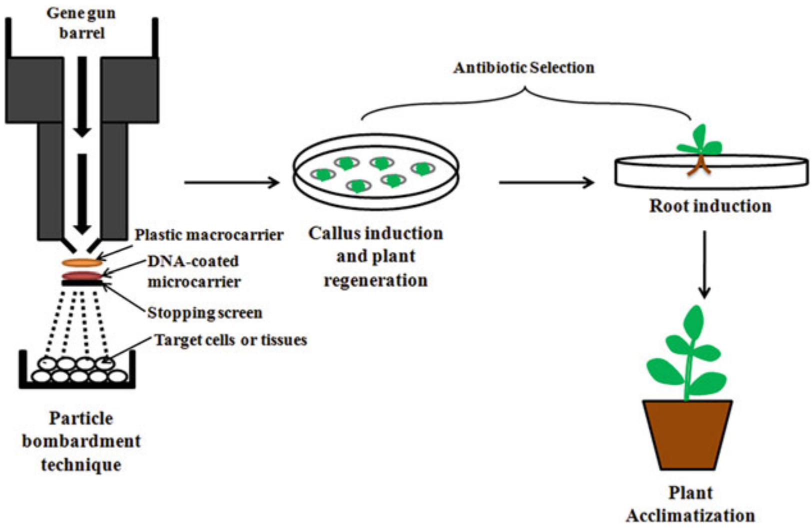


Fig. 9.2 Plant genetic transformation using biolistic technique

presence of divalent cations (usually calcium) that facilitates integration and transformation. The PEG is a polyether compound that has high affinity towards DNA and along with Ca^{2+} penetrates through the membrane and facilitates transfer of DNA into the target. In this method usually, leaf mesophyll is used to prepare protoplasts. The leaf disc is treated with cellulose and protease enzymes to degrade the cell wall (Sahab et al. 2019). After enzymatic degradation, the protoplast is purified with a mannitol solution. The prepared protoplast is suspended in the solution of PEG/Ca^{2+} and the DNA solution of concentration 60 μm. The solution of DNA and protoplast is incubated for a brief period of time. The DNA penetrates into the protoplast then enters the nucleus and integrates into the genome. Finally, the protoplast treated with DNA is spread over a suitable medium and the protoplast containing the transferred DNA is isolated by using a marker. Thus, regeneration of the whole plant from the transferred protoplast results in the transgenic (Fig. 9.3). Though the methodology is simple and devoid of costly equipment, it suffers difficulty in the regeneration of plants from a protoplast. The concentration of various chemicals and DNA is standardized based on the target.

9.2.1.3 Electroporation

Electroporation is a transformation technique that applies a high electrical field to facilitate pore formation on the cell membrane as a result of polarity shifts induced by the applied electrical field (alternated or pulsed). When electric field is applied, the hydrophobic lipid bilayer of the cell membrane gets charged which results in the alignment of positive charge on the outer membrane and negative charge towards the inner membrane leading to a pore formation in the cell membrane called hydrophobic pore which mediates insertion of the foreign DNA into the target cells or tissues.

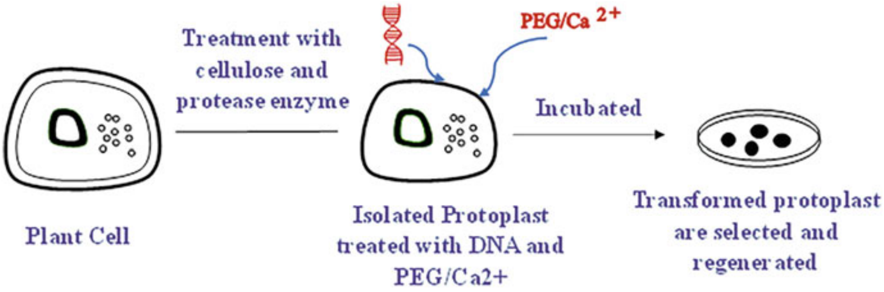


Fig. 9.3 Protoplast transformation

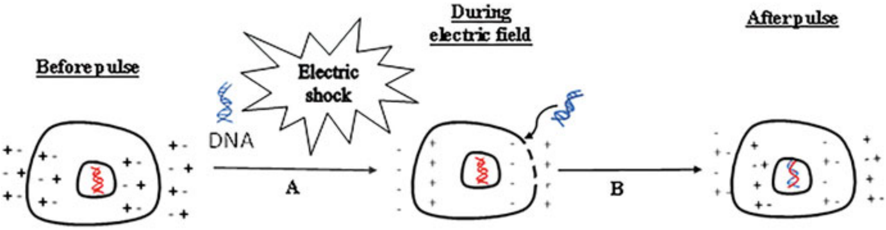


Fig. 9.4 Electroporation technique in plant genetic transformation. (**a**) A DNA of interest is introduced into the protoplast. (**b**) Application of electric field facilitates integration of DNA into the genome

The DNA gets aligned to the anode pole and gains entry into the cell by the pore, resulting in the formation of protoplast with the transgene from which transgenic plants can be generated (Fig. 9.4).

An electroporator device is used for electroporation. It is divided into three major components, including (1) a pulse power supply (it houses control units such as electrical pulse settings, field strength, and time), (2) electroporation cuvettes (these are glass cuvettes in which the target cell suspension is transformed and electroporated), and (3) electrodes (electrodes are electrical conductors that facilitate the direct contact between the cell suspension and the electrodes. Upon application of the adjusted electric pulse settings to the cells via electrical conductors and electrodes, a direct contact between cell suspension and electrodes facilitates integration of foreign DNA into the target cells (Bullmann et al. 2015)). The electro-permeabilization of the cell membrane is mediated by different pulse characteristics (including voltage, resistance, and capacitance) and an optimum field strength implicated in transmembrane voltage. During the electro-transformation of nucleic acids, three distinct types of electrical wave pulses (time constant, square wave, and exponential decay) are often applied to electroporators. The two important variables, viz. field strength (kV cm^{-1}) and time constant, distinguish the pulse that is being delivered. These variables can be altered to achieve ideal efficiency in transfection of various cell types (Ibrahim and Kuaybe 2020). The electroporation technique is accurate, generative, swift, extremely effective, and easy to use and results in stable transgenics. Nevertheless, one of its key drawbacks is that the cells may die when subjected to elevated electric voltage.

9.2.2 Indirect Gene Transfer into Plant Cells

9.2.2.1 *Agrobacterium*-Mediated Genetic Transformation

Agrobacterium tumefaciens is a soil-dwelling gram-negative bacterium that infects plant wound sites. This bacterium triggers crown gall disease in plants by transferring (T)-DNA into target plant cells via the type IV secretion system (T4SS) of bacteria. The oncogenes in the T-DNA region of Ti plasmid can be replaced with the desired gene to perform plant genetic transformation. The Ti plasmid consists of two genetic components—the T-DNA region which is transferred to the host cell and the Vir region that helps in transferring T-DNA into the target host. The border sequences of T-DNA, namely, left border (LB) and right border (RB), are described by the presence of conserved 25 base pair imperfect repeats towards the end of the T-region. The genes that code for auxin, cytokinin, and opine are present in T-DNA. Auxin and cytokinin produce uncontrolled cell division that results in tumour formation and opines act as a nutrient to bacterial growth. The Vir region consists of Vir genes (virulence genes) that code for Vir proteins involved in tumour formation. The Vir protein recognizes the LB and RB sequence and creates nick to produce a single strand of linear T-DNA that gets transferred to the host cell to produce pathogenicity. This natural mechanism was exploited to transfer a gene of interest to the target cell by inserting the sequence to be transferred in place of T-DNA (Christine Desfeux and Bent 2000).

In nature, injured plant tissues exude chemical substances such as high levels of various phenolics, including lignin and flavonoid precursors, which serve as chemotactic agents. These chemicals draw *A. tumefaciens* to the injured areas and trigger infection in the plants. Plant phenolics directly or indirectly interact with the transmembrane sensory protein VirA/VirG and initiate the signalling pathway which promotes the coding of Vir protein to plant phenols. The peak expression of Vir genes takes place when chromosomal virulence (chvE) gene attaches to monosaccharide released by plants.

The T-DNA is produced by a complex of proteins called the relaxosome protein complex. The protein complex consists of VirD2, VirD1, VirC1, and VirC2 attached to the border sequence of T-DNA. The linear single-strand T-DNA is produced by VirD2 and it remains covalently attached to $5'$ end of T-strand (single strand of T-DNA) to produce VirD2-T-DNA nucleoprotein complex. The action of VirD1 is to cleave Vir D2 from superhelical T-border (type I DNA topoisomerase). VirC1 and VirC2 act as T-DNA production enhancer (Meyer et al. 2018). *A. tumefaciens* adhere to the host cell by two types of binding system—unipolar polysaccharide (UPP)-dependent polar attachment and T pilus-mediated attachment.

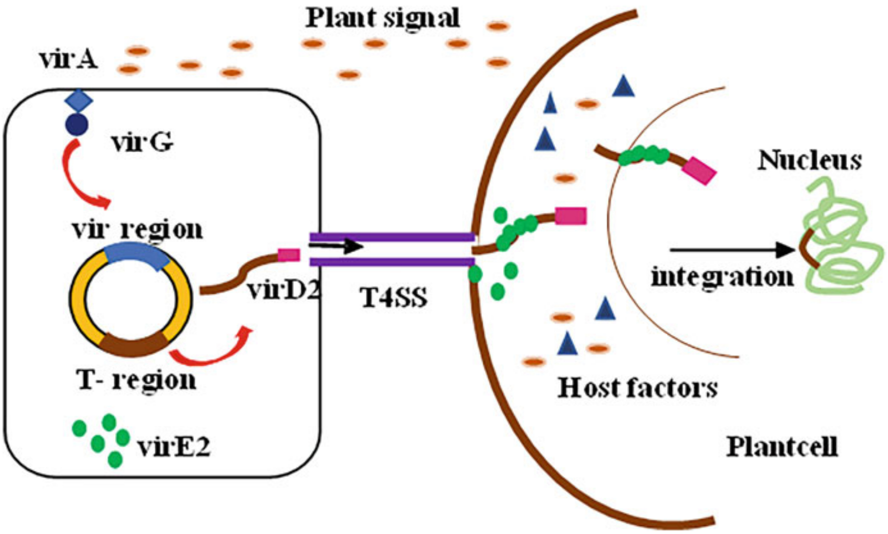


Fig. 9.5 *Agrobacterium*-mediated genetic transformation

For the transport of T-DNA, a pore channel across cell envelope is produced by type IV secretion system (T4SS) (Lacroix and Citovsky 2018) that consists of 11 Vir B proteins and Vir D4 represented as VirB/VirD4 T4SS. The VirD2 T-DNA nucleoprotein complex is transferred via VirB/VirD4 T4SS along with four Vir proteins (VirD5, VirE2, VirE3, and VirF). The VirE2 acts as a single-strand binding protein and protects the complex from host nucleases (Fig. 9.5). The VirD2-T-DNA nucleoprotein complex along with VirE2 coat is called "T-complex" and together with other translocated Vir protein called "super T-complex" (Guo et al. 2019). The integration of T-DNA into the host genome is through the use of the host DNA repair mechanism but it is not proved completely. The T-DNA containing the only exon gets integrated into the actively transcribed region of the host genome. The 3′ end of T-DNA has a promoter of eukaryotes called microhomologies that helps in the expression of T-DNA. The integrated T-DNA shows two types of expression: One is a transient expression in which the gene is transcribed without integration into the genome due to which the gene is not transferred to progeny and the second is a stable transformation, that is, the transferred DNA can be passed to progeny and this is through the integration of the transferred DNA into the host genome. Plant genetic transformation mediated by *Agrobacterium tumefaciens* is summarized in Fig. 9.6. Transgenic rice with highly tolerant to severe drought stress in both the vegetative and reproductive stages was developed using the AtDREB1A transcription factor through *Agrobacterium*-mediated transformation (Ravikumar et al. 2014). Recently, success has been achieved in developing in planta transformation methods against abiotic stress (Varalaxmi et al. 2015). An alternative approach, pyramiding of two or more genes through *Agrobacterium*-mediated transformation, is now in application to enhance the resistance in plants (Ahmad et al. 2010).

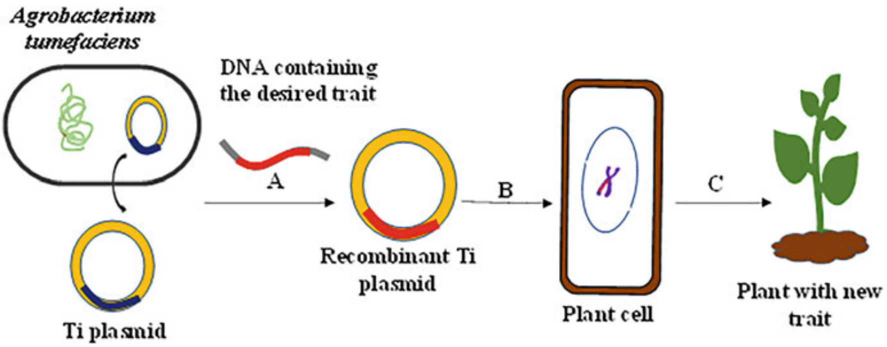


Fig. 9.6 Schematic representation of *Agrobacterium*-mediated genetic transformation against abiotic stress. (**a**) Insertion of gene into plasmid using restriction enzyme and DNA ligase. (**b**) Introduction into plant cell. (**c**) Regeneration of plant

9.2.3 RNAi Technology

The RNA interference or RNAi is the mechanism of suppression of gene expression at post-transcriptional or translational level mediated through small non-coding RNAs such as small interfering RNA (siRNA), short hairpin RNA (shRNA), and microRNA (miRNA). This technique has been successfully used for engineering plants for abiotic stress tolerance such as drought, salt, cold, and heat in many important cereal crops. The RNAi technology is not only used for developing abiotic stress tolerance in plants but also to understand the specific function of a gene involved in abiotic stress (Fig. 9.7).

(a) **Transgenic approach for siRNA-mediated abiotic stress tolerance**
The small non-coding RNAs are the resulting product of double-stranded RNA breaks. The dsRNA is cleaved by the enzyme known as dicer in plants into siRNAs (Kumar et al. 2012).

(b) **Transgenic approach for abiotic stress tolerance by regulating stress-responsive miRNAs**
miRNAs are small RNAs that regulate gene expression post-transcriptionally. During abiotic stress conditions, the stress-responsive miRNAs genes undergo transcriptions into primiRNAs. The primiRNAs are cleaved into premiRNAs by endonucleases such as Drosha and exported into cytoplasm by Exportin 5 protein. Dicer then cleaves the premiRNAs into miRNAs. The matured miRNAs are then loaded into RISC complex and activate AGO1. The microRNA-RISC complex then complements with target gene mRNA, leading to either degradation or translational repression of the target mRNA.

(c) **RNAi-mediated gene regulation for abiotic stress tolerance in major cereals**
Overexpression of stress-responsive miRNAs in cereal crops results in abiotic stress tolerance. Many miRNAs have been reported to be abiotic stress responsive. Small RNAs antagonistically work with transcription factors during abiotic

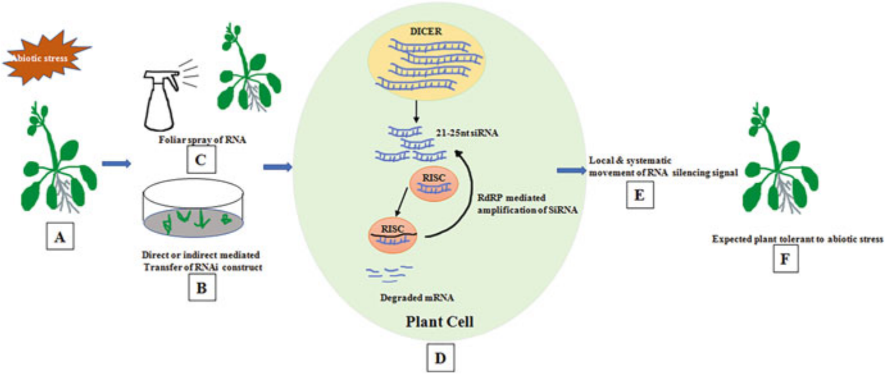


Fig. 9.7 Simplified procedure of RNAi in plants against abiotic stress. (**a**) Plant is susceptible to abiotic stress. (**b**, **c**) The RNAi construct is transferred to the plant through direct (gene gun-mediated plant transformation) or indirect (*Agrobacterium*-mediated transformation) or through the foliar spray of RNA. (**d**) The transferred RNA is recognized by DICER enzyme and cleaves into 21–25 small nucleotide RNAs known as the siRNA. The siRNA along with the RISC complex degrades the target mRNA. (**e**, **f**) The silencing signal is locally and systematically transferred to the entire plant resulting in expected plant tolerance to the said abiotic stress

stresses. In rice, plants overexpressing miR156 showed negative regulation of SPL9 to increase abiotic stress tolerance such as salinity and drought (Cui et al. 2014). miR166 drives the enlargement of roots in plants overexpressing *OsNAC10*, resulting in drought tolerance with increased yield (Jeong et al. 2010), whereas the knockdown of miRNA166 in rice conferred drought tolerance by reducing transpiration rate (Zhang et al. 2018). Overexpression of another rice miRNA, Osa-MIR319, resulted in cold tolerance in rice by changing the leaf morphology (Yang et al. 2012). In wheat, nutrition partitioning and grain yield were improved under abiotic stress by decreasing the expression of *TaNAM* through RNAi (Guttieri et al. 2013). *mir169* controlled the expression of transcription factor NUCLEAR FACTOR-Y subunit A coding genes in maize leaves in response to abiotic stress such as drought, abscisic acid, or salt stress (Luan et al. 2015).

9.2.4 Genome Editing

Genome editing techniques such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/CRISPR-associated system (CRISPR/Cas) have been utilized for improving various traits such as cold, heat, drought, and salt in cereals. More extensive applications in cereal crop improvement were observed using TALEN and CRISPR/Cas9. Further availability of more advanced variants of

CRISPR/Cas such as CRISPR/Cpf1 and Cas9-derived DNA base editors provides possibility of wider application of these techniques.

9.2.4.1 Zinc Finger Nucleases (ZFNs)

ZFNs are sequence-specific nucleases that enable targeted mutation. ZFN is in general zinc finger protein (ZFP) which is fused to the cleavage domain of *Fok*I restriction enzyme (Fig. 9.8a). Target-specific ZFN can be designed by manipulating ZFP. The designed ZFN induces cleavage in the targeted genomic region and repair through either non-homologous end joining (NHEJ) or homology-directed repair (HDR), thereby creating indels or new transgene insertion, respectively. ZFN has not been extensively used for cereal crop improvement against abiotic stress.

9.2.4.2 Transcription Activator-Like Effector Nucleases (TALENs)

TALEN is the next advanced technique of genome editing after ZFN. The basic structural assembly is similar to ZFN where a DNA binding protein domain (TAL) is fused with the cleavage domain of *Fok*I restriction enzyme resulting in targeted double-strand break (Fig. 9.8b). This TAL region is the naturally occurring region unlike ZFN. Manipulating the TAL region of interest which consists of repeat variable diresidues (RVD) enables a target of different sequences for customized nucleases. The application of TALEN technology was not explored for abiotic stress tolerance in cereals.

9.2.4.3 CRISPR-Related Endonuclease Cas9 (CRISPR/Cas9)

Clustered regularly interspaced short palindromic repeats/CRISPR-associated system (CRISPR/Cas) is the most advanced and effective GE technique. Since its discovery, it has been extensively used in various fields including crop improvement due to its ability to edit genes at a precise location. It is an RNA-guided nuclease system and often engineers to modify specific sequences in the target genome (Fig. 9.8). Previously, its use was limited to model plants like *Arabidopsis* and tobacco; however, it is currently being expanded to cereal crops like as rice, maize, and wheat. Initially, CRISPR/Cas9 system was used to identify the function of a gene by generating mutant lines in cereal crops. Gene-specific targeting of rice genes such as *OsPDS*, *OsMPK2*, *OsBADH2*, *OsMPK5*, *OsMPK2*, *OsDEP1*, *OsDERF1*, *OsPMS3*, *OsMYB5*, *OsAOX1a*, and *OsAnn3* was reported to be involved in various abiotic stresses through CRISPR/Cas technique (Shan et al. 2014; Zhang et al. 2014a, b; Xu et al. 2015; Shen et al. 2017). The mutants of miRNA gene *OsMIR528* generated through CRISPR/Cas9 in rice revealed its role as a positive regulator in salt stress (Zhou et al. 2007). CRISPR/Cas9 technique was used for multiple abiotic stress tolerance in rice where the gene drought and salt tolerance (DST) were targeted resulting in moderate tolerance to osmotic stress and salt stress in the seedling stage (Kumar et al. 2020). Another gene, *OsRR22*, was targeted through CRISPR/Cas9 which conferred salt stress tolerance in rice (Zhang et al. 2019). In maize, drought stress tolerance was achieved with increased crop yield by targeting *ARGOS8* through CRISPR/Cas9 (Shi et al. 2017). The guidelines for the GE regulations are still to be designed clearly in India as generation of GE through

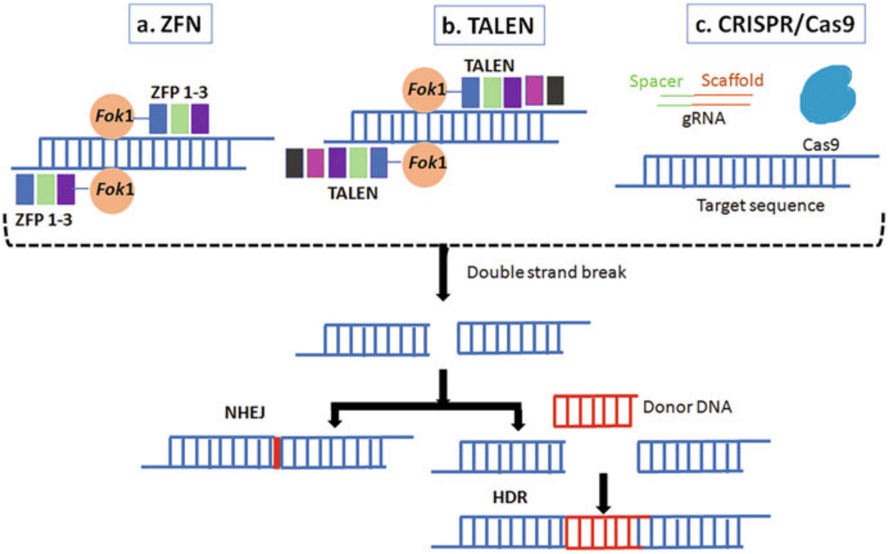


Fig. 9.8 Targeted double-stranded DNA break and repair either through NHEJ or HDR pathway with genome editing techniques. (**a**) ZFN, (**b**) TALEN, and (**c**) CRISPR/Cas9

CRISPR/Cas introduces Cas9 transgene in the target genome. However, Cas9 can be removed in the subsequent generation through self- or back-crossing (Huang et al. 2016).

9.2.5 Cisgenic Approach

The cisgenic approach for gene transfer is genetic manipulation through recombinant DNA technology between the same species or closely related sexually compatible groups of plants. Cisgenic is one of the genetic engineering strategies to increase abiotic stress tolerance in crop plants. The cisgenic approach is considered safer and one of the techniques for food security in future as compared to transgenics crops and conventional breeding. Despite its tremendous potential for crop improvement, GM crops are linked with societal concerns about human health and the environment. Cisgenics overcome the problem such as linkage drag associated with conventional breeding. On the other hand, due to its similar way of selecting gene as classical breeding, cisgenic plants should be treated as classical bred plants. Therefore, cisgenic crops provide a future new generation of genetically modified crops with the possibility of wider acceptance.

9.3 Abiotic Stress Tolerance in Cereal Crops

Plants are incessantly exposed to a range of environmental stressors due to their sessile nature (Suzuki et al. 2014). Nonetheless, there remained a dearth of insights of the mechanisms by which crops uphold production in the face of abiotic stresses until recently. Climate change has a multifaceted impact on the consequences of abiotic stresses, putting agriculture's long-term sustainability and production at risk. Drought, heat, low temperature, and salinity are some of the most significant abiotic stresses that have had a significant impact on agricultural output in recent years (Tardieu and Tuberosa 2010). Numerous techniques have been devised regularly to maximize agricultural output while minimizing pre-harvest and post-harvest losses inflicted by abiotic challenges (Gust et al. 2010). Plants react to multiple stresses through intricate and specialized cellular and molecular mechanisms to prevent loss and increase survival. In response to each stimulus, these mechanisms cause morphological and growth pattern alterations, as well as changes in biochemical and physiological processes.

9.3.1 Drought Stress

Drought, caused by inadequate precipitation or an undersupply of irrigation water, is a significant hazard to plant productivity throughout the globe (Khan et al. 2016). Owing to paucity of water, 33% of the world’s farming land is dry or semi-arid (Rady 2011). Drought stress, in conjunction with other environmental changes, results in a substantial diminution in agricultural production (Huang et al. 2018). Researchers have been studying structural, physiological, biochemical, and molecular underpinnings for more than 20 years in attempt to understand the mechanisms of plants’ drought stress responses. In attempt to discover prospective genes implicated in drought stress resilience, genetic engineering or breeding methodologies are crucial (Mishra et al. 2017; Noman et al. 2017). To uncover potential genes (which might govern a specific trait), several molecular methods such as marker-assisted selection (MAS), genomic selection (GS), and QTL mapping are being used, and CRISPR/Cas9 has been exploited to establish transgenic lines for drought stress tolerance in cereals.

The impacts of drought stress on cereal crops have been discussed in a number of study papers. Drought stress repercussions vary significantly from anatomical to subcellular level and are witnessed at all stages of development, regardless of when the moisture shortage occurs. Drought stress, in general, has a detrimental effect on germination of seeds, resulting in subpar agricultural productivity (Anjum et al. 2011). Drought stress lowers the plant’s cellular water potential and turgor pressure, resulting in higher solute concentration gradient in the cytosol and extracellular matrix. As a consequence of the drop in turgor pressure, cell development is hindered (Lisar et al. 2012). Furthermore, plant wilting is caused by an increased accumulation of abscisic acid (ABA) and compatible osmolytes like proline. At the same time, reactive oxygen species (ROS) such as H_2O_2 are overproduced.

Overproduction of ROS, despite their role as signalling molecules, may inflict significant cellular oxidative damage and photosynthetic slowdown.

Plant drought stress adaption is a multifaceted phenomenon featuring many genes and signalling networks. In order to adapt to drought conditions, plants have evolved many interwoven networks of signalling pathways to regulate different groups of drought-responsive genes and generate various classes of proteins, such as enzymes, molecular chaperones, TFs, and other functional proteins. These proteins function in a particular manner to aid plants in coping with drought. Hundreds, if not thousands, of genes that modulate plant stress response pathways have been discovered using different functional genomics methods. Single function genes and regulatory genes are two types of genes involved in drought stress responses, based on their biological function (Shinozaki and Yamaguchi-Shinozaki 2007). Osmolyte-accumulating enzymes, reactive oxygen species (ROS) scavengers, ion and water transporters and channels, and lipid biosynthesis enzymes are all coded for by single-function genes (Reguera et al. 2012). Transcription factors (TFs), protein kinases, protein phosphatases, and proteinases are examples of regulatory genes that are involved in signalling pathways and transcriptional or post-transcriptional regulation of gene expression (Shinozaki and Yamaguchi-Shinozaki 2007; Roychoudhury et al. 2008). Additionally, regulatory genes have been shown to play a crucial role in plant drought stress acclimation. As a consequence, changing the transcription of a regulatory gene is more advantageous and is anticipated to be increasingly used in GM crops that can survive abiotic stress (Reguera et al. 2012). Ectopic expression or inhibition of regulatory genes may cause a variety of stress tolerance responses. Furthermore, transcription factors are an important family of proteins that control gene expression at the transcriptional level, enabling plants to endure drought. Multiple transcription factors implicated in drought responses in plants include APETALA2/ethylene-responsive element-binding protein (EREBP), NAM-ATAF1/2-CUC2 (NAC), MYB, basic leucine zipper (bZIP), and zinc finger (Ariel et al. 2007; Ciftci-Yilmaz and Mittler 2008; Fang et al. 2008; Yamaguchi-Shinozaki and Shinozaki 2006; Fang and Xiong 2015). ARBs/ABFs (ABA-responsive element-binding proteins/factors) are bZIP TFs that have been shown to operate in ABA-dependent regulatory pathways during drought stress (Banerjee and Roychoudhury 2017). Employing transgenic strategies such as overexpression and knockdown, many of these genes are being genetically modified (GM) or altered to study their effect on increasing drought resistance in crops. Table 9.1 provides a list of genes that have been transferred to several cereal crops to enhance drought stress tolerance utilizing genetic engineering methods.

9.3.1.1 Rice (*Oryza sativa*)

As the impacts of drought stress become more pronounced, a greater number of rice genes alter expression, with about 5000 transcripts being upregulated and 6000 transcripts being downregulated (Bin Rahman and Zhang 2016). The vast majority of these genes belong to three categories: those that influence the membrane transport, those that affect signalling, and those that regulate transcription (Upadhyaya and Panda 2019; Kim et al. 2020). During drought conditions, their

Table 9.1 List of transgenic cereal crops developed through genetic engineering for improved abiotic stress tolerance

Crop name	Gene of interest (GOI) and source	Response of transgenic cereals	References
Drought stress			
Rice (*Oryza sativa*)	*OsCDPK1* from rice	Drought stress tolerance	Ho et al. (2013)
	OsLEA3-2 from rice	Drought and salt stress tolerance	Duan et al. (2012)
	TSRF1 from tomato	Drought and osmotic stress tolerance	Quan et al. (2010)
	CaMSRB2 from pepper	Drought and salt stress tolerance	Kim et al. (2014a, b)
	OsCPK4 from rice	Drought and salt stress tolerance	Campo et al. (2014)
	*OsAREB1*from rice	Drought and heat stress tolerance	Jin et al. (2010)
	BvMTSH from *Brevibacterium velvovum*	Drought stress tolerance	Joo et al. (2014)
	AtDREB1A from *Arabidopsis thaliana*	Drought and salinity stress tolerance	Ravikumar et al. (2014)
	OAT from rice	Drought and oxidative stress tolerance	You et al. (2012)
	OsSIK2 from rice	Drought and salt stress tolerance	Chen et al. (2013a, b)
Wheat (*Triticum aestivum*)	*GmbZIP1* from soybean	Drought and salt stress tolerance	Gao et al. (2011)
	TaDREB2/TaDREB3 from wheat	Drought stress tolerance	OGTR (2008)
	TaNAC69 from wheat	Drought stress tolerance	Xue et al. (2011)
	TaERF3 from wheat	Drought and salinity stress tolerance	Rong et al. (2014)
	SNAC1 from rice	Drought and salinity stress tolerance	Saad et al. (2013)
	SeCspA from *Escherichia coli*	Drought stress tolerance	Yu et al. (2017)
	P5CS from wheat	Drought stress tolerance	Pavei et al. (2016)
	TaSHN1 from bread wheat	Drought stress tolerance	Bi et al. (2018)
Barley (*Hordeum vulgare*)	*OsMYB4* from rice	Cold and drought stress tolerance	Soltész et al. (2012)
	TaDREB2/TaDREB3 from wheat	Drought and frost tolerance	Morran et al. (2011)
Maize (*Zea mays*)	*TsCBF1* from *Thellungiella salsuginea*	Drought stress tolerance	Zhang et al. (2010)
	LOS5 from *A. thaliana*	Drought stress tolerance	Lu et al. (2013a, b)
	betA and *TsVP* from *E. coli* and *Thellungiella halophila*, respectively	Drought stress tolerance	Wei et al. (2011)

(continued)

Table 9.1 (continued)

Crop name	Gene of interest (GOI) and source	Response of transgenic cereals	References
Sorghum (*Sorghum bicolor* L.)	*mtlD* from *E. coli*	Drought and salinity stress tolerance	Maheswari et al. (2010)
Salinity stress			
Rice (*O. sativa*)	*OsTPS1* from rice	High salinity and drought stress tolerance	Li et al. (2010a, b)
	P5CS from *Vigna aconitifolia*	Salinity stress tolerance	Karthikeyan et al. (2011)
	P5CSF129A from *V. aconitifolia*	Salinity stress tolerance	Kumar et al. (2010)
	OsNAP from rice	High salinity, drought, and low temperature stress	Chen et al. (2014)
	ZFP182 from rice	Salt, drought, and cold stress tolerance	Huang et al. (2012)
	kanE from *E. coli*	Enhanced production of catalase and increased salinity stress tolerance	Moriwaki et al. (2008), Prodhan et al. (2008)
	OsPP1 from rice	High salinity and drought stress tolerance	Ge et al. (2008)
	GSMT and *DMT* from *Aphanothece halophytica*	Salt and cold stress tolerance	Niu et al. (2014)
Wheat (*T. aestivum*)	*betA* from *E. coli*	Salinity stress tolerance	He et al. (2010)
	BADH from *A. hortensis*	Salinity stress tolerance	Tian et al. (2017)
	TaERF3 from wheat	Salinity and drought stress tolerance	Rong et al. (2014)
Maize (*Z. mays*)	*BADH* from *Atriplex micrantha*	Improved salinity stress tolerance	Di et al. (2015)
Heat stress			
Rice (*O. sativa*)	*mtHsp70* from rice	Improved heat tolerance in rice	Qi et al. (2011)
Wheat (*T. aestivum*)	*TaFER* from wheat	Heat stress tolerance	Zang et al. (2017)
	TaHsfA6f from wheat	Enhanced thermotolerance	Xue et al. (2015)
	TaHsfC2a from wheat	Enhanced thermotolerance	Hu et al. (2018)
	TaMBF1c from wheat	Enhanced thermotolerance	Qin et al. (2015)
	ZmPEPC from maize	Heat stress tolerance	Qi et al. (2017)
Maize (*Z. mays*)	*OsMYB55* from rice	Heat stress tolerance	Casaretto et al. (2016)
Cold stress			
Rice (*O. sativa*)	*OsMYB2* from rice	Enhanced thermotolerance	Yang et al. (2012)
	OsMYBS3 from rice	Enhanced thermotolerance	Su et al. (2010)
	ZmCBF2 from maize	Enhanced thermotolerance	Xu et al. (2011)
	OsWRKY76 from rice	Cold stress tolerance	Yokotani et al. (2013)

(continued)

Table 9.1 (continued)

Crop name	Gene of interest (GOI) and source	Response of transgenic cereals	References
	OsNAC5 from rice	Cold stress tolerance	Song et al. (2011)
	OsTPS1 from rice	Cold stress tolerance	Li et al. (2011)
	OsRAN2 from rice	Cold stress tolerance	Chen et al. (2011)
Wheat (*T. aestivum*)	*GhDREB* from cotton	Cold stress tolerance	Gao et al. (2009)
Barley (*H. vulgare*)	*HVA1* from barley	Enhanced thermotolerance	Checker et al. (2012)
Maize (*Z. mays*)	*ZmDREB1* from maize	Enhanced thermotolerance	Hu et al. (2011)

transcription regulates the majority of biochemical, physiological, and molecular processes in rice (Gupta et al. 2020). Multiple genes/transcription factors have been reported to be differentially expressed in rice and are being incorporated in transgenic plants to significantly improve drought stress responses (Kumar et al. 2017; Upadhyaya and Panda 2019). ABA-independent and also ABA-dependent regulatory mechanisms influence the majority of genes affected by drought (Gupta et al. 2020). *OsJAZ1* reduces drought tolerance in rice by regulating ABA signalling, which concocts growth and development of plants under drought stress circumstances (Fu et al. 2017). Various genes associated with osmotic adjustment and late embryogenesis abundant (LEA) proteins have been described as being important in drought stress resilience in rice (Roychoudhury and Nayek 2014). Overexpression of *Rab16A* (group 2 *LEA*) gene in transgenic rice developed salinity and drought tolerance (Ganguly et al. 2012, 2020). In GM rice, the gene *DRO1* stimulates lengthening of roots and deeper rooting (Uga et al. 2013). Certain genes, like *EcNAC67* and *OsPYL/RCAR5*, slow down leaf rolling and increase root and shoot volume in water-stressed rice (Kim et al. 2014a, b; Rahman et al. 2016). Overexpression of *OsDREB1F*, *CYP735A*, and *OsDREB2B* improves root architectural plasticity in rice plants during drought stress (Kim et al. 2020). DREB2-like gene *OsDRAP1* imparts drought resilience in rice, according to Huang et al. (2018). Enhanced grain production in rice during the episodes of water scarcity is critical, and it may be accomplished through transgenic strategies involving the incorporation of genes like *OsWRKY47* (Raineri et al. 2015), *OsbZIP71* (Liu et al. 2014), *OsbZIP46* (Tang et al. 2012), *OsNAC10* (Jeong et al. 2010), *OsLEA3-1* (Xiao et al. 2007), and *OsNAC5* (Hu et al. 2006). Introgression of genes like *EDT1/HDG11* (Yu et al. 2013), *AtDREB1A* (Ravikumar et al. 2014), *OsMIOX* (Duan et al. 2012), and *OsTPS1* (Duan et al. 2012) results in relatively high water use efficiency, cumulation of osmolytes, elevated antioxidant enzymatic activity, and augmented photosynthetic activity in transgenic rice (Li et al. 2011). In transgenic plants, *OsCPK9* enhances drought stress tolerance via improving stomatal conductance and maintaining osmotic pressure (Wei et al. 2014). Transgenic plants overexpressing *OsDREB2A* have been shown to thrive better in conditions of severe drought and salinity (Cui et al. 2011). *CDPK7* and *CIPK03*/*CIPK12* modulate a number of regulatory enzymes, signalling pathways, and protein kinases in rice (Xiang et al. 2007). The WRKY proteins perform key functions in plant growth by responding to drought stress (Sahebi et al. 2018). Multiple genes are being studied in the laboratory or under glass house settings to understand if they may impart drought tolerance in rice. Nevertheless, prior to actually integrating such genetic traits in molecular breeding approaches, they should indeed be validated in the field.

9.3.1.2 Wheat (*Triticum aestivum*)

The diminution of resistance genes induced by genetic deterioration must be compensated for by using such efficient and consistent methods, which can transfer genes in a short span of time. It is worth noting that recombinant DNA technology has emerged as a potent tool for accomplishing this objective. It also has the advantage of stripping away genetic barriers, enabling genes from any wild relatives, land race, or other species to be introduced in the target species (Hussain 2015). TFs that control the primary enzymes of signal transduction pathways; genes that produce defensive chemicals including reactive oxygen species (ROS), proline, JA, and SA; and genes that manufacture defence protein are indeed strong candidates for dehydration stress resistance in wheat (Yang et al. 2010). It has been demonstrated that dehydration-responsive element-binding (DREB) transcription factors improve drought resilience in GM wheat (Saint Pierre et al. 2012). Using the RD29A and ubiquitin promoters, a DREB gene (*GmDREB)* from soybean (*Glycine max*) was particle bombarded into wheat, and transgenic plants with both promoters exhibited improved drought and salinity stress tolerance (Shiqing et al. 2005). Such heightened drought resilience has been ascribed to twice as much proline synthesis, the stay-green phenomena during drought, and survival and recovery on re-watering (SURV) following a dry period (Wang et al. 2006), implying a role for the signal transduction cascade downstream of proline biosynthesis. Owing to enhanced accumulation of soluble sugars and photosynthetic pigments (chlorophyll) in leaves, wheat engineered with a cotton-derived DREB (*GhDREB*) enhanced drought, salt, and cold tolerance (Gao et al. 2009). In greenhouse conditions, genetically engineered wheat plants containing the *DREB1A* gene showed substantially greater tolerance to dehydration stress than wild type, as indicated by slower wilting and leaf discolouration when water was discontinued (Pellegrineschi et al. 2004). *HDG11*, an HDZip IV transcription factor, was identified to improve drought tolerance by enhancing the expression of a multitude of drought-responsive proteins, notably genes implicated in calcium signalling pathway and the ABA biosynthesis (Cao et al. 2009). Following 30 days of exposure to drought stress environments, transgenic wheat overexpressing the *AtHDG11* gene was analysed. Not only did transgenic plants overexpressing *AtHDG11* gene improve physiological parameters, but they also significantly increased production, had a reduced water loss rate and stomatal density, and synthesized more proline.

Many crops have been genetically engineered with osmolytes including trehalose, mannitol, and glycine betaine to strengthen abiotic stress tolerance by stabilizing essential macromolecules. Proline, which is produced under stress, protects biomolecules from denaturation, helps reduce oxidative stress by scavenging ROS, and modulates cytosolic activity (Hayat et al. 2012). Drought tolerance has been documented in transgenic wheat plants overexpressing proline biosynthesis genes (Sawahel and Hassan 2002). GM wheat has been developed in yet another research by incorporating the *betA* gene, which encodes choline dehydrogenase, under the control of maize ubiquitin promoter (He et al. 2011). Although wheat crops do not generally cumulate mannitol, transgenic wheat developed via overexpressing *mtlD* gene transferred from the bacteria *Escherichia coli* cumulated it (Abebe et al. 2003). Drought, salt, and ABA stimulate late embryogenesis abundant (LEA) proteins, which are hydrophilic in nature. In barley, one of these proteins is called late embryogenesis abundant 3 (*LEA3*), which is transcribed by the *Hordeum vulgare* abundant protein 1 (*HVA1*) gene. GM crops that have the *HVA1* gene and have been treated with ABA exhibited increased dehydration and salinity stress tolerance (Nguyen and Sticklen 2013). Genetic engineering approaches have been extensively utilized for introduction/incorporation of multiple genes including *SNAC1*, *TaFER-5B*, *PEPC* (phosphoenolpyruvate carboxykinase), and *PPDK* (pyruvate orthophosphate dikinase), *AtOTS1* (OVERLY TOLERANT TO SALT-1) and *TaPEPKR2* (phosphoenolpyruvate carboxylase kinase-related kinases) into transgenic wheat which showed increased tolerance to drought stress environments (Saad et al. 2013; Zang et al. 2017; Zhang et al. 2014a, b; Le Roux et al. 2019; Krugman et al. 2010).

9.3.1.3 Barley (*Hordeum vulgare*)

The prime end goal of genetic modification is to generate drought-tolerant plants with a single or many desirable traits that would be transmitted down through generations. Overexpression of *TaDREB2* and *TaDREB3* in barley transgenic lines, for example, improved drought tolerance by safeguarding cells from dehydration and damage (Morran et al. 2011). The *HvP5CS* gene, which encodes delta-1-pyrroline-5-carboxylate synthase (*P5CS*), has previously been cloned in barley as the principal drought-tolerant gene (Abu-Romman et al. 2011). The relative expression profile of *HvP5CS* gene was examined in the transgenic plants using semi-quantitative reverse transcription-PCR assay following drought, salinity stress, and abscisic acid (ABA) treatments. *HvP5CS* expression was shown to be elevated in leaf tissue following exposure to drought and salt stress, and this expression was greater in both scenarios than it was in response to just ABA treatment. Additionally, overexpression of *HvSNAC1* in barley improved drought resistance and tolerance to other biotic stressors, like fungal infection of *Ramularia collo-cygni*, as per another study (Al Abdallat et al. 2014).

9.3.1.4 Maize (*Zea mays*)

Maize is one of the most essential crops; however, escalating drought stress across the globe is putting its production at risk. Regardless of the fact that there have been relatively few reports of drought-tolerant transgenic maize to date, research conducted in the private sector, in particular, seem to be very compelling. The very first drought-resilient GM crop is MON 87460, a maize (*Zea mays* L.) variety engineered by Monsanto Company in 2009 and reportedly planted in the United States in 2013. From then on, plantings have grown 5.5-fold, from 50,000 ha in 2013 to 275,000 ha in 2014 (http://www.isaaa.org/resources/publications/briefs/49/toptenfacts/default.asp). Drought tolerance is imparted by the inclusion of cold shock protein B (*CSPB*) from *Bacillus subtilis* in transgenic maize variety MON 87460. Furthermore, increased expression of a Nicotiana protein kinase (*NPK1*) gene with a potential function in oxidative stress management was shown to improve photosynthesis process and hence drought stress resilience in GM maize (Shou et al. 2004a, b). Overexpression of maize transcription factor, *ZmNF-YB2*, improved dehydration stress tolerance and productivity under drought conditions, as per a Monsanto Company study (Nelson et al. 2007). Virlouvet et al. (2011) showed that under both well-watered and drought-stressed circumstances in the field, overexpressing the *ZmASR1* is manifested in higher dry leaf mass and total chlorophyll content and also enhanced maize kernel production. The study of Li et al. (2008) showed that integration of *TsVP* gene (vacuolar H^+-pyrophosphatase ($V\text{-}H^+$-PPase) from the dicotyledonous halophyte (*Thellungiella halophila*) into the maize (*Z. mays* L.) crop resulted in enhanced drought resistance of genetically engineered plants. In yet another genetic modification method, bacterial RNA chaperones imparted dehydration stress resilience in maize plants (Castiglioni et al. 2008). Wang et al. (2016) demonstrated that GM maize with *ZmVPP1* gene overexpression is more drought resilient than untransformed counterparts.

9.3.1.5 Sorghum (*Sorghum bicolor* L.)

In response to environmental stress, sorghum exhibits physiological changes such as a deeper and more pervasive root system, leaf curling, and reduced stomatal conductance, as well as decreased metabolic activity, allowing it to withstand drought conditions (Schittenhelm and Schroetter 2014). As a result, sorghum is significantly tolerant to drought compared to other cereal crops. There have been barely a few reports of transgene introduction in sorghum to generate drought-tolerant cultivars. For instance, to initiate the mannitol biosynthetic cycle, the *mtlD* gene from *E. coli*, which encodes mannitol-1-phosphate dehydrogenase, was introduced into *S. bicolor* L. Moench cv. SPV462. Leaf samples from GM sorghum exhibited enhanced leaf water potential when challenged to polyethylene glycol 8000 (−2.0 MPa) and 1.7- to 2.8-fold greater shoot and root multiplication when subjected to NaCl stress (200 mmol/L) as compared to control plants (Maheswari et al. 2010). Nevertheless, a variety of drought stress tolerant genes originating from sorghum have been introduced to other model and agricultural crops. *SbER2-1*, a leucine-rich repeat-receptor-like kinase gene from sorghum (*Sorghum bicolor* L.), has been found to improve drought tolerance in maize (Li et al. 2019). Analogously, overexpression of *SbSNAC1*, a sorghum NAC transcription factor, conferred drought tolerance in transgenic *Arabidopsis thaliana* (Lu et al. 2013a, b). Furthermore, utilizing a population from BT × 642 and RT × 7000, four QTLs (*stg1*, *stg 2*, *stg 3*, and *stg*

4) for the stay-green phenotype were identified in sorghum. Under drought stress environments, RT × 7000 NILs with the *stg2* gene from BT × 642 had greater green leaf area than other NILs, as per physiological analysis (Harris et al. 2007).

9.3.1.6 Pearl Millet (*Pennisetum glaucum*)

There have been no published studies on the establishment of a GM pearl millet variety that is resistant to abiotic stress. As a result, despite its economic importance, the development of GM pearl millet cultivars is still in its early stages, necessitating a concerted effort to produce and evaluate transgenic lines under a range of stress conditions (Shivhare and Lata 2017).

9.3.2 Salinity Stress

Cereals are cultivated in essentially every region of the world, and they are exposed to a multitude of environmental stressors that obstruct their growth and productivity. One of the most detrimental abiotic threats cereal crops face is salinity. Food security requires the deployment of countermeasures to this challenge. Plant scientists have leveraged a variety of approaches for improving crop yields on salt-affected soils. In the development of salt stress adaptable agricultural crops, traditional breeding, genetic engineering, marker-assisted selection, and, lately, genome editing have all proven effective. Improvements in salinity tolerance have been observed in rice and wheat, but there are few good instances for other cereals. Challenges to the generation of salt-tolerant cultivars/lines have been reported as a dearth of understanding regarding crop genetic structure, biochemical and physiological characteristics, vast diversity in environmental circumstances, and the intricate polygenic nature of the salinity tolerance trait. The application of transgenic methods to create salt-tolerant grain crops is discussed further below. A list of genes that have been incorporated to various cereal crops to enhance salt stress tolerance utilizing genetic engineering methods is shown in Table 9.1.

9.3.2.1 Rice (*O. sativa*)

In the large portion of studies, scientists introduced genes for vacuolar Na^+/H^+ transporters from various sources into rice and observed increased salt stress resilience (Zhao et al. 2006; Verma et al. 2007). The introduction of the vacuolar Na^+/H^+ antiporter *AgNHX1* gene from *Atriplex gmelinii*, for instance, contributed in an eight-fold increase in the activities of the vacuolar-type Na^+/H^+ antiporter, improving rice seedling survivability from 50% to 100% under salinity environments (Ohta et al. 2002). *PgNHX1* from *Pennisetum glaucum* (L.) R. Br., another vacuolar Na^+/H^+ antiporter gene, demonstrated expansive root architecture in GM rice seedlings and exhibited approximately 81% greater shoot and root lengths compared to untransformed control seedlings (Verma et al. 2007). Analogously, in salinity environments, GM rice seedlings with the yeast Na^+/H^+ antiporter SOD2 gene thrived effectively (Zhao et al. 2006). The second important approach that has been employed is genetic transformation to produce higher concentrations of compatible solutes. Proline over-accrual is also regarded as a considerable selection criterion for salinity tolerance. The *P5CS*, an essential proline biosynthesis intermediate gene isolated from moth bean (*Vigna aconitifolia*), was incorporated into rice, resulting in GM rice with higher proline contents than wild types in both control and salinity environments (Su and Wu 2004). Trehalose overproduction is another essential feature of salt-tolerant plants. Transgenic rice bearing the yeast trehalose gene (*TPS1*) exhibited improved salt tolerance as well as dehydration and cold stress tolerance (Garg et al. 2002). Furthermore, the chimeric gene *Ubi1::TPSP* in another GM rice variety led to significant concentrations of trehalose, thus providing salinity tolerance (Jang et al. 2003). The salt tolerance potential of GM rice lines was improved by overexpression of wheat *TaSTRG* in rice (Zhou et al. 2009). Recently, the *A. thaliana AtDREB1A* TF was introduced into rice under the control of two distinct promoters, CaMV35S and Lip9. The resulting transgenic plants were exposed to both normal and drought-stressed conditions. Plants with the Lip9-*DREB1A* and 35S-*DREB1A* genes were more fertile than wild-type plants; however, when salt stress was applied, Lip9-*DREB1A* plants outperformed 35S-*DREB1A* plants in terms of grain production (Hussain et al. 2018).

9.3.2.2 Wheat (*T. aestivum*)

On moderately saline soils, wheat productivity losses are significant (Shahbaz et al. 2012), and growth is considerably retarded (Perveen et al. 2012). GM crops, generated with the use of a range of genetic engineering techniques, have been described as a speedy way to produce crops that are resistant to unfavourable environmental conditions. Despite the fact that few experiments on the efficacy of stress-tolerant GM plants in natural settings have been undertaken, scientists are holding out hope to make headway in this area (Ashraf and Akram 2009). Salt tolerance is a multifaceted characteristic governed by a multitude of quantitative traits. Researchers have attempted to introduce specific genes to generate resistant GM varieties/lines of wheat to enhance salinity tolerance. For example, in wheat, higher expression of the vacuolar Na^+/H^+ antiporter gene *AtNHX1* (from *A. thaliana*) boosted germination, biomass production, and productivity (Xue et al. 2004). Upon being exposed to salinity stress, the transgenic lines accumulated less Na^+ in the leaves and more K^+ in the leaves and had a 68% greater shoot dry mass and a 26% higher root dry weight than control plants (Xue et al. 2004). Bread wheat has a greater Na^+ ion exemption capacity than durum wheat, which enhances its salinity tolerance capability. The *TmNax2* locus from *T. monococcum* was transferred into durum wheat, which escalated salinity tolerance and production by up to 25% compared to control plants grown in high salinity soil (Munns et al. 2012). In wheat, high-affinity K^+ transporters (*HKTs*) play a pivotal part in regulating Na^+ accumulation (Munns and Gilliham 2015). Salinity tolerance was bolstered in GM wheat and *Arabidopsis* lines with high expression levels of *TaAOC1*, an allene oxide cyclase implicated in the α-linolenic acid metabolism pathway (Zhao et al. 2014). Indeed, genetically engineered wheat genotypes with higher *TaCHP* (a zinc finger protein) expression levels are more salt tolerant than the salinity-sensitive cultivar Jinan 177 (Li et al. 2010a, b). In genetically engineered wheat varieties, with higher expression level of *TaOPR1*, a 12-oxo-phytodienoic acid reductase, improves salinity stress tolerance (Dong et al. 2013).

9.3.2.3 Barley (*H. vulgare*)

Barley is an excellent model crop plant for understanding the physiological and molecular mechanisms underpinning salt stress tolerance in crop plants, because it is one of the most resistant crops when it comes to salt stress. Remarkably, there are few instances of transgenes being incorporated into barley to strengthen salt stress endurance. Several barley-derived genes, on the other hand, have been introduced into a variety of transgenic crops with the purpose of investigating salt stress resistance. Environmental stress tolerance in barley has stirred up interest in uncovering stress-responsive genes, which might be achieved using a number of omics methods, such as comparative genomics and genetic transformation to express stress-responsive genes (Gürel et al. 2016). Numerous barley transcription factors, including *HvWRKY38* (Xiong et al. 2010), *HvCBF4* (Oh et al. 2007), and *HvDREB1* (Xu et al. 2009), are being constitutively overexpressed in transgenic plants, resulting in a significant increase in drought and salt stress tolerance, perhaps by modulating the transcription levels of stress tolerance genes, which have a greater affinity for DNA (Gürel et al. 2016). In addition, it has been shown that introducing the *Arabidopsis* vacuolar H^+-pyrophosphatase gene (*AVP1*) into genetically engineered barley enhances shoot biomass and grain production under salinity stress conditions (Schilling et al. 2014). *AVP1* gene is primarily engrossed in the production of compatible solutes (like glycine betaine and proline) and also enzymes implicated in the elimination of ROS, all of which contribute to improved crop salt stress tolerance (Roy et al. 2014). Similarly, it has been demonstrated that calcineurin-B-like interacting protein kinases (*CIPK*) are crucial in the modulation of Na^+ buildup in *Arabidopsis* shoots. The *A. thaliana CIPK* gene has been overexpressed in barley, which resulted in decreased accumulation of Na^+ ion in shoot and increased biomass yield under salt stress (Roy et al. 2013).

9.3.2.4 Maize (*Z. mays*)

Maize is grown under a variety of environmental situations. Genetic engineering has been used effectively in maize to incorporate salt stress-tolerant/stress-resistant genes from multiple sources. For instance, Yin et al. (2004) incorporated the vacuolar Na^+/H^+ antiporter *AtNHX1* gene from *A. thaliana* into *Z. mays*, and the putative transgenic seedlings demonstrated significantly increased germination efficiency (80%) under salinity conditions (0.5% NaCl) than parental wild-type plants (13–57%). Li et al. (2010a, b) found that abolishing the *als* genes in *AtNHX1* transgenic maize (marker-free transgenic maize) resulted in surprisingly high salinity tolerance, as evidenced by increased grain production, and boosted many important physiological features when compared to their respective control parental plants. Chen et al. (2007) had previously succeeded in developing salt-resilient maize by transferring the rice Na^+/H^+ antiporter gene (*OsNHX1*) into maize. When compared to non-engineered control plants, the GM line produced more biomass at a salt concentration of 200 mM. *BADH* gene originating from *Suaedaliaotungensis* Kitag and *Atriplex micrantha* has been incorporated into the maize genome through the pollen tube pathway and under the control of the maize ubiquitin promoter, separately, and conferred salinity stress tolerance (Wu et al. 2008; Di et al. 2015). Beltagi (2008) reported that GM maize containing a gene from *Bacillus thuringiensis* (Bt Corn) exhibited improved salt stress tolerance when subjected to varying concentrations of NaCl (50 mM, 100 mM, and 150 mM). The chlorophyll a concentration and chlorophyll stability index (CSI) of Bt transgenic maize were shown to be unaltered when compared to non-transgenic control plants.

9.3.2.5 Sorghum (*S. bicolor* L.)

Owing to the slow pace of advancement in developing transformation methodologies, the literature on genetically engineered sorghum to strengthen saline stress tolerance is limited (Shahbaz and Ashraf 2013). Primarily due to tissue culture constraints, a dearth of reference genomes, poor regeneration efficiency, and the difficulty to maintain regenerated plantlets via sub-cultures, progress in developing transgenic sorghum has lagged behind that of other cereals (Madhusudhana et al. 2015). Nevertheless, genetic modification has presented modest achievement in generating salt-tolerant sorghum. The *mtlD* gene, which encodes mannitol-1-phosphate dehydrogenase from *E. coli*, was introduced into the sorghum cv. SPV462 with the objectives of increasing tolerance to salt stress conditions (Maheswari et al. 2010). When exposed to NaCl stress (200 mmol L^{-1}), the *mtlD* gene harbouring transgenic plants showed a 1.7–2.8-fold increase in root and shoot growth, respectively, compared to non-transgenic controls, exemplifying that integrating the mannitol biosynthetic process into sorghum can bestow elevated salt stress tolerance. *SbDREB2*, a dehydration response element-binding (*DREB*) transcription factor gene from sorghum, was identified by Bihani et al. (2011) and was found to be activated particularly during drought/salt stress.

9.3.2.6 Pearl Millet (*P. glaucum*)

Pearl millet (*P. glaucum*) is a staple grain and the world's sixth most commonly cultivated cereal grain. It is acclaimed for its remarkable abiotic stress resistance and nutritional composition. Shinde and colleagues identified *PgNAC21* TF as a potential salt stress-responsive candidate gene in *P. glaucum*. Plants with enhanced levels of expression of the PgNAC21 TF exhibited a greater germination rate, fresh weight, and root length under salt stress than their non-transgenic counterparts (Shinde et al. 2019). Higher levels of *PgNAC21* TF expression in *Arabidopsis* plants enhanced transcription of stress-responsive genes like *GSTF6* (GLUTATHIONE-S-TRANSFERASE 6), *COR47* (COLD-REGULATED 47), and *RD20* (RESPONSIVE TO DEHYDRATION 20). *PgNAC21* functions as a stress-responsive NAC TF, according to Shinde et al. (2019), and may be utilized in GM techniques to improve salinity stress tolerance in agricultural plants.

9.3.3 Heat Stress

While traditional plant breeding methods and popular molecular biological strategies, such as molecular markers and genetic modification, have helped to functionally validate and/or revitalize plants with improved heat stress tolerance, documentation on the genetic core principle of heat stress tolerance is largely lacking. Transcriptional activation of specific proteins has been shown to enhance high temperature tolerance. Several GM plants with different degrees of heat stress tolerance are being developed, alongside scientific knowledge of the expression of heat shock proteins (HSPs)/chaperones and the manipulation of HSF expression patterns. Nonetheless, in comparison to experiments aimed at reconfiguring drought, salt, or cold stress tolerance, such investigations have been limited (Hemantaranjan et al. 2018). Table 9.1 shows a list of transgenes introduced into various cereal crops for heat stress tolerance.

9.3.3.1 Rice (*O. sativa*)

A substantial number of genes are involved in the synthesis of heat shock proteins (HSPs), which are activated by high temperature and play an essential role in heat stress recovery (Liu et al. 2007). Engineering of HSPs in GM plants has the ability to enhance temperature stress resilience and has a major effect on rice's intrinsic genetic potential (Zou et al. 2011). Rice varietal reinforcement for thermotolerance via genetic engineering is a prospective methodology for rice production under undulating environmental circumstances (Zou et al. 2011). Nevertheless, high temperature tolerance in transgenic rice has been reported in just a few instances. Heightened thermotolerance has indeed been witnessed in engineered plants constitutively expressing HSPs. The significance of HSPs in conferring heat stress tolerance was substantiated by empirical observations from mutants and genetically engineered species (Queitsch et al. 2000). The cDNA of *A. thaliana* HSP101 (*AtHSP101*) was adeptly integrated into the *indica* rice variety Pusa basmati-1 (Katiyar-Agarwal et al. 2003). In terms of sustenance and development at high temperatures, T2 generation GM lines outperformed non-transformed plants. Analogously, the GM rice variety 'Hoshinoyume' that overexpresses HSP (*sHSP17.7*) exhibited enhanced thermal stress tolerance (Murakami et al. 2004). High temperature resilience has been seen in transgenic rice harbouring the *FAD7* (dienoic fatty acid) gene, which enhanced growth rate and chlorophyll pigment concentration (Sohn and Back 2007). Thermal resistance was bolstered in GM rice plants with higher levels of expression of the *mtHsp70* (mitochondrial gene), as evidenced by diminished programmed cell death and significantly lower ROS generation (Qi et al. 2011).

9.3.3.2 Wheat (*T. aestivum*)

Elevated temperature tolerance may be improved through genetic engineering, which can aid in mitigating the negative consequences of thermal stress (Chapman et al. 2012). It involves introducing beneficial traits into target plants in order to strengthen the plants' adaptability to heat stress (Zheng et al. 2012). However, wheat genetic engineering efforts are difficult due to the complexity of the genomic architecture. Persistent heat stress increases elongation factor (EF-Tu) protein synthesis in chloroplasts, which is related to wheat's resistance to severe temperatures. Overexpression of EF-Tu in GM wheat reduced leaf proteins from thermal agglomeration, reduced thylakoid membrane disintegration, enhanced photosynthetic efficiency, and safeguarded the plant from devastating microbial invasion (Fu et al. 2012). Wheat cultivars having higher EF-Tu levels tolerated temperature distress better than those with lower EF-Tu levels (Ristic et al. 2008). Overexpression of the maize phosphoenolpyruvate carboxylase gene (*ZmPEPC*) in wheat increased photocatalytic and antioxidant enzymatic activities, induced photosynthesis-related gene expression, slowed chlorophyll degeneration, altered proline and other metabolite concentrations, and improved heat tolerance (Qi et al. 2017). Plants that synthesize more glycine betaine after being transformed with the BADH gene have been suggested as a potential method for improving plant heat tolerance (Yang et al. 2005). Better osmotic adjustments and an improved antioxidative defence response have been shown to augment heat tolerance in GM wheat line T6 due to glycine-betaine overproduction (Wang et al. 2010).

9.3.3.3 Barley (*H. vulgare*)

One of the most significant abiotic stress factors influencing the production capacity of temperate cereal crops like barley is temperature. Certain heat shock factors (*HSFs*) and heat shock response regulators (HSRs) stimulate heat-responsive gene transcription in plants, enabling them to tolerate thermal stress. A recent study has shown that overexpression of a wheat heat shock factor (*TaHSFA6b*) in barley is linked with heat stress resilience (Poonia et al. 2020). Aside from incorporating genes from other origins into barley to strengthen heat stress tolerance through transgenic approach, a handful of *H. vulgare* genes have indeed been studied in various model plants, including tobacco. Higher levels of *HvSHN1* [*APETAL2/Ethylene Responsive Factor (AP2/ERF) family*] expression in GM tobacco plants under the regulation of the 35S promoter bolstered resilience to salt, water, and heat stress (Djemal and Khoudi 2021). The resulting transgenic lines exhibited altered cuticle permeability and reduced stomatal density. *HvSHN1* transgenic lines had enhanced catalase (CAT) and superoxide dismutase (SOD) activity and decreased MDA and H_2O_2 levels when challenged to thermal stress compared to non-transformed control plants (Djemal and Khoudi 2021). Increased expression levels of the *HvAPX1* (ascorbate peroxidase 1) gene from barley elevated high temperature tolerance in *A. thaliana* (Shi et al. 2001).

9.3.3.4 Maize (*Z. mays*)

Numerous strategies have been undertaken to generate heat-tolerant transgenic maize by modulating (upregulation/downregulation) known pertinent genes obtained from various crop species, including maize. For instance, the overexpression of *OsMYB55* in GM maize resulted in the upregulation of stress-responsive genes and improves heat and drought tolerance (Casaretto et al. 2016). Furthermore, a variety of maize-derived genes were tested using a transgenic approach by expressing them in various model plants to verify their potential role in heat stress endurance. When subjected to drought stress conditions, increased levels of *ZmWRKY106* expression drastically enhanced drought and heat resilience in GM *A. thaliana* by inducing stress-related genes via the ABA signalling cascade and curbing ROS generation, which was accompanied by increases in SOD, peroxidase (POD), and CAT activities (Wang et al. 2018). Analogously, compared to untransformed plants, overexpression of *ZmHSP16.9* in transgenic tobacco imparted heat and oxidative stress tolerance, as shown by higher seed germination rate, root length, and antioxidant enzyme activities (Sun et al. 2012).

9.3.3.5 Sorghum (*S. bicolor* L.)

Grain sorghum tolerates not only droughts but also severe temperatures better than other crops. Temperature stress has been shown to have a negative impact on sorghum reproductive development. However, at the time of drafting this book chapter, we have been unable to pinpoint any published literatures describing the use of genetic engineering techniques for the introgression of genes from other species into grain sorghum in order to enhance thermotolerance.

9.3.3.6 Finger Millet (*Eleusine coracana* L.)

Agricultural production is diminished as a consequence of the occurrence of numerous unfavourable circumstances as a result of global climate change. The identification and eventual transfer of genes from stress-tolerant plant species to cultivated cultivars may aid in agricultural production stabilization and improvement. Finger millet (*E. coracana* L.), a climate-resilient crop, could be a potential source of novel stress-tolerant cereal genes. Ramakrishna et al. (2018) reported *EcbZIP17*, a novel endoplasmic reticulum (ER) membrane-anchored bZIP transcription factor from finger millet. Transgenic tobacco plants overexpressing this gene exhibited greater vegetative growth and seed production than wild-type (WT) plants under optimal growth conditions, indicating activation of brassinosteroid signalling genes. The engineered plants outperformed WT plants in terms of germination rate, biomass production, primary and secondary root growth, and recovery rate when subjected to abiotic stressors such as water deprivation, heat stress, 400 mM mannitol, 250 mM NaCl, and 10% PEG6000 (Ramakrishna et al. 2018). In a similar line, *EcDREB2A* gene expression in transgenic tobacco increased resilience to heat stress 42 °C lasting up to 7 days by altering physiology and biochemical processes (Singh et al. 2021). *EcDREB2A* transgenics exposed to heat stress had higher stomatal conductance, chlorophyll and carotenoids levels, and other photosynthetic indices than WT plants (Singh et al. 2021).

9.3.4 Cold Stress

Plants' gene expression patterns and protein product levels change significantly when they are exposed to cold temperatures. Non-freezing cold damages or kills a variety of tropical and subtropical plant species, including cereals, with symptoms such as discolouration, necrosis, and reduced development. Plants that are cold tolerant, on the other hand, may flourish in such conditions. Traditional breeding methods, such as inter-specific or inter-generic hybridization, have made little headway in improving the cold tolerance of important agricultural crops. Researchers have gained a better grasp of the complex mechanism that occurs under cold stress, thanks to recent study that combines whole genome profiling/ sequencing, mutagenesis, and analyses of GM plants. When challenged to freezing conditions, changes in gene expression are accompanied by increases in the synthesis of hundreds of compounds (metabolites), the most of which have been shown to defend against the negative effects of cold stress. A number of low temperature inducible genes have been uncovered in plants. The bulk of the genes involved in cold stress endurance have been revealed, and many of them are controlled by transcription factors (TFs) called C-repeat binding factor/dehydration-responsive element-binding (*CBF/DREB1*). Significant modifications occur at the physiological and molecular levels during cold adaptation, implying that cold tolerance is a more complicated process entailing more than one mechanism. A list of transgenes incorporated into different cereal crops for cold stress tolerance is shown in Table 9.1.

9.3.4.1 Rice (*O. sativa*)

Genetic engineering, which involves the incorporation or disruption of certain gene sequences in rice plants, is a potential strategy to enhance cold stress resilience. The application of effective gene transfer methods and breakthroughs in recombinant DNA technology have culminated in speedy transformation and development of transgenic plants (Sanghera et al. 2011). The overexpression of a number of low-temperature stress-inducible genes resulted in GM rice plants with stress-tolerant characteristics. CBF/DREB1 TFs are among the most significant findings in the area of low-temperature acclimatization and signalling pathways (Sanghera et al. 2011). Overexpression of *OsDREB1* or *AtDREB1* in GM rice plants enhanced resilience to cold temperature, dehydration, and salt stress, as well as the accumulation of osmoprotectors like free proline and other soluble sugars (Ito et al. 2006). The rice DREB1 gene (*OsDREB1D*) was overexpressed in *A. thaliana* plants, producing GM plants with a link between cold stress and *OsDREB1D* transcription (Zhang et al. 2009). Increased levels of expression of a maize CBF gene (*ZmCBF3*) in rice plants resulted in GM plants that exhibited growth deceleration only at the nursery stage and had no production loss in outdoor environments. GM plants, as envisaged, were indeed cold stress resilient (Xu et al. 2011). TFs from a variety of families have indeed been introduced into rice plants in an effort to create transgenics that are more resistant to cold stress. In GM rice plants, expression of *OsCOIN* (*O. sativa* cold inducible), a TF from the bZIP zinc finger protein family, is manifested in improved tolerance to cold, salt, and drought stress treatment, as well as increased proline levels following cold treatment, than WT plants (Liu et al. 2007). Table 9.1 shows a list of genes that have been incorporated into rice through genetic engineering to improve cold stress resilience.

9.3.4.2 Wheat (*T. aestivum*)

Unexpected temperature fluctuation is linked to a high probability of devastating low-temperature episodes. Wheat is an important crop for addressing world food demands. Extreme temperatures caused by climate change significantly impact wheat's vegetative and reproductive development, resulting in a reduction in productivity. Cold temperatures cause a variety of changes in the morphophysiological, biochemical, and molecular composition of wheat (Hassan et al. 2021). Crop plants power up their cold tolerance regimes in response to these changes which include the accumulation of soluble sugars, signalling pathways, and transcription of cold tolerance genes (Hassan et al. 2021). Among the various methods used for development of cold/freezing stress-tolerant wheat, transgenic approach is the most widely used. The transgenic strategy is the most routinely adopted of the many approaches used to produce cold/freezing stress-resilient wheat. Not only have genes from other sources been used to develop GM wheat, but a variety of wheat-derived genes have also been transferred to other crops. For instance, drought, high salt, and cold stress tolerance were substantially enhanced in GM wheat harbouring the cotton DRE-binding TF (*GhDREB*) maintained under the regulation of the ubi1 or rd29A promoters (Gao et al. 2009). Analogously, cold stress endurance was improved in GM wheat plants containing the barley lipid transfer protein, BLT101 (Choi and Hwang 2015). Huang et al. (2014) documented that the wheat aquaporin gene *TaAQP7* imparts cold stress resilience in transgenic tobacco. Under cold exposure, *TaAQP7* overexpression in tobacco plants culminated in enhanced root extension and growth in comparison to the wild-type (WT) plants. In GM strawberry leaves, overexpression of the wheat *WCOR410*, an acidic dehydrin gene, increases freezing stress resilience. In GM *A. thaliana*, overexpression of the wheat MYB TF gene *TaMYB56-B* improves resilience to chilling and salinity stress conditions (Zhang et al. 2012).

9.3.4.3 Barley (*H. vulgare*)

Strengthening the cold hardiness of winter grains is one of the most important challenges confronting plant breeders today. Therefore, analysing the genes that regulate cold acclimatization processes is critical. In a few instances, transgenic methodologies have been seamlessly used for functional studies of different cold stress-responsive regulatory genes. Many studies in a number of plant species have shown the involvement of CBF genes in cold adaptation. For example, GM barley with enhanced level of expression of *TaCBF14*, *TaCBF15*, and *OsMYB4* boosts frost resilience and germination efficiency in cold environments (Soltész et al. 2012, 2013). In a related research, the cold-inducible expression of wheat *TaDREB3* in barley and rice was optimized using two stress-inducible gene promoters, *OsWRKY71* and *TdCor39*, with the intent of enhancing cold stress tolerance in barley. The promoters exhibited varying characteristics, such as activation period, strength, and ABA sensitivity (Kovalchuk et al. 2013). The stress-inducible promoters HDZI-3 and HDZI-4 were used to drive the transcription of two DREB/CBF genes, *TaDREB3* and *TaCBF5L*, in GM wheat and barley. The promoters were obtained from durum wheat genes encoding HD-Zip class I subfamily c-clade TFs.

Dehydration and cold resilience of GM barley seedlings increased with expression of the *DREB*/*CBF* genes under both promoters, as did frost endurance of GM wheat seedlings (Yang et al. 2019).

9.3.4.4 Maize (*Z. mays*)

Based on the existing literature, it can be inferred that relatively few researches have been conducted to date utilizing transgenic technology to improve cold stress tolerance in maize. According to oxidative stress and growth rate evaluations, evidence suggests that introgression and overproduction of *A. thaliana* iron superoxide dismutase (FeSOD) may result in higher methyl viologen concentrations as well as enhanced freezing tolerance in maize (Van Breusegem et al. 1999). When the *E. coli*-derived *beta* gene was introduced into maize via *Agrobacterium*-mediated plant transformation, the majority (four out of five) of the maize lines tested showed decreased cell membrane damage; increased photosynthetic activity; increased levels of amino acids such as alanine, glutamic acid, glycine, and serine; and improved survival rates under freezing stress as compared to their untransformed counterparts (Quan et al. 2004). In GM maize, low-level yet constitutive heterologous expression of a tobacco active mitogen-activated protein kinase (NPK1) gene is manifested in heightened freezing stress tolerance (Shou et al. 2004a, b). Drought and cold stress tolerance in *A. thaliana* transgenic plants were improved by enhanced expression of the maize *ZmDBP3* gene, a candidate of the A-1 subgroup of the CBF/DREB subfamily (Wang and Dong 2009).

9.3.4.5 Sorghum (*S. bicolor* L.)

In temperate zones, soil temperatures <15 °C impede germination and establishment of sorghum seedlings during early-season planting. Producing short-duration sorghum cultivars is an essential breeding objective for temperate regions since low spring temperatures result in a protracted juvenile development (Anami et al. 2015). The dearth of relevant literature indicated that just a few efforts had been undertaken, with limited success in improving cold/freezing stress tolerance in grain sorghum crops thus far.

9.3.4.6 Pearl Millet (*P. glaucum*) and Finger Millet (*E. coracana* L.)

We did not find any noteworthy publications on the use of genetic engineering technology for transferring cold stress-responsive genes from other sources into pearl millet and finger millet at the time of preparing this book chapter.

9.3.5 Heavy Metal Tolerance

Among the various abiotic stresses to plants, heavy metal contamination is a serious environmental problem which affects the growth, productivity, and genome stability (Saini and Westgate 1999). The major cause of heavy metal contamination is the use of urban waste for crop production. In Asia, the urbanized or nearby urban areas are under major concern of increasing heavy metal toxicity because of soil and water contamination (Simmons et al. 2010). Exposure to high level of heavy metals results in the overproduction of ROS and ultimately leads to oxidative stress in crops.

Hyperaccumulation of heavy metal inside the plants and hypertolerance to it are the most practical means of combating heavy metal toxicity without affecting their viability (DalCroso et al. 2013). Though the research findings of heavy metal stress tolerance are majorly limited to the model plants such as tobacco, *Arabidopsis*, etc., the major outcomes of transgenic development in the case of cereals are limited in number. The transgenes or transcription factors used in these research findings are of great importance to understand the heavy metal tolerance and their downstream pathways. The major breakthrough achieved using transgenic approaches to develop heavy metal tolerance is summarized in Table 9.2.

9.3.6 Anoxia Stress/Oxygen Deficiency Stress Tolerance

Since plants are obligate aerobes, the deficiency of oxygen (hypoxia) or its absence (anoxia) causes various ecological stresses. The reason of stress condition may be due oxygen deprivation on hydromorphic and flooded soils leading to the poor solubility of oxygen and low diffusion rate in the water (Jackson et al. 2009; Vissere et al. 2003). Anoxia stress may cause the wilting and even can lead to the total damage of the crop. It appears that increased flooding tolerance is likely to result from ethanolic fermentation pathway upregulation in rice which is evident from several studies. The use of transgenic approach to oxygen deficiency has been employed in various cereals as well as in model plants. Important transgenic cereals targeting the ethanolic fermentation pathways and several other genes/TFs with improved hypoxia tolerance/resistance have been summarized in Table 9.3.

9.3.7 Ultraviolet-B (UV-B) Stress Tolerance

Plants respond to ultraviolet-B light (UV-B), which is one of the significant abiotic stresses and triggers their genetic programme and decrease in biomass production (Ulm and Jenkins 2015; Yin and Ulm 2017). The UV-B exposure induces an oxidative burst produced by higher level of ROS (Brosché and Strid 2003; Frohnmeyer and Staiger 2003). In the UV-B stress conditions plants accumulate ROS in the apoplast which is triggered by NADPH oxidase *AtRBOHD* and *AtRBOHF* subunit activation. The accumulation happens in the chloroplast as well because of the effect of UV-B on photosystem (PS) II function (Kulandaivelu and Noorudeen 1983; Larkum et al. 2001). The UV-B stress in crops has been mostly studied in combination with other abiotic stresses, and there is very limited information available on the development of transgenic cereals targeting UV-B stress tolerance. In some model plants and other plant species, transgene expression and their impact on the ROS and other oxidative accumulation have been elucidated. A few examples of transgenic approach used for UV-B stress tolerance development have been comprised in this section. In the report of Nagy et al. (2016), the

Table 9.2 Examples of transgenic cereals for heavy metal tolerance and accumulation

Transgenic cereals	Gene/TF and its source	Response of target transgenic cereal/improved trait	References
Rice (*Oryza sativa*)	*MTH1745* from *Methanothermobacter thermoautotrophicum*, protein disulphide isomerase-like protein (PDIL)	Increase in Hg tolerance, photosynthesis rate, activity of SOD and POD, and decrease in superoxide radicals, H_2O_2, and lipid peroxidation compared to the wild type	Chen et al. (2012)
	TaCNR2 (cell number regulator 2 from common wheat (*Triticum aestivum*))	Enhanced stress tolerance to Cd, Zn, and Mn and increased translocation from roots to shoot due to overexpression in *Arabidopsis* and rice	Qiao et al. (2019a, b)
	TuCNR10 from diploid wheat, *Triticum urartu*	Overexpression in *Arabidopsis* and rice leads to enhanced tolerance and translocation from root to shoot of Cd, Zn, and Mn	Qiao et al. (2019b)
	OsLEA4 late embryogenesis abundant (LEA) proteins	Suppress the oxidative stress associated with drought, salt, and heavy metal stresses	Hu et al. (2016)
	TaPCS1	Cd hypersensitivity	Wang et al. (2012)
	OsMT1	Zn accumulation	Yang et al. (2009)
Oryza sativa L. cv. Zhonghua No.11	*GST* (glutathione-S-transferase) and CAT1 (catalase1) transgene	In particular, co-expression of GST and CAT1 had a significant effect on the whole glutathione-ascorbate cycle. The GST and CAT1 transgenes, as well as the coordination of the whole glutathione-ascorbate cycle, contributed to low oxidative damage in the transgenics induced by Cd and the combined stress	Zhao et al. (2009)
	HsCIPK from Tibetan Plateau annual wild barley (*Hordeum spontaneum C. Koch*)	In rice (*Oryza sativa* L. cv Nipponbare), ectopic expression of multiple *HsCIPKS* led to enhanced root tolerance to salt, heavy metal (Hg, Cd, Cr, and Cu), and drought stress in transgenic plants	Pan et al. (2018)

(continued)

Table 9.2 (continued)

Transgenic cereals	Gene/TF and its source	Response of target transgenic cereal/improved trait	References
Wheat (*Triticum aestivum*)	NAC Tfs from *Ae. markgrafii*	Overexpression of AemNAC2 in the wheat cultivar 'Bobwhite' led to reduce Cd concentration in the root, shoot, and grains	Du et al. (2020)
	Isopentenyltransferase *(ipt)* gene	Under Cd stress, transgenic wheat isoline shows less seminal root growth impairment and a different metabolite profile	Gomez Mansur et al. (2021)
	MATE gene named *HvAACT1* (codes for a membrane-bound citrate transporter)	Wheat, barley, and rice displayed increased HvAACT1 expression which leads to a faster citrate efflux from roots and increased Al (3+) tolerance	Zhou et al. (2013)
Maize (*Zea mays*)	*ZmAT6* (a novel protein)	Transgenic plants show increased root growth and reduced Al accumulation, lower levels of malondialdehyde, and reactive oxygen species (ROS) in the roots, but higher levels of proline. Compared with wild-type plants, they have a reduced absorption of Evans blue in their roots	Du et al. (2020)
Barley (*Hordeum vulgare* L.)	AKR enzymes derived from either thale cress (*A. thaliana*) (AKR4C9) or alfalfa (*Medicago sativa*) (MsALR) aldo-keto reductase (AKR4C9) from *A. thaliana*	Improved heavy metal (Cd) and salt tolerance compared to WT, which was considered to be an effect of the reduction of reactive aldehyde molecules. Enhances tolerance of barley to oxidative stress and cadmium stress by detoxifying reactive aldehydes in vivo	Éva et al. (2016)
	ALMT1 gene of wheat (*Triticum aestivum*) encodes for malate transporter	An Al-activated efflux of malate was found with similar properties to the Al-tolerant wheat. Transgenics showed high Al tolerance in hydroponic as well as acid soils	Delhaize et al. (2004)
	Two *MATE* genes (which encode for citrate transporters: SbMATE (major Al(3+) tolerance gene from sorghum and FRD3 (Fe nutrition in *Arabidopsis*))	In hydroponic and short-term soil trials, lines expressing SbMATE exhibited Al(3+)-activated citrate efflux from root apices as well as greater tolerance. FRD3 transgenic lines revealed phenotypic similarities except that citrate release from roots was constitutive	Zhou et al. (2014)

Table 9.3 Examples of transgenic cereals for improved hypoxia/oxygen stress tolerance

Transgenic cereal	Gene/TF and its source	Response of target transgenic cereal/improved trait	References
Rice (*Oryza sativa*)	SmAPX ascorbate peroxidase (APX) (*Solanum melongena*, SmcDNA (eggplant)	Transgenic rice seedlings exhibited higher flood tolerance and less oxidative injury and grew faster than non-transgenic plants upon exposure to flood treatment	Chiang et al. (2015)
	Pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH)	PDC1 polypeptide level increased in shoots but not in roots and shoots of transgenic lines expressing the rice Pdc1 CDNA derived from an anaerobic promoter (6XARE). An increase in PDC activity and ethanol production was observed in shoots of these lines under anoxic conditions, as well as decreased growth when submerged in water	Rahman et al. (2001)
		Transformants with reduced *adh1* showed reduced ethanol production and coleoptile growth under hypoxia	
		A transgenic line expressing cotton *adh2* cDNA showed three to four-fold more ADH activity	
Barley (*Hordeum vulgare* L.)	Rice *Osmyb4* gene	Germination under hypoxia conditions is improved and tolerance to frost is enhanced	Soltész et al. (2012)
Wheat (*Triticum aestivum*)	Isopentenyltransferase *(ipt)* gene from *Agrobacterium tumefaciens*	Ipt-transgenic wheat plants (*Triticum aestivum* L.) were more tolerant to flooding than wild-type plants	Tereshonok et al. (2011)

transgenic barley lines expressing *Medicago sativa* aldose reductase (*MsALR*) gene showed accumulation of MsALR in the cytosol as well as chloroplast, which can increase the abiotic stress tolerance caused by ROS in the plants. The report of Wang et al. (2006) elucidates the enhanced tolerance to UV-B and heat stress overexpressing the cytosolic ascorbate peroxidase (cAPX) in transgenic tomato. While exposing the plants to UV-B, heat, and drought stresses, a several-fold higher level of APX activity was observed in the leaves of cAPX transgenic plants. The presence of higher APX activity in plants suggests the enhanced tolerance to the ROS as plant develops antioxidant enzymes such as SOD and APX to detoxify the ROS. Another interesting study on carrot by Jayaraj and Punja (2008) has been demonstrated to understand the expression of an algal β-carotene ketolase gene (*bkt*). The constitutive expression of pigments like astaxanthin, adonirubin, canthaxanthin, echinenone, adonixanthin, and β-cryptoxanthin in transgenic plants resulted in significant better growth than the wild type while exposed to the UV-B radiation. The transgenic tissue also accumulated lower amount of H_2O_2 following exposure to oxidative stresses, which suggests the quenching of ROS by the ketocarotenoids. Thus, it is well understood that the UV-B stress and oxidative stress caused by it have been attempted using transgenic plants, though it has not been studied in cereals.

9.4 Major Challenges of Transgenic Approach

The major challenge in the development of abiotic stress-tolerant transgenic cereal crops is the changing environmental condition. A particular genotype compatible in one particular environment may not be compatible in another. Moreover, all abiotic stresses can affect a plant depending upon its genetic makeup and adaptive response. The specific genotype × environment interaction leads to varied effects in response to different environments. Molecular genetic analysis of specific genes conferring tolerance against different abiotic stresses has produced many successful transgenic cereals. The challenge ahead lies in the identification of the natural variation in wild-type populations and using them to develop promising transgenic climate-resilient cereals. Another bottleneck in the development of future-ready abiotic stress-tolerant transgenic cereals lies in the crosstalk between many of the abiotic stresses. Combinations of these stresses like heat and drought can occur in the field causing unique effects that cannot be predicted individually (Suzuki et al. 2014). This may lead to varied physiological interactions which need novel interventions. Transgenic pyramiding can be a viable approach to solve this problem. For example, stacking of candidate genes for drought as well as salinity has been successfully done in rice (Gupta et al. 2018; James et al. 2018) and maize (Nguyen et al. 2013). Another challenge in the development of abiotic stress tolerant transgenic cereals is to understand the stress-responsive signalling pathways and the genes involved (Yoshida et al. 2014). Overexpression of transcription factors and other regulatory genes has been successfully used to develop transgenic cereals with improved stress tolerance and productivity (Mickelbart et al. 2015). Nevertheless, overexpression analysis might not fully represent the natural function of genes in the plant which may pose another difficulty.

9.5 Conclusion

Abiotic stresses will continue to be a challenge in agriculture. In the present scenario, with competing uses of land and the ever-increasing world population, the challenge is to produce the maximum in a limited area with depleting natural resources, confronted with climate change adversely affecting crop productivity. The transgenic approach has been successfully employed to engineer climate-resilient cereals. There are many instances where genetic engineering has come handy in producing environmentally stable crops that can yield more. Recent techniques like RNAi and

CRISPR are now being used to develop future-ready cereals that can remain stable under changing climatic conditions. The challenge before us in crop improvement is to integrate information on abiotic stress response pathways, identify and fish out the different candidate genes, and use these to engineer environmentally stable cereals that can yield more under climatic constraints, to feed the growing population.

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Genetically Engineered Cereals Tolerant to Abiotic Stress

10

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Abstract

Plants unlike animals do not have the privilege to escape the external changes arising from biotic and abiotic factors. In most cases, these stresses can intervene in the growth, reproduction, and productivity of crop plants. Abiotic stresses such as temperature, drought, salinity, and heavy metal exposure can compromise the growth and development of cereal plants. These factors have catastrophic effects on the agriculture and socioeconomic condition of a region. Although evolutionary timelines have shown several crops to have developed mechanisms to adjust with the severity of these ecological barriers, there are lots of cereals in existence which are susceptible to abiotic stresses. These cereals (maize, rice and wheat, etc.) lack the ability to withstand such unfavorable conditions and are jeopardizing the economical pathways of world's communities. In recent decades, scientists are trying to understand the responses of plants to external stresses and molecular mechanisms underlying those responses. A number of model plants have been used for the investigation of genomics, proteomics, and metabolomics leading to the idea of development of cereals with abiotic stress tolerance. An interdisciplinary study consisting of molecular techniques, biotechnological tools, and recent scientific advancements has made it possible to identify and exploit the set of genes responsible for stress tolerance in cereal crops. Genes for osmoprotectants, detoxifying proteins, molecular chaperons (heat shock proteins), antioxidant compounds, and regulatory proteins

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_10

(transcription factors) have been isolated and exploited for their tolerance-related effects in cash crops. Transgenic plants carrying genes against stress have been developed using a broader range of approaches such as CRISPR-CAS 9 technology, alternative splicing, seed priming, and molecular priming. This chapter will discuss the recent strategies involving transgenic technology in inducing abiotic stress tolerance in cereals.

Keywords

Transgenics · Abiotic stress · Cereals · Quantitative trait loci · Late embryogenesis abundant proteins · Heat shock proteins · Transporters · Transcription factors

10.1 Introduction

Increasing the growth and production of a particular crop in an area where it is threatened by abiotic stresses is an art of labor for all plant researchers. The inconsistency in the duration and intensity of the stress indicate the absence of exact way to apprehend the molecular and physiological understandings of growth and productivity. To make a clear perception out of the physiological responses of the stress and then transforming this effort towards molecular evaluation, it is imperative to point out the most critical aspects of growth development and production. Plants are inducible species; their molecular and developmental process is activated in response to external stimuli. Significant scientific studies have been invested on the influence of abiotic stresses on the initial growth and survival mechanism in the crop plants. However, a comparatively lesser attention has been given to understand the effects of abiotic stress on the reproductive life of crops, regardless of the significant impact on grains (Paul and Roychoudhury 2019). Abiotic stresses are the major constraints in cereal production and economic growth. In fact, a slight dose of abiotic stress can impose an irreversible change in cereal output, without interfering the vegetative cycle of the plant. A substantial amount of research has investigated how grain filling and size are influenced by post-anthesis stress, while fewer sources have focused on the pre-flowering effects of abiotic stress on grain count (Yang and Zhang 2006; Sinclair and Jamieson 2006). Interestingly, it is not grain size but grain number that primarily contributes to yield crises upon experiencing abiotic stress. The grain number is the most susceptible aspect of crop yield. The prominence of grain number over grain size has also been evident from history where studies have documented a successful increase in yield through breeding. In each case, it was grain number that could determine the abundance of yield and grain size did not matter much (Bingham 1966). The surge of global population accompanied by climatic deterioration has become a matter of serious concern for well-beings of plants. This has resulted in imminent threat for breeders to produce cereal crops which can yield maximum grain under appropriate cultivation system. In order to acquire this, cereal crops must cope with fluctuating environmental conditions (Powell et al. 2012). Creating cereal varieties with sublimed production rate that can keep up with extreme surviving regimes will need a thorough understanding of effects of environmental stresses on plant growth and plant response to these abiotic intruders.

Abiotic stresses including low temperature, high temperature, and water deficit influence specific phases of plant growth. In moderate climate regions, where the period of growth is comparatively shorter, or at higher altitudes, cold interferes with the initial seedling at the start of the period and reproductive phase during the end of the season. This is the subject of great concern for cereals like rice, which is widely grown in temperate regions with limited growing seasons. Upon exposure to extreme low temperatures (cold), rice receives indigent seedling initiation at season's beginning, leading to hampered heading and extensive grain deprivation at the season's climax when the temperature falls under a threshold, usually around 16 °C (Dolferus et al. 2013). However, certain cereals in temperate locations such as barley and wheat have developed tolerance system to escape the stressful effects of cold surrounding the reproductive stage of the crops. Such plants perceive changes in temperature and day extent via vernalization and photoperiodism, signaling the plant not to flower in extremely low temperature (Greenup et al. 2009; Distelfeld et al. 2009). Cold stress can be unforeseeable and prior flowering can cause a huge damage to crop yields. On the other hand, drought stress is more challenging for a plant to adapt to. Water-deficit crises can happen all the time in a plant lifetime, particularly in the hottest season when plants are prepping to flower. Drought stress is often partnered with heat stress and studies have documented that plants' tolerance to these stresses has co-evolved (Jagadish et al. 2011). In regions with temperate climate such as Australia, effective reproductive technology for grain production of wheat needs a pragmatic equilibrium between encouraging delayed flowering to evade frost injury and avoiding too-late flowering to fight heat and drought injury. Reproductive phase is, undoubtedly, the most critical factor of cereal life, and stress affecting the vegetative part of the plant growth can also contribute to crop productivity. Biomass productions are vulnerable to abiotic stress and indirectly affect the grain number. Numerous traits for enhanced vegetative-phase resistance of cereal have been studied. To exemplify, traits expressing response to drought include yield potential, harvest index (HI), water use efficiency (WUE), enhanced transpiration performance, and root penetration ability to approach water and nutrients in soil. Despite the fact that these traits are crucial for thriving reproductive comfort, they do not obliterate the actions of abiotic stresses. It is peremptory to mention that a mild hit of abiotic stress during reproductive cycle can have a calamitous and irreparable consequence on grain yield (Dolferus et al. 2013). The development of abiotic stress tolerance in cereal is the main agricultural issue in the past few decades and has caught the interests of agriculture scientists and economists now than ever before. With the advancement in molecular and biotechnological techniques, genetically modified crops have been developed with tolerance to both biotic and abiotic stresses along with qualitative enhancement of the crops. Herbicide-resistant, insecticide-resistant, pathogen-resistant, and nutritionally enhanced crops have been introduced with both biological and economic benefits. This chapter will explain transgenic strategies involved in the development of abiotic stress-tolerant cereal crops.

10.2 Importance of Grain Number in Cereals

Two important factors that are the determinants of grain yield are grain number and grain weight. Both these elements are susceptible to abiotic stresses and thus greatly interrupted when exposed to an external stressor. Grain number is an accepted standard for the estimation of wheat yield, and a drop in grain number is the primary indicator of compromised grain yield submitting to abiotic stresses. Grain number is a sophisticated genetic trait. Numerous agronomic factors are involved in grain number per square meter which include seed growing rate, methods of managing the cultivation, and number of tillers per square meter. Furthermore, the grain number is determined by pre-anthesis growth, while grain filling as a result of grain weight is controlled by post-anthesis conditions (González et al. 2003; Ji et al. 2010; Dolferus et al. 2013). There is a strong interconnection between grain number and weight; their survival depends on the availability of nitrogen and resource designation from vegetative parts. Another determinant for the maintenance of grain size under stress (drought), when photosynthesis halts, is the presence of water-soluble carbohydrates (Dolferus et al. 2013). As the grain number and grain weight are governed at various stages of reproductive cycle, they might be affected by different environmental conditions during development (Acreche and Slafer 2006).

The ecological and geographical factors that have impacts on grain number and size are photoperiodic changes, edaphically behaviors, rainfall, level of atmospheric carbon dioxide, heat, and elevation. These elements are indirectly involved with other factors that exhibit direct influence. For instance, resource limitations and an external stressor may conduct changes in leaf area, carpel weight, and quantity of fertile florets resulting in restraint size and weight of grains. Not only small cereal crops but plants which possess large seed that would normally resist abiotic stress conditions are reported to have loss in size of seeds (Leishman et al. 2000). Multiple physiology-related changes also influence grain size by affecting either rate or duration of grain filling process. These aspects include rate of photosynthetic process, transportation of reserve food, and cellular number and size in the forming grains (sink capacity) (Gupta et al. 2006).

10.3 Mapping Qualitative Trait Locus (QTL) for Abiotic Stress Tolerance in Cereals

Propagation for reproductive stage tolerance to abiotic stresses in cereals has not enjoyed good rating of success for numerous reasons. In undomesticated view, gain production is a strategy used by cereal crops to help them withstand the severity of environmental stresses. Habituated plants such as cereals have always been a subject of experimental adaptation programs to optimize the growth conditions which can contribute to high gain yield in cereals. However, using such breeding techniques might have affected the intrinsic property of the plants to survive nonoptimal conditions. Another issue with these programs is they pay less attention to abiotic stress management compared to prioritizing commercial gains such as qualitative and quantitative potential of grains. This mismanagement may have ended in the partial selection of beading lines that have zero tolerance in terms of abiotic stresses (Forster et al. 2000). There was no significant development in genetic maps until the arrival of DNA-based molecular markers. With the introduction of DNA markers, advancements were made in marker-assisted technologies producing large number of markers. The main characteristic of the genetic markers is that they can be tracked at every developmental stage of plant and are entirely free of being interrupted by environmental stresses. In order to locate a gene or QTL involved in abiotic stress tolerance (Roychoudhury and Chakraborty 2013), the first step is to construct a comprehensive genetic map with maximum genomic coverage. The persisting enhancement of DNA markers and spotting of markers lined with genes or QTLs responsible to phenotype of tolerance to abiotic stresses will support introgressive hybridization of tolerant genes into novel crop cultivars. QTL evaluation can be exploited to detect, situate, and investigate the phenotype of gene and interplay of a set of specialized genes. For analysis of QTL, two basic requirements are obligatory: a genetic marker map and information about phenotype imparting a particular trait of interest. Choosing breeds with enhanced tolerance to abiotic stresses is a challenging task in the field, where duration, intensity, and occurrence of the stress amidst reproductive cycle cannot be handled. The absence of effective screening mechanisms has impeded breeding headway in abiotic stress management of reproductive development. A struggle to select the most suitable breeding lines under sustained environmental conditions along with appropriate physiological and morphological perception of the impact on reproductive development indicates a crucial move towards introducing stress-tolerant cereals. Assessing cereals under controlled environmental conditions will offer phenotypic information that is authentic and reproducible for QTL evaluation (Dolferus et al. 2013). The use of QTLs mapping is one of the approaches to dissect the convoluted subject of abiotic stress tolerance (Vinocur and Altman 2005).

With advancement in molecular genetics applications, QTLs have been identified which can help the plant under abiotic stresses in either of the two ways: by increasing the yield of plant or upregulating the expression of genes that have the potential to resist abiotic stresses. Such genes can be exploited either as QTLs or as transgenic sequences (Cattivelli et al. 2008; Forster et al. 2004). In maize, restriction fragment length polymorphism (RFLP) is employed to map linkage between molecular markers and genes for qualitative traits. The genomic region that accounts for tolerance to water-deficit (drought) stress in maize was located on chromosomes 1, 3, 5, 6, and 8. In recent scientific approaches, 45 QTLs were identified for yield and yield-related compartments under stressful condition of drought (Agrama and Moussa 1996; Nikolić et al. 2013). The cultivation of crop plants can be accelerated through effective rooting system; profound rooting system will scarcely be affected by compromised water providence (Hamada et al. 2012; Spielmeyer et al. 2007). A genotype in rice, known as *Moroberekan*, provides the plant with drought managing strategy due to enormous rooting system. This system enables rice to develop penetrating roots deeper down the land layer to access water. The mapping of different QTLs has been uncovered so far; among them the most loci that have influenced the roots of rice (also common in wheat) are QRA.qgw-3D, QRA.qgw-2A, and QRA.qgw-5D for angles and qRN.qgw-1B for root number (Christopher et al. 2013). Moreover, the QTLs for length are QRl. ccsu2B, QRdw, ccsu-2A.2, and QRdw.ccsu-2A.1 for dry weight of roots and were localized to chromosomes 2a and 2b, respectively (Bharti et al. 2014).

Excess of salts in soil where plants have affinity to grow can extremely affect growth rate, metabolic activities, and productivity (Lutts et al. 1995). Salt-induced stress interrupts cereal germination, reduces viability in seedlings, destructs photosynthetic organelles, and ultimately declines grain yield (Saleem et al. 2020). The sodium ion elimination in wheat crop has been mapped on chromosome 2A (Lindsay et al. 2004). Two such sodium-resistant QTLs mapped in rye are OsHKT1;5 and TmHKT1;5-A and OsHKT1;5 and TmHKT1;5-A, a QTL present in wheat (James et al. 2006). *Trihelix* genes (transcription factor family) in wheat provide tolerance against abiotic conditions, cold and salinity stresses in particular (Xiao et al. 2019). Recent investigations have identified four QTLs associated with salt tolerance for STI (NL) on different chromosome regions: 3A, 4B, 5A, 5B, 6A, and 7A. In rice crop, the QTLs that carry salt resistance characters have been mapped and named as qNaSH8.1, whereas potassium and sodium cation levels are regulated by QTLs, qSKC9, and qSNC9 located on chromosome 9 (Pandit et al. 2010; Xu et al. 2012). In addition, 41 QTLS have been mapped with the capacity to tolerate salinity-induced stress at seedling phase in maize cereals (Luo et al. 2019).

Crops in their lifetime have to survive extreme temperature environments and these two temperature shocks, high and low temperature, can have catastrophic impacts on cereals. Global warming is continually causing temperature to surge up day by day which has been the most serious concern a plant has to confront (Wahid et al. 2007). Extreme temperature pushes the nutritive cells in the anther (tapetal cells) into early abortion which ends with pollen impotency (Parish et al. 2012). Up to date, numerous QTLs have been mapped in cereals which are developed in response to high and low temperatures. During blooming season of rice, four QTLs for stress tolerance to temperature were mapped which include qhr1, qhr3-1, qhr4-3, and qhr8-1 (Talukder et al. 2014; Ye et al. 2012, 2015). In rice, QTLs have been mapped to counteract the damage caused by low temperature which involves two QTLs named as qCTB7 and qCTB8 (Kuroki et al. 2007; Zhou et al. 2010). Other QTLs, which enable seeds to germinate under low temperatures, qLTG3-1, qLTG11-1, and qLTG3-2, are also mapped (Satoh et al. 2016). On chromosomes 4, 5, 6, 7, and 9, about six QTLs were found to be involved in germination in cold conditions and length of roots in the cereal. Essential cereals are being affected severely by male sterility during onset of reproductive stage. Hence, two QTLs (qCTR5 and qCTR12) were detected on chromosomes 5 and 12 of maize's DNA which are the controlling centers for the abovementioned phenotype at reproductive cycle (Ahmad et al. 2018).

Waterlogging is a common problem in some regions of the world where excess of water surrounds the roots of plants which can result in its dislocation from its erected orientation permanently. It is recognized as a critical problem for cereals and severe logging can jeopardize the whole agricultural land, compromising the production of cereals. Logging stress destabilizes the yield and quality of cereals by shattering or twisting the stem. Unfortunately, very little is known about the enhancement of cereals against waterlogging stress. A QTL resistant to logging stress, lrt5, and a submersion QTL (SUB1) were identified in rice. Similarly, in maize, an immersing liked QTL (Subtol6) has been investigated on chromosome 6 (Ahmad et al. 2018).

10.4 Chronology of Transgenic Cereal Development

Following the successful commercial development of herbicide- and insect-resistant cereal crops, researchers migrated towards the complicated issues of producing tolerant cereals to widely stressful abiotic conditions such as drought, salinity, extreme temperatures, and nitrogen-phosphate deficiencies. In this regard, the first commercial breakthrough was the development of Monsanto genetically modified maize DroughtGard™ variety expressing cspB, an RNA chaperone gene isolated from *Bacillus subtilis* (Castiglioni et al. 2008). This gene which increases crop yield under water-deficit conditions is also introduced into maize adapted to African climate as part of a project called water-efficient maize for Africa (WEMA). Besides, there is a list of approaches that are being investigated at present in an effort to enhance and encourage the growth of cereals under abiotic stresses (Saint Pierre et al. 2012). For instance, wheat with overexpression of 12-oxo-phytodienoic acid gene (TaOPR1) considerably escalated the level of tolerance to saline conditions (Dong et al. 2013). It is believed that this gene is involved in the midst of episodic response of abiotic stress as the signaling compound is concerned with the regulation of ABA-arbitrated signaling pathways. Moreover, enhanced salinity tolerance was achieved when mitogen-activated protein kinase, HvMPK4, was expressed in barley plant (Abass and Morris 2013). In transgenic rice, overexpressing phytochrome-interacting factor-like protein, OsPIL1, advanced the length of internodes (Todaka et al. 2012). This data further documented that OsPIL1 works as the main regulator of restricted height in plant through cell wall-related genes against drought condition, and this strategy can be used to improve plant regrowth in such unfavorable conditions.

Upon the overexpression of TF osbZIP16 in transgenic rice, a positive relation was seen in the level of osbZIP16 expression and enhanced tolerance to drought stress (Chen et al. 2012). Similar results appeared when transgenic rice GM rice expressed Oshox22, which is a member of homeodomain-leucine zipper (HD-Zip) family I of 357 transcription factors (Zhang et al. 2012). Both the authors agreed that Oshox22 influences the bio-production of ABA and manages water-deficit and salinity stress via ABA-arbitrated signal transduction pathways. There are evidences for other similar results exploring the overexpression of a large number of genes in genetically modified rice. These cases include a putative helix-loop-helix known as OrbHLH001 providing salt resilience (Chen et al. 2012); a TFIIIA-type 362 zinc finger protein known as ZFP182 that contributes towards increasing tolerance against diverse abiotic stresses such as cold, drought, and salt exposures (Huang et al. 2012); a LEA protein, ZFP182, that demonstrated improved growth under salinity and water-deficit stress (Duan and Cai 2012); and a DEAD-box 366 helicase that enhances growth in 200 mM salt (Gill et al. 2013). Studies on GM rice also coincided with the suggestion that by overexpression a gene encoding salt-induced protein (TaSIP) in wheat and a sheepgrass gene (LcSain1) may be the best gene candidates for enhanced salt-tolerance system (Du et al. 2013; Li et al. 2013). A parallel experiment reported that GM oats containing CBF3 gene from *Arabidopsis* displayed better growth, sustenance of leaf area, chlorophyll components, transpiration and photosynthetic rates, and elevated level of proline and soluble sugar under extreme salt conditions (Oraby and Ahmad 2012).

In another study, two wheat CBF TFs (TaCBF14 and TaCBF15) were cloned in spring barley, and the result expressed that transgenic lines possessed forbearance to freezing acclimation several degrees down than the temperatures which were supposed to be harmful wild-type spring barley (Soltész et al. 2013). There are also examples on improving cereal growth under limited nutritional supply as a result of overexpression of H^+-pyrophosphatase gene from *Thellungiella halophila* in maize crops (Pei et al. 2012). With the presence of proper phosphate supply, genetically enhanced plants exhibited much more robust root growth than wild-type cultivar, and under constricted phosphate availability, they established a more effective root network. This manipulation supports phosphate uptake and GM crops could uptake more phosphorus. A resembled study exploiting the overexpression of phosphate transporter (*pht1*) encouraged phosphate absorption in GM rice (Sun et al. 2012). Moreover, metal ions are also impediments to normal development of plants. In a recent study on genetic evaluation of root hairs of wheat, the inhibitory effects of extra aluminum presence in acidic soil were the center of the study (Delhaize et al. 2012). An analogous approach is to evaluate multidrug and toxic compound extrusion (TaMATE1B) gene in wheat and barley (Tovkach et al. 2013; Zhou et al. 2013). Upon expression of iron-phytosiderophore transporter (HvYS1) (isolated from barley) in GM rice, it showed improved growth in alkaline soils. This gene helps barley crop with the ability to take up iron (Fe) from alkaline soils, and GM rice cultivated in alkali-rich soil showed prompt growth, yield, and iron accumulation compared to control group (Gómez-Galera et al. 2012).

10.5 Transgenic Strategies to Counteract Abiotic Stresses

The whole human race is facing the dreadful challenge of global warming and climate change. As humans are mainly dependent on plants and animals for their survival, the effects of environmental variables can be catastrophic to both plants and animals. Among all the factors, abiotic factors are relentlessly competing with crops' yield and putting both the health and economy at stake. Nowadays, engineering crops with abiotic stress tolerance has become a hot subject for agricultural researchers and economists. Green biotechnology which includes genome-assisted breeding and genetic engineering is helping the concerned people to understand the complex relation of plants and abiotic stresses, and providing methods for the enhancement of crops against the hostile behaviors of environmental stresses. Plants cope with environmental stresses by activating a molecular hub comprising of stress communication, signal processing, gene regulation, and production of functional metabolites. Using approaches of functional genomics, scientists have succeeded in identifying sets of genes involved in stress regulation in model plants and non-model species. Hence, developing strategies of genetic engineering that can trigger the expression of other stress-associated genes or mediate crucial functions in protection and sustenance of cellular machineries may drive plant-enhancing initiatives. Numerous of the candidate's genes have already been cloned in crops to enhance abiotic stress resistivity. However, genetic engineering establishes not only single gene stress tolerance and gene pyramiding in crop plants but also manipulation of regulatory system involving transcription factors (Lata and Shivhare 2021).

When plants are exposed to certain doses of abiotic stress, the expression of different genes is activated and increases in level of specific metabolites and proteins are regulated. Some of them have protective role in plants to stress conditions. The logic towards enhanced breeding crops is to apprehend the overall cellular, biochemical, and molecular changes in living machinery in response to stress. Advance molecular technology consists of the identification and application of molecular markers for the augmentation of breeding programs. Nonetheless, the manipulation of QTLs (genomic segments) with tolerance characteristics often introduces unwanted agronomic traits from the donor plant. The reason is the absence of genuine knowledge about genes behind the QTLs. That is why the most viable choice will be the production of genetically engineered cereals by introduction or upregulation of candidate genes to accelerate the breeding of "super-tolerant" plants. Consequently, genetic engineering is the most rapid way to clone the desirable genes compared to conventional and molecular breeding. It will also make possible the phenomenon of inter-specific breeding in which a native gene of interest can be inserted into non-native, distant wild species. Using such methods, numerous plants with improved stress tolerance have been developed (Allen 1995) (Fig. 10.1).

Stress-instigated expression of genes can be classified into three main classes: genes producing proteins with detectable enzymatic or structural functions, proteins with unspecified functions, and proteins with regulatory functions. Initially, transgenic development involved single action genes (e.g., tobacco) for abiotic tolerant plant; such genes produce a single metabolite that propagates abiotic tolerance in plants to salt or drought conditions. The primary targets of the transgenic technology were stress-responsive proteins with known activities such as water, production of biocatalysts for osmolytes, enzymes for detoxification, and transport proteins. In addition, metabolic pathways involving few enzymes have been a soft target for genetic manipulation as compared to structural and developmental traits. Unfortunately, this approach is not of much success as abiotic stress and tolerance response involve multigenic association at a time and single gene approach is likely to be futile. The second transgenic revolution started with the aim to modify plants with third category of stress-driven genes, more properly termed as regulatory genes/proteins. With the help of regulatory proteins, a number of genes mediating stress response can be activated by stress-associated transcription factors (Kasuga et al.

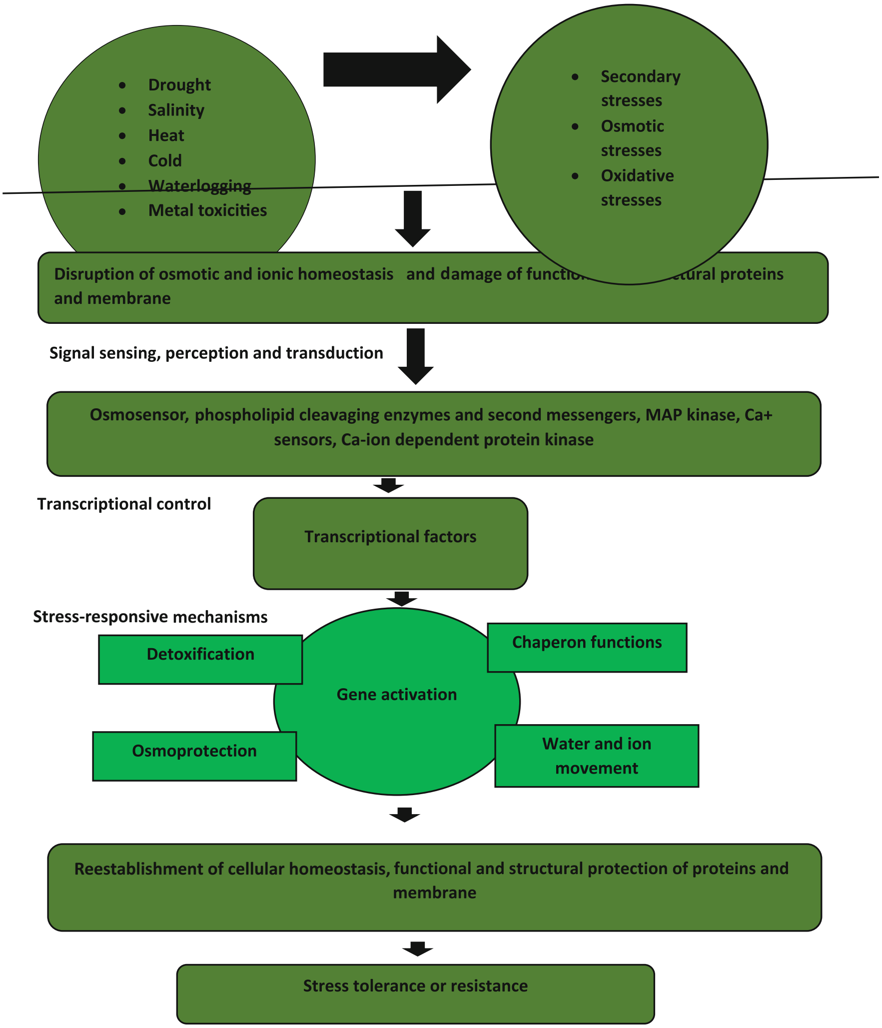

Fig. 10.1 Response of plants to abiotic stresses (Roy et al. 2011)

1999), thus making a possible way for enhanced tolerance towards corresponding stresses such as water-deficit, salt, and hard cold conditions. Interestingly, this revolutionary technology has coexisted with better incorporation in genetic engineering and plant physiology. Genetic engineering has also made it possible to regulate timing, tissue-specific expression, and rate of expression of the inserted genes for optimal activity. These factors are very countable if the expression of a gene or transcription factor is required at specific time, in specific tissue, or upon exposure to a particular stress environment. The research outcomes about stress promoters have resulted in change in pattern for genetically modified stress-tolerant crops (Katiyar-Agarwal et al. 1999). To develop transgenic plants, the most applicable promoters are constitutively expressed, i.e., they are expressed all the time through plant's life cycle. However, when the expression of a gene needs to be tissue-specific or time-specific, under such circumstances the use of constitutive promoters is not a pragmatic option, given the condition of stress-induced genes. The reason of this statement is that the expression of stress-related genes in constitutive manner could have disastrous implications in the crop plants. Consequently, a more effective effort to produce transgenic plants is via exploiting gene cassettes steered by stress-instigated promoters. With the advancement in the uncovering of abiotic stress genes and the genetic transformation getting more accessible, identification of stress-activated promoters is acquiring utmost attention than ever before (Bhatnagar-Mathur et al. 2008). It is obligatory to evaluate transgenic plants and how improved traits can be adopted to cereals crops just like model crops. Although lots of experimental work have been published assessing transgenic plants against abiotic stresses under controlled growth conditions, the effects of transgene in natural environment are yet to be understood. The following sections will discuss some accomplished milestones in plant transgenic technology for enhancement of abiotic stress tolerance with examples targeting various stress conditions.

10.5.1 Single Action Genes

10.5.1.1 Osmoprotectants

Occurrence of lethal changes in cellular components as a result of osmotic stress is inevitable. In the development of stress-resistant plants, numerous genes mediating the production of osmoprotectants (organic compounds such as amino acids, amine, sugar, and sugar alcohols produced in response to osmotic changes) have been used till date (Vinocur and Altman 2005). Unfortunately, a number of crops are unable to produce osmoprotectants that are synthesized in stress-tolerant plants. Hence, osmoregulation is expected to be a viable option to enhance abiotic stress tolerance by stimulating the osmoregulatory genes against stress conditions (drought, salinity, and extreme cold). Consequently, the engineering of specific osmolytes or overexpression of osmolytes in plants is the best transgenic strategy to develop stress-resistant crops. Multiple approaches are being exploited to genetically manipulate osmoprotection in crops. To develop a stress-tolerant plant, the first step is to engineer genes/pathways that are involved in the synthesis of enzymes for certain osmolyte production (Bray 1993). This strategy has contributed in the identification and engineering of osmoprotectants which include glycine-betaine and proline (Roychoudhury et al. 2015; Roychoudhury and Banerjee 2016). Moreover, some sugar alcohols such as mannitol, trehalose, myo-inositol, and sorbitol have been engineered to prevent membrane deterioration and protein sequences during stress conditions. Transgenics modified with the ability to overexpress polyamines have been documented in several crops (Bhatnagar-Mathur et al. 2008). A substantial research has focused to identify-isolate-clone genes that could contribute to enhanced flood-stress tolerance, based on the enzymes involved in glycolytic and alcohol fermentation processes. This also correlates respiratory pathways and anaerobic stress affecting these pathways. Submergence tolerance has been extensively investigated with the role of genetically altered levels of *pdc* and *adh* in tobacco and rice crop. Transgenic rice, up- and downregulating pyruvate decarboxylase 1 (pdc1) gene, has been produced, indicating a direct correlation of elevated PDC activities with tolerance to submergence (Quimio et al. 2000). In most of the mentioned case above, the transgenic manipulation of biosynthetic and metabolic pathways has shown that enhanced stress management and production of appropriate solutes can also prevent damage imposed by reactive oxygen species (ROS) along with chaperone resembling activities by avoiding interruption in protein integrity and function. However, there are lots of studies contradicting the beneficial effects of the above conditions. The engineering in endogenous metabolic pathways can lead to necrosis and restricted growth (Bhatnagar-Mathur et al. 2008). A study on the effects of osmoprotection over crop yield in response to water stress did not prove any beneficial outcome as it suggests that it can be the plant's intrinsic abilities to withstand root development under extreme stress conditions (Voetberg and Sharp 1991). Another study on chickpea also confirmed no positive effects of osmotic regulation in plant under water-deficit stress (Turner et al. 2007). Considering these views and lots of other results on the negative behaviors of osmotic adjustment in crops, it is not wise to use the overexpression of osmoprotectants for greater metabolic modifications to avoid pleotropic effects but the over-synthesis of competent solutes should be tissue specific, stress inducible, or both.

10.5.1.2 Detoxifying Genes

In many organisms, reactive oxygen species (ROS) are accumulated in response to environmental stress, and effective elimination of such ROS is crucial for plant survival. To maintain the amount of ROS and protect the plant from oxidative stress, plants have adopted specialized antioxidant strategies to neutralize the threats posed by free radicals. These systems consist of an enzymatic unit and non-enzymatic unit that perform a vital role in ROS scavenging in plants (Vranová et al. 2002; Das and Roychoudhury 2014). A substantial level of progress has been made to genetically engineered plants with abiotic stress tolerance using detoxification strategy. These include development of transgenic plants which are engineered to overexpress enzymes involved in antioxidant activities such as glutathione peroxidase, superoxide dismutase, ascorbate peroxidases, and glutathione reductases (Lieceng et al. 1999; Roxas et al. 1997). The most common problem in rice in response to abiotic stress is the accumulation of higher amount of methylglyoxal (MG) and reactive oxygen species (ROS). Multiple researches on rice have documented a very close connection between MG detoxifying glyoxalase pathway and the ROS detoxifying ascorbate-glutathione (AsA-GSH) pathway in battling abiotic stress (Roychoudhury and Basu 2012). A thorough study is needed to apprehend the mechanism of action of these pathways in order to develop stress-resistant rice varieties and transgenic modification can be a good choice for such developments (Hossain et al. 2021a). In wheat, tolerant genotypes could alleviate excessive ROS activities via transcription of genes encoding SOD (superoxide dismutases), CAT (catalase GR glutathione reductase), APX (ascorbate peroxidase), GPX (glutathione peroxidase), and GST (glutathione-S-transferase) enzymes under abiotic stress. The SOD serves as the main shield of defense against elevated level of ROS (Hossain et al. 2021b). Genetically modified tobacco upregulating SOD in cytosol, chloroplast, and mitochondria has been produced (Bowler et al. 1991; Van Camp et al. 1996); the results have explored enhanced resilience to oxidative stress imposed by methyl viologen (MV) in leaf disc assays. A significant improvement was noticed in the photosynthetic ability of transgenic tobacco and potato plant overexpressing Cu/Zn SOD in chloroplast upon exposure to extreme cold condition (Gupta et al. 1993; Perl et al. 1993). Another study found that transgenic tobacco plants upregulating alfalfa aldose reductase gene (MsALR) exhibited decreased concentrations of reactive aldehydes and accelerated resilience against oxidative species and water-deficient conditions (Oberschall et al. 2000).

10.5.1.3 Late Embryogenesis Abundant (LEA) Proteins

LEA is a group of proteins produced in higher concentrations during late embryogenesis and the accumulation of these proteins occurs in two conditions, i.e., seed dehydration and water-dearth conditions (Galau et al. 1987; Banerjee and Roychoudhury 2016). Out of all the five groups of LEA proteins, the members of group 3 are supposed to mediate a role in confiscating ions that are abundant in cellular desiccation. These proteins consist of 11 monomers of amino acid motifs with 13 times repeated consensus sequence of TAQAAKEKAGE (Dure III 1993; Roychoudhury and Nayek 2014). The group 1 LEA proteins are estimated to be involved in increasing water-binding ability, whereas sequestration of ions during water loss is thought to be the function of group 5 LEA proteins. Transgenic rice plants overexpressing HVA1 (group 3 LEA member isolated from barley) constitutively have been reported to exhibit resilience to water limiting conditions in soil and presence of excessive salt (Xu et al. 1996). In another case, when HVA1 gene was expressed in constitutive manner or stress-motivated under salinity and drought stress, a significant enhancement was seen in growth parameters and stress management in transgenic wheat and rice (Sivamani et al. 2000; Rohila et al. 2002). In spite of significantly lower water use efficiency (WUE), transgenic rice known as TNG67 containing a wheat LEA group 2 protein, PMA80 gene, or the wheat LEA group 1 protein, PMA1959 gene, provides improved tolerance to stresses induced by desiccation and salinity (Cheng et al. 2002). In addition, the protective potential of chaperone resembling LEA proteins serving against cellular injury has been documented (Vinocur and Altman 2005), confirming the role of LEA proteins in anti-coagulation of enzymes in response to dehydration and severe arctic stress (Goyal et al. 2005). *LEA* gene known as *OsLEA* has been successfully engineered in rice under stress-inducible promoters. When the transgenic rice lines expressing LEA proteins gene were transferred into field conditions, they exhibited enhanced resistance against water-deficit stress (Bakhsh and Hussain 2015). Transgenic spring rice expressing *HVA1* gene bestowed water-deficit tolerance, introducing maximum biomass accumulation and water use efficiency (WUE) under drought stress in greenhouse (Sivamani et al. 2000). Another study revealed the phenomenon of improved drought resistance in four autonomous transgenic wheat lines (T4) investigated in nine field trials over six different growing seasons. This also showed the performance of each line was associated with the expression level of *HVA1* transgene (Bahieldin et al. 2005). According to Xu et al. (1996), upon the expression of the same transgene in rice cv. Nipponbare in constitutive pattern, tolerance against water deficiency and salinity was improved characterized by the absence of damaging signs and enhanced recovery. Moreover, the third-generation transgenic line of rice cereal (cv. Pusa Basmati 1) demonstrated maintenance of cellular integrity under salinity and drought stress (Rohila et al. 2002). Overexpression of group 2 *LEA* gene, *Rab16A* from rice, enhanced salt tolerance in transgenic tobacco (Roychoudhury et al. 2007) and rice (Ganguly et al. 2012) and also conferred tolerance to drought stress in an aromatic rice variety (Ganguly et al. 2020).

10.5.1.4 Transporter Genes

An important approach for acquiring maximum abiotic stress tolerance is to support plants in maintaining homeostatic regulation under stress conditions, achieving both ionic and osmotic homeostases. This is currently the most functional approach and is expected in the future to be more effective to improve plant tolerance towards salt stress using genetic engineering, where the aims of transgenic manipulation are to pump the sodium ions out of the roots or their preservation in cell's vacuoles. Numerous tolerant transgenic plants have been produced which can survive abiotic conditions by improving cellular proteomic machinery such as vacuolar antiporter proteins which maintain transport in cells (Bordas et al. 1997). Transgenic barley varieties upregulating a subfamily HKT transporter, HvHKT2;1, demonstrated elevated biomass accumulation under salt stress conditions via sodium ions elimination or storage of extra sodium ions in leaves (Mian et al. 2011). In another study on aluminum toxicity in plants grown in acid-abundant soil, overexpression of barley *HvAACT1* gene, producing a citrate transporter protein, administered improved aluminum resilience in barley and wheat (Zhou et al. 2013). Similarly, toxicity caused by boron can also restrict crop yield and is effectively managed with engineered tolerant varieties. Sutton et al. (2007) documented that by multiplying the copy number of *Bot1* gene responsible for the production of boron efflux transporter, boron toxicity tolerance was induced into African barley landrace with four replicas of the corresponding gene. In alkaline soil, iron retardation is the primary obstacle in plant productivity. Hence, the strategy of improving uptake of iron from the alkali-rich soil was accomplished through constitutive expression of a barley iron-phytosiderophore transporter (*HvYS1*) in transgenic rice line (Gómez-Galera et al. 2012).

10.5.1.5 Heat Shock Protein Genes (HSPs)

Heat shock protein and their allied sequences are the main defenders of cell stress. With increasing climate change and extreme rise in global temperature, the heat shock system is helping to revolutionize the suitability of organisms to fluctuating temperatures (Chen et al. 2018). The heat shock response is brought about by the accelerated expression of specific set of genes in order to neutralize heat jolt or other lethal agent and is a common biological retaliation in all life forms (Waters et al. 1996). The amount of stress-responsive genes such as heat shock is abundant in plants than any other organism due to entire genome duplication and gene conservation throughout evolutionary ladder (Sterck et al. 2007). Plants have a unique mechanism of HSP regulation during stress, and they discourage the cells to produce normal proteins and advance the biosynthesis of stress-associated proteins (HSPs) (Khan et al. 2019). Heat shock proteins having high molecular weight such as HSP70, HSP90, and HSP101 have been identified with distinctive sequence similarities in Kingdom Plantae (Boston et al. 1996). The use of genetic engineering to enhance thermotolerance by overproducing heat shock proteins (HSPs) has been accomplished in several plants (Malik et al. 1999; Li et al. 2003; Katiyar-Agarwal et al. 2003). A few cases of successful introduction of HSP genes to confer thermotolerance to rice cereal have been revealed. For instance, there was an increase in heat tolerance when a transgene HSP102 from *Arabidopsis* was overexpressed in transgenic rice (Katiyar-Agarwal et al. 2003). Overexpressing of sHSP17.7 gene provided thermotolerance and UV-B resilience to rice crop (Murakami et al. 2004). Although a substantial scientific literature has been available about transgenic development and heat shock proteins in some plants, research on genetic exploitation of heat shock genes in main cereals is still in need of insightful investigations. Table 10.1 describes the summary of HSPs in cereals in response to abiotic stresses (Khan et al. 2019).

10.5.2 Regulatory Genes

Some genes that are activated in response to some stresses like dehydration and cold stress via transcription are stimulated by abscisic acid (ABA), which prevents the cells from the adverse impacts of dehydration (Mundy and Chua 1988; Dure et al. 1989; Skriver and Mundy 1990). To maintain cellular integrity and provide plants with stress tolerance, transforming a single gene encoding a specific protein conferring resilience against stress may not suffice the need and further reinforcement must meet to achieve targeted tolerance (Bohnert et al. 1995). In order to compensate this requirement, the most productive strategy will involve a gene encoding stress-inducible transcription factor that itself regulates a chain of other genes, thereby increasing plant tolerance towards diverse stresses (Yamaguchi-Shinozaki et al. 1994; Chinnusamy et al. 2005). Table 10.2 describes the genes encoding proteins for abiotic stress response in different cereals (Jeyasri et al. 2021).

10.5.2.1 Transcription Factors

A fascinating subject for genetic modification and gene regulation is a small group of transcription factors that have been studied to bind promoter sequences in genes that are affected by abiotic stresses (Bhatnagar-Mathur et al. 2008). Such transcription factors stimulate a cascade of genes that works coherently in pacing up tolerance to a variety of abiotic stresses. A significant number of transcription factors are involved

Table 10.1 Summary of HSPs in cereals in response to abiotic stresses

Abiotic stress factor	Plant	Type of HSP	Expression pattern	Technique used
High temperature	Wheat	HSP70	Up	qRT-PCR
		HSP26	Up	qRT-PCR
	Rice	HSP100	Up	WB
		HSP90	Up	q-PCR
	Maize	HSP101	Up	SDS-PAGE
		HSP70	Up	SDS-PAGE
Low temperature	Maize	HSP70	Up	MA, qRT-PCR
	Wheat	HSP70	Up	MS
		HSP90	Up	MS
		HSP60 HSP21	Down	qRT-PCR
	Rice	HSP75	Up	MS
		HSP95 HSP90	Up	MS qRT-PCR
	Barley	HSP70	Up	MS
Salinity stress	Wheat	HSP70	Up	MS
		All HSPs	Up	MS
	Rice	HSP70	Up	qRT-PCR
		HSP40	Up	qRT-PCR
		ClpD1	Up	qRT-PCR
Drought stress	Barley	HSP17.5	Up	qRT-PCR
	Rice	HSP70	Up	MA, MS, qRT-PCR
		HSP101	Up	MS
		HSP17.7	Up	qRT-PCR
	Maize	HSP70 HSP26	Up	MS
Flooding stress	Rice	HSP70	Up	qRT-PCR
	Maize	HSP70	Up	qRT-PCR

in mediating dehydration stress tolerance in plants (Vinocur and Altman 2005; Bartels and Sunkar 2005). These large numbers of transcription factors are grouped together into large families which predominantly include AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2 zinc finger, and WRKY (Banerjee and Roychoudhury 2015, 2017). Although belonging to the same family, different members of certain family can behave in different ways to stress crisis. However, a similar kind of transcription factors may contribute to multiple stress-responsive genes due to the appreciable overlapping of gene expression profiles that are instigated in response to various stresses (Seki et al. 2001; Chen and Murata 2002). The possible transcriptional stimulation of stress-driven genes in transgenic plants has been achieved via overexpression of one or more transcription factors that detect regulatory elements in promoter region of these genes. Among all the listed families, bZIP and MYB families are associated with ABA signaling pathways and its activation. Numerous ABA-inducible genes have a common (C/T) ACGTGGC consensus and cis-acting ABA-responsive element (ABRE) in their promoter regions (Guiltinan et al. 1990; Mundy et al. 1990; Roychoudhury et al. 2008). One approach using genetic

Table 10.2 Genes encoding proteins for abiotic stress response in different cereals

Gene category	Gene	Cellular response	Species
Osmolyte compounds			
Glycine betaine	*BADH*	Heavy metal stress	Rice
	CodA	Salt, cold, and drought stress	Rice
Proline	*P5CS*	Drought	Wheat
Regulatory genes			
bZIP	*bZIP4*	Salinity stress	Maize
	HBP1b	Drought, salt, cold	Rice
	bZIP16	Dehydration, salt, and ABA	Rice
Transporters			
Na^+-H^+-dependent K^+ transporter	*ZmHKT1*	Salt stress	Maize
Na^+-K^+-symporter	*HKT1*	Salt stress	Wheat
	HKT1	Salt stress	Rice
Stress-responsive genes			
Transcription factors	*SAP7*	Abiotic stress	Rice
	DREB	Abiotic stress	Maize
	MYB6	Drought and salt	Rice
Antioxidants			
Ascorbate peroxidase	*APX*	Drought, salt, and cold	Rice
Catalase	*CAT*	Drought stress	Wheat
CAT, superoxide dismutase	*MnSOD*	Abiotic stress	Rice

engineering is to enhance stress tolerance to introduce transcription factor genes in ABA signaling pathways. Transcriptional studies have pointed out hundreds and thousands of uniquely expressed genes triggered by a single or simultaneous abiotic stresses proposing sophisticated stress management and gene expression mechanisms (Watt et al. 2020). A number of TF families have been linked to development of abiotic stress response such as DREB (dehydration-responsive element binding), ABRE/ABF (ABA-responsive element), MYB (myeloblastosis), NAC, bZIP (basic leucine zipper), and WRKY gene families (Ambawat et al. 2013; Nuruzzaman et al. 2013; Gujjar et al. 2014; Rahman et al. 2019). A transcription factor in wheat known as *TaNAC2-5A* is stimulated by stresses like cold, drought, salinity, and ABA exposure. Overexpression of this gene in model plant *Arabidopsis* boosted tolerance to salinity, drought, and freezing cold acclimation concurrently (Mao et al. 2012). Additionally, stress-instigated *TaNAC2-5A* function improved the transcriptional activities of DREB*2A* and *ABI5* transcription factors. It has been reported that both stress-inducible and constitutive regulations of DREB*2A* in wheat and barley increased stress management efficiency to water-deficit and high temperature stress due to enhanced expression of LEA genes. These LEA genes encode dehydrins and cold-responsive proteins that are involved in the stabilization of membrane integrity and other members of DREB gene families (Morran et al. 2011). Likewise, transcription factor *ZmSNAC1* has been studied for tolerance to abiotic stresses and has shown that transcription factor can discourage dehydration in wheat, rice, and barley, most probably via NAC-DREB-LEA regulatory module (Lu et al. 2012; Hong et al. 2016). Currently, the most effective transcription factors in transducing abiotic stress tolerance include DREB/CBF, ABF, AP2/ERF, bZIP, NAC, MYB, MYC, HD-ZIP, bHLH, NF-Y, EAR, and WRK and several of them have already been cloned and exploited both at genomic and proteomic level in transgenic plants. The characterization of most of the transcription factors' promoter binding sites has been accomplished and precise changes in these motifs seemed to be responsible for deciding the binding affinity of transcription factors (Singh and Laxmi 2015). As barley owns intrinsic property to neutralize abiotic stresses due to the superior DNA binding affinity and specificity of transcriptional factor regulation of stress-responsive genes, it is indeed a wise notion that barley's TFs could be appraised as promising candidates to strengthen abiotic resilience in other cereal crops. WRKY transcription factors are a large family of zinc finger TFs known for the transcriptional activation of genes during growth and development along with responding to environmental stresses such as physical injuries, pathogenic invasion, and abiotic stresses. A number of studies have focused on the role of WRKY TFs in different plant species including barley (Li et al. 2014). DREB1/CBF and DREB2, produced in response to freezing stress and dehydration, stand in dehydration-responsive element binding protein/C-repeat binding factor family of TFs controlling the gene expression of abiotic stress-associated sequences. Another study, ortholog of DREB1A isolated from xeric (wild barley (*H. spontaneum* L.) under the transcriptional effect of stress-inducible *HVA1* promoter, demonstrated better survivability and biomass production under extreme salt stress and consecutive cycles of severe water loss in bahiagrass (James et al. 2008). Cereal lip19 genes encoding bZIP-type TFs are supposed to play a regulatory function in the expression of stress-responsive genes during cold acclimation. In a study, Wlip19 (a homologue of lip19 in wheat) was triggered by low temperature stress in seedlings and appeared to be higher in a freezing tolerant cultivar compared to freezing sensitive cultivar. The expression of GUS reporter gene under the watch of promoter sequences of four wheat *Cor/Lea* genes (Wdhn13, Wrab17, Wrab18, and Wrab19) was accelerated by Wlip19 expression in wheat callus, suggesting that WLIP19 is a potential candidate/transcriptional regulator of *Cor/Lea* genes in the procurement of abiotic stress tolerance in cereals (Kobayashi et al. 2008).

10.6 Role of Bioinformatics and Functional Omics to Address Abiotic Stress Tolerance Mechanism

As mentioned earlier, the dynamic gene regulatory mechanisms in different cellular, physiological, and biochemical processes within plants are affected by environmental factors. In order to examine the phenomenon involved in these regulatory pathways, multiple functional omics projects have been commenced in the agricultural world since then (Jeyasri et al. 2021). In addition, several omics and bioinformatics approaches have been exploited for the development of sustainable crop plants that are resistant to abiotic stresses via molecular breeding, genetic modification, and advancements in genetics, genomics, and molecular physiology (Ismail and Horie 2017; Tiwari et al. 2018). Therefore, the need to work on novel functional omics and bioinformatics software and other tools is the center of all modern researches in biological sciences to identify abiotic stress-responsive genes from gene pools. In this regard, reinforcements have been used such as synthetic promoter trapping approaches, random and targeted mutagenesis, gene shifting, complementation, and expression reads by RNA sequencing method to ensure functional analysis of stress-responsive genes and tolerance phenomenon (Chantre Nongpiur et al. 2016). It is also important to restate that TFs are utmost important in acknowledging molecular pathways that help plants to grow and survive under abiotic stresses. Apprehending the post-translational degradation of proteins and interactions of non-coding miRNA (microRNA) allow inflection of target protein sequences. Moreover, some of small interfering RNAs (siRNAs) mediate an important function as stress inducers and influence protein translation with alternative splicing. Genome-wide association studies (GWAS) have risen to fame as they provide novel strategies for the identification and characterization of unique stress-responsive genes which are introduced into crop plants for establishing resilience to abiotic stress conditions. As a result, certain stress-responsive genes along with respective promoters have been identified and characterized for specificity. Integrated omics and computational tools are being used to investigate stress-responsive genes, growth regulation, and corresponding multidimensional metabolic network. Due to advancing knowledge, the functional omics can be widely classified into two prominent approaches involved in modifying gene pools for increased abiotic tolerance systems. These two approaches are (1) identification of stress-responsive genes followed by genetic enhancement to develop stress-tolerant cereals and (2) mining of markers associated with agronomically satisfied genes and their applications in marker-assisted breeding in initiatives (Muthuramalingam et al. 2018, 2020; Le et al. 2021).

10.7 Conclusions

Plants, whether cereals or non-cereals, are significant as food sources, medicinal reservoirs, and agro-economic stabilizers. Given their fragile nurture, they are mostly affected by biotic and abiotic environmental factors. Improvement of crop plants has been a result of decades of study and lots of information in this regard are available for agricultural expeditions in the future. All the disciplines of omics, genomics, proteomics, transcriptomics, and metabolomics, are being approached to find ways for the improvement of abiotic stress tolerance in cereals. Although there exist certain limitations to the use of these areas due to lack of prior knowledge, there is a great possibility that a multidiscipline approach may have a potential place for the production of stress tolerance cultivars. Transgenic technology has, undoubtedly, been the most important approach to compete with any other existing plant-improving strategy. Based on pure genomics and molecular biology, genetic engineering can pave the way for future research on crop improvement instigated by identification of genes, underlying proteins, and mechanism of physiological expressions in cereals. The future progress in this area will demand an eagle eye view of diverse approaches targeting the catastrophic effects of climate change which contribute to most severe effects of abiotic stresses.

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Genome Editing and CRISPR-Cas Technology for Enhancing Abiotic Stress Tolerance in Cereals

11

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Abstract

Cereals comprise an indispensable part of the human diet. However, their production encounters various limitations, including reduced farming areas and low yield and productivity mainly due to abiotic and biotic stress factors. Abiotic stresses such as drought, salinity, extreme temperatures, anoxia/hypoxia, and heavy metal toxicity contribute to the limiting crop productivity worldwide. With a rocketing increase in the human population, challenges to accomplish the demand for food security amidst the adverse effects of climate changes are of paramount importance. In this regard, technological advancements in plant breeding and genetic engineering approaches to improve abiotic stress tolerance are the call of the hour. With the introduction of genome editing technologies like meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR-Cas), precise and efficient double-strand break (DSB) in the targeted gene sequences aids in manipulating the genome. Successful genome editing to overcome/mitigate various abiotic stresses has been demonstrated in major cereal crops such as rice, maize, and wheat but remains unexplored in barley, sorghum, and millets. Genome editing (GE) technologies including the much-acclaimed "CRISPR/Cas" are the most promising options for precise

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_11

engineering of desired genes into cereal crops with enhanced tolerance to single or multiple abiotic stresses. This chapter highlights the recent advancements, challenges, and prospects of GE and CRISPR-Cas technologies in mitigation of abiotic stresses in cereals. It also sheds light on the potential of GE technologies to be exploited for crops like barley, sorghum, and millets.

Keywords

Abiotic stresses · Cereals · Genome editing · TALEN · ZFN · Clustered regularly interspaced short palindromic repeats (CRISPR)

11.1 Introduction

Agriculture and food safety are jeopardized by extreme weather conditions (Piao et al. 2010; Hasegawa et al. 2018). An increased level of greenhouse gases is accountable for agricultural stress caused by the recurrent occurrence of extreme heat and drought (Asseng et al. 2015). A one-degree Celsius rise in ambient temperature is anticipated to diminish wheat, rice, and maize productivity by 6%, 10–20%, and 21–31%, respectively, negatively impacting world agricultural production (Asseng et al. 2015; Yang et al. 2017; Wang et al. 2019). Notably, the detrimental effects of these abiotic pressures are exacerbated in Africa and South Asian countries, where food scarcity has already become a challenge (Hasegawa et al. 2018). Developing climate-smart crops that can withstand abiotic pressures like persistent high-temperature stress, water scarcity, and/or salinity would be a durable solution to such significant issues (Roychoudhury and Chakraborty 2013). Ever since the beginning of agriculture, various technologies have been developed to improve agricultural productivity and quality. Scientists are constantly probing the broad spheres of biological science for novel approaches to enhance agricultural production in the current scenarios, where a rapidly mounting global population, dwindling arable land, and drastically changing climate pose severe challenges to sustainable agriculture. Despite the fact that traditional breeding approaches have made substantial contributions to crop improvement for abiotic stress resilience, more efficient and advanced techniques with instantaneous impacts are undoubtedly necessary to overcome these challenges (Driedonks et al. 2016). Over the past several years, discoveries in genome engineering techniques have overhauled crop improvement efforts, allowing for incredibly fast and precise gene editing down to the nucleotide level (Lu and Zhu 2017; Zong et al. 2017). The diverse genome editing (GE) technologies include meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (CRISPR/Cas9), which give a mechanism for achieving specific genome alterations in a wide array of living organisms utilizing customized nucleases (Jain 2015; Banerjee and Roychoudhury 2018, 2020). The formation of double-strand breaks (DSBs) at specified positions of the target sequence to be modified distinguishes these tailored GE techniques (Carroll 2011). The repairing of these DSBs in DNA is facilitated by the non-homologous end joining (NHEJ) and homology-directed repair (HDR) processes. NHEJ arbitrarily generates insertion and deletions (indels) of different length that eventually induce frameshift mutations in both coding and regulatory DNA regions (Cristea et al. 2013). A homologous sequence to the DSB has to be accessible in the instance of HDR, which might be exogenously provided by donor DNA (Bortesi and Fischer 2015). Among all the GE techniques, CRISPR/Cas9 is the most advanced, successful, and straightforward technology in plant science (Ma et al. 2016). We describe the CRISPR/Cas9 GE strategies in this chapter, which could efficiently be deployed to develop abiotic stress-resilient crops expeditiously. Owing to its accuracy and "foreign DNA-free engineering" strategy, such procedures may help to encourage and streamline the usage of genetically modified products in terms of public acceptance. While some nations have explicitly embraced genome-edited crops, many others are currently debating the topic. Nonetheless, the technology would most certainly witness a mass acceptance, thereby ensuring the approval of genetically modified crops in many countries.

11.2 First-Generation Genome Editing Tools

Genome editing tools (GETs), which are also collectively referred to as genome editing with engineered nucleases (GEEN), target specifically desired DNA regions. Several GETs were discovered with their potential applications in genome engineering in plants. One such tool is the zinc finger nucleases (ZFNs) that was reported in 1996 (Kim et al. 1996). ZFNs are artificial endonucleases that can site-specifically cleave DNA that consists of zinc finger protein (ZFP) connected to the cleavage domain of *FokI* restriction enzyme (Fig. 11.1a).

Earlier ZFN was the basis of genome editing in various organisms. However, ZFN is associated with some disadvantages such as complexity and requirement of high cost for ZFP construction. Most of the disadvantages with ZFN can be overcome by the discovery of transcription activator-like effector nucleases (TALENs) with relatively simple construction and higher efficiency in diverse cell types and organisms. The structural assembly of TALEN is similar to ZFN which comprises of nonspecific *Fok*I nuclease domain fused with a customizable DNA binding domain. The DNA binding domain comprises of highly repeating units of TALE from *Xanthomonas* bacteria. ZFN and TALEN are considered first-generation GETs, whereas CRISPR/Cas system is the second-generation GETs. The designed ZFN and TALEN induce cleavage in the targeted genomic region and repair either by non-homologous end joining (NHEJ) or homology-directed repair (HDR), thereby creating indels or new transgene insertion, respectively.

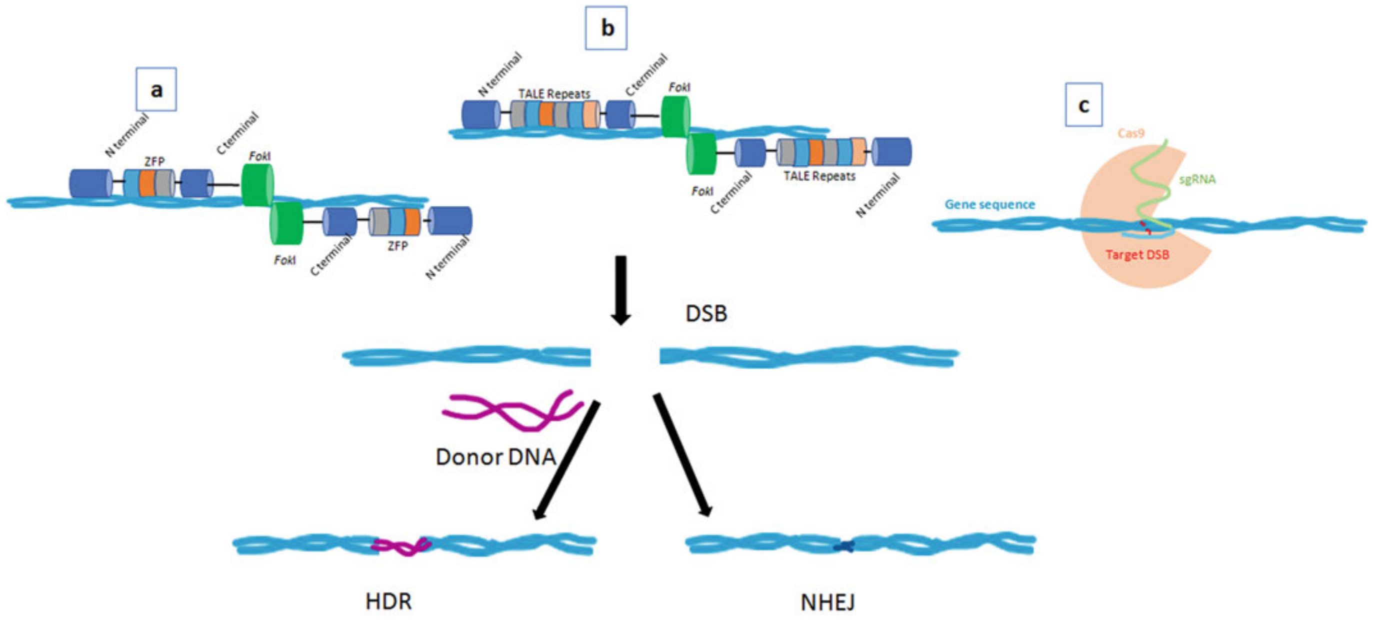

Fig. 11.1 Different generations of nucleases used for crop improvement. (**a**) ZFN, (**b**) TALEN, and (**c**) CRISPR/Cas9. The nucleases create double-strand breaks (DSB) in the target sequence. The break in the nucleotide sequence is repaired by the cell's own mechanism either through NHEJ or HDR

11.3 Second-Generation Genome Editing Tools

The rapid advancement in the GE area leads to the discovery of the second-generation GETs which is the CRISPR/Cas GET. CRISPR/Cas system was developed 2 years after the discovery of TALEN. The CRISPR repeats were detected in the bacterial system as early as 1987 with unknown functions (Ishino et al. 1987). Later in 2005, it was reported that the spacer sequence has similarities with bacteriophages that attach bacteria (Bolotin et al. 2005). Bacteria use CRISPR/Cas system as an adaptive defence mechanism against invading bacteriophages. CRISPR/Cas system is more specific than TALEN or ZFN for targeting genes as it uses the noncoding RNA complements with the target sequence for DSB (Fig. 11.1c). The various components of CRISPR/Cas9 complex are presented in Fig. 11.2.

The two principal components are single guide RNA and cas9 protein. The single guide RNA is the combination of tracrRNA and crRNA. The crRNA is composed of a spacer sequence that is complementary to the target sequence. The tracrRNA and crRNA combine to form functional guide RNA which has the ability to interact with Cas9 protein. The Cas protein has double-stranded specific nuclease activity. The DSB in the target DNA is repaired by the cell's repair machinery either through

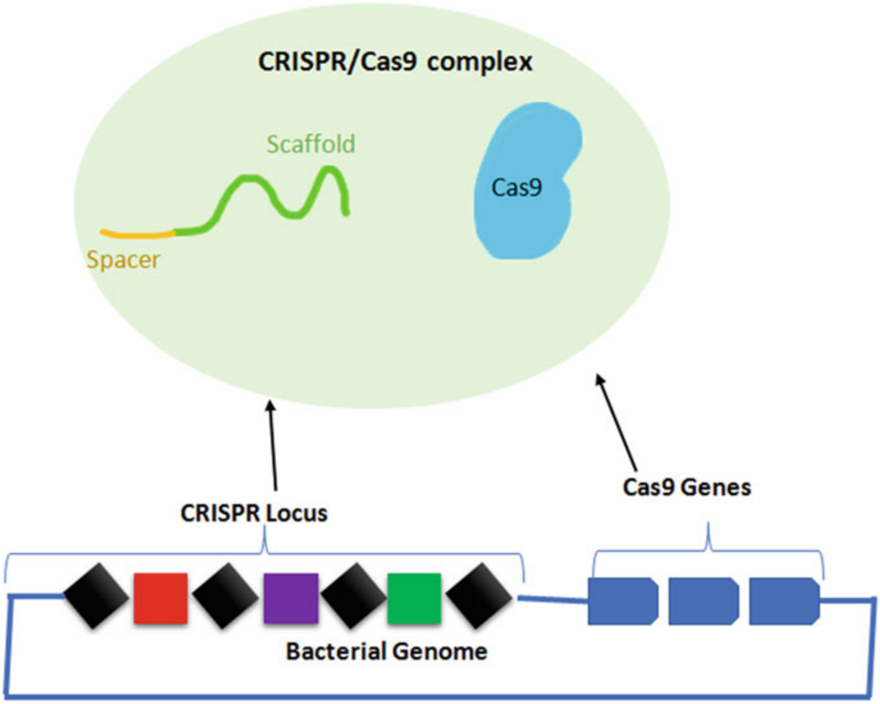

Fig. 11.2 A schematic representation of CRISPR/Cas9 complex. The bacterial genome is composed of CRISPR locus (spacers 🟧 🟪 , 🟩), repeats (⬛), and Cas9 genes (🟦)

non-homologous end joining (NHEJ) or homology-directed repair system (HDR) causing indels or substitution, respectively. CRISPR/Cas system is preferred over the transgenic approach due to its targeted genetic manipulation, thus marking its importance in crop improvement. Compared to the transgenic crops, products of GE can be used in breeding programmes with more acceptability and less regulatory issues (Waltz 2018).

CRISPR/Cas system has been divided into two classes, viz. class I and class II based on effector design. Class I has multisubunit effectors, whereas class II has single effector protein. These two classes are further subdivided into six types where class I includes types I, III, and IV, while class II comprises types II, V, and VI. Among them, the most popular system is type II which consists of Cas9 protein and is found naturally in *Streptococcus pyogenes*. CRISPR/Cpf1 is another efficient class II type V system found in *Prevotella* and *Francisella* bacteria (Zetsche et al. 2017). Compared to the Cas9, theCpf1 DSB results in cohesive ends with 4–5 nt overhangs with less off-target effect in plants and animals. Another improved CRISPR/Cas system is the C2c2 nuclease from *Leptotrichia shahii* with dual nuclease activity and single-stranded RNA as target (Zaidi et al. 2017).

11.4 Implication of Genome Editing in Cereals: Problems and Opportunities for Betterment

Cereals are grown and consumed as a staple food in almost all parts of the world since they are a source of a vast majority of carbohydrates, protein, B vitamins, and minerals. Throughout many countries, a single grain is the mainstay of the diet. Rice, wheat, and maize are the most common cereals, accounting for more than 90% of total cereal calories, while barley, rye, sorghum, oat, triticale, and millet are a few of the other cereals. Though cereals are a rich source of carbohydrates, their protein fractions are insufficient in amount. The bioavailability of minerals from cereals is considerably less due to the presence of phytic acid and other antinutritional factors. Cereals are cultivated on more than 73% of the world's harvested land and account for more than 60% of global food output (Srikaeo 2020).

Though a significant amount of research has been conducted for improvement of cereal crops through conventional and/or molecular breeding, there lies immense scope of improving the same through genetic manipulation. However, genome editing faces several challenges in cereals; a main reason being its polyploid nature that makes the genome complex. Due to repeated sequences and greater genome size, they pose a challenge for sequencing. Functional annotations are particularly complicated by the increased number of gene copies and the various functions of duplicated genes that have evolved as a result of neofunctionalization. It is difficult to achieve the necessary mutations because of the intricacy. Many times, mutations notably knockout or knockdown of a gene may lead to no phenotypic changes due to the dosage impact of additional paralogous copies of genes. Another reason is the transformation efficiency of the particular crop as the exploration of current advancements in plant genome editing techniques for agricultural enhancement is mostly dependent on it. *Agrobacterium*-mediated transformation and particle bombardment have found wide applicability in plant science in the last three decades. Despite its widespread use, it is ineffective for many agricultural crops due to issues as follows: (1) generating plants harbouring transgenes by tissue culture takes a long period, (2) events altered exhibit a reduced frequency over time, (3) there is lower DNA concentration, and (4) gene transfer mediated by particle bombardment is less precise. For advancement of genome editing in agricultural crops, a model resolution that might be employed in many labs would be the simplification of the current transformation technique (Ansari et al. 2020).

11.4.1 Advancement During Pre-CRISPR Era (2000–2011)

The first-generation genome editing tools are already discussed in brief in the previous section. Here are a few examples of the application of these tools in various cereals.

11.4.1.1 ZFNs

The use of ZFNs for genome editing was initially reported in *Arabidopsis* and tobacco (Ansari et al. 2020). The application of this technology was reported in maize where *inositol-1,3,4,5,6-pentakisphosphate* (phytic acid biosynthesis gene) was targeted. This resulted in the desired modification of the inositol phosphate content in developing seeds and herbicide resistance (Shukla et al. 2009). Another study mentions ZFNs mediated gene modification in the *SSIVa* gene that codes for starch biosynthesis pathway leading to generation of transgenic rice. Thus, the engineered ZFNs were successful in cleaving and stimulating mutations at SSIVa locus in rice to affect starch content, grain filling, and plant height (Jung et al. 2018). Ran et al. (2018) reported another successful application of ZFNs in allohexaploid wheat where the coding region of *AHAS* (acetohydroxyacid synthase) gene was addressed to provide resistance against imidazoline herbicides.

11.4.1.2 Transcription Activator-Like Effector Nucleases (TALENs)

TALEN-based genome editing technology is used to enhance qualitative and quantitative traits as well as to understand the role of many genes whose functions remain unidentified. Previously ZFNs were used to improve agronomic traits in multiple crop plants, but their high complexity in technical procedure limited their wider use. To overcome this drawback of ZFNs, TALENs were used as an alternative to ZFNs. Li et al. (2012) reported the TALEN-based disruption of the promoter region for rice bacterial blight susceptibility gene *OsSWEET14* that encodes a member of the SWEET sucrose-efflux transporter family. In rice, TALENs have also been used to improve seed storability by disrupting *lipoxygenase*, *LOX3* (Ma et al. 2015a), to create fragrant rice by mutating betaine aldehyde dehydrogenase gene (*OsBADH2*) (Shan et al. 2015) and to develop resistance against *Xanthomonas oryzae* by knocking out *TFIIAγ5* (basal transcription factor) (Han et al. 2020). A study was conducted by Haun et al. (2014) in soybean seed by targeting two genes of fatty acid desaturase 2 (*FAD2-1A* and *FAD2-1B*) which aid in the conversion of monounsaturated fat, oleic acid, to polyunsaturated fat, linoleic acid. They engineered TALENs for the recognition and cleavage of the conserved DNA sequences in both genes. An increase in oleic acid content from 20 to 80% and decrease in linoleic acid from 50% to less than 4% were observed in the mutant plants.

11.4.2 Advancement During the CRISPR Era (2012 to Till Date)

A key enzyme that belonged to a specific CRISPR-based system was discovered in the year 2012, known as clustered regularly interspaced palindromic repeats (CRISPR)-associated proteins (Cas9). This marked the beginning of the age of CRISPR leading to a seismic shift in the field of genome editing. There have been many progressions in the CRISPR studies (in both plants and animals) which are mentioned below in detail.

11.4.2.1 Multiplex Genome Editing

Targeting numerous related or unrelated targets/genes simultaneously is known as multiplex genome editing. Wang et al. (2014) reported the earliest multiplex genome editing studies in wheat providing resistance to powdery mildew using TALENs which is unusual. In terms of CRISPR/Cas system, the method is more straightforward as numerous guide RNAs can be delivered as autonomous expression cassettes along with their own promoters or as polycistronic transcripts processed into mature gRNAs. Mostly the exonic or coding region is selected but a few recent examples also mentioned promoter modification for the generation of mutants with different levels of gene expression.

One of the early examples of CRISPR/Cas9 multi-targeting vectors with separate gRNAs for each gene was reported for monocots and dicots. Each construct carried one or multiple (up to eight) sgRNA expression cassettes that were driven by OsU3 and OsU6 promoters in rice. The other constructs carried one to three sgRNA expression cassettes driven by the AtU3 and/or AtU6 promoters for targeting genes in *Arabidopsis* (Ma et al. 2015b). Several researches reported multiplex genome editing in plants like tomato (Li et al. 2018b), tobacco (Mercx et al. 2017), potato (Nakayasu et al. 2018), cotton (Wang et al. 2018), wheat (Li et al. 2021), barley (Lawrenson et al. 2015), sorghum (Li et al. 2018a), etc. that focus on hormone biosynthesis, plant development, herbicide resistance, metabolic engineering, and molecular farming. The very recent addition to the genome editing tools is CRISPR/Cpf1 whose versatile application has been widely reported in plant and animal systems. Wang et al. (2018) and Xu et al. (2019) reported of multiplexing in rice by CRISPR/Cpf1 system.

11.4.2.2 Off-Target Mutations

Off-target activity is another significant challenge for CRISPR/Cas9 technology adoption. ZFNs and TALENs have extensive recognition sequences that must be at a specific distance from the cleavage location; they rarely cleave at off-target sites.

The CRISPR/Cas9 system can tolerate mismatches between the sgRNA and the target and has been used in the majority of off-target mutation studies. Several software tools, such as CRISPR-P, CHOP-CHOP, Benchling, cCTOP, CRISPR GE, etc., are used to design guide RNAs with high specificity which can help avoid any probable off-target sites. However, there are software tools to detect off-targets in the genome such as CasOffinder. Moreover, the use of engineered variants of Cas is also another alternative to reduce the off-target mutations.

Mismatches within the first 12 bp proximal to the PAM often prevent DNA cleavage (Cong et al. 2013) and this has been reported in rice (Xu et al. 2015), wheat (Upadhyay et al. 2013), and maize (Zhu et al. 2016). A study conducted in rice demonstrated that a sgRNA with 65% GC content targeting the IPA1 locus detected 47.5% off-target mutations at a region with one mismatch at eight positions upstream from the PAM. However, in the same study, sgRNA targeting DEP1 detected only 2.5% off-targets at six positions from the off-target site (Li et al. 2016b). In another study that reported editing in barley, the sgRNA having an overall GC content of 60%, 4.2% off-target mutation was observed in the T1 progeny, where the tolerated mismatch was a C replacing T in CTGGGGC sequence (Lawrenson et al. 2015). sgRNAs with a GC content (>70%) may encourage off-target mutations (Tsai et al. 2015). Therefore, as the GC content appears to affect the efficiency and specificity of CRISPR/Cas9, this could limit the number of targets in GC-rich genomes, suggesting a balanced GC content to overcome this limitation.

11.4.2.3 Engineered Cas9 Variants

The CRISPR/Cas9 system has been extensively used for genome engineering in both prokaryotes and eukaryotes. Cas9 recognizes and cleaves the target DNA via extensive base pairing between single guide RNA (sgRNA) and the target DNA. This also requires a short protospacer adjacent motif (PAM) sequence flanking the target site.

One of the imminent problems associated with CRISPR/Cas technology is off-target mutations. The sgRNA sometimes identifies an almost similar non-target region with few base mismatches leading to unwanted, off-target mutations within the cell. To decrease the off-target mutations, mutated Cas9 (nickase) was used with pairs of sgRNAs having close target sites in opposite strands. The mutant version has only one active domain (HNH or RuvC) that introduces nick only in one strand at the target region resulting in a double-strand break. This leads to the occurrence of mutation at the target site via NHEJ repair pathway. However, in the case of the off-target nick, DSB does not prevail due to the absence of another close nick. This off-target nick was repaired by a high-fidelity base excision repair pathway (Ran et al. 2013). Another variant of Cas9 known as dCas9 (nuclease deficient Cas9) was reported by Gilbert et al. (2014). It cannot introduce any break in the genome but can bind specifically to any sequence with the help of sgRNA. The study has mentioned various transcription activators or repressors that are being fused to CRISPR-dCas9 system bringing about changes in the regulation of the target genes or enhancing/inhibiting transcription, respectively. There have been several success stories regarding the versatility of CRISPR-Cas system to introduce precise modifications in the genome in the past. It is further being improved to increase its efficiency and specificity. The CRISPR systems were improvised by using engineered Cas9 versions, for example, enhanced specificity Cas9s (eSpCas9) (Slaymaker et al. 2016), high-fidelity Cas9s (Cas9-HF) (Kleinstiver et al. 2016), and xCas9 (Wang et al. 2017b).

The most commonly used Cas9 is derived from *Streptococcus pyogenes* (SpCas9). It requires NGG protospacer adjacent motif (PAM) at its DNA target site which is a key limitation of the technology. Recent studies by Nishimasu et al. (2018) have shown a SpCas9-NG variant that can also recognize relaxed NG PAMs. This mutated variant has been used for the disruption of gene and cytosine base editing in rice. Another report of the gene editing experiments and all of the base editing work was carried out in rice calli (Endo et al. 2019). A study conducted in rice by Hua et al. (2019) demonstrated that Cas9-NG was more efficient in indel formation and base editing at non-NGG than xCas9. Legut et al. (2020) benchmarked Cas9 and two recently developed PAM-flexible variants (Cas9-NG and xCas9), showing that PAM flexibility of the mutant Cas9 comes with reduced editing efficacy. The authors combined Cas9-NG and xCas9 resulting in a functional hybrid enzyme “xCas9-NG” demonstrating superiority over both the variants for transcriptional activation. Chatterjee et al. (2020a) engineered a hybrid Cas9 variant, iSpyMacCas9, that allows targeted mutagenesis through base editing of A to G and C to T at A-rich PAMs. Sretenovic et al. (2021) used this same platform for genome editing in rice.

11.4.2.4 PAM-Free Nuclease

PAM plays a vital role in the natural function of CRISPR/Cas systems. The PAM helps the adaptive immune system of prokaryotes to distinguish between the nonself (DNA target in non-host genetic material) and self (the same DNA sequence comprised of CRISPR arrays that produce the RNA guides). In the PAM-less environment, CRISPR-Cas systems would target their CRISPR arrays which can lead to a potentially fatal autoimmune response. At present, the recognized PAM sequences are not common among all the Cas nucleases and instead differ widely with different sequences, complexities, lengths, orientations, and distances from the target. This requirement limits the ability to target any sequence with CRISPR and has led to widespread efforts to ease the PAM requirement with the advancement and expansion of CRISPR technology, and the need for a flexible targeting or mining for PAM-free nuclease was initiated.

The major advantage of a PAM-free nuclease is quite clear: the capability to target any sequence. Thus, the selection of sites with high on-target but low off-target activity is simplified (Chakrabarti et al. 2019). During multiplexing, it would be more beneficial as only one nuclease is required to simultaneously target any set of sequences. However, there are serious downsides that should be considered. The first disadvantage is for gRNAs expressed from DNA constructs; self-targeting of this DNA would be prompt, inevitable, and likely catastrophic. Secondly, a PAM-free nuclease would most likely scan every sequence in the genome which can increase off-targets and can be time-consuming for target recognition.

Accordingly, Walton et al. (2020) designed specific variants of the *Streptococcus pyogenes* Cas9 enzyme called SpG (targets an expanded set of NGN PAMs) and near-PAM less SpCas9 SpRY (targets NRN and to a lesser extent NYN PAMs) that could recognize relaxed PAMs, but exhibited increased off-targets compared to their parent proteins. Fortunately, the addition of mutations that decrease mismatch tolerance could neutralize this effect and even enhance the recurrence of on-target editing. This was applied to develop high-fidelity variants of SpRY, Sc++, and enAsCas12a (Chatterjee et al. 2020b; Kleinstiver et al. 2019; Walton et al. 2020). Therefore, it can be assumed that a PAM-free nuclease may not be extensively suited for every CRISPR technology and rather comes with actual compensation that could adjust some applications.

11.4.2.5 Prime Editing (PE)

The recent development in gene editing is prime editing which is also referred to as "search and replace" technology. This tool can be used to introduce targeted insertion, deletion, and all the base transversions without DSBs in the genome. Here prime editing guide RNA (peg RNA) replaces the sgRNA scaffold and modified Cas9 protein is combined with reverse transcriptase altogether. The special characteristic of peg RNA is that it incorporates a primer binding site (PBS), the desired sequence that will be added to the target gene, and a spacer sequence complementary to one strand of DNA. The key advantage of prime editing is the flexibility of the required PAM sequences as it can introduce mutations (>30 bp) away from the site of nick (Anzalone et al. 2019). Furthermore, PE using Cas9 protein is found to reduce off-target mutation in the genome in comparison to Cas9-based mutations. A very recent study reports the versatility of PE in plants by showing that prime editors were successful in inducing point mutations, deletions, and insertions in cereals such as rice and wheat (Lin et al. 2020).

11.4.2.6 Base Editing (BE)

Base editing is a type of genetic engineering that can be achieved without DSBs and the NHEJ process. Generally, base editors comprise of (1) CRISPR/dCas9 (catalytically inactive), (2) cytidine deaminase (converts C to U and is single-stranded specific), (3) a uracil glycosylase inhibitor, and (4) a nickase (cleaves the nonedited DNA strand in the wobble of DNA duplex made by dCas9) (Komor et al. 2016). Base editing of C • G to T • A and A • T to G • C is most widely explored. Using the base editors, Zong and his colleagues (Zong et al. 2017) have converted cytosine to thymine with precision and succeeded to achieve mutations in rice, wheat, and maize (Zong et al. 2017).

A new ABE (adenine base editor) system using SpCas9-NGv1 has been designed for relaxing the prerequisite of PAM sequence. This tool was successfully implied to generate mutations in the rice genome by inducing A • G base substitutions at target sites (Negishi et al. 2019). The diversity of base editing was increased using a combination of cytidine deaminase, adenosine deaminase, nCas9, and uracil DNA glycosylase inhibitor to achieve C:G > T:A and A:T > G:C simultaneously (Li et al. 2020). The translational application of the base editing system is phenomenal in the improvement of low amylose rice cultivars by targeting the promoter region of *Wx* gene (Xu et al. 2021).

11.4.2.7 CRISPR-CasΦ (Cas Phi)

Recent advancement in CRISPR technology is the discovery of a minimal functional CRISPR/Cas system that comprises a single ~70 kDa protein, CasΦ, and a CRISPR array. It is encoded solely in the genomes of large bacteriophages. Unlike Cas9, CasΦ engages a single active site for CRISPR RNA (crRNA) processing and crRNA-guided DNA breakage to target non-host genetic material. This recent high-tech machinery is useful for genome editing and DNA detection. CasΦ is advantageous for cellular delivery due to its low molecular weight, which is half that of Cas9 and Cas12a, thereby expanding the genome editing toolbox (Pausch et al. 2020).

11.5 Application of Genome Editing Technology in Imparting Abiotic Stress Tolerance in Cereals

Abiotic stresses are the most imminent threat to crop production. With the upsurge in global food demand, a major emphasis of existing research is to increase crop yield under abiotic stresses like drought, salinity, extreme temperatures, anoxia/hypoxia, heavy metal toxicity, herbicide toxicity, etc. These abiotic stresses have been reported to cause significant agricultural losses each year especially in cereal crops. The tolerance of plants to such abiotic stresses is conferred by a network of complex traits involving several genes, proteins, transcription factors, microRNAs, transporters, hormones, metabolites, and ions (Budak et al. 2015; Hussain et al. 2017). Several researchers have made substantial efforts to improve crop productivity by targeting these traits through conventional breeding and transgenic approach, the progress of which has however been slow owing to its limitation. Excitement sneaked in with the advancements in genome editing tools, especially the CRISPR-Cas system, which brought opportunities for precise and efficient manipulation of desired genes for enhancing abiotic stress tolerance in crop plants. The potential of using genome editing for the development of abiotic stress-tolerant cereal crops is reflected in the numerous studies that have been reported. These studies predominately report on rice while quite a few on other cereals including maize, wheat, and barley. Some comprehensive information regarding the application of genome editing in cereals for abiotic stress tolerance is listed in Table 11.1.

11.5.1 Drought Stress Tolerance

Under drought or water-stressed conditions, maintenance of physiological functions in plants is compromised and can negatively impact crop growth or yield. Several genes have been targeted using transgenic approaches for imparting drought tolerance in plants (summarized by Ali et al. (2017)). Being extremely sensitive to

Table 11.1 Application of genome editing for abiotic stress tolerance in major cereals

Type of abiotic stress	Cereal crop	Targeted gene(s)	Targeted trait	System used for genome editing	Type of mutation	Effect on edited lines	References
Drought	Rice	*OsDERF1*	Drought-responsive ERF genes	TALEN	Deletion mutation	Heritable mutation	Zhang et al. (2016)
	Rice	*OsSAPK2*	Osmotic stress/ABA-activated protein kinase 2	CRISPR/Cas9	Frameshift mutation	Drought sensitive	Lou et al. (2017)
	Rice	*OsNCED3*	ABA biosynthetic pathway, 9-cis-epoxycarotenoid dioxygenase (NCED)	CRISPR/Cas	Deletion of 18-amino-acid protein and a frameshift mutation caused by one-nucleotide insertion	Increased sensitivity to water and salt stress	Huang et al. (2018)
	Rice	*OsPYL9*	ABA receptor gene	CRISPR/Cas	Deletion, insertion, or substitution	Enhanced drought tolerance	Usman et al. (2020)
	Rice	*OsERA1*	B-subunit of farnesyltransferase; regulates dehydration response	CRISPR/Cas	Frameshift mutations	Enhanced drought tolerance	Ogata et al. (2020)
	Rice	*OsDST*	Zinc finger transcription factor	CRISPR/Cas	Deletion mutation	Improved drought and salt tolerance and grain yield	Kumar et al. (2020)
	Rice	*OsDEP1*	Encodes a G protein γ subunit	CRISPR/Cas	Gain-of-function mutation	Enhanced agronomic trait (potential candidate for drought stress)	Xu et al. (2016), Li et al. (2016b)

(continued)

Table 11.1 (continued)

Type of abiotic stress	Cereal crop	Targeted gene(s)	Targeted trait	System used for genome editing	Type of mutation	Effect on edited lines	References
	Rice	*SRL1; SRL2*	Encodes a putative glycosylphosphatidylinositol-anchored protein	CRISPR/Cas9-based multiplexing	Deletion mutation	Improved water stress tolerance and panicle number	Liao et al. (2019)
	Maize	ARGOS	Negative regulators of ethylene response	CRISPR/Cas	Deletion mutation	Improved maize grain yield under drought stress	Shi et al. (2017)
	Wheat	TaERF3/TaDREB2	Abiotic stress-responsive transcription factor	CRISPR/Cas	Deletion mutation	Enhanced drought tolerance	Kim et al. (2018)
Salinity	Rice	*OsRR22*	Transcription factor	CRISPR/Cas	Insertions, deletions, and substitutions mutations	Enhanced salinity tolerance	Takagi et al. (2015), Zhang et al. (2019a)
	Rice	*OsRAV2*	Transcription factor	CRISPR/Cas	Deletion mutation	Enhanced salinity tolerance	Duan et al. (2016)
	Rice	*OsMIR528*	miRNA; positive regulator of salt stress	CRISPR/Cas	Deletion mutation	Enhanced salinity tolerance	Zhou et al. (2017)
	Rice	*OsBBS1*	Receptor-like cytoplasmic kinase	CRISPR/Cas	Deletion and frameshift mutation	Enhanced salinity tolerance	Zeng et al. (2018)
	Rice	*OsSAPK2*	ABA-activated protein kinase 2	CRISPR/Cas	Frameshift mutation	Enhanced salinity tolerance	Lou et al. (2017)
	Rice	*OsOTS1*	Specific components of SUMOylation	CRISPR/Cas	Insertion or deletions leading to frameshift mutation	Sensitive to salinity	Zhang et al. (2019b)

High temperature	Rice	*HSA1*	Encodes fructokinase-like protein 2	CRISPR/ Cas	Deletion mutation	Heat stress caused delay in chloroplast biogenesis	Qiu et al. (2018)
	Rice	*OsPYL*	Abscisic acid receptor gene	CRISPR/ Cas	Deletion mutation	High-temperature tolerance, increased grain yield, and reduction in preharvest sprouting	Miao et al. (2018)
Low temperature	Rice	*OsANN3*	Annexins (Ca^{2+}-dependent phospholipid binding proteins)	CRISPR/ Cas9	Deletion mutation	Involved in cold tolerance	Shen et al. (2017)
	Rice	*OsMYB30*	Nuclear protein and negative regulator of cold tolerance	CRISPR/ Cas	Single base mutation	Cold tolerance and higher yield	Zeng et al. (2020a, b)
	Rice	*OsPRP1*	Proline-rich proteins	CRISPR/ Cas	Single base insertion and deletion	Involved in cold tolerance	Nawaz et al. (2019)
Herbicide tolerance	Maize	IPK1	Encodes inositol-1,3,4,5,6-pentakisphosphate 2-kinase	Zinc finger nucleases	Insertion and deletion mutation	Herbicide tolerance; phytate reduction in developing seeds	Shukla et al. (2009)
	Rice	*OsALS*	Biosynthesis of branched chain amino acids	TALENs	Double point mutation	Resistance towards bispyribac-sodium	Li et al. (2016c)
	Rice	*OsEPSPS*	Encodes 5-enolpyruvylshikimate-3-phosphate synthase	CRISPR/ Cas9	Insertion	Tolerance to glyphosate	Li et al. (2016a)
	Rice	OsALS	Biosynthesis of branched chain amino acids	Cas9-based base-editing-mediated gene evolution (BEMGE)	Substitution	Tolerance to bispyribac-sodium	Kuang et al. (2020)
	Rice	*ALS*	Biosynthesis of branched chain amino acids	Cytosine base editors (CBEs)	Missense mutation	Tolerance to bispyribac-sodium, flucarbazone-sodium, imazapic (IMP),	Zhang et al. (2020)

(continued)

Table 11.1 (continued)

Type of abiotic stress	Cereal crop	Targeted gene(s)	Targeted trait	System used for genome editing	Type of mutation	Effect on edited lines	References
						nicosulfuron, and pyroxsulam	
	Wheat	ALS and acetyl-coenzyme A carboxylase	Biosynthesis of branched chain amino acids	Base editing	Point mutation	Tolerance to sulfonylurea-, imidazolinone-, and aryloxyphenoxy propionate	Zhang et al. (2019c)
	Maize	*ALS1* and *ALS2*	Biosynthesis of branched chain amino acids	CRISPR/Cas9	Insertion and deletions mutation	Tolerance to chlorsulfuron	Svitashev et al. (2015)
	Rice	*ALS*	Biosynthesis of branched chain amino acids	CRISPR/Cas9	Substitution, deletion, and frameshift mutation	Tolerance against imazapic and imazethapyr	Wang et al. (2021)
	Rice	*ACCase*	ACCase catalyzes the first step of fatty acid biosynthesis	Base editing by CRISPR-Cas9	Point mutation-induced substitution	Tolerance to aryloxyphenoxypropionate (APP) group ACCase-inhibited chemical, haloxyfop-R-methyl	Liu et al. (2020)
	Rice	*OsDEP*; *OsALS*; *OsACC*	Biosynthesis of branched chain amino acids	Prime editing	Single-base mutation	Herbicide resistance	Xu et al. (2020b)
	Rice	*ALS*; *APO1*	Biosynthesis of branched chain amino acids	Prime editing	G-A base transition in *ALS*	Resistant to imidazolinone herbicides	Hua et al. (2020)
	Rice	*ACC1*; *PDS1*; *WX1*	ACCase catalyzes the first step of fatty acid biosynthesis	Prime editing	+1 G-to-C mutation in the OsACC site	Aryloxyphenoxypropionate herbicide tolerance	Xu et al. (2020a)

	Rice	*ALS*	Biosynthesis of branched chain amino acids	CRISPR-Cas9 and Target-AID	C-to-T point mutation	Imazamox resistance	Shimatani et al. (2017)
Heavy metal toxicity	Rice	*OsNramp5*	Metal transporter	CRISPR/Cas	Insertion and deletion mutations	Low cadmium accumulation	Tang et al. (2017)
	Rice	*OsATX1*	Antioxidant (copper chaperone)	CRISPR/Cas	Deletion mutation	Facilitates root to shoot translocation of copper leading to reduced Cu concentrations in roots	Zhang et al. (2018)
	Rice	*OsARM1*	Transcription factor	CRISPR/Cas	1-bp insertion and 1-bp deletion mutation	Improved tolerance to arsenic by facilitating As transport	Wang et al. (2017a)
Anoxia/hypoxia	Rice	RBOH (Respiratory Burst Oxidase Homolog)	NADPH oxidase	CSISPR/Cas	Frameshift mutations	Formation of lysigenous aerenchyma in roots and reduces ROS accumulation	Yamauchi et al. (2017)

drought at any stage of growth is a major limitation to rice production especially in rainfed ecosystems (Lafitte et al. 2004; Pandey and Shukla 2015). Genome editing techniques such as TALENs and CRISPR/Cas9 approach have been applied for improving drought tolerance in rice. *OsDERF1* is a drought-responsive ERF gene that is triggered by stressors like drought, ethylene, and abscisic acid (Wan et al. 2011). This gene was targeted using TALEN to create mutation in rice together with an aim to optimize the TALEN scaffolds to improve the efficiency of mutation in rice and study their heritability (Zhang et al. 2016).

Abscisic acid is a key hormone that plays a pivotal role in plant responses to adverse environmental stimuli, the most common event being elevated ABA during various abiotic stresses (Swamy and Smith 1999). It is well established that plants respond to drought stress through ABA-dependent or independent signalling cascades (Budak et al. 2015). Lou et al. (2017) described the role of *OsSAPK2* (osmotic stress/ABA-activated protein kinase 2) in rice by creating a loss-of-function mutants through the CRISPR/Cas9 approach where the third exon of *OsSAPK2* was targeted for sgRNA designing. The *sapk2* mutants were insensitive to ABA and more sensitive to drought stress than the wild type. The mutant lines (*sapk2*) thus obtained exhibited more sensitivity to drought and reactive oxygen species (ROS) compared to their wild-type counterparts. Expression analysis of the edited plants confirmed increased gene expression under drought stress along with a better survival rate establishing the significance of *OsSAPK2* for the response to drought conditions in rice. In the ABA biosynthetic pathway, 9-cis-epoxycarotenoid dioxygenase (NCED) is a key rate-limiting enzyme. The rice *OsNCED3* is responsive to multi-abiotic stress tolerance including drought, salinity, and consequent H_2O_2 stress. In their attempt to study this gene, Huang et al. (2018) generated two independent homozygous lines *nced3-1* and *nced3-2* having a deletion of 18-amino-acid protein and a frameshift mutation caused by one-nucleotide insertion resulting in early termination of the protein translation. The *nced3* mutant rice plants showed increased sensitivity to water and salt stress due to its lower ABA content, while the overexpression of *OsNCED3* in rice by CRISPR/Cas system increased ABA accumulation and tolerance to salinity and drought stresses (Huang et al. 2018). Similarly, the ABA receptor gene, *OsPYL9*, was targeted using CRISPR/Cas9 for creating precise mutation by Usman et al. (2020). The edited rice plants showed a reduction in the number of stomata, stomatal conductance, transpiration rate, vascular bundles, and malondialdehyde content under stress conditions. Moreover, enhanced cuticular wax and chlorophyll and increased content of abscisic acid, catalase, superoxide dismutase, and antioxidant activities contributed towards improved drought tolerance. Their study demonstrated that *OsPYL9* mutants generated through CRISPR/Cas9 had the potential to enhance drought tolerance as well as yield of rice. Another regulator of ABA signalling is the ERA1 (Enhanced Response to ABA1) that encodes the β-subunit of farnesyltransferase and regulates dehydration response. CRISPR/Cas9 was used for targeted inactivation of ERA1 homolog in rice to induce frameshift mutations. The *osera1* mutant lines displayed an increase in primary root growth along with enhanced response to water stress through regulation of the stomatal conductance (Ogata et al. 2020).

Introgression of desirable alleles into elite cultivars can be achieved by CRISPR-Cas technology. The *indica* rice cv. MTU1010 was edited using this technology to generate mutant alleles of *drought and salt tolerance* (*DST*) gene using two sgRNA. Loss-of-function mutants of *Osdst* had reduced stomatal density that enables more water retention in leaves under dehydration stress and high level of salt stress during the seedling stage. Therefore, *dst* mutant allele can be a potential candidate for improving drought and salt tolerance and grain yield in *indica* rice cultivars (Kumar et al. 2020). The DENSE AND ERECT PANICLE 1 (DEP1) encodes a G protein γ subunit in rice that is reported to play an important role in the regulation of erect panicle, grains per panicle, uptake of nitrogen and its metabolism, ABA response, and drought tolerance (Xu et al. 2016). Although CRISPR/Cas9 constructs to target DEP1 in exon 5 were used to study panicle density, grain size, and plant height (Li et al. 2016b), it could be a potential candidate for editing rice for water stress tolerance.

An interesting strategy is the alteration in leaf morphology for improved water stress tolerance in plants, especially in drought-prone areas. The functional role of *SRL1* and *SRL2* (Semi-Rolled Leaf 1 and 2) in rice was demonstrated using CRISPR/Cas9-based multiplexed editing, and mutant plants exhibited curled leaves, reduced number of stomata, stomatal conductance, transpiration rate, and malondialdehyde (MDA) content, as compared to wild-type plants. Additionally, mutants had a higher panicle number, abscisic acid (ABA) content, catalase (CAT), superoxide dismutase (SOD), and survival rate (Liao et al. 2019).

ARGOS (auxin-regulated gene involved in organ size) genes are negative regulators of ethylene response and play a critical role in enhancing drought tolerance. Shi et al. (2015) reported that constitutive overexpression of ARGOS8 produces more grains in maize under drought stress conditions. Therefore, to attain a robust constitutive expression of ARGOS8, CRISPR/Cas9 system was used to insert or substitute the 5′-untranslated region or the native maize GOS2 promoter of *ARGOS8* gene. The novel ARGOS8 variants resulted in improved maize grain yield under drought stress (Shi et al. 2017). Such studies could aid advanced breeding technology to target multiple tissues at different developmental stages for mitigating drought stress in maize.

Kim et al. (2018) successfully applied CRISPR/Cas9 genome editing system in polyploid wheat by transient expression of small guide RNA and Cas9 protein in wheat protoplast to target two abiotic stress-responsive transcription factor genes, wheat ethylene responsive factor 3 (TaERF3) and wheat dehydration responsive element binding protein 2 (TaDREB2). This is a potential target for manipulating the wheat genome for improving tolerance towards drought stress.

11.5.2 Salinity Stress Tolerance

Soil salinity can severely affect crop productivity by triggering various molecular responses via ion toxicity, nutrient deficiency, and osmotic and oxidative stress that are unfavourable for the growth and development of plants (Farhat et al. 2019).

Transcription factors (TFs) are potential targets for genome editing applications for abiotic stress tolerance in plants as they regulate the expression of genes on promoter regions (Roychoudhury et al. 2008). Among many salt-related genes, the *OsRR22* gene encodes a transcription factor that has a significant role in cytokinin signal transduction and metabolism. Takagi et al. (2015) reported that there was an increase in salt tolerance due to loss of function of this gene. An enhanced tolerance towards salinity was achieved via CRISPR/Cas9-targeted mutagenesis of the *OsRR22* gene by using Cas9-OsRR22-gRNA expression vector in rice by Zhang et al. (2019a). T2 homozygous mutant lines/edited lines obtained in their study had improved salinity tolerance compared to that of wild-type plants, and their agronomic traits such as plant height, flowering, tillers per plant, grains per panicle, spikelet fertility, 1000-seed weight, and yield per plant under normal field conditions showed no significant difference with the wild plants. The RAV transcription factors are reported to act as negative regulators of drought and salt stress in *Arabidopsis* (Fu et al. 2014). Apparently, there are five *RAV* genes in the rice genome, of which the *OsRAV2* gene, a transcription factor, is involved in saline stress response (Duan et al. 2016). A specific regulatory region in this promoter was the GT-1 element which is involved in the salt-induced regulation of OsRAV2. In order to establish the regulatory role of the GT-1 element, CRISPR/Cas9-mediated targeted mutagenesis was performed. The edited lines were unable to overexpress the *OsRAV2* gene under the condition of high salt, which confirmed its role in salinity stress.

In plants, miRNAs are known to regulate many developmental processes as well as biotic and abiotic stress responses. Zhou et al. (2017) demonstrated the application of CRISPR-Cas9 to target rice miRNA genes for obtaining mutant plants and established that *OsMIR528* is a positive regulator of salt stress. They confirmed that *OsMIR528-sgRNA01-10* edited lines matched the phenotype of the wild rice plants during salinity stress which indicated that 1-bp indels in the mature miRNA did not impact its function, while a 4 bp or 54 bp in *OsMIR528-sgRNA01-15* T1 lines rendered mature miRNA non-functional. Their study suggested that CRISPR-Cas9 is an effective tool for knocking out plant miRNAs and *OsMIR528* is a potential candidate in rice for salt stress tolerance. Similarly, the role of rice gene *OsBBS1* in early leaf senescence and salt stress sensitivity was studied by Zeng et al. (2018). *OsBBS1* encoded a receptor-like cytoplasmic kinase and its expression was greatly induced by salt stress. *bbs1* mutant was generated by CRISPR/Cas technology where two 20 bp sequences were targeted for designing guide RNA (gRNA) near the start codon and the tyrosine protein kinase domain of *OsBBS1*. The *bbs1* mutant displayed hypersensitive salt stress response which led to the conclusion that *OsBBS1* played a critical role in the salt stress response in rice. Similarly, McLoughlin et al. (2012) showed that two SNF 1-related protein kinases (SnRKs), SnRK2.4 and SnRK2.10, were triggered in response to salt stress and were involved in the maintenance of root architecture under saline conditions. In a study describing the function of *OsSAPK2* in osmotic stress, the generated loss-of-function mutants in rice developed through CRISPR/Cas also exhibited salinity tolerance (Lou et al. 2017).

In another attempt to decipher the role of specific components of SUMOylation (post-translational modification), CRISPR gene editing was used to study the *OsOTS* (Overly Tolerant to Salt1) class of SUMO proteases that plays a critical role in salt and drought stress in rice. gRNA-mediated CRISPR/Cas9 gene editing system was used to create mutations in the *OsOTS1* gene. The edited lines of *OsOTS1* were sensitive to saline conditions, further confirming its role in imparting salt tolerance in rice (Zhang et al. 2019b).

11.5.3 Heat Stress Tolerance

Another abiotic stress that has a considerable negative impact on the yield and quality of crops is high temperature. Components of the photosynthetic apparatus and mechanisms that regulate chloroplast development are highly vulnerable to heat stress. Chloroplasts can play the role of sensors to perceive an increase in temperature due to components of the photosynthetic apparatus being prone to thermal damage (Yu et al. 2012). Mechanisms regulating chloroplast development under heat stress were studied by Qiu et al. (2018), by generating an HSA1 (heat-stress sensitive albino 1) deletion mutant by CRISPR/Cas9 editing. The second exon of the HSA1 gene was targeted by constructing a CRISPR/Cas9 vector (pHSA1cas9-1) and introduced into wild-type rice. The loss of function of HSA1 in the independent *hsa1cas* (mutant alleles of HSA1) rice lines exhibited a delay in chloroplast biogenesis during heat stress. Their experiments were conclusive of the fact that HSA1 is critical for early chloroplast development and is essential for heat tolerance in rice. In another study, Miao et al. (2018) developed triple knockout rice pyl1/4/6 by CRISPR/Cas9 editing during their research on the OsPYL abscisic acid receptor gene family. The pyl1/4/6 showed better performance than the wild type with respect to high-temperature tolerance, increased grain yield, and reduction in preharvest sprouting. Though several studies have mentioned the use of various heat-responsive QTLs and genes (summarized by Ni et al. (2018)) for conventional breeding and transgenic technique, the use of genome editing for high-temperature tolerance remains obscure.

11.5.4 Cold Stress Tolerance

Temperature plays a very crucial role in agricultural production. Low temperatures can affect the growth of cereal crops and can also influence their productivity rate. Exposure to a cold temperature usually results in poor germination, restricted seedling growth, withering and yellowing of leaves, and reduced tillering. A significant reduction in grain yield results due to delayed heading and pollen sterility caused by cold stress (Suzuki et al. 2008). To overcome this problem and to produce cold stress-tolerant cereals, various approaches have been considered to provide cold resistance to the crops. One such new yet promising approach involves the use of CRISPR/Cas technology. Scientists have first identified the genes playing an important role in helping the plants adapt to cold stress either by upregulation or downregulation of the gene expressed during stress. The role of the rice annexin gene *OsANN3* was studied by Shen et al., where the knockdown of the *OsANN3* gene was created in the Japonica rice variety. The study indicated that the rice annexin gene significantly decreased the ability of the plant to adapt to cold stress. Another study revealed that knockout of the proline-rich proteins (PRPs) can induce cold sensitivity in rice. Therefore, the *OsPRP1* gene could be exploited for improving cold tolerance in rice, and CRISPR/Cas9 technology is helpful to study the function of a gene by analysing the phenotypes of knockout mutants generated (Nawaz et al. 2019). CRISPR/Cas9 gene editing system was also used to produce Nipponbare variety of rice with higher yield and cold tolerance. The simultaneous CRISPR/Cas9 editing of the yield-related genes (*OsPIN5b* and *GS3*) and the cold stress-related gene (*OsMYB30*) induced high yield and cold tolerance in mutant rice lines (Zeng et al. 2020a). Since cereals, mainly rice, are sensitive to cold temperatures, improved desirable traits can be achieved by targeting and editing cold-responsive genes such as *COLD1* which confers chilling tolerance in rice (Ma et al. 2015c) and *WLT10* which encodes a member of the cereal-specific low-temperature-responsive/cold-responsive protein family in wheat (Ohno and Takumi 2015).

11.5.5 Heavy Metal Toxicity Stress Tolerance

Heavy metal contamination can be very harmful to human health. High concentrations of heavy metals such as cadmium (Cd), copper (Cu), lead (Pb), nickel (Ni), and arsenic (As) in the soil get absorbed by the plants which can cause many diseases including cancers, gastrointestinal disorders, and neurological problems. Heavy metal-contaminated soil and ground water enter into the plant system via transporters and membrane-bound ion channels (Roychoudhury et al. 2012). Many bacterial and plant genes have metal remediation effects which can be used to target plants via CRISPR/Cas technology. Therefore, genome editing techniques could be exploited to reduce the toxicity caused by heavy metals and can be utilized to edit genes responsible for the accumulation or transport of heavy metals in the plant system. A study revealed that *OsNramp5* which is a metal transporter gene could be knocked out using the CRISPR/Cas9-mediated mutagenesis in the male sterile line 638S and restorer line HZ of *indica* hybrid rice to develop Cd pollution-free *indica* rice variety lines. The high Cd accumulation trait of *indica* rice was modified, with no impairment in yield and main agronomic traits (Tang et al. 2017). Another study showed that antioxidant protein *OsATX1* reported to be a Cu chaperone found in *Oryza sativa* was knocked out using the CRISPR/Cas gene editing technique which reduced Cu concentration in roots but increased its accumulation in shoots, suggesting its role in aiding root-to-shoot Cu translocation and simultaneous redistribution from old leaves to developing tissues and seeds in rice (Zhang et al. 2018). Bioaccumulation of toxic arsenic (As) in rice poses a serious threat to human health (Wang et al. 2017a). The R2R3 MYB transcription factor OsARM1 (ARSENITE-

RESPONSIVE MYB1) regulates As tolerance in rice. Knockout of OsARM1 enabled As transport and improved tolerance, while its overexpression increased sensitivity to As. Therefore, CRISPR/Cas system can be utilized in a variety of ways by targeting and editing stress-responsive genes involved in the accumulation and transport of heavy metal in the plant system.

11.5.6 Herbicide Stress Tolerance

In a span of just a few years, the genomic editing approach has created an entirely new perspective for editing genes like acetolactate synthase (ALS) and 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) for generating herbicide resistance in plants. Many studies have reported specific mutations in these genes that can confer resistance to certain herbicides. Zinc finger nucleases (ZFNs) can be used to induce double-strand break at any target locus to modify endogenous genes in crop species like *Zea mays*. Shukla et al. (2009) in their study used zinc finger nucleases for precise genome modification to target the IPK1 gene (encodes inositol-1,3,4,5,6-pentakisphosphate 2-kinase) in maize. When the target locus IPK1 was disrupted, it led to herbicide tolerance along with variation in the profile of inositol phosphate leading to simultaneous phytate reduction in developing seeds. Acetolactate synthase (ALS) plays a key role in the biosynthesis of branched chain amino acids and is a major target for many agriculturally important herbicides. In an attempt to demonstrate the use of TALENs to create gene replacement in plants, Li et al. (2016c) targeted the rice acetolactate synthase gene (*OsALS*) to generate double point mutations using TALEN-mediated homologous recombination (HR) in the bispyribac-sodium (BS)-sensitive rice cultivar Kitaake. The HR-mediated herbicide-resistant rice lines displayed strong resistance towards bispyribac-sodium (BS), a pyrimidinyl carboxylate type herbicide. Moreover, these mutations were heritable to the T1 generation progeny and did not deviate from the morphological characteristics of the wild plants.

NHEJ has been exploited to create small insertions and/or deletions (indels) and thereby generate targeted gene knockouts in various organisms including plants. On the other hand, the homologous recombination process can generate precise modifications in the target gene by replacements and insertions using a homologous donor DNA but remains challenging (Gorbunova and Levy 1997; Kim and Kim 2014). In a study conducted by Li et al. (2016a), CRISPR/Cas9 system was used to introduce intron-mediated site-specific gene replacement and subsequent insertion in the rice endogenous gene 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) for generating glyphosate-resistant rice plants. A pair of sgRNAs was designed to target the C3 site in the first intron and the C5 site in the second intron of the *OsEPSPS* gene. Gene replacements were performed by targeting adjacent introns and a donor DNA template with the same pair of sgRNA sites, while gene insertions were obtained by targeting single intron and a donor DNA template within the same sgRNA site.

Sun et al. (2016) reported that CRISPR/Cas9-mediated homologous recombination can efficiently introduce multiple point mutations in the rice *ALS* gene. Kuang et al. (2020) in a proof-of-concept study developed novel herbicide-tolerant rice germplasms by using a novel technique of base-editing-mediated gene evolution (BEMGE). This process uses a Cas9n-based cytosine and adenine base editors along with a tiled sgRNA library to target any genomic locus for inducing mutations. A loss-of-function mutation of the rice *OsALS1* gene led to the development of tolerance towards the herbicide bispyribac-sodium. Moreover, a rice allele with a P171F substitution was successfully introduced into a commercial rice cultivar Nangeng 46 rendering this *OsALS1*-edited rice plant to tolerate high level of bispyribac-sodium. This study was conclusive of the fact that BEMGE is a robust tool with a potential for application into agricultural breeding programmes in the near future. Similarly, cytosine base editors (CBEs) were used to generate a series of missense mutations in two codons (P171 and/or G628) of *OsALS*. Different combinations of mutation exhibited diverse tolerance to a group of five herbicides, viz. bispyribac-sodium, flucarbazone-sodium, imazapic (IMP), nicosulfuron, and pyroxsulam. However, a triple mutant (P171F/G628E/G629S) displayed the highest tolerance to all five tested herbicides which establishes its potential to be used in rice breeding (Zhang et al. 2020).

In another study (Zhang et al. 2019c), base editing of acetolactate synthase (ALS) and acetyl-coenzyme A carboxylase genes of wheat induced tolerance to multiple herbicides, viz. sulfonylurea-, imidazolinone-, and aryloxyphenoxy propionate-type. They reported that the characteristic tolerant traits towards these new herbicides can have a great scope for weed management if introduced into elite wheat varieties. Furthermore, they established a selectable co-editing strategy to detect the edited plants by coupling a sgRNA of interest with the wheat ALS Pro-174 codon (TaALS-P174) sgRNA that was confirmed by resistance to nicosulfuron herbicide in vitro.

The ALS gene family comprises of the two genes, *ALS1* and *ALS2*, which were targeted for gene editing to confer resistance to sulfonylurea class herbicides. In a study conducted by Svitashev et al. (2015) in maize, when both *ALS1* and *ALS2* were targeted by sgRNA, biallelic knockout mutations led to the development of unstable edited lines. Subsequent editing by using different repair templates (double-stranded vector DNA and two single-stranded oligos) successfully resulted in editing of maize ALS2. However, the specificity of the ALS-CR4 gRNA in editing the maize ALS2 gene was conclusive of the fact that if there are nucleotide polymorphisms between different genes of the same family, single or multiple genes within a family may be targeted. In yet another report, CRISPR/Cas9-mediated knockout of the acetolactate synthase (*OsALS*) gene resulted in generating a novel herbicide-tolerant allele (G628W) due to a G-to-T transversion at position 1882 of *OsALS*. The mutant rice plants were conferred with a high level of herbicide tolerance against imazapic (IMP) and imazethapyr (IMT) (Wang et al. 2021). In another study, functional assessment of acetyl-CoA carboxylase (ACCase) variants in rice was screened by CRISPR-Cas9-mediated base editing (Liu et al. 2020). ACCase catalyzes the first step of fatty acid biosynthesis and its loss-of-function mutations can cause developmental errors or may be lethal to plants (Baud et al. 2004). As many as 141 sgRNAs of the rice *OsACC* gene were designed from the carboxyltransferase (CT) domain ACCase for the base editing screening (Liu et al. 2020). Such research can initiate immense scope to facilitate in planta CRISPR-Cas9-mediated base editing screens for crop improvement. Xu et al. (2020b) demonstrated the use of prime editing in rice for introducing a range of single or multiple nucleotide edits by targeting three rice genes, viz. *OsDEP1* (involved in nitrogen-use efficiency), *OsALS*, and *OsACC* genes (confers herbicide tolerance). Similar studies were conducted by Hua et al. (2020) by using the prime editing system to target the ALS and Aberrant Panicle Organization 1 (APO1) and by Xu et al. (2020a) to target the *ACC1*, *PDS1* (phytoene desaturase), and *WX1* (waxy) genes in rice. To overcome the difficulty of delivery of template DNA in plants, Shimatani et al. (2017) used a fusion of CRISPR-Cas9 and Target-AID (target-activation induced cytidine deaminase) to introduce point mutations into rice *ALS* gene for inducing herbicide (imazamox) resistance. The C287T gene mutation confers resistance to rice plants towards herbicide imazamox (IMZ). Target-AID is a base editing system that includes nuclease-deficient Cas9 (dCas9) or nickase CRISPR/Cas9 (nCas9) fused to *Petromyzon marinus* cytidine deaminase (PmCDA1)1, indicating that base editing could be a potential technology for crop improvement.

There has been only limited research on barley (*Hordeum vulgare* L.) with loss-of-function experiments for altered root morphology and cytokinin metabolism (Gasparis et al. 2019), increased starch, amylose, and beta-glucan content (Yang et al. 2020), and reduced tocopherol and tocotrienol content (Zeng et al. 2020b) by targeting *HvCKX1* and *HvCKX3*, *Hv d-Hordein*, and *HvHPT* and *HvHGGT* genes, respectively. However, with many abiotic stress-responsive genes reported in transgenic studies of barley, there is immense scope for using gene editing technologies for improvement of barley.

11.5.7 Anoxia/Hypoxia Stress Tolerance

Plants are dependent on oxygen for their growth and development. Free oxygen can act as a limiting factor for plant growth. The response of plants under oxygen limitations depends on the functioning of signalling molecules and transcriptional factors, which trigger signalling cascades at the cellular level for enhancing plant hypoxia/anoxia tolerance (Zahra et al. 2021). Reactive oxygen species (ROS) produced during anoxic/hypoxic conditions can trigger signal transduction in plants and thereby aid them in adapting to low oxygen conditions. ROS regulates the adaptation by developing certain kinds of structures such as lysigenous aerenchyma in shoots in rice (Colmer and Pedersen 2008) and lysigenous aerenchyma in roots of maize and wheat (Rajhi et al. 2011; Yamauchi et al. 2014). A study showed that the formation of these structures in plants was found to be induced by ethylene. A homolog of NADPH oxidase known as Respiratory Burst Oxidase Homolog (RBOHH) had a role in the formation of lysigenous aerenchyma in roots of rice. Knockout of RBOHH by CRISPR/Cas9 reduced ROS accumulation and induced aerenchyma formation in rice roots (Yamauchi et al. 2017). The expression of certain genes such as *TaMyb1 Triticum aestivum* Myb transcription factor 1 in wheat (Lee et al. 2007) can be used as a potential target for editing via CRISPR/Cas to produce anoxia-/hypoxia-tolerant cereals.

11.6 Challenges and Future Prospects of Genome Editing/CRISPR Technology in Mitigation of Abiotic Stress

Although CRISPR/Cas9-mediated genome editing has made significant strides in crop improvement, there are certain barriers to its adoption, and to boost its usability in cereals and other crops, these barriers must be cleared so as to endorse the successful deployment of GE technologies for crop genetic improvement with long-term prospects. Compiling pangenomes for crop improvement, using functional genomics to programmatically specify potential locations in the genome to be edited, developing highly efficient gene editing cargo delivery techniques, minimizing the likelihood of off-target editing, decoding novel mechanisms for reducing unintended modifications, and optimizing the Cas9 protein function are all part of this. One of the many challenges that the CRISPR-based genome editing technique confronts is transformation efficiency, which must be optimized for different cultivars. Although transformation methods have been developed for the majority of cereal crops, such processes are limited to particular genotypes. Genomic modification in the genetic background of high-yielding commercial varieties cannot be studied successfully in such a situation. There are other minor problems, such as the need for PAM, which may make genome editing difficult for genes missing the required PAM sequence. Another significant disadvantage of the CRISPR/Cas9 technology is its ineffective delivery mechanism for plant genome editing. The wrapping of Cas proteins into delivery vectors creates substantial barriers to efficient CRISPR/Cas apparatus delivery. Several novel cargo-vector systems with promising potential for successful delivery techniques, on the other hand, have been presented. For instance, carbon nanotubes have been utilized to deliver CRISPR/Cas9 editing complexes to plant leaves. Additional nano-structures, such as mesoporous silica nanoparticles and layered double hydroxides, with significant transformation efficiencies and minimal toxicity and cellular impairment, have the prospects to expand the availability of delivery systems. Delivery method optimization would be essential for more customized and more accurate genome editing (GE) techniques for crop improvement. Further, since there are many safety concerns connected with CRISPR/Cas9-derived bio-products, the probability of off-target consequences must be examined more rigorously. Off-target changes in plants and mutants are generally acceptable, and off-target effects may be determined and mitigated via segregation through many generations. Cas9 with a longer PAM sequence and sgRNA with higher sensitivity for the targeted area of DNA to be edited may help to reduce off-target effects in the future. Further attempts are expected in the future to overcome these limitations and extend the experimental freedom and applied skills of the CRISPR/Cas9 toolkit (Razzaq et al. 2019). Furthermore, polyploidy, which arises as a consequence of the acquisition of one or more entire sets of chromosomes, is one of the major challenges with successful genome editing in cereal crops (such as wheat and ryegrass). Autopolyploidy, allopolyploidy, and segmental allopolyploidy are the three forms of polyploidy. Polyploidy genomes are intricate in many aspects. They are challenging to sequence because of their repetitive regions and larger genome size. The increase in the number of gene copies, as well as the varied functions of duplicated genes created by neofunctionalization, makes functional annotation difficult. It is challenging to achieve desired mutations due to their complexities. Certain mutations, particularly knockdown or knockout of genes, may occasionally result in no subtle change in phenotype due to the dosage impact of alternate paralogous versions of genes. Aside from such obvious faults, the presence of many variations of genes makes editing any specific copy very challenging. On the other hand, occasionally desired trait manipulation requires altering all paralogs, which may decrease efficacy considerably (Braatz et al. 2017). Aside from that, the increasing use of genome editing technologies has created regulatory issues, since there is debate over whether or not genome-edited plants should be classified as GMOs. Governments across the globe take various methods to regulation, with some favouring a product-based strategy and others preferring a process-based one. As a consequence, there is a need for global collaboration on genome editing crop management to evaluate the potential hazards associated with it. Proactive conversations between academics, the general public, and regulatory authorities might improve the regulation and social acceptance of genome-altered crops.

11.7 Conclusion

Crop improvement has witnessed an entirely new dimension with the advancement in genome editing technologies like the use of mega nucleases, zinc finger nucleases, TALENs, and CRISPR/Cas. Since its inception in 2013, CRISPR/Cas genome editing system has been tested in many plants and is currently the most common tool for functional as well as agronomic studies. Despite being a faster, cost-efficient, and precise tool for achieving desired crop improvement, its applicability in cereals for mitigation of abiotic stresses is very less. However, to enhance its applicability in cereals crops, some challenges need to be addressed at the earliest, especially the optimization of transformation efficiency and regulation of laws for public acceptance of edited crops. With the budding intensive research in genome editing, its implementation in the field of agriculture is indicative of a revolution that has the potential for generating global food security.

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Abiotic Stress Tolerance in Cereals Through Genome Editing

12

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Abstract

Improving crop yield and developing new crop varieties are the top priorities in the twenty-first century due to its important agricultural and socioeconomic values. Recent revolutions in genome editing have paved the way to improve the desirable characteristics in various crops by targeting the related genes precisely. Clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR-associated protein (Cas) technology is a newly emerged, highly effective, and robust site-specific genome editing tool used for precise mutagenesis induction in various crop species. From comparison of this technology with two other currently used sequence-specific nuclease genome editing tools: transcription activator-like effector nucleases (TALENs) and zinc finger nucleases (ZFNs), we summarize that CRISPR-Cas technology has more potential to edit genomes efficiently. The availability of genome sequences for numerous crops, along with rapid advancements in genome editing techniques, has created opportunities to breed for almost any given desirable trait. CRISPR-Cas technology has recently been widely used to study the functions of several genes in different cereals including rice, wheat, and maize and to determine their potential in quality improvement. Designing of most efficient and accurate tools of CRIPSR has motivated and enabled researchers to modify cereal genes for improved traits. In addition to summarizing various experimental applications and successfully edited genes in cereals by single and multiplex gene editing

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_12

systems of CRISPR-Cas technology for enhancing abiotic stress tolerance, we also highlight the diversity of proteins and mechanisms in these systems. We also consider future challenges and potential directions for research developments in the era of CRISPR-Cas technology.

Keywords

Genome editing · CRISPR-Cas · Cereals · Abiotic stress · Genome engineering · Multiplex genome editing system

12.1 Introduction

For the human race, today's main issue is feeding an ever-increasing population of the world. The world's population is growing at a fast pace and is expected to reach ten billion by 2050 (Jaganathan et al. 2018). In order to meet this need, global food production must expand by 60–100% from current levels (Hunter et al. 2017). In addition to the growth in human population, the proportion of different abiotic stresses including heat, cold, drought, salinity, and herbicide stresses rises, which have negative impacts on plants' growth rate and their productivity (Khan et al. 2021a, b). Increased frequency of abiotic stresses has a major impact on plants from germination to maturity (Bhat et al. 2021). Abiotic stresses such as heat, cold, drought, salinity, and herbicides (acting singly or in combination) have arisen as a serious threat to the survival of plants (Paul and Roychoudhury 2019). Adaptations to stress (both structural and metabolic) are required for plant survival in such harsh conditions that are integrated into the endogenous developmental program towards restoring cellular homeostasis (He et al. 2018). Despite the fact that abiotic stresses have shaped plant evolution, they are also causing adverse changes and impairment of physiological, metabolic, and molecular processes with severe implications on plant growth and development, as well as a decrease in overall productivity (Boscai and Fita 2020). Environmental stress is a major issue in the twenty-first century and is an unpredictable constraint that exacerbates the difficulties in ensuring an adequate food supply for an ever-increasing human population (Nadeem et al. 2018). In such circumstances, crop varieties with greater growth adaptability under a variety of environmental conditions become essential. Though traditional breeding has been successful in reaching the goal to increase the production to a significant extent, it has the disadvantage of losing fitness and genetic diversity. Aside from being time-consuming, relying on natural allelic variants makes it inefficient for getting a desired characteristic and ensuring production sustainability (Wang et al. 2019). In the last two decades, both classical mutant breeding and molecular breeding have been utilized to boost crop productivity. Plant breeders have concentrated on enhancing selection efficiency in crop breeding programs through technologies such as high-throughput marker-assisted selection, precision phenomics-based selection, and genomic selection in order to generate better genetic improvement in terms of better yield potential in the shortest possible time. Underlying consumer preferences for better physical, nutritional, and cooking traits presents a challenge for researchers, and therefore increased focus on experiments based on genome editing could produce new varieties with premium qualities (Liu et al. 2021; Valverde-Arias et al. 2018). Advancement in the era of genomics brings many opportunities and solutions to help researchers to address these challenges. Now it is possible for researchers to identify and locate the genes underlying yield quality traits and to mine for mutagenesis in the alleles with genome information (Roychoudhury et al. 2011). One of the initial motivators and evoking interests for sequencing rice genome is that it can possibly be used as a model for other cereal genomes such as maize, wheat, and barley (Jackson 2016; Liu et al. 2018). Plant breeders have always attempted to envision an ideal haplotype; however, the chance of obtaining such an individual through "recombinational breeding" is remote due to unfavorable associations with a large number of such favorable alleles (Bhattacharya et al. 2020). Novel strategies are being investigated to overcome the limits of traditional breeding procedures in identifying and removing the roadblocks that cause chronic losses in agricultural output or altering crop plants to improve desirable features (Chen et al. 2019). Genome editing has been done for the last two decades with a variety of programmable and site-specific nuclease tools. Among them, transcription activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and the CRISPR-Cas9 system are among the most promising and well-studied ones (Li et al. 2020). A programmable DNA-binding domain confers sequence specificity in TALENs and ZFNs genome editing tools, while another customized domain of the FokI nuclease mediates the cleavage (Gaj et al. 2013). In contrast, in CRISPR-Cas9 system sequence specificity is conferred by a 20-nucleotide-long sequence in the chimeric gRNA and DNA cleavage is performed by the Cas9 nuclease (Khan et al. 2021a, b).

There are some potential advantages of CRISPR-Cas system over TALENs and ZNFs mutagenesis strategies which make CRISPR-Cas system the most promising and applicable editing tool (Table 12.1). The target specificity of the CRISPR-Cas system is based on ribonucleotide complex formation, and gRNAs can be easily and cheaply designed to target nearly any sequence in the genome specifically (Waryah et al. 2018). However, re-targeting TALENs and ZFNs requires protein engineering of the DNA-binding domain which is particularly challenging and difficult. CRISPR-Cas system with modified Cas protein and gRNA led to modification of chromosomal targets efficiently with low off-target mutations as compared to TALENs and ZFNs. Custom designing of ZFNs is considered difficult and labor intensive due to the complex nature of the interaction between DNA and zinc fingers (Maggio and Goncalves 2015; Turchiano et al. 2020). Another potential disadvantage of ZFNs is that target site selection is limited, thus imposing off-target cleavage (Gupta and Musunuru 2014). There are one-to-one recognition rules in TALENs between nucleotide sequences and protein repeats; therefore, it's easy and cheap to design and their construction has been robust and simplified by prominent and efficient DNA assembly tools such as Golden Gate cloning; however, TALENs are made up of highly conserved and repetitive sequences that can significantly promote homologous recombination in vivo. Moreover, TALENs are sensitive to

Table 12.1 Comparison of ZFNs, TALENs, and CRISPR genome editing approaches

Property	ZFNs	TALENs	CRISPR	References
DNA-recognition mechanism	Protein-DNA interactions	Protein-DNA interactions	RNA-guided protein-DNA interactions	Zhang et al. (2019a, b, c)
DNA-recognition site	18–36 nts	20–40 nts	22–44 nts (RNA guide + protein)	Porteus (2016)
Recognition module	Each ZF motif recognizes 3 bp	Each module recognizes 1 bp	PAM sequence	Rusk (2014)
Nuclease	Fok1	Fok1	Cas	Sakuma and Woltjen (2014)
Mechanism of DNA cleavage and repair	Double-strand break induced by FokI	Double-strand break induced by FokI	Single- or double-strand break induced by Cas	Char and Yang (2020)
Design	Challenging	Easy	Easy	Shan et al. (2013)
Work mode	Pair	Pair	No pair	Chen et al. (2016)
Efficiency	Variable	Moderate	High	Hui et al. (2018)
Off-target rate	High but variable	Low	High but controllable	Lee et al. (2016)
Multiplexing	Low	Moderate	High	Zhang et al. (2017a, b)
Cost	High	Moderate	Low	Chen et al. (2016)

cytosine methylation, especially at CpG dinucleotides, which is a common and well-known mechanism for DNA silencing, and in this situation TALEN pairs provide little to no mutagenesis activity (Tsuji and Imanishi 2019). The most desirable feature of CRISPR-Cas editing tool is its multiplexed mutation property as by using this system, mutations can be introduced into multiple genes at the same time by injecting multiple modified gRNAs into multiple genes in parallel to the target sites in the same cell (Cobb et al. 2015). We concentrated our efforts in the current work on food security and abiotic stress, with a special focus on recent advancements in CRISPR-Cas-mediated crop plant editing such as rice, wheat, and maize to solve the phenomenological quandary of different abiotic stresses.

12.2 CRISPR-Cas System for Plant Genome Editing

CRSIPR-Cas technology shows great promise to introduce an accurate and safer change in the genome by introducing, replacing, or removing a DNA sequence. This technology accelerated the development of plant biotechnology as it has been used as a key source for trait improvement and to develop novel plant varieties (Razzaq et al. 2019). Programmable nucleases are used in CRISPR-Cas technology to generate single-strand breaks (Cas9 nickase) (Osakabe and Osakabe 2015) or double-strand breaks (DSBs) at target region precisely and in a predictable manner (Arora and Narula 2017). The breaks can subsequently be repaired by either the non-homologous end-joining (NHEJ) or the homology-directed repair (HDR) pathways or both NHEJ and HDR (Yeh et al. 2019). NHEJ is a pathway that repairs DSB existing in all types of cells at different points in the cell cycle that first modifies the two broken ends and then ligates them together leading to insertions or deletions (indels) at the cut site of the DNA molecule (Zhou et al. 2017). NHEJ repair pathway usually induces mutations or generates null alleles by frame-shifting which leads to knockout gene function and therefore widely employed in the generation of loss-of-function mutants (Jin et al. 2019). HDR is a template-dependent pathway occurring only in the S and G2 phases of cell mitosis for DSB repair which has been clearly exploited in many species to insert non-native genes of interest or for single-nucleotide substitutions at a target locus. To guide the DNA repair HDR utilized a DNA donor repair template (DRT) which is flanked with sequences homologous to those adjacent to the DSBs. HDR can be utilized for replacement of targeted allele or the insertion of markers to specific loci, making it a valuable tool for crop improvement (Jayavaradhan et al. 2019; Li et al. 2018). CRISPR-Cas system is used widely as a novel approach for captivated genome editing, due to its flexibility and ease (Tang et al. 2016).

12.3 Application and Potential of CRISPR-Cas in Crop Improvement

Cereals are the most important food source on the planet. Yield is a universally important characteristic of cereals. Yield is a quantitative trait that is determined by the number of traits such as the number of panicles or tillers, grain number per panicle, and grain weight (Paul et al. 2020). The CRISPR-Cas system has become an enormously important genome editing tool in agricultural research and its area of utilization and impact increases drastically every day. During the last two decades, the CRISPR-Cas genome editing technology has been utilized effectively to create new crop types with enriched characteristics such as improved yield, stress tolerance, nutritional enhancement, and disease resistance. The CRISPR-Cas genome editing system is a popular and powerful tool for targeted genetic mutagenesis. In the past few years, this technology has revolutionized the precise genetic manipulation. It has been widely used to study functions of various genes and to breed rice and other crops accurately, due to its simplicity, consistency, flexibility, and high efficiency (El-Mounadi et al. 2020). In the last two decades, significant progress has been made in improving and optimizing the CRISPR-Cas systems in cereal crops, particularly for targeted mutagenesis in desired genes (Mishra et al. 2018). This method might potentially be used to successfully integrate non-native genes into cereal crop genomes. Homology-directed repair (HDR) mechanism allows non-native genes to be inserted into a safe locus, such as intergenic regions (IGR), where there is negligible effect on the neighboring genes (Rozov et al. 2019). As genome editing research is still in its early stages, current efforts are being made to increase the effectiveness of the CRISPR-Cas technology. Over the last decade, research efforts have concentrated on editing endogenous agricultural genes that have previously been extensively characterized and are easy to locate. It was observed that in the majority of the studies, the mutations produced by selecting a region for editing in the crops' genomes were bi-allelic in nature. The changes created by the CRISPR-Cas system were then seen and evaluated to be stable in subsequent generations of the altered crops, showing that crops can be edited effectively using CRISPR-Cas technology.

In this chapter, we summarize those genes which have been edited in the last decade for improved cereal crop qualities with abiotic stress tolerance using CRISPR-Cas technology. We also speculate on the high profitability and future uses of CRISPR-Cas technologies for genome editing in cereal crops.

12.3.1 Application of CRISPR-Cas Technology for Enhancing Abiotic Stress Tolerance in Cereals

Every day, new research in the innovative era of CRISPR-Cas technology predicted that it will improve all the desired characteristics in major crops in a tremendous way. It can be used to manipulate crops genetically for dense nutrition, resistant to drier and hot weather, salinity, and drought conditions. CRISPR-Cas9 genome editing has been used successfully in a variety of crops, including rice, wheat, and maize (Haque et al. 2018). However, there are few studies on the targeting of abiotic stress tolerance genes. This is due to the fact that previous research has concentrated on biotic stresses like diseases and insect pests. CRISPR-Cas technology has been successfully optimized to introduce multiple genes in different crops such as in rice, wheat, and maize (Mishra et al. 2018). Table 12.2 contains a few recent examples of how the CRISPR-Cas technology has been used to identify the functioning of a target gene as well as conferring abiotic stress tolerance in cereal plants.

12.3.1.1 CRISPR-Cas Genome Engineering in Cereal Crops for Heat Stress Tolerance

Temperature increases of 5–15 °C over the usual range are referred to as heat shock or heat stress (Fragkostefanakis et al. 2015). It is a major abiotic stress that damages cellular structure and affects various metabolic pathways especially those relating to plant development, morphology, and production (Bita and Gerats 2013). As part of the plant defense, genes encoding heat shock proteins, scavenger proteins, enzymes for synthesis of reactive oxygen species (ROS), and enzymes that activate the accumulation of metabolites that are essential for cellular activity are denatured in response to varying degrees and durations of heat stress (Bhat et al. 2021). Therefore, it is important to address heat stress using biotechnological techniques. Genetic engineering for high temperature tolerance in plants can be accomplished by overexpressing heat shock protein genes or changing the level of heat shock

Table 12.2 Genes targeted by the CRISPR-Cas system to confer resistance to abiotic stresses

Crop	Stress	Target gene	Description of functions	Reference(s)
Rice (*Oryza sativa*)	High/low temperature	COLD1	COLD1 works as a sensor for cold signal. It triggers calcium influx together with RGA1 protein by receiving the chilling signal and leads to activation of cold-responsive genes	Dong et al. (2019)
		OsNAC006	It is a transcription factor in rice; by knocking out this gene via CRISPR-Cas9 system, it is characterized that this gene has a key role in drought and heat stress tolerance	Wang et al. (2020)
		OsMYB30	Mutant of *Osmyb30* gene produced via CRISPR-Cas technology exhibits enhanced cold tolerance	Zeng et al. (2020)
		Ann3	Mutated rice cultivar with OsAnn3 gene using CRISPR-Cas technology showed enhanced cold tolerance	Shen et al. (2017)
		OsWRKY11	Overexpression of the *OsWRKY11* gene in rice seedlings enhances heat and drought resistance	Wu et al. (2009)
		Hv-WRKY38	In transgenic rice, the transcription factor Hv-WRKY38 confers cold and drought resistance	Mare et al. (2004)
		ICE1	ICE1 regulates the cold-induced transcriptome. Its overexpression gives transgenic rice with cold tolerance	Zuo et al. (2019)
		$\mathrm{H_{SP}}$70	$\mathrm{H_{SP}}$70 is involved in a variety of cellular processes and predominantly expressed as forms of chaperon and co-chaperon under heat stress conditions to confer high temperature tolerance	Shanmugavadivel et al. (2019), Truman (2018)

(continued)

Table 12.2 (continued)

Crop	Stress	Target gene	Description of functions	Reference(s)
		TamiR159	Plants that overexpressed TamiR159 were more sensitive to heat stress than wild-type plants	Ni et al. (2018)
Wheat (*Triticum aestivum*)		TaPEPKR2	Its overexpression conferred heat stress tolerance both in wheat and *Arabidopsis*	Zhang et al. (2018)
		TaHsfA6f	Overexpressing of TaHsfA6f gene enhances thermotolerance in transgenic wheat	Xue et al. (2015)
		TaFER-5B	Heat tolerance is increased by overexpression of the wheat ferritin gene TaFER-5B	Zang et al. (2017)
		TaGASR1	Wheat plants that overexpress TaGASR1 gene are more resistant to heat and oxidative stress	Zhang et al. (2017a, b)
		TaHsfC2a	This gene functions as a transcriptional activator and promotes heat stress tolerance by modulating ABA signaling	Hu et al. (2018)
Rice (*O. sativa*)	Drought stress	SRL1, SRL2	CRISPR-Cas9-based mutagenesis was used to create rolled leaf mutant plants by knocking out the SRL1 and SRL2 genes, which displayed drought tolerance phenotypes	Liao et al. (2019)
		OsNAC14	Drought tolerance is mediated by OsNAC14, which recruits components involved in DNA damage repair and defense response, leading to enhanced drought tolerance	Shim et al. (2018)
		SAPK2	Through a CRISPR-Cas method, mutant lines (sapk2) were generated in rice that were more susceptible to reactive oxygen species (ROS) and drought stress than wild-type plants	Lou et al. (2018)

(continued)

Table 12.2 (continued)

Crop	Stress	Target gene	Description of functions	Reference(s)
		OsWRKY30	Overexpression of OsWRKY30 in rice significantly improves drought resistance	Chen et al. (2017)
		OsWRKY80	Drought-induced senescence might enhance the expression of OsWRKY80 which is thought to be regulated by amino acid adenosine monophosphate (ABA)	Ricachenevsky et al. (2010)
		OsDREB	To improve drought tolerance in rice, CRISPR-Cas technology was employed to edit the drought-responsive *OsDREB* gene	Bhat et al. (2021)
		OsGRAS23	In comparison to wild-type, rice plants with overexpressed *OsGRAS23* gene showed enhanced drought resistance, oxidative stress tolerance, and lower accumulation of H_2O_2	Xu et al. (2015a, b)
		OsPYL9	OsPYL9 mutants produced by CRISPR-Cas9 have the potential to improve both drought tolerance and rice yield	Usman et al. (2020)
		OsSKIPa	Its expression is induced during drought stress and has a considerably enhanced reactive oxygen species-scavenging capacity	Hou et al. (2009), Jiang et al. (2021)
Wheat (*Triticum aestivum*)		TaDREB2, TaERF3	Drought tolerance in wheat was enhanced by CRISPR-Cas editing for dehydration responsive element binding protein 2 (TaDREB2) and ethylene responsive factor 3 (TaERF3)	Kim et al. (2018)
Maize (*Zea mays*)		ARGOS8	This gene's CRISPR-Cas9-altered plants have improved drought tolerance	Shi et al. (2017)

(continued)

Table 12.2 (continued)

Crop	Stress	Target gene	Description of functions	Reference(s)
		ZmWRKY40	The CRISPR-Cas9 technology was used to investigate the potential of ZmWRKY40 genes in maize. The resulting lines demonstrated increased drought resistance	Wang et al. (2018a, b)
Rice (*O. sativa*)	Salinity stress	OsGTγ-2	A trihelix transcription factor involved to confer salinity tolerance	Liu et al. (2020)
		OsBBS1	In rice, a multifunctional gene called *OsBBS1* was discovered to be involved in salt sensitivity and early leaf senescence	Zeng et al. (2018)
		OsRR22	The *OsRR22* gene was altered using CRISPR-Cas9 technology, demonstrating that this gene has the potential to increase salt resistance in rice	Zhang et al. (2019a, b, c)
		OsERF922	OsERF922-overexpressing lines had decreased tolerance to salt stress and a higher Na^+/K^+ ratio in the shoots	Wang et al. (2016)
		OsPQT3	*OsPQT3* knockout mutants (ospqt3) were found to be more resistant to oxidative and salt stress	Alfatih et al. (2020)
		OsRAV2	OsRAV2 was successfully altered using CRISPR-Cas9, and its expression analysis indicated that it has the potential to help rice plants survive in high salt concentrations	Duan et al. (2016)
		DST	It is a transcription factor with a zinc finger. This gene mutation produces rice cultivars with improved drought and salt tolerance	Ganie et al. (2021)

(continued)

Table 12.2 (continued)

Crop	Stress	Target gene	Description of functions	Reference(s)
		NADPH	The knocking out of the coding sequences of NADPH oxidase by CRISPR-Cas9 resulted in a salt-sensitive phenotype	Prior et al. (2016)
		OsNAC041	The CRISPR-Cas9 technique was used to create a targeted *osnac041* mutant, which was found to enhance seed germination under salt stress	Bo et al. (2019)
		FLN2	FLN2 knockout lines were created using CRISPR-Cas9 gene editing. The results showed that FLN2 is involved in salinity stress tolerance because the mutant lines were hypersensitive to stress	Chen et al. (2020)
		OsDOF15	A transcription factor that regulates cell elongation and controls primary root elongation under salt stress via ethylene biosynthesis	Khan et al. (2021a, b)
		SPL10	It is transcription factor that has a negative impact on salt tolerance	Gao et al. (2018)
		SnRK2	On expression, SnRK2s act as key regulators of ABA signaling, mediating salt tolerance in plants	Lou et al. (2017)
		OsOTS1	CRISPR-edited lines of *OsOTS1* exhibit increased salt sensitivity with decreased root and shoot biomass, showing that this gene plays an important role in salt stress tolerance in rice	Zhang et al. (2019a, b, c)
		OsRMC	OsRMC encodes a receptor-like kinase that has been described as a negative regulator of rice salt stress responses	Serra et al. (2013)

(continued)

Table 12.2 (continued)

Crop	Stress	Target gene	Description of functions	Reference(s)
		SAPK1	In rice plants, SAPK1 acts as an ABA pathway regulator and activated in response to salt stress	Lou et al. (2018)
		OsMIR528	CRISPR-Cas9 genome editing demonstrates that constitutive expression of rice microRNA528 improves plant growth and increases salt tolerance	Khan et al. (2021a, b)
Maize (*Z. mays*)		HKT1	It is a high-affinity potassium transporter that regulates Na^+ entrance into plant roots	Khan et al. (2020)
Maize (*Z. mays*)	Multiple stress tolerance	PSY2	Under salt stress, PSY2 expression is increased via the ABA signaling pathway	Oleszkiewicz et al. (2021)
Rice (*O. sativa*)		ERF genes	In rice plants, the genes *OsBIERF1*, *OsBIERF3*, and *OsBIERF4* were edited. The mutant plants grew better in different types of abiotic stressors	Wang et al. (2016)
		OsAOX1a, OsAOX1b, OsAOX1c	These are breeding stress markers which improve tolerance to frost, drought, and salinity	Khan et al. (2021a, b), Xu et al. (2015a, b)
		OsSRFP1	OsSRFP1 could be a promising engineering target for developing rice plants with enhanced stress tolerance using genome editing techniques such as CRISPR technology	Fang et al. (2015)
Rice (*O. sativa*)	Herbicide resistance	OsALS	Synthesize acetolactate to confer herbicide resistance	Butt et al. (2017)
		BEL	CRISPR-Cas9-mediated genome editing may be efficiently used to create herbicide tolerance within rice cultivars by altering BEL	Xu et al. (2014)

(continued)

Table 12.2 (continued)

Crop	Stress	Target gene	Description of functions	Reference(s)
Wheat (*T. aestivum*)		TaDEP1, TaGW2	Through CRISPR-Cas mutagenesis was used to generate wheat mutants of *TaDEP1* and *TaGW2* genes to enhance herbicide resistance	Mishra et al. (2020)
Maize (*Z. mays*)		ALS genes	The *acetolactate synthase* genes (*ALS1* and *ALS2*) in maize were the primary targets of the CRISPR-Cas system in developing herbicide-resistant crop variants	Endo et al. (2016)
		MS26, MS45	Targeted mutagenesis using the modified I-CreI homing endonuclease or the CRISPR-Cas9 system resulted in the creation of new ms26 and ms45 male sterile lines	Chen et al. (2018)
		LIG1	Herbicide-resistant maize has been conferred by CRISPR-Cas9-mediated knock-in and replacement in the *liguleless1* (*LIG1*) gene	Hussain et al. (2018)
		EPSPS	The enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) has a conserved motif in all plants that serves as the binding site for its substrate pyruvate. To generate herbicide tolerance, the second exon of EPSPS is changed in rice using CRISPR/Cas9	Dong et al. (2017a, b)

transcription factor, as well as increasing osmolyte levels. Heat stress tolerance in cereals is a quantitative characteristic that is regulated by a number of genes (Ni et al. 2018). The CRISPR-Cas system has proven to be the most effective method genome editing approach for modifying these genes required for the creation of thermotolerant crops (Biswal et al. 2019). Some of the genes that confer heat tolerance in wheat include TamiR159 (Ni et al. 2018), TaPEPKR2 (Zang et al. 2018), TaHsfA6f (Xue et al. 2015), TaFER-5B (Zang et al. 2017), and TaHsfC2a (Hu et al. 2018).

12.3.1.2 CRISPR-Cas Genome Engineering in Cereal Crops for Cold Stress Tolerance

Chilling (0–20 °C) or freezing (0 °C or low) cold temperature can disrupt a regular metabolic functioning of plants. Plants are sensitive to both high and low temperatures (Yadav 2010). Rice and maize are both susceptible to cold stress. In plants rapid wilting of leaves, followed by the formation of sunken pits, which resulted in patches of necrosis on the lead surface of tissues is a very common symptom of cold stress. When plants are exposed to cold temperatures, water stress is the most common symptom, which occurs when leaf water drops owing to a decrease in root hydraulic conductivity, resulting in stunted development (Parkash and Singh 2020). Several significant cold stress resistance genes in rice were discovered as a result of these comprehensive studies. Some genes which have been verified for cold tolerance after being targeted for mutagenesis using the CRISPR-Cas method include COLD1 (Dong et al. 2019), OsMYB30 (Zeng et al. 2020), Ann3 (Shen et al. 2017), OsWRKY11 (Chen et al. 2017), Hv-WRKY38 (Mare et al. 2004), ICE1 (Zuo et al. 2019), and $H_{SP}70$ (Truman 2018).

12.3.1.3 CRISPR-Cas Genome Engineering in Cereal Crops for Drought Stress Tolerance

Drought is a significant abiotic stress which poses a serious threat to plant survival (Kapoor et al. 2020). It arises over time as a result of changes in the global climate and reduces plant production by affecting plant growth in various stages of life cycle of a plant from germination to maturity. Drought occurs often in rain-fed environments at any stage of plant development, resulting in reduced crop yields (Raza et al. 2019). However, progress in genetically improving crops for drought tolerance using traditional methods has been sluggish. Drought tolerance QTLs have been identified in major crops such as in rice (Sahebi et al. 2018). The CRISPR-Cas9 method is being used to enhance the drought tolerance of major crops. For example, Lou et al. (2018) used CRISPR-Cas9 to create loss-of-function mutants of OsSAPK2 in rice. The *sapk2* mutant lines were more susceptible to dehydration and reactive oxygen species (ROS) than the wild-type plants. According to phenotypic and expression analyses, the mutated plants exhibited higher survival and gene expression rate under drought stress than the control plants. CRISPR-Cas was utilized in rice to improve drought resistance by editing *OsDREB* gene, which is a dehydration-responsive element (Bhat et al. 2021). *OsPYL9* mutants created by CRISPR-Cas9 have the potential to show high tolerance to drought resistance and to improve rice yield (Usman et al. 2020). Drought-tolerant indica mega rice cultivar (MTU1010) dst mutant produced by CRISPR-Cas9 mediating editing has wider leaves with decreased stomatal density, which contributes to improved leaf water retention in drought-stressed conditions (Ganie et al. 2021). CRISPR-Cas edited ethylene responsive factor 3 (TaERF3) and drought tolerance dehydration responsive element binding protein 2 (TaDREB2) in wheat have improved drought stress tolerance (Kim et al. 2018). The interruption of the DNA ligase-IV gene prior to CRISPR-Cas-mediated gene targeting (GT) by *Agrobacterium*-mediated transformation improves GT efficiency and resulted in the generation of bi-allelic mutants with a high *ALS* gene frequency (Endo et al. 2016). As maize is mostly cultivated using dryland farming methods, drought-tolerant cultivars must be developed (Mashingaidze 2006). The ethylene response factor ARGOS8 was edited using CRISPR-Cas9 technology that resulted in a drought-tolerant maize cultivar. ARGOS8 transcript levels were much higher in the mutant ARGOS8-v1 and ARGOS8-v2 lines than in the wild plants. Furthermore, the ARGOS8 varieties demonstrated a significant increase in grain production in drought circumstances while retaining yield at optimum growth conditions (Shi et al. 2017). Abiotic stress-related response of *WRKY* gene family in rice and maize was studied. The CRISPR-Cas system was used to investigate the potential of many *WRKY* genes, including *OsWRKY11*, *OsWRKY30*, and *OsWRKY80* in rice and *ZmWRKY40* in maize. The resulting lines were found to be drought tolerant (Bhat et al. 2021; Phukan et al. 2016; Shahzad et al. 2021; Wang et al. 2018a, b).

12.3.1.4 CRISPR-Cas Genome Engineering in Cereal Crops for Salinity Stress Tolerance

Every day, salinization causes a productivity loss of 2000 hectares of arable land worldwide (Shahid et al. 2018). The main sources of salinity are natural phenomena such as water evaporation and transpiration or seawater drift, which lead to salt accumulation and precipitation in the soil. Accumulated salts disrupt the root system's nutrient absorption and have an impact on plant development. Human interventions such as fertilizer use, over-irrigation, and irrigation of salty water all contribute to salinity. Several efforts have been made to increase crop production under high salt conditions using traditional breeding and transgenic approaches (Khan et al. 2021a, b).

The CRISPR-Cas genome editing technique was used to identify the mechanism of salt tolerance in rice (Farhat et al. 2019). Several rice genes have been edited with CRISPR-Cas technology to confirm their role in salt tolerance, but further research is needed to understand their precise functions and functional mechanisms. For example, the CRISPR-Cas9 method was utilized to create *OsBBS1* (a multifunctional gene) rice mutants that are resistant to induced salt stress (Zeng et al. 2018). Furthermore, the *OsMIR528* gene was found to be a salt stress positive regulator (Khan et al. 2021a, b). OsRAV2 was successfully altered using CRISPR-Cas9, and expression analysis revealed that mutated lines adopt resistance to high salt concentrations (Duan et al. 2016). Loss-of-function mutations of SnRK2 (Lou et al. 2017) and osmotic stress/ABA-activated protein kinases (SAPK1 and SAPK2 genes) (Lou et al. 2018) induced by CRISPR-Cas system conferred salinity resistance in rice. The *OsRR22* gene was altered using CRISPR-Cas9 technology, demonstrating that this gene has the potential to increase salt resistance in rice (Zhang et al. 2019a, b, c). Using the CRISPR-Cas9 technique, a targeted osnac041 mutant was created, with greater plant height and tolerance to salty environments than the wild type (Bo et al. 2019). OsGT-2 is a transcription factor with trihelixes; its CRISPR-Cas9-based studies show that it is involved in salinity adaptation (Liu et al. 2020). Researches indicate that modifying or knocking out the *OsERF922* gene using CRISPR-Cas9 is an effective way to improve blast resistance in rice (Wang et al. 2016). CRISPR-Cas9 was utilized to create dst mutant alleles in rice, which resulted in improved drought- and salt-tolerant cultivars (Kumar et al. 2020). CRISPR-Cas technology with multiplexing and co-targeting of many loci at the same time might be utilized to produce new rice varieties by knocking out whole cassettes of unwanted genes that hinder or may cause problems in rice development in salty environments (Arora and Narula 2017). For example, utilizing a multiplex method of the CRISPR system, a transcription factor OsRMC that negatively regulates salt stress is knocked out to improve salinity tolerance in rice (Serra et al. 2013), while this technique is used to achieve allelic variation in many transporters, such as HKT, which is involved in reducing Na^+ accumulation in rice to cope with salt stress (Khan et al. 2020). CRISPR-Cas9-mediated knockout of the coding region of NADPH oxidase resulted in decreased root apex H_2O_2 and K^+ levels, as well as GRF12, AHA1, and HAK5 expression, resulting in a salt-sensitive phenotype (Prior et al. 2016). MicroRNAs (miRNAs) play an important regulatory function in plants, and a few miRNAs have been shown to be involved in the salt stress tolerance process (Gao et al. 2018). These negatively regulating miRNAs have the potential to be used in gene editing to improve salt tolerance. For example, CRISPR-Cas9 system was used to edit highly conserved miRNAs, i.e., miR393a and miR396c, to confer salt tolerance in rice (Chaudhary et al. 2021).

12.3.1.5 CRISPR-Cas Genome Engineering in Cereal Crops for Herbicide Stress Tolerance

Herbicides are chemicals that are used to control weeds that grow alongside crop plants. Weeds that are resistant to herbicides might have a detrimental impact on crop plant development in some circumstances. It is essential to eliminate weed since their presence reduces crop yield, which in turn affects human nutrition. Using CRISPR-Cas genome editing technology, various genes have been inserted or deleted to establish herbicide stress tolerance in cereal crops (Dong et al. 2021). Point mutations caused by nuclease-deficient Cas9 (dCas9) or nickase CRISPR-Cas9 (nCas9) variant coupled to *Petromyzon marinus* cytidine deaminase (PmCDA1) resulted in the creation of herbicide-resistant rice mutant lines (Mishra et al. 2020). Genes responsible for acetolactate synthase (ALS1 and ALS2) are significant targets of genome editing to create chlorsulfuron-resistant crop cultivars. CRISPR-Cas was used to target the *ALS1* and *ALS2* genes in maize to develop herbicide-tolerant mutant lines (Svitashev et al. 2015). Moreover, maize plants with resistance to bispyribac-sodium herbicide are produced by combining two gRNAs and a repair template (Hussain et al. 2021). To produce herbicide-resistant rice cultivars, NHEJ-based intron targeting using chimeric sgRNAs (Butt et al. 2017), two separate sgRNAs targeting the repair template (Sun et al. 2016), and disruption of DNA ligase-IV implicated in NHEJ repair (Alexander et al. 2016) are used. In addition, CRISPR-Cas in combination with single-stranded oligonucleotides produces herbicide resistance in flax (Sauer et al. 2016). CRISPR-Cas9-mediated gene editing has recently been shown to be an effective method for introducing herbicide resistance into rice cultivars by editing BEL (Xu et al. 2014), EPSPS

(Dong et al. 2017a, b), OsALS (Butt et al. 2017), OsAOX1a, OsAOX1b, and OsAOX1c (Xu et al. 2015a, b).

12.4 Development of Multiplex Genome Editing Systems

Many researchers have effectively used CRISPR-Cas9 and other orthologs of this system, such as SaCas9 and StCas9 in rice, to perform single gene editing. However, in many situations, many genes must be targeted at the same time. CRISPR-Cas systems, which are based on Gibson Assembly or Golden Gate ligation, have been designed and developed to target numerous genes with homologous sequences utilizing just one or two nucleases (Xu and Li 2020). Due to the limited effectiveness of co-delivery, popular plant genetic transformation techniques such as *Agrobacterium*-mediated transformation and biolistic bombardment are seldom utilized for co-transformation of multiple vectors. To achieve high-efficiency multiplex targeting, several sgRNAs must be co-expressed within a single construct. Mao et al. (2019) developed a system that could integrate several gRNAs into a single vector at the same time using the isocaudomer method. These gRNAs designed by intermediate vectors were then integrated into a binary vector such as pC1300-Cas9 and pRGEB32 to form the final binary vector of enormous potential that has been successfully used to create multiple mutants of rice cultivar at once (Lacchini et al. 2020). In addition, researchers have developed several techniques for co-expressing Cas9 protein and gRNAs from a single Pol II promoter (Jun et al. 2019). PolyA sequences were utilized as a linker to co-express the Cas9 protein and gRNA cassettes from a Pol II promoter. Multiple gRNAs were released using a self-cleaving hammerhead ribozyme (RZ). Currently for simultaneous targeting of multiplex endogenous genes, another approach which is based on the mechanism of endogenous mRNA splicing and tRNA processing has been established (Wang et al. 2018a, b). To produce numerous gRNA expression cassettes, the polycistronic tRNA-gRNA (PTG) was integrated into the intron of Cas9. Genome editing has been lauded properly using this multiplex method, since a number of genes have been effectively targeted in recent times (Dong et al. 2017a, b; Oh et al. 2020).

12.5 Conclusions and Future Prospects

Abiotic stressors have severe consequences on plant growth and development in the face of increasing challenges posed by climate change. With severe concerns regarding yield reductions, it frequently fails to ensure food security for the world population. In such situations, it becomes critical to implement strategic policies aimed at achieving agricultural sustainability in order to meet the expanding food needs of human population. The aforementioned issues have prompted the scientific community to seek solutions, such as creating improved cultivars through traditional breeding procedures or utilizing advanced ways of altering the genome for desirable characteristics that can assure agricultural sustainability. Conventional breeding methods improve progeny for desired qualities by assigning natural phenotypic and agronomic features to them. However, conventional breeding techniques are inadequate having two shortcomings: (1) the usage of marker genes (MG) during transformation process and (2) inefficient transgenic transformation. The genome engineering techniques have overcome the obstacles faced by traditional breeding procedures, which resulted in the transition from domestication to a settled agricultural production system. As progress continues in the era of CRISPR-Cas technology, which has the ability to bring a new revolution in functional genomic research, which will demonstrate the potential future of agriculture in crops for sustainable production, CRISPR-Cas technology has the potential to not only alter current crop species but also to generate new varieties for production of specialized chemicals and biomaterials (Hsu et al. 2014; Zaidi et al. 2020). Applications of this technology enable targeted mutagenesis in the genomes and improved transformation of agricultural species for higher yield. This method might be used in crop plants to improve nutritional value, insect resistance, and adaptability to changing climates. Plant biotechnologists use the CRISPR-Cas system as a genome editing technique to create agricultural plants with excellent root systems capable of capturing unevenly distributed water and nutrient resources under climatic instability and resource shortages (Khatodia et al. 2016). CRISPR-Cas technology, which is capable of multiplexing and targeting several loci at the same time, might be used to knock out whole gene cassettes of undesirable genes that inhibit or may create trouble in rice growth (Tang et al. 2017; Yin et al. 2017). Large inheritable chromosomal deletions have been found, which may be used to delete whole gene clusters, allowing for the functional investigation of previously unknown genes, which may aid in the introduction of novel characteristics (Zhou et al. 2014). Exons can be replaced with exons of choice via the NHEJ repair process by targeting the introns next to them and inserting a changed sequence to take their place. This approach of intron targeting may result in rice with better characteristics and qualities (Li et al. 2016). Development and improvement in CRISPR-Cas system has resulted in the creation and use of new Cas9 variant proteins such as Cas9-VQR, Cpf1-RR, Cas9-VRER, SaCas9, Cpf1-RVR and many more similar proteins, which has considerably expanded the genome editing range (Jun et al. 2019). Other optimization methods, including the use of an endogenous promoter and the enhancement of sgRNA expression elements, have substantially increased the editing effectiveness of the rice CRISPR-Cas9 system. Furthermore, numerous studies have demonstrated that the CRISPR-Cas technology's off-target effect is generally controlled, with the emergence of Cpf1 which has lower off-target activity on the rice genome than Cas9 (Mei et al. 2016; Xie et al. 2015).

The emergence of advanced CRISPR-Cas-based genetic operating systems, such as gene insertion, deletion, replacement, and transcriptional control, will revolutionize both fundamental and applied crop breeding research (Zhang et al. 2018). However, still there are several pressing issues for rice genome editing, such as efficiently delivering the CRISPR-Cas system without incorporating it into the rice genome. The ability to precisely knock in and replace endogenous genes via HDR repair is relatively limited (Jun et al. 2019). In conclusion, this approach is becoming more accurate and efficient with the passage of time. Future prospects of the CRISPR-Cas technology, such as inducible Cas9 expression and direct Cas9 protein delivery, are being investigated in many species and different cell types by biologists across the world. By expressing the Cas9/sgRNA only when needed, these new improvements can help to avoid the boosting of off-target effects.

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Varietal Physiology, Metabolic Regulation, and Molecular Responses of Rice Genotypes to Diverse Environmental Stresses

13

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Abstract

Rice, the world's second largest cereal grain, is a staple meal for more than half of the world's population. The unprecedented growth of the world's population has compelled mankind to expand food production. While rice yields have improved due to modern technologies, the current average yield is still 10–15% below potential. Rice plants are highly susceptible to different abiotic stresses. Nutrient deficiency, heavy metals, salinity, heat, and drought are all abiotic stresses that contribute to the dramatic decline in rice production under existing climatic changes. Plants respond to various environmental pressures by a sequence of biochemical and molecular modifications that are coordinated by a variety of phytohormones. Since plants cannot escape abiotic stress by shifting, they have evolved a variety of pathways for stress tolerance. Plants undergo diverse changes in biochemical mechanisms to better tolerate abiotic stresses. For instance, plants accumulate different osmolytes as a stress response, including inositol, mannitol, sorbitol, trehalose, and glycine betaine which carry out osmotic adjustment under osmotic stress. Besides their role in osmotic adjustment, these organic compounds are involved in stabilizing proteins, reducing ions toxicity, preserving membrane integrity, scavenging reactive oxygen species, shielding cellular parts, balancing cellular redox, and strengthening antioxidant compounds. Besides, all plant species are incapable of accumulating or producing large quantities of these organic compounds under stress. Therefore, genetically modified plants

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_13

containing transgenic genes encoding osmolytes synthesis could become a viable option for better rice production. Recent advances in plant molecular biology based on physiological stress response studies have allowed the discovery of several genes associated with stress resistance.

Keywords

Cereals · Oxidative defense · Ions homeostasis · Gene expression · Physiology · ROS metabolism

13.1 Introduction

The main food crops cultivated worldwide are cereals including barley, corn, wheat, and rice. Rice is an exceptional model plant for studying the biology of all grains. It has genome size of 430 Mb (Pradhan et al. 2019). In addition, rice supplies nourishment for most of the world population, particularly in Eastern and Southern Asia, the Middle East, the West Indians, and Latin America. Rice is cultivated under many climatic conditions, with varied temperatures, soil moisture levels, and climates. It can also grow in such areas, which are not suitable for the growth of other crop plants (Pradhan et al. 2019). There are over 400,000 accessions of rice germplasm in gene bank across the world, which show that rice species has enormous chemical diversity (Kusano et al. 2015). Unfavorable conditions affect the production of rice significantly and generate significant losses globally even on the most productive irrigated areas. Abiotic stress is a significant element that restricts agricultural yield in wide parts of the world. Abiotic stresses, including salinity, water supplies (excess or less water), high and low temperature, metal/metalloids, and nutrient deficiency, significantly threaten the rice production in different parts of the world (Faseela et al. 2019; Paul and Roychoudhury 2019). The magnitude of losses caused by abiotic stress varies according to the duration and severity of the stress. Metabolomics has aided in finding the metabolite biomarkers linked with rice tolerance to stress, particularly abiotic stress. Approximately, 38 million km^3 water, which makes 2.7% of the total water in earth, is suitable for consumption by the terrestrial plants and animals. Approximately, 76% of this fresh water is trapped in permanent glaciers and ice caps, with remaining 11% stored underground at depths greater than 1000 m. Almost 4.5 million km^3 of fresh water is accessible for use, with 97% available as groundwater at 1 km depth. There are just 0.14 million km^3 of fresh water in the atmosphere, rivers, and lakes that may be used for irrigation. Significant population expansion, on the other hand, increases fresh water demand for the residential uses, while decreasing available land and fresh water for agricultural, leading large-scale irrigation development to stall drastically for the last few decades (Fahad et al. 2019; Rasheed et al. 2020). Rice thrives and yields better in flooded conditions compared with dry soil conditions. Rice, unlike other crops, suffers from water stress at soil water levels that are much higher than the field capacity. As a result, proper water availability is critical for optimum rice development and harvests. Rice production potential is limited by water shortage. Likewise, salinity, being a major abiotic stress, exerts negative impact on crop development and yield production (Arif et al. 2020). Soil having higher concentration of soluble salts such as anions and cations with an electrical conductivity of greater than 4 dS/m is referred to as saline. Salinity is a frequent issue in arid and semiarid locations where rainfall is low and evaporation is substantial, causing salts to be transported to the root zone by capillary rise. Salinity can arise in coastal locations as a result of seawater interference. Salinity has ravaged over 900 million ha of land, making around 6% of the total world area and 20% of the total arable land in the world (Fahad et al. 2019). Of the entire worldwide cultivated land, 23% is saline and 37% is sodic, and it is estimated that half of the world's irrigated fields are severely impacted by waterlogging and salinity. The response of cereals to salinity varies, with rice being the most susceptible and barley being the most tolerant. Presently, salinity is the second most important abiotic limitations in the production of rice after drought (Munns and Tester 2008).

Inadequate agricultural practices, overgrazing, and deforestation all contribute to soil degradation, including chemical pollution, salinization, acidification, nutrient depletion, and loss of organic matter alongside deterioration of physical characteristics such as wind erosion and water. These processes have a negative impact on about 2000 million ha of arable land throughout the world. Several chemical and physical soil issues, such as inefficient N utilization and K, Zn, and C shortage in soil, are regarded as important hindrances to rice and wheat production (Adnan et al. 2017). These limitations are mostly responsible for low crop production in rice and wheat cropping systems (Rahman et al. 2016). Problems such as iron, zinc, or phosphorus deficiency as well as overabundance of salts, aluminum, or iron have lowered rice production on roughly 50 million ha of rice area in Asia. Zn insufficiency is the most serious and common nutritional issue in lowland rice since flooding abridges Zn solubility. Of various crops, rice is considered to be more sensitive to drought stress; it is regarded as a negative element for rice plants since it impacts lengthening and prolongation in the growth time.

13.2 Effect of Abiotic Stresses on Plant Morphological Attributes

Salinity stress stops seeds from germinating and affects the plant's physiological properties. Moving salt water from surrounding places through flooding or a low-quality water system causes salt to collect in the soil (Fahad et al. 2015a, b; Kozlowski 2000). Both Cl^- and Na^+ ions cause problems in the growth of rice root system. The broad leaf cutting edge of Cl^--activated damage indicates burning, whereas the accumulation of Na salts generates leaf mottling and rolling. The presence of salt in the soil reduces the plant ability to receive water, resulting in diminished development, which is known as the water shortage impact of salt. When large levels of NaCl enter the plant by transpiration, it damages the leaves, slowing the rate of development even more (Greenway and Munns 1980). As evidenced by their various development reactions, plants differ greatly in their tolerance to salt stress. The majority of commonly grown crops are sensitive to salt stress (Flowers and Colmer 2008). Among the major cereal crops, rice is thought to be the most vulnerable crop (Akram et al. 2019; Maas and Hoffman 1977). The development of leaves is slowed, the stomata close, and the photosynthetic rate reduces as a result of salt stress (Rahnama et al. 2010). Reduced salt uptake or salt avoidance, enhanced K^+/Na^+ proportion, tissue resistance, stomata closure, antioxidant system control, water utilization proficiency, flowering, and active growth to weaken the NaCl concentration in crop are the major components that regulate salt resilience (Akram et al. 2017a; Ismail et al. 2007).

Rice is more susceptible to drought stress than other crops (Showler 2016). It is thought to be harmful to rice plants because it affects lengthening and lengthens the growth time (Shao et al. 2008). Seed germination and basic seedling development are considered the most sensitive stages of growth under water deficit conditions (Ahmad et al. 2009). The rice crop has been the subject of numerous studies on how plants respond to drought stress in terms of seed germination and early seedling development (Chen and Arora 2011; Gao et al. 2008). Seed germination was significantly influenced by the dry circumstances, with the germination rate in particular declining as the polyethylene glycol content increased (Kaya et al. 2006). Furthermore, seed germination capacities, hypocotyl length, and root/shoot biomass comprising fresh/dry masses all reduced as a result of the polyethylene glycol-induced water deficit, whereas root measurement increased (Zeid and Shedeed 2006; Roychoudhury et al. 2008). Drought stress significantly slowed rice plant growth and development during vegetative growth phases (Akram et al. 2019; Manickavelu et al. 2006). Due to a lack of turgor pressure, cell growth halted during drought stress. Active meristematic cell division fueled the growth of early seedlings. However, when there is a severe lack of water, cell expansion can be slowed by water spilling out of the xylem into the surrounding expanding cells (Nonami 1998). Drought stress inhibited mitosis, and cell enlargement resulted in decreased rice development and yield qualities (Hussain et al. 2008). Drought stress affects soil water content, which in turn impacts plant cell turgidity, disrupting the normal growth process (Hsiao and Xu 2000) by morphological changes (Jabran et al. 2017b; Rahdari and Hoseini 2012). Drought stress affects rice fresh root/shoot mass and soil-plant water potentials (Jaleel et al. 2009). Because soil supplements are delivered to the roots through water, the drought season has an impact on their accessibility and transportation. Therefore, drought stress inhibits transportation of essential nutrients including NO_3^-, SO_4^{2-}, Ca, Mg, and Si that are important for plant normal growth (Nasim et al. 2017; Selvakumar et al. 2012). Drought stress is a multifaceted stress that affects all cell units, cell tissues, and the entire plant (Rahdari and Hoseini 2012). It also generates free radicals, which disrupt antioxidant defense mechanisms and lead to the formation of reactive oxygen species (ROS), which causes oxidative stress. ROS can affect the rice plant at various levels of connection, for example, it can cause lipid peroxidation or protein and the membrane layer degradation (Nair et al. 2008). Overall, this stress reduces the rate of photosynthetic pigmentation, which is a sign of photooxidation (Anjum et al. 2016, 2011), and also affects biochemical processes such as nitrate reductase due to reduced NO_3^- uptake from dry soil, which limits plant growth and development (Ali et al. 2014; Awais et al. 2017a, b). Water scarcity reduces the number of leaves per plant, as well as the size and life span of leaves. For development and growth, the leaf area index relies on leaf turgor pressure, temperature, and acclimation. Drought reduces the leaf zone, which is due to the hiding of leaf expansion, which reduces photosynthetic rate. The reduction of fresh and dry biomass is a common adverse effect of water stress on rice plants (Zhang et al. 2006; Rabara et al. 2021). In former ages, drought was the most harmful abiotic factor affecting world food security (Nasim et al. 2016a). These dry conditions might range from direct and indirect to extreme and delayed in nature, affecting product yields (Jabran et al. 2017a; Nasim et al. 2011). By 2050, the dry season is expected to inflict damage on plant development in more than half of arable land (Kasim et al. 2013). Rice grain filling is a process of carbohydrate starch production. This method is thought to involve at least four chemicals: (1) sucrose synthesis; (2) ADP-glucose pyrophosphorylase synthesis; (3) starch synthesis; and (4) starch branched compound synthesis (Taiz and Zeiger 2002). Reduced sucrose synthase activity caused a decrease in grain filling rate, whereas development was halted due to inactivation of ADP-glucose pyrophosphorylase under dry circumstances (Ahmadi and Baker 2001; Nasim et al. 2016c). During fertilization, a lack of water increased the frequency of grain production. Water scarcity has a deleterious impact on rice grain filling, which resulted in the remobilization of stored carbohydrates into rice grains (Nasim et al. 2016b; Yang et al. 2012). In brief, the dry season slows plant growth and development and reduces blooming rate, grain filling, and, as a result, grain yield. The decrease in grain filling is due to a reduction in sucrose and starch-producing chemical activities (Mahla et al. 2017). Drought-responsive strategies including morphological (reduction in germination and plant biomass), molecular (altered gene expression), and physiological changes (photosynthesis, chlorophyll fluorescence, and plant water relations) can be used for determining rice yields. Yield does an excellent job of coordinating a large number of these procedures. In this way, it is difficult to figure out how plants accumulate, consolidate, and display the constantly changing and inconclusive operations throughout the rice life cycle. The articulation and connection of a few plant development stages determine grain production. Lack of water causes a significant reduction in yield, most likely through disrupting leaf characteristics by limiting the size of the source and sink tissues, as well as inhibiting phloem stacking, absorb translocation, and drying tissue (Farooq et al. 2009; Hammad et al. 2017). Drought spells suppress drying tissue formation to a large extent by inhibiting leaf expansion, leaf advancement, and, as a result, lower photosynthesis (Nam et al. 1998). Blooming in the dry season usually results in insufficiency. A decrease in absorption flux at the optimal level required for grain growth was one notable factor but far from the only one (Yadav et al. 2004). Because of the low soil water content, dry circumstances produce stomatal closure, which reduces CO_2 intake and so reduces photosynthesis (Akram et al. 2019; Flexas et al. 2004), and as a result, plant development and advancement are hampered, flower formation and grain filling are hampered, and grain yields are reduced.

Among all heavy metals, cadmium (Cd) is the most toxic metal, reducing root/shoot growth rate and productivity, uptake of essential nutrients, homeostasis, and bioaccumulation in important cereal crops. Cadmium (Cd) can be ingested by animals and humans through our food chain, resulting in a variety of health problems (Akram et al. 2017b; Di Toppi and Gabbrielli 1999). High levels of Cd in soil generate stress symptoms such as stunted growth and development, particularly in roots, disruption of nutrition and glucose metabolism, and a reduction in biomass and yield (Moya et al. 1993; Roychoudhury et al. 2012). Lead (Pb) is also one of the most abundant metals on the planet, and human exposure to it can cause major health concerns. Even at modest levels, increased Pb in soil affects yield output and disrupts germination, photosynthetic rate, mitosis, plant water status, nutrition, and enzyme activity (Patra et al. 2004). The knowledge of heavy metal activity on H^+-ATPase in the outer layer is limited. Enzymatic activities were discovered to be altered in the presence of heavy metal (Cd, Cu, Ni, Al) stressors in a number of studies. The effect of heavy metals on plasma layer H^+-ATPase movement is determined by the time they were introduced to the plant, as well as the type and amount of metals present (Javaid et al. 2017; Kabała et al. 2008). Heavy metals have a considerable impact on rice seed germination, vegetative growth, and yield when the rate of heavy metals is increased. Heavy metal stress during seed germination has deleterious consequences at many phases, including the root/shoot length ratio and seedling height. Cu, Zn, and Pb are widely found in rice plants, causing deleterious effects (Mahmood et al. 2007; Mushtaq et al. 2017).

The current temperature in rice-producing areas is nearing the optimum for rice productivity. Increases in mean temperature during vulnerable phases could be damaging and reduce grain output significantly. Temperature spikes are anticipated to reduce rice yields by 41% by the end of the twenty-first century (Ceccarelli et al. 2010). If these extreme climate predictions are right, rice yields in their current cropping zones could be completely wiped off (Aghamolki et al. 2014). The optimal temperature for normal rice growth and development is between 27 and 32 °C (Akram et al. 2019; Yin et al. 1996). Temperatures higher than this have an impact on all stages of rice development, from development to maturity and harvesting.

Rice plants can only withstand a certain range of temperatures, especially during the flowering, preparation, and seed development stages, or they will be injured, resulting in lower yields (Porter 2005). During the vegetative stage, a high day temperature can cause problems on leaf gas exchange qualities. Even a little period of warmth can produce significant stress, resulting in the premature production of botanical buds and blooms during the conceptive stage (GUILIONI et al. 1997). Rice flowering and booting periods are shown to be the most vulnerable to high temperatures (Ali et al. 2016; Shah et al. 2011). The rice vegetative stage is more vulnerable to heat than the conceptual stage (Akram et al. 2019; Peng et al. 2004).

13.3 Changes in Metabolism, Physiology, and Gene Expression in Rice Under Salinity

Salinity is known to abridge rice growth and productivity (Chattopadhayay et al. 2002). For instance, Basu et al. (2017) reported rice as the main staple cereals that are extremely vulnerable to drought and salinity. These two abiotic constraints (drought and salinity) severely limit rice cultivation. The aim of the study was to appraise the effects of salinity stress on selected drought tolerant rice genotypes at the seedling stage in order to identify the possible tolerant rice genotypes. In all traits related to salt and drought vulnerability, there existed a significant genetic variation in rice cultivars (Basu and Roychoudhury 2014). The rice genotype IR84895-B-127-CRA-5-1-1 showed a remarkable drought and salinity tolerance. Different parameters such as K^+/Na^+, lipid peroxidation, proline accumulation, and photosynthetic performance showed a significant variation in rice genotypes under salinity. Cellular and subcellular membrane degenerated by the accumulation of ROS leading to decrease in photosynthetic activity. Thus, buildup and detoxification of ROS were considered as the major mechanisms for salinity tolerance in rice. The bettered detoxification of ROS was ascribed to enhanced antioxidant defense system in rice under salinity. The findings reveal a direct link of ROS with enhanced physiological activity in rice under salinity (Basu and Roychoudhury 2021).

Controlling xylem Na^+ loading is frequently cited as a key component of salinity tolerance, though it is unclear how much of a difference in this characteristic influence salinity tolerance in different species. Plants of barley and rice were grown at two salinity levels in this study. After the imposition of stress treatments, Na^+ and K^+ in the sap were measured at different intervals. For several days, salt-exposed rice plants avoided the Na^+ loading in xylem, but plants were unable to continue the process for long run, resulting in a significant Na^+ accumulation in shoot. Barley plants raised xylem Na^+ concentrations and transported to the shoot. Plants maintained higher root Na^+ contents that resulted in non-toxic levels of Na^+ in shoots in rice under salinity. Rice plants showed higher K^+ in shoot, while barley plants had greater K^+ in roots. Rice and barley plants control xylem Na^+ loading in quite different ways, which may differentiate the levels of salinity tolerance between two grass species. This trait should be used in breeding programs aiming at improving crop salinity tolerance (Ishikawa and Shabala 2019).

In another study, the relative potential for saline-alkaline tolerance was evaluated in two rice cultivars in terms of nutrient accumulation under 50 mM NaCl stress. Salt-tolerant cv. Pokkali and a salt-sensitive cv. PTT1 were exposed to three levels of saline-alkaline conditions, namely, mild, moderate, and severe stress. Pokkali exhibited comparable stronger stress tolerance than PTT1 because of its increased biomass production according to findings under stress conditions. Higher expression of *OsHKT1;5* encoding Na^+ transporter in the shoot *OsNHX1* encoding Na^+/H^+ antiporter at the tonoplasts in roots allowed Pokkali shoots to maintain lower Na^+/K^+ ratio. The authors suggested under all stress conditions higher expression of Fe deficiency-responsive genes, namely, *OsNAS2*, *OsNAS1*, *OsYSL5*, *OsIRO2*, and *OsIRT1*, in two rice cultivars contributed to Fe homeostasis in shoots. Furthermore, the concentration of Cu, Zn, Mn, and Ca increased in roots but dropped in shoots in both rice cultivars under stress conditions. Higher rhizospheric pH has an impact on the uptake of nutrient and transportation to the aerial parts in rice under stress conditions (Nampei et al. 2021).

European rice grown in river deltas has a crucial quality of salinity tolerance. A panel of 235 temperate japonica rice accessions genotyped with 300 single nucleotide polymorphism (SNP) markers was evaluated under salinity stress. At seedling stage, the panel of accessions was subjected to salinity (6 dS/m). For both normal and stress treatments, different growth characteristics were examined alongside Na^+ and K^+ mass fractions which were determined with atomic absorption spectroscopy. Plant growth was influenced by salt treatment, especially the shoot parameters (Frouin et al. 2018). In plants, cytokinins played an important role in regulating grain yield. The irreversible degradation of cytokinins is catalyzed by cytokinin oxidases, thereby regulating the cellular cytokinin concentrations. The reduction in yield production under salinity stress conditions and its association with inflorescence meristem-specific rice cytokinin oxidases (OsCKX2) was studied. The authors used RNAi-based methodology to analyze the role of OsCKX2 in retaining grain production under salinity. The inflorescence of OsCKX2 knockdown plants showed significant increase in cytokinins. The authors compared the growth, physiology, and grain yield of OsCKX2 knockdown with the wild type to examine if there is any association between OsCKX2 levels and grain yield under salinity stress. In salinity stress, OsCKX2 knockdown plants showed improved photosynthesis rate, relative water content, and vegetative growth alongside minimal electrolyte leakage compared with the wild-type plants. Importantly, the authors found a negative relation between OsCKX2 expression and plant yield as measured by the agronomical parameters, for example, filled grain per plant, harvest index, and panicle branching under normal or stress conditions. These findings indicated that OsCKX2 adjusted floral primordial activity via regulating rice grain yield under salinity stress (Joshi et al. 2018).

In India, abiotic stress limiting plant growth in rice in coastal regions is soil salinity. Alternative ways have been offered to monitor soil status on such a big scale, because it is time-consuming and expensive. Instead of directly detecting soil conditions, the crop conditions can be utilized as a reference to identify salinity-affected rice crops. Vegetation images produced from satellite with great temporal resolution such as moderate resolution imaging spectroradiometer offer the ability to detect soil salinity in a cost- and time-efficient manner. This study evaluated the temporal pattern of salinity as well as behavior of rice crop phenology markers at various salinity levels. The authors examined the application of satellite imaging approach for soil salt detection in rice field (Paliwal et al. 2019). Genes with DUF221 domains perform essential part in the responses to abiotic stress, hormonal signaling, and developmental biology. The authors performed a genome-wide analysis of this significant gene family in rice to better understand the structure and evolutionary relationship under salinity. Due to segmental duplication process in some of the gene families, there is an accretion of the *DDP* gene family in rice. According to the findings, a highly clear selection was found acting on all orthologous and paralogous *DDP* gene pairs. Multiple *OsDDP*s were differentially expressed in saline conditions. The microarray analysis and qRT-PCR analysis indicated that *OsDDP6* expression was greater at all the developmental stages in the salinity-tolerant rice cultivar FL478. Intriguingly, protein network analysis revealed that OsDDP6 is involved in the proline metabolism pathway under salinity. The distinct gene structure, different transmembrane topologies with different expression patterns indicated the functional diversity in *DDP* genes in rice under salinity. In this investigation, the in-depth evolutionary analysis of *DDP* genes from different rice and *Arabidopsis* species was performed, and the generated data will provide foundation for future studies on *DDP* genes in other plant species (Ganie et al. 2017). Understanding the molecular and physio-biochemical features that contribute to salinity tolerance as well as morphological traits is significant for producing salt-tolerant rice cultivars. In this study, the rice genotypes CSR10 and M-148 with differential salinity tolerance were compared. CSR10 restricted Na^+ in roots, maintained lower Na^+/K^+, and manifested greater physio-biochemical indices under salinity. The higher stress tolerance of CSR10 was associated with the greater expression of LEA3 and DREB2A, as well as SOS1 and HKT2;1. The tolerant rice genotype showed higher solute accumulation and better ionic homeostasis that resulted in higher salinity tolerance (Singh et al. 2018). Throughout the world, there are about 955 million ha of arable land, and 49% of the agricultural land is facing Zn deficiency due to excess salt effects. Plant abiotic stress is intensified by Zn deficiency and soil salinity. However, the mechanism by which Zn can lessen salinity effects on plant functions is not clear. The authors performed an experiment in order to know how Zn deficiency and salinity affected the growth, grain yield, membrane integrity, chlorophyll contents, and Zn^{2+} and K^+ contents. The results indicated that Zn significantly improved salinity tolerance in rice plants by improving Zn^{2+}/K^+ retention, restricting Na^+ uptake, and enhancing chlorophyll biosynthesis and cell membrane integrity (Tufail et al. 2018).

In another study, it was examined whether there exists a link between better antioxidant activities and ROS balance in cell in rice under salinity. Rice genotype BRS Bojuru (salinity tolerant) and BRS Pampa (salinity sensitive) were exposed to salinity for different time intervals. In the tolerant genotype, there was a prominent decline in MDA contents. In contrast, the sensitive rice genotype showed enhanced lipid peroxidation measured as MDA levels under salinity. The expression of antioxidant enzymes was greater in salinity-tolerant genotype over salinity-sensitive rice genotype. The strengthened antioxidant enzyme activities displayed a significant role in improving salinity tolerance of rice plants (Vighi et al. 2017). Subramanyam et al. (2019) reported that Na_2SeO_4 administration as seed priming and foliar spray significantly improved salinity tolerance of rice plants reflected as higher growth, chlorophyll content, and better ion homeostasis due to induced expression of *OsNHX1*. The ROS generation was also minimal in plants administered with Se that significantly boosted the antioxidant enzyme activities in rice under salinity. Tang et al. (2019) reported higher tolerance to salinity and drought in rice plants which had enhanced expression of *OsMYB6* and *MYB* Genes. Formentin et al. (2018) advocated that metabolic adjustments and regulation of ionic transporters are important hallmarks in predicting salinity tolerance potential of rice plants. Kim et al. (2018) reported that rice plants with salicylic acid administration displayed better salinity tolerance reflected in the form of minimal oxidative damage due to strengthened oxidative defense. Liu et al. (2020) reported that rice plants supplemented with melatonin had higher tolerance to salinity due to better K^+ homeostasis and expression of plasma membrane K^+ transporters mediated by NADPH oxidase. Liu et al. (2019) found that genetic differences for salinity tolerance in rice genotypes are related to tissue-specific regulation of K^+ and Na^+ transporters. Sagar et al. (2020) reported higher salinity tolerance in rice inoculated with halophilic rhizobacteria containing ACC deaminase activity.

13.4 Chromium Stress in Rice Plants

Chromium stress causes a significant abridge in growth and yield production in rice (Ahmad et al. 2011). Two different rice cultivars were exposed to 0.01 μM level of brassinosteroids (EBL) to mitigate the harmful impacts of Cr (100 μM). The results demonstrated that seeds primed with EBL showed improved plant germination characteristics, leaf pigment contents, and growth in response to Cr stress, compared to control. Cr stress boosted the plant enzymatic antioxidant activities and ROS generation. However, the Cr-stressed plants treated with EBL further enhanced the plant antioxidant system to effectively quench the ROS production. The application of EBL also triggered the gene expression of *SOD* and *POD* under Cr stress. Besides, the seeds primed with water (control) showed more Cr uptake than EBL-treated plants under Cr toxicity. EBL treatment protected the rice plants from Cr-mediated oxidative injury (Basit et al. 2021).

Sahoo et al. (2021) observed the potential of *Azotobacter vinelandii* strain against different dosses of Cr (50, 100, 150, 200, and 250 μM) in rice plants. Rice plant inoculation with *Azotobacter vinelandii* displayed higher growth, leaf pigment contents such as net photosynthetic rate, intracellular CO_2, stomatal conductance, and enzymatic antioxidant activities under Cr (200 μM) toxicity.

The efficacy of salicylic acid (SA) and silicon (Si) was assessed against Cr stress in rice plants. The combined application of SA (10 μM) and Si (5 μM) in growth media containing Cr (100 μM) improved the rice plant growth. Both SA and Si triggered the efficiency of total chlorophylls and carotenoids, osmolytes accumulation, and water holding capability and, in turn, enhanced the plant growth and biomass. Further, both SA and Si increased the nutrient transportation and improved the enzymatic activities being involved in glutathione-ascorbate cycle. Rice plants also exhibited lower oxidative injury in the form of lessen ROS production and membrane recovery in response to Cr toxicity (Yang et al. 2021).

In this study, the application of microRNA was advocated to reduce the toxic impacts of Cr toxicity in rice plants. The gene expression level showed that microRNA improved the plant defense system and effectively detoxified the Cr through ATP-binding cassette transporters, response of auxin, heat shock proteins, transcription factors, and metal transportation in plants. The differential expression of chosen miRNAs and their probable target genes was confirmed using real-time PCR analysis (Dubey et al. 2020).

Cr stress inhibited the metabolism, growth, and development of plants. Thus, it is crucial to increase the plant tolerance against Cr stress. In this context, this study was conducted to evaluate the impact of citric acid in rice plants in response to Cr stress. It was showed that citric acid improved the plant growth by ameliorating the growth attributes, total protein contents, and recovered membrane structure under Cr toxicity. Besides, citric acid addition to growth media resulted in lowered Cr accumulation in roots and higher translocation of Cr towards aerial parts owing to their chelating attributes in plants. Moreover, citric acid regulated the SOD, POD, and GR activity along with higher glutathione and proline contents in rice plants under Cr stress. However, citric acid did not display any substantial role in iron (Fe) accumulation in plants under Cr stress (Khatun et al. 2019).

Metallothioneins (MTs) are considered as metal ion-binding proteins that reduced the metal toxicity in plants. The effect of MTs in binding of metal and ROS quenching was checked. The rice plants were supplemented with either Cr^{3+} or Cr^{6+} at three different levels by using Agilent 44K rice microarray and real-time PCR method. Rice plants accumulated both Cr species in the roots than aerial parts. The plants accumulated higher amount of ROS (H_2O_2 and superoxide) in their tissues with maximum availability of H_2O_2 and MT contents. They reported that analysis from PCR showed that ten specific *OsMT* genes were involved in ROS quenching both in plant tissues and Cr species. They showed different pattern in this regard. Metallothioneins enhance chromium detoxification through scavenging ROS and stimulating metal chelation in *Oryza sativa*.

Chromium (Cr) is thought to be as hazardous environmental element owing to its toxic impacts on living beings. The impact of exogenously applied calcium (Ca) was observed for demolishing Cr negative impacts in rice plants, since Ca play role as signaling molecule at plant cellular level. The findings showed that Ca application improved the growth attributes, membrane structure, and protein contents of rice plants under Cr stress. Further, Ca application hindered the Cr transportation from root to aerial parts of rice plants. It might be due to overaccumulation of phytochelatin that resulted in vacuolar sequestration of Cr in plant roots. Plant enzymatic activity (POD, CAT, GR, and GSH) was also improved after exposure to Ca under Cr stress (Mukta et al. 2019).

It was reported that Cr stress inhibited the metabolism of carbohydrate in rice plants and disturb their productivity. The transcriptome map of sucrose metabolism in rice plants subjected to both Cr^{3+} and Cr^{6+} was reported using Agilent $4 \times 44K$ rice microarray analysis. Cr stress after 3-day interval displayed higher sucrose accumulation and changed the activities of sucrose synthase, phosphatase, amylosynthase, and sucrose phosphate in rice plant tissues. The authors found 119 different regulated genes that are involved in encoding 17 sucrose mobilizing enzymes. The expression of genes was more evident in roots compared to aerial parts of plants under both Cr stresses (Feng et al. 2019).

Rice plants were subjected to nutrient medium either having 100 μM Cr^{6+} or Cr^{6+} along with *Phyllanthus emblica* aqueous extract for 4–8 days. Cr^{6+} toxicity halted the plant growth attributes including root as well as shoot length, fresh biomass, relative water content, and leaf pigments. Cr^{6+} ameliorated the root membrane permeability, ROS generation, lipid peroxidation, and enzymatic antioxidant system of rice plants. More Cr^{6+} was accumulated in plant roots than aerial parts. However, addition of 5 mg/L level of *P. emblica* aqueous extract remarkably improved the Cr^{6+}-mediated reduced plant growth, leaf contents, and biomass. However, Cr^{6+}--mediated higher antioxidant activity was decreased in response to *P. emblica* aqueous extract application. They advocated that *P. emblica* aqueous extract showed better results in terms of low Cr^{6+} uptake, oxidative injury, and modulation of plant defense system, thereby improving plant growth (Pandey et al. 2019).

In another study, rice plants were subjected to varying levels of Cr (0, 100, 500 mg/kg). To alleviate Cr stress, plants were supplemented with different doses such as 0, 10, 20, and 30 mg/L of lysine chelated with zinc (Zn-lys) under Cr stress. Cr stress inhibited the morphological, physiological, and antioxidant system of rice plants. Plants showed more oxidative injury under Cr stress. However, the foliar application of Zn-lys enhanced the plant photosynthetic contents, enzymatic activities, biomass, and Zn contents under Cr stress. For instance, 30 mg/L dose of Zn-lys under 500 mg/kg of Cr level showed higher plant height (50%), dry weight of shoot (70%), as well as root (129%) and reduced Cr contents in roots. Further, 30 mg/L level of Zn-lysin improved the plant growth and inhibited oxidative injury and Cr level by enhancing the antioxidative defense system, photosynthetic contents, and Zn uptake under Cr stress (Hussain et al. 2018).

A soil and hydroponic study was established to determine the impact of iron plaque formation on Cr uptake and accumulation by growing rice seedlings. Rice plants were supplemented with three doses of Fe^{2+}, viz., 0, 30, and 100 mg/L in hydroponics and 0, 1, and 2 g/kg of Fe^{2+} in soil medium. Different doses of Cr (0, 0.5, 2, 5, 10, and 20 mg/L) in nutrient medium and 300 mg/kg in soil media were given by using chromate as Cr source. Elevated level of Cr suppressed the rice plant growth and biomass. However, application of Fe^{2+} boosted the Cr proportion in iron plaque but reduced in rice plants under Cr stress. Plants grown in nutrient medium displayed iron plaque production with Fe^{2+} addition at 100 mg/L that remarkably minimized the Cr accumulation in aerial parts supplied with Cr (10 and 20 mg/L). Similar results were obtained in plants grown in soil medium. Therefore, it was suggested that an appropriate amount of iron plaque plays positive role in terms of reducing Cr uptake and accumulation in rice plants (Xu et al. 2018).

Among different enzymes, PAL (phenylalanine ammonia lyase) actively involved in secondary metabolism of plants. The expression of individual isogene from the *PAL* gene family is varying with plant species under various environmental constraints. For that purpose, the transcriptome analysis of the *PAL* gene family in rice plants subjected to Cr^{6+} (potassium chromate) and Cr^{3+} (chromium nitrate) was held through using agilent 44k rice microarray and real-time quantitative RT-PCR. After 3 days of Cr treatment, more Cr was accumulated in roots than aerial parts of plants. The expression of individual isogene from the rice *PAL* gene family was different in response to both Cr species. Among two Cr treatments, only *osPAL2* and *osPAL4* genes displayed their expression in similar way. Further, gene expression pattern was conflicting in both plant tissues. It was evident from results that the gene expression is tissue and stimulus specific in response to varying Cr levels, advocating their different implementation for Cr removal in plants (Yu et al. 2018).

Cytochrome c is the redox active molecule involved in plant mitochondrial electron transport. It played effective role in metal interactions with mitochondrial proteome. It is also important for insight into electron transfer during oxidative stress. In this study, rice plants were supplemented with either Cr^{3+} or Cr^{6+} for evaluating the cytochrome c interaction with metal ion accumulation and linked cytochrome modulation with ROS production in plants. The results showed that rice plants accumulated both species of Cr. Cr stress suppressed the plant growth by ROS production. Both root and shoot maintained alterations of cytochrome c contents under both Cr species in plants. The transcriptional level of rice cytochrome c genes after real-time quantitative RT-PCR was conflicting in rice tissues. Results showed that ROS-mediated oxidative injury unequally disturbs the cytochrome c contents and expression patterns of *cytochrome c* genes under Cr stress. It was advocated that reduction in root cytochrome c content was not responsible for ROS accumulation that might be owing to metal-binding attributes. Higher shoot cytochrome contents might be resulting in ROS accumulation that, in turn, disturbs plant normal growth under Cr stress.

13.5 Conclusion

Rice is among the essential cereals grown throughout the world. However, rice is susceptible to abiotic constraints, including salinity, drought, temperature, and heavy metals. However, because of unfavorable environmental conditions, its cultivation and productivity are under enormous pressure. Salinity stress in rice is one of the significant abiotic stresses that hamper growth and yield production. It is estimated that various abiotic constraints cause 30–60% loss in rice production worldwide each year. For decades, deliberate attempts have been undertaken to mitigate the adverse impacts of abiotic stresses such as diverse cultural practices, the adoption of tolerant rice cultivars, and remedial water management. There is a direct need to gather data on changes in physiological, morphological, and molecular attributes in rice under abiotic constraints in rice plants. The information on how rice plants undergo physiological and biochemical alterations under abiotic stress could pave the way for stress-tolerant rice varieties through genetic engineering or conventional breeding programs.

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Breeding and Omics Approaches to Understand Abiotic Stress Response in Rice

14

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Abstract

Rice (*Oryza sativa* L.) is the major sources of food and calories for more than half of the world population. Productivity of rice is adversely affected by various abiotic stresses such as salinity, drought, submergence or flooding, high and low temperatures, and heavy metal. They are also responsible for significant yield loss and threaten global food security across major rice-producing countries. Climate change has aggravated the frequency of abiotic stress and may even threaten survival of plants. By 2050, at least 50% more rice production is required to feed nine billion people. Thus, it is important to understand plants response against adverse conditions of paddy fields with the hope of improving better tolerance to environmental stress. The rice plant has comparatively smaller genome size which is useful to understand the effect of abiotic stress on the physiology, biochemistry, and genomic levels. Molecular breeding approaches had improved our basic understanding about abiotic stress tolerance in rice through identifying and introgressing major effect QTLs. On the other hand, advances in "omic" studies such as genomics, transcriptomics, proteomics, metabolomics, etc. and

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_14

genome editing tools have paved the way of identifying the causal genes and their expression patterns during the period of stress perception and response. As a whole, more combined efforts are required to develop stress tolerance crops to boost rice yield even under stress. In this chapter, we attempt to discuss the recent advances in abiotic stress tolerance in rice by exploiting molecular breeding, "omic" approaches, and modern biotechnological tools.

Keywords

Abiotic stress · Stress tolerance · Marker-assisted breeding · Quantitative trait loci (QTL) · Omic approach · Genetic engineering · Rice

14.1 Introduction

Rice (*Oryza sativa*) is one of the most significant cereal crops in the world and provides food to almost half of the world's population. About 90% of the world's rice is cultivated and consumed in Asia, where it creates a major income source for the 60% of the earth's people who live in rural areas (Datta 2004). It is the most diversified crop and grown in a wide range of environments characterized by climatic regions and ecological conditions under four major ecosystems, viz., irrigated, rain-fed lowland, flood-prone, and rain-fed upland. Abiotic stresses are the major threat towards the plant which severely affect the productivity of the plant and become the major cause of crop yield loss (Canter 2018; Zörb et al. 2019). Initially, it creates ionic imbalance and hyperosmotic pressure in the plant cell and then produces reactive oxygen species, which is toxic to the plant cell at higher concentration causing damage to lipids, DNA, and proteins that triggers/promotes cell death (Debnath et al. 2011; Das and Roychoudhury 2014). The major abiotic stresses are anticipated to increase in response to rapid climate change conditions, namely, high and low temperature, excess and deficient water stress, salinity, and heavy metals that cause significant rice yield loss and threaten worldwide food safety (Pareek et al. 2010; Mantri et al. 2012; Roychoudhury and Basu 2012). In defense of these abiotic stresses, plants altered different survival strategies in molecular, cellular, tissue, anatomical, and morphological levels, but the prediction of a signaling pathway is very difficult (Witcombe et al. 2008). Thus, it is important to understand the defense mechanisms which help to identify the proper management strategies for increasing significant productivity under stress conditions. Marker-assisted selection (MAS) and map-based cloning need to use for the rapid development of improved rice varieties. Besides, modern biotechnology for gene transformation has also provide a lot in the area of crop improvement both in understanding the mechanisms of stress and developing stress tolerance plants for stress conditions. Compared to breeding and marker-assisted selection approaches, transformation of target gene seems to be more attractive, time, labor, and cost saving process for developing stress-tolerant crops without affecting their genetic background (Ahmar et al. 2020). These days, genetic engineering has been intensively used for two decades to develop abiotic stress-tolerant plant (Cui et al. 2018, 2020). Considering the above aspects, combined strategies of molecular breeding and modern biotechnological tools are required for developing stress-tolerant improved lines (Varshney et al. 2011; Roychoudhury et al. 2007). However, understanding the physiology and molecular biology of stress tolerance and developing improve tolerance rice varieties, this chapter describes an overview on recent progress primarily focusing on adaptation and tolerance mechanism, breeding approaches, omics technologies, genome editing tools, and genetic engineering techniques towards different types of abiotic stresses.

14.2 Adaptation and Tolerance Mechanism of Rice Under Abiotic Stress Condition

Rice is an important cereal crop for the majority of the world's population. Crop growth and productivity are negatively affected by the various abiotic stresses in the worldwide. Rice plants are also sensitive to various abiotic stresses, viz., submergence, drought, salinity, high temperature, low temperature, and heavy metal stress. Growth and production of rice are hampered due to extreme environmental conditions outranging acceptable tolerance limit. Thus, plants have developed certain mechanisms to manage different environmental stresses. So, understanding the physiological and molecular mechanisms of stress response in rice is essential to develop new rice varieties with improved tolerance (Roychoudhury et al. 2008). In this section, a review of adaptation and tolerance mechanism of rice against abiotic stress is described, with a special focus to the major abiotic tolerance genes and relevant pathways.

14.2.1 Submergence Tolerance Mechanism in Rice

Submergence is a significant constraint on rice production in Southeast Asia. Annually, complete submergence affects around 12 million hectares of rice in South and Southeast Asia (Ismail et al. 2013). The degree of damage produced by temporary submergence is determined by flood water parameters such as temperature, turbidity, dissolved gas content, and light penetration (Das et al. 2009). The primary characteristics related to submergence tolerance include the preservation of high stem carbohydrates, optimal rates of alcoholic fermentation, aerenchyma production, energy supply via underwater photosynthesis, and root aeration (Sarkar et al. 2006; Colmer et al. 2014). During submergence, the most critical variables impacting plant development and survival include light intensity, turbidity, temperature, pH, gas diffusion, aerenchyma, and leaf shape (Banerjee and Roychoudhury 2020). Light intensity reaching the leaves is reduced after submergence due to suspended phytoplankton or silt in the water and dissolved organic materials. In case of flash floods, the water is remaining turbid, allowing a small quantity of solar radiation to reach the canopy of plant, reducing the rate of photosynthesis. Light intensity is also a crucial factor for influencing the concentrations of carbon dioxide and oxygen during submergence, which substantially affects the physiological condition of the submerged plant (Sarkar et al. 2006). Plants, in general, create oxygen through photosynthesis. However, the absence of an adequate oxygen transport route to non-photosynthesis organs implies that these organs lack oxygen when physiological activity inhibits oxygen diffusion to the outsides (Loreti et al. 2016). Furthermore, plants respond differently to low oxygen levels, restricting respiration (hypoxia) and a total lack of oxygen (anoxia), both of which are harmful to plant life (Loreti et al. 2016). Flooding circumstances can have a significant impact on plant survival. According to Pucciariello et al. (2014), there are significant differences in survivability between plants submerged under dark condition and that of partially submerged in light, indicating the significance of photosynthesis under submerge condition. Waterlogging impedes the development and function of roots and shoots by limiting respiration owing to a lack of oxygen. The effects of photosynthetic blocking and root aerobic respiration are caused by the amount of light in the water, CO_2, and O_2 (Voesenek and Bailey-Serres 2013).

The two primary problems limiting rice plant survival during prolonged submergence are (1) a lack of oxygen and (2) a lack of light intensity; these result from the plant's inability to grow leaves and significant injury done to the older leaves. Plants are subjected to low oxygen conditions while submerged, and the final electron acceptor O_2 in the electron transport chain in mitochondria is restricted. To continue glycolysis, plants must recycle NADH via an alternate route. This is mainly achieved by ethanolic fermentation, which converts pyruvate to ethanol, which regenerates one molecule of NAD^+. This is preceded by the temporary conversion of pyruvate to lactate, which causes a decrease in cellular pH and allows acetaldehyde to develop as a hazardous intermediary. Detoxification of acetaldehyde, most likely by the mitochondrial aldehyde dehydrogenase OsAdh2 (Nakazono et al. 2000), is thus critical to avoiding cell death during prolonged submersion. Various additional biological functions are changed in low oxygen circumstances.

Submerged plants fall shortage in oxygen and subjected to high light tension, resulting in the formation of reactive oxygen species (ROS) such as hydrogen peroxide, hydroxyl radicals, and superoxide anion, which, if not moderated, can seriously damage the cellular organization and lead to plant death. Rice cultivars effective in ROS detoxification following de-submergence can retain chlorophyll, support plant development, regenerate new leaves, and keep older leaves. As a result, rice plants protect themselves from oxidative damage via two mechanisms: (1) antioxidant enzyme systems and (2) natural antioxidants. Natural antioxidants of low molecular weight, such as phenols, a-tocopherol, ascorbate, carotenoids, and glutathione, have been reported to decrease oxidative damage in plants. The amount of ascorbate is increased in the root system under hypoxia condition, whereas it dropped after reaeration (Hancock and Viola 2005; Das et al. 2004). Ascorbate, therefore, plays a crucial function in protecting plants against ROS damage.

Submergence tolerance is related to considerably greater transcript levels and activity of key enzymes in the ethanolic fermentation pathway, delayed starch breakdown, and the persistence of a higher level of soluble sugars until 14 days after submergence (Fukao et al. 2006). Plants use less energy due to reduced elongation of submergence tolerance. Cell elongation suppression has been linked to decreased cell wall loosening expansion genes in Sub1 near-isogenic lines. As a result, Sub1 provides tolerance by optimizing metabolic maintenance and suppressing the energy-consuming escape reaction. This allows plants to remain underwater for around 14 days while retaining sufficient carbohydrate stores for growth regeneration once the water recedes. The existence of three ethylene-responsive transcription factor (ERF) genes (Sub1A, Sub1B, and Sub1C) was discovered by sequencing the Sub1 locus on Chr. 9, and the Sub1A-1 allele has been identified as the primary predictor of tolerance (Xu et al. 2006).

14.2.2 Mechanisms of Drought Tolerance in Rice

In general, stress is a change in physiological circumstances brought on by a variety of events that tend to disrupt the stability of the plant (Singhal et al. 2016). Drought resistance is a plant's capacity to generate its optimum economic yield under water-stressed conditions than a water stress-free field. Drought resistance is caused by various morphological, biochemical, and physiological responses and their interactions (Mitra 2001). The rice crop primarily responds to drought stress conditions by leaf rolling, stomatal closure, and enhanced abscisic acid (ABA) production (Price et al. 2002). Furthermore, photosynthesis and metabolism are severely disrupted in extreme drought circumstances, eventually leading to the plant's death (Ozga et al. 2017). According to Anjum et al. (2017), drought stress hinders cell enlargement compared to cell divisions. Ion absorption, respiration, photosynthesis, growth stimulants, carbohydrate, source-sink connection, and nutrient metabolism are all affected by this stifling of plant development (Fahad et al. 2017). According to physiological studies, drought resistance in rice is primarily dependent on water use efficiency (WUE), which allows for the lowest water consumption for maximal output, and osmotic adjustment, which helps plants maintain turgor and preserve the meristem (Nguyen et al. 2004). Plants may adapt to drought stress through various processes, including drought escape, drought avoidance, and drought tolerance; they adapt through molecular responses and morpho-physiological alterations (Fukai and Cooper 1995; Basu et al. 2010).

14.2.2.1 Drought Escape Mechanism of Rice

Drought escape is a plant's capacity to finish its life cycle before significant soil water shortages emerge. It consists primarily of two distinct mechanisms: fast phenological growth and developmental flexibility. Plants with rapid phenological growth can produce flowers with minimal vegetative growth, producing seeds on a limited water supply. In developmental flexibility, plants can generate an abundance of vegetative growth, blooms, and seeds during rainy seasons. This allows desert ephemerals to avoid dryness and live extended periods without rain. Drought escape is an essential process that permits rice to produce grains despite inadequate water supply in drought-prone areas (Kumar et al. 2008).

14.2.2.2 Drought Avoidance Mechanism of Rice

Drought avoidance is plants' capacity to retain comparatively high tissue water potential in a water deficit situation. Plant tissue has two options for maintaining a high water level during drought: minimizing water loss or sustaining water supply. Drought avoidance is also conferred via mechanisms for enhancing WUE and lowering water loss. Drought-resistant rice cultivars often feature deep, coarse roots with a high aptitude for branching and soil penetration, a more excellent root-to-shoot ratio, flexibility in leaf rolling, early stomatal closure, and solid cuticular resistance (Wang et al. 2006).

14.2.2.3 Drought Tolerance Mechanism in Rice

Drought tolerance refers to a plant's capacity to survive under low tissue water content (Fleury et al. 2010). Drought tolerance in rice is a complex trait regulated by polygenic effects. It involves complex morpho-physiological mechanisms (Li and Xu 2007), such as turgor maintenance by osmotic adjustment, enhanced cell flexibility, decreased cell size, and desiccation tolerance via protoplasmic resistance. Osmotic adaptation is carried out by accumulating proline, sucrose, glycine betaine, and other solutes in cells to promote water absorption. Proline has been the most widely studied due to its significant stress-relieving ability under adverse conditions (Roychoudhury et al. 2015). Under various adverse conditions, proline acts as an osmolyte in plants (Liang et al. 2013). The difference in proline accumulation in rice under normal and stress conditions has been reported (Nezhadahmadi et al. 2013; Chourasia 2017; Luo 2010).

Antioxidants are essential scavengers of reactive oxygen species (ROS) in crops, and their expression increases the drought tolerance of rice (Fahramand et al. 2014). Under drought stress, the imbalance between the creation and extinction of ROS is a common scenario (Lum et al. 2014). ROS are composed of hydroxyl free radicals, superoxide radicals, hydrogen peroxide, and singlet oxygen, which cause protein denaturation, lipid peroxidation, disturbance of cellular homeostasis, cellular oxidative damage, and DNA mutations (Banerjee and Roychoudhury 2019). A complex antioxidant system that includes enzymatic antioxidants and non-enzymatic molecules protects plants from the adverse effects of ROS. Enzymatic antioxidants include dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), superoxide dismutase (SOD), guaiacol peroxidase (GPX), glutathione reductase (GR), catalase (CAT), ascorbate peroxidase (APX), and ascorbate-glutathione cycle enzyme (Sahebi et al. 2018), while ascorbate (AsA) and glutathione (GSH) function as intracellular non-enzymatic antioxidants. Increased drought stress levels cause enriched rice enzymatic and non-enzymatic antioxidant activity (Lum et al. 2014). The tendency of these antioxidant defense enzymes to increase their activity indicates a protective action that neutralizes oxidative damage caused by drought stress.

Polyamines (PA) are tiny positively charged molecules that can respond to drought stress in rice (Capell et al. 2004). PA in plants includes putrescine (Put), spermidine (Spd), and spermine (Spm). It can interact with various signal networks and regulate membrane stability, ion permeability potential, and homeostasis. The increase in PA content during drought stress is directly related to the increase in photosynthetic capacity, the decrease in water loss, and better detoxification and osmotic adjustment. The functions of AP include regulating gene expression by maintaining ion balance, promoting DNA binding of transcription factors, stabilizing membranes, removing free radicals, and preventing aging through DNA conformation conversion and protein phosphorylation (Sahebi et al. 2018). A study on rice shows that rice produces more putrescine under stress, which promotes the synthesis of spermidine and spermine, and ultimately protects plants from drought (Capell et al. 2004).

The main plant hormones that play an essential role in responding to drought are abscisic acid (ABA), cytokinin (CK), jasmonic acid, ethylene, etc. Drought stress is considered to be a kind of hydraulic pressure. Due to the dryness of the soil, the pressure gradient from the soil to the plant will generate this pressure. When hydraulic pressure is detected, the concentration of ABA signaling hormone will change (Raghavendra et al. 2010). ABA levels usually increase to indicate drought stress, while other hormones (such as CK) can be reduced by inhibiting gene expression, degradation caused by oxidase activity, or stress damage. In addition, the duration and severity of drought stress may affect endogenous concentrations of certain hormones and may vary across plant organs. For example, ABA has an indirect function in water shortage signaling by reducing the synthesis of the hormone ethylene (Li et al. 2017a). ABA-dependent and ABA-independent signaling pathways are used to elicit responses to drought, and rapid accumulation of ABA is associated with enhanced drought tolerance (Roychoudhury et al. 2013; Sahebi et al. 2018). In a study on drought-tolerant revived plants (Craterostigma Wilmsii), researchers discovered that drought stress significantly influenced ABA concentrations (Raghavendra et al. 2010). ABA and other hormonal signaling pathways lead to significant changes in plant growth, defense responses, and key drought tolerance mechanisms.

14.2.3 Salinity Stress Adaptation and Tolerance Mechanism in Rice

Soil salinity is considered as major constrain to cereal production worldwide. Rice is a salt-sensitive crop. Despite this, remarkable variation in salinity tolerance was found in rice (Akbar et al. 1972; Flowers and Yeo 1981). Rice is tolerant to salt stress during germination, active tillering, and maturity. In contrast, rice shows higher sensitivity during early seedling and reproductive stages. The physiological reason behind salinity tolerance during early seedling stage includes vigorous seedlings, prohibiting salt inclusion at the root level, ions reflux in structural and older tissues, and upregulation of antioxidant systems, especially ascorbate/glutathione pathway of oxidative stress tolerance. In case of reproductive stages, tolerant genotypes exclude salt from flag leaves and develop panicles (Yeo and Flowers 1986). Although above traits are independent, none of the known salt-tolerant landraces contain more than a few characters in their background. There are remarkable differences in terms of expression of particular traits among rice varieties, suggesting the probability of identifying promising donors for salinity tolerance. As seedling and reproductive stages are weakly related, thus, a pyramiding of promising traits at both stages is required for developing salt-tolerant cultivars (Moradi et al. 2003).

14.2.3.1 Morphological and Anatomical Adaptation Under Salinity Stress in Rice

The response of plants to salinity has a specific morphology, anatomy, and ultrastructure. Roots play a significant role in adapting to salinity. The length, diameter, and the number of roots will affect root performance and improve plant performance by increasing the absorption of water and nutrients during stress. During salinization process, the roots will grow and become larger. So it goes deep underground to reduce salinity. In the root zone, in order to overcome the toxic effects of salt, the density and conductivity of the root system are increased, which further leads to higher ion retention and slows down the accumulation of toxic ions (Acosta-Motos et al. 2017; Kheloufi and Mansouri 2019; El Ghazali GEB 2020). The plant has undergone several anatomical changes to make it salt-tolerant. The exodermis and endodermis of plant roots are respond to salinity by forming significant apoplast barriers (such as casparian bands and corn flakes) (Krishnamurthy et al. 2018). In certain aerial parts of plants, such as leaves, they are modified, for example, by strengthening the palisade cells and reducing the cells of the spongy parenchyma, which promotes the full diffusion of gas under conditions that minimize the stomatal apertures. Some plants also form a thick epidermis in the above-ground parts (stems and leaves); this leads to reduced sweating and keeps water swelling (El Ghazali GEB 2020). Under salt stress, the chloroplast becomes unstable and changes the chloroplast; this leads to a decrease in stomatal conductivity and reduces photosynthesis; in particular, a stable size structure and an increase in the number of chloroplasts are observed to counteract salinization and increase plant resistance (Acosta-Motos et al. 2017).

14.2.3.2 Ionic Balance and Compartmentation Mechanism Under Salinity Stress in Rice

The salinity tolerance mechanism of the rice plants is heavily reliant on ionic balance. When rice is subjected to salt stress, dissolved salt ions, mainly Na^+, K^+, and Cl^-, are typically taken up together with water through the transpirational stream. A high K^+/Na^+ ratio is required for proper cell activity, which is generally the situation under a non-salt environment. However, in a salt-stressed climate, rice consumes an excessive quantity of Na^+ at the expense of energy-consuming K^+ and Ca^{2+} absorption. Increased passive absorption and load of Na^+ in the xylem eventually penetrates the tissues/cells, disrupting physiological and biochemical processes. This buildup causes a disrupted Na^+/K^+ ratio in plant cells, as well as hazardous amounts of Na^+, which inhibits enzyme function inside the cell, ultimately leading to cell, tissue, or organ death (Yeo et al. 1990; Horie et al. 2001). Rice can withstand 50–100 mM Na^+ in cystol, after which the cell must either sequester the Na^+ to the tonoplast or cell membrane via antiporters or succumb to excessive salt concentration. Precision management of the ion transport system in the plant cell, tissue, or whole plant level is essential for salt tolerance to adapt to the adverse environment and limit the degrading consequences. To survive in saline soil plant, maintain optimum K^+/Na^+ ratios in the cytosol, including K^+ uptake regulation and/or Na^+ entry prevention and Na^+ efflux from the cell. Plant growing in saline soil uptakes excessive salt, and it increases salt accumulation in cell and tissue.

Plants cannot withstand under high salt concentration in the cytoplasm, so the excess salt will be secreted into the old part of the plant tissue or transferred to the vacuole to remove it from the cytoplasm. The sodium ion (Na^+), which enters the plant through the roots, enters the cytoplasm and is then transported into the vacuole with the help of various pumps (Na^+/H^+ antiporters) (Gupta and Huang 2014; Fahad et al. 2015). The main type of H^+ pumps found in tonoplasts is H^+ ATPase and VPPase. H^+ ATPase, also called VATPase, dominates in a plant and thus maintains the equilibrium, transport, and growth of solutes (Parihar et al. 2015). NHX, like NHX1 and NHX2, mediates the binding of Na^+ in the vacuole; it participates through the tonoplast Na^+/H^+ exchanger (Ismail and Horie 2017). Salt Overly Sensitive (SOS) also plays an important role in reducing salt toxicity and maintaining ion balance. The Na^+/H^+ antiporter (encoded by SOS1) regulates the release of Na^+ from the cytoplasm and promotes the transport of toxic ions from roots to buds (Gupta and Huang 2014). SOS2 and SOS3 participate in the Ca^{2+} mechanism responsible for the release of Na^+. In addition, the HKT family proteins act as Na^+/K^+ symporters. Selective Na^+ transporters maintain the balance of Na^+ and K^+ in plant cells by eliminating Na^+. In addition, various research reports claim that maintaining the K^+/Na^+ ratio contributes to the salt tolerance of plants. Therefore, maintaining a large amount of K^+ helps to exclude Na^+ from the cell (Tang et al. 2015). In salt-tolerant plants, the correct ion balance (Na^+, Cl^-, K^+) in the chloroplast is maintained for proper photosynthesis (Ismail and Horie 2017).

14.2.3.3 Oxidative Stress Management and Antioxidant Defense Mechanism Under Salinity Stress in Rice

Under salt stress, the buildup of reactive oxygen species such as superoxide radicals, H_2O_2, and hydroxyl radicals has been identified as a major cause of plant cell damage (Apse et al. 1999). Alleviating these oxidative stressors lowers cell-level damage and improves salt tolerance. Tolerant plants generate more antioxidants such as ascorbic acid, whereas sensitive plants produce less glutathione and other reactive oxygen scavenger enzymes. In particular stress condition, up-regulation of the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and ascorbate peroxidase (APX) and the increased non-enzymatic antioxidant is glutathione (GSH), ascorbic acid (ASC) and their derivatives and photosynthesis assistants pigments, such as carotenoids have been produced in all cellular compartments (Mbarki et al. 2018). Antioxidants are powerful ROS inhibitors and free radical scavengers. Therefore, they act as powerful salt mitigators. Detoxification in plant cells is a continuous process. First, plants enrich the production of SOD, which leads to the conversion of superoxide anions to H_2O_2. Then POX and CAT help break down the toxic H_2O_2 in plant cells (Zhang and Dai 2019). The ascorbate glutathione cycle participates in removing reactive oxygen species from various plant organelles by increasing the level of ascorbate-specific peroxidase. The salinity of halophytes leads to increased antioxidants and soluble sugars (Singh et al. 2015; Krishnamurthy et al. 2018). The plant synthesizes a variety of osmolytes, such as proline, glycine betaine, and soluble sugars. Proline (Pro) is an amino acid and osmotic protectant and an important signal molecule. It accumulates in the cytoplasm of plants and acts as a stabilizer and protectant for membranes and protein enzymes. Various proteins regulate plant metabolism during stress periods by activating membrane proteins and ROS scavengers and maintaining cytosolic homeostasis. Many researchers report that Pro under salinity can improve water absorption and antioxidant mechanisms. In addition, it can also reduce the accumulation of toxic ions (Tang et al. 2015; Ismail and Horie 2017; Zhang and Dai 2019).

14.2.4 Physiological and Biochemical Mechanisms of Heat Tolerance in Rice

Heat tolerance is a plant's capacity to thrive and generate an economic yield under high temperatures (Wahid et al. 2007). Rice plants deal with heat stress primarily through escape or avoidance mechanisms, such as the timing of heading and blooming concerning the stress, and the actual tolerance of key processes, such as anther dehiscence, to stress. The capacity of a variety to reduce canopy temperature with decreasing RH through transpiration cooling might be regarded as an avoidance mechanism. Plants use various methods to cope with heat stress, such as changing leaf orientation, transpirational cooling, and adjusting membrane lipid compositions (Adams et al. 2001). According to Wang et al. (2004a), primary HT (heat tolerance) mechanisms in plants include ion transporters, free radical scavengers, osmoprotectants, abundant proteins in late embryogenesis, and different characteristics involved in signaling cascades and transcriptional regulation. According to Larkindale et al. (2005), changes in salicylic acid, abscisic acid, ethylene, calcium, and hydrogen peroxide-like hormones also impact plant thermostability via mutation. When these hormones are artificially given, they can improve HT (Larkindale and Knight 2002), primarily through increased antioxidant capacity and membrane thermal stability (MTS), reducing the degree of damage caused by ROS (Mohammed and Tarpley 2009). The development of antioxidant enzymes and changes in membrane structure are other essential processes that neutralize heat stress (HS) (Stone 2001). Some cultivars can experience evaporative cooling, which can lead to HS tolerance under irrigated circumstances. Heat tolerance in rice is mainly comprised of escape or avoidance (Weerakoon et al. 2008). Plants can escape heat stress during anthesis by changing their blooming habits, such as flowering in the early morning. True heat tolerance at critical phases may be given by preventing heat damage to structural proteins, enzymes, and membranes. The function of heat shock proteins (HSPs) and other components in these processes is critical (Maestri et al. 2002).

Isoprene and other volatile molecules also play an essential role for heat tolerance in plants (Monson et al. 2013). HT plants' leaves may produce volatile chemicals like isoprene, which act as signaling molecules in communications between plants in ecosystems (Vickers et al. 2009). This was demonstrated by the presence of isoprene-induced thermotolerance (Singsaas et al. 1997), even in species or transgenic plants that do not generate isoprene (Sharkey et al. 2001). Cyclic electron transport is also essential in rice thermotolerance. It supplies enough ATP even if the thylakoid membrane leaks owing to HS (Bukhov et al. 1999), preventing ATP depletion under high temperature stress (Schrader et al. 2004). Many scientists have shown that plant hormones cytokinin and photosynthetic machinery enzymes have a role in HT, with delayed leaf senescence and improved stress tolerance in many plant species (Xing et al. 2009). In plant cells, HS promotes ROS formation, namely, hydroxyl radicals, superoxide radicals, and hydrogen peroxide (Wise and Naylor 1987). These ROS wreak havoc on proteins, DNA, and cell membranes (Apel and Hirt 2004). Under HS, HT plants use both non-enzymatic and enzymatic mechanisms for hunting ROS. Enzymes such as catalase, superoxide dismutase, ascorbate peroxidase, peroxidase, glutathione reductase (Mittler 2002), and non-enzymatic antioxidants such as glutathione, ascorbic acid, and tocopherols (Noctor and Foyer 1998) influence ROS and therefore protect plants.

14.2.5 Heavy Metal Toxicity Adaptation and Tolerance Mechanism in Rice

To maintain trace element homeostasis, plants have evolved tolerance mechanisms to metals such as exclusion, chelation, and compartmentalization (Hall and Williams 2003; Shahzad et al. 2016, 2017). Compounds such as H_2S can aid in the reduction of heavy metal toxicity in rice. Pb buildup, for example, reduced rice seedling development while increasing ROS production. Furthermore, increased Ca^{2+} buildup, MBP (myelin basic protein) kinase activity, and CDPK (calcium-dependent protein kinase) activity prevented cell death caused by lead toxicity and activated the MAP (mitogen-activated protein kinase) (Huang and Huang 2008). Under Cd stress, high endogenous H_2S levels in rice enhanced biochemical and physiological characteristics. Tolerance to Cd stress via H_2S usage may be attributed to reduced Cd-induced oxidative stress due to a more significant redox state and increased activity of enzymatic antioxidants that detoxified the methylglyoxal and ROS. In addition, H_2S preserved nutritional and Cd homeostasis in the leaves and roots of Cd-stressed plants. In contrast, the addition of hypotaurine (an H_2S scavenger) reversed the beneficial impact of H_2S, bolstering evidence of H_2S' participation in Cd toxicity tolerance in rice (Mostofa et al. 2015). The upregulation and enhanced expression of genes involved in heavy metal transport and localization also help in heavy metal stress tolerance (Yuan et al. 2012).

Hormonal therapy may assist in mitigating the harmful effects of heavy metal toxicity. Si treatment has been shown to decrease the adverse effects of Cd/As toxicity by reducing their concentration and increasing rice production (Meharg and Meharg 2015). Foliar Si treatment was beneficial in lowering As/Cd concentrations (Liu et al. 2013a, b). Foliar treatment of both inorganic and organic

Si reduced heavy metal concentrations (Cd, Cu, Pb, and Zn) and bio-concentration factors in grains and heavy metal translocation factors from root to shoot and shoot to grain. The use of nano-Si was the most effective at reducing Cd absorption and translocation (Wang et al. 2016a). Many studies have focused on the genetic differences between rice genotypes in heavy metal absorption and translocation in the roots, shoots, and grains (Li et al. 2003; Liu et al. 2013a, b). Choosing appropriate germplasm might be an intelligent approach. The expression of heavy metal tolerance genes and proteins using molecular and transgenic methods is also a viable alternative for the creation of heavy metal resistant rice genotypes. Zhang and Liu (2017) investigated the discovery, characterization, and localization of OsMTP11, a metal stress-resistant protein in rice. When rice was treated to Cd, Ni, Zn, and Mn stress, the expression of OsMTP11 rose considerably, indicating that this protein may play a role in heavy metal stress resistance.

14.3 Molecular Breeding Conferring Abiotic Stress Tolerance in Rice

Molecular breeding is the utilization of genetic manipulation performed at the level of DNA to improve characters of interest in plants and animals, and it may also involve genetic engineering or gene manipulation, molecular marker-assisted selection, and genomic selection (Ribaut et al. 2010). In the last decades, we have seen the favorable outcome of progressive molecular and genomic tools such as molecular markers, expressed sequence tags (ESTs), microarrays, and genetic transformations to scrutinize the genetic basis of stress tolerance and ultimately to evolve crop cultivars upgraded for stress tolerance. The new advancements in DNA marker technology have assisted to develop the ideas of QTLs mapping, marker-assisted selection (MAS), and genetic transformation to generate plants of better quality. Moreover, molecular markers can be utilized for estimating genetic diversity, fingerprinting genotypes, separating hybrids from individual progeny, and other purposes. The real recognition of candidate DNA markers related to resistance genes utilizing fine-mapping may well allow rice breeders to proficiently transfer these genes from donor cultivars into new, elite rice cultivars utilizing marker-assisted selection (MAS). There is likewise a need to link knowledge about genes and gene function to generate new fruitful varieties that are an essential component of a sustainable food security for the future (Ashkani et al. 2015).

14.3.1 Identification of QTL and Gene Towards Abiotic Stress Tolerance in Rice

Quantitative trait loci (QTL) are regions of the genome at which genetic variation is identical with a specific quantitative trait. QTLs are recognized by appearing a statistical association between genetic markers and measurable phenotypes. For QTL mapping, researchers are need to (1) choose and/or develop appropriate mapping population (experimental populations for linkage-based mapping or natural/breeding populations for association mapping); (2) phenotype the population for the characters of interest (morphological characters, agronomic traits, disease and pest scores, drought resistance, etc.) under greenhouse, screen house, and/or field conditions; (3) decide the type of molecular marker(s) and the genotyping approach (entire population, selective genotyping, or bulked segregant analysis) and generate the molecular data for adequate number of uniformly spaced polymorphic markers; (4) recognize molecular markers related to the trait(s) of interest utilizing statistical programs (linkage-based QTL mapping methods need construction of genetic linkage map); and (5) test the pertinence and reliability of the markers connected with major QTLs in predicting the trait(s) in associated families (marker validation or verification) for QTLs of medium to large effect. The availability of a vast range of molecular markers and powerful statistical methods has significantly simplified QTL mapping. Both QTL mapping methods begin with the assortment of genotypic and phenotypic data from either segregating or natural population, followed by statistical analyses to expose all possible marker loci wherever allelic variation associates with the phenotype. Nowadays, QTL mapping is being intensively utilized to find the specific location of the concerned regions/genes. To classify the abiotic stress-resistant QTLs, a lot of work has been done by plant biologists, but recognized QTLs proved unstable across diverse environmental conditions because of their complex inheritance mechanism of abiotic stress tolerance. In this part, we will describe and summarize the various major and minor QTLs or genes identified in rice for different abiotic stresses that would be helpful for the researchers to find the desirable QTLs in future work.

14.3.1.1 QTLS Related to Submergence Tolerance in Rice

A lot of QTLs have been identified by crossing varieties which have anaerobic germination (AG)-tolerant properties (Angaji et al. 2010; Zhang et al. 2017; Yang et al. 2019). Several QTL gene accounts for AG tolerance (qAG9-2) were first detected as trehalose-6-phosphate phosphatase gene, OsTPP7, through biparental linkage mapping including the anaerobic germination-tolerant variety of Japonica-oriented subspecies (spp.), Khao Hlanon (Kretzschmar et al. 2015) (Table 14.1). In an AG-intolerant variety (IR64) as for linkage mapping, the genomic region enveloping OsTPP7 was omitted (Kretzschmar et al. 2015). TPP activates the reformation of trehalose-6-phosphate (T6P) to trehalose (Figueroa and Lunn 2016). It has been suspected that T6P contributes as a negative assessment regulator of sucrose levels in plants; the T6P/sucrose proportion is a focal homeostatic framework in sugar signaling (Figueroa and Lunn 2016). Kretzschmar et al. (2015) recommended that modulating T6P/sucrose proportions via OsTPP7 activity in germinating tissues accelerates resource allocation from the endosperm conserve to the coleoptile, resulting in embellished growth in the coleoptile during AG. Therefore, the OsTPP7 gene is disappeared in several cultivated rice varieties by chromosomal deletion (Kretzschmar et al. 2015); an incorporation of the OsTPP7 region may enhance AG tolerance and grain yield in these rice varieties by further breeding.

Table 14.1 QTL/gene identified on submergence tolerance properties in rice

Gene name	QTL name	Trait	Chromosome	Encoding protein annotation	Parentage	Reference
OsTPP7	qAG-9-2	Anaerobic germination	9	Trehalose-6-phosphate phosphatase	Khao Hlanon × IR64	Kretzschmar et al. (2015), Angaji et al. (2010)
SUB1A	qSUB1 (Sub1)	Flash flood submergence tolerance	9	AP2/ERF family transcription factor	FR13A × M-202	Xu et al. (2006), Xu and Mackill (1996)
SK1 (SNORKEL1, SK2)	qTIL2	Internode elongation under submergence	12	AP2/ERF family transcription factor	C9285 (Dowai38/9) × Taichung 65	Hattori et al. (2009), Hattori et al. (2007)
SDI (OsGA20ox2)	qTIL1	Internode elongation under submergence	1	Gibberellin 20-oxidase	C9285 (Dowai38/9) × Taichung 65	Kuroha et al. (2018), Hattori et al. (2007)

Source: Modified from Kuroha and Ashikari (2020)

The local cultivar FR13A, a submergence-tolerant rice variety of India, having Submergence QTLs (Sub1), is responsible for up to 70% of the phenotypic variation of the flash flood tolerance properties (Xu and Mackill 1996) (Table 14.1). Biparental linkage mapping reduces the related region, which includes a group of transcription factor genes affiliated with the APETALA2 (AP2)/ethylene response factor (ERF) superfamily SUB1A, SUB1B, and SUB1C (Xu and Mackill 1996). In between, SUB1A is the gene which accounts for flash flood tolerance (Xu et al. 2006). The flash flood-tolerant allele SUB1A-1 has an SNP liable for an amino acid substitution at a site indispensable for its protein function (Xu et al. 2006; Singh and Sinha 2016) (Table 14.1). Afterward introgression of the allele actively serves flash flood tolerance without any breakdown on grain yield and quality; SUB1A-1 has been extensively accustomed to breed flash flood-tolerant varieties in many other countries in Asia (Bailey-Serres et al. 2010).

AP2/ERF transcription factor SNORKEL1/SNORKEL2 (SK1/2) genes were first recognized as seminal genes for internode prolongation held down submergence undulating the QTL on chromosome 12 (Hattori et al. 2009). The QTL SK1/2 are alone obtained in deepwater rice varieties but are not present in other cultivated rice (Hattori et al. 2009; Zhao et al. 2018). SK1/2 transcription is precisely speculated through OsEIL1b, which is homologous to ETHYLENE INSENSITIVE 3 (EIN3), a transcription factor equitable aside ethylene signaling (Hattori et al. 2009). The expression QTL analysis proving recombinant inbred lines from non-deepwater and deepwater rice identified candidate downstream genes of SK1/2 (Kuroha et al. 2017). Impressively, SK1/2 also exist to the ERFVII family, which consist of SUB1A-1 (Hattori et al. 2009). A consolidation of GWASs and linkage mapping recently detected SEMIDWARF1 (SD1) as a causal gene for the deepwater response undulating the QTL on chromosome 1 (Kuroha et al. 2018). SD1 encodes *O. sativa* gibberellin 20 oxidase 2 (OsGA20ox2), an enzyme engaged in GA biosynthesis. The loss of function of SD1 negotiates a semi-dwarf phenotype along a short internode (culm) and was liable for a better output harvest index and greater grain yield during the Green Revolution (Sasaki et al. 2002).

14.3.1.2 QTLs for Drought Tolerance in Rice

After ineffective efforts with secondary characters in breeding for drought tolerance, detailed studies have been executed to detect meta-QTLs for yield under stress which characterize the hotspot regions for drought tolerance in rice (Courtois et al. 2009; Dixit et al. 2012, 2014; Vikram et al. 2011). This governed to the identification of QTLs from diverse drought-tolerant sources, namely, Nagina 22, Vandana, Way Rarem, and Apo elucidating a more than 30% phenotyping modification. Among the local germplasm, the major-effect QTL, $qDTY_{1.1}$ on chromosome 1 recognized from crosses including Nagina 22 with high-yielding cultivars (i.e., Swarna, IR64, and MTU1010) is the furthermost reliable QTL for grain yield under drought (Vikram et al. 2011). The similar QTL has likewise been recognized in another mapping populations such as CT9993-510 to 1-M/IR62266-42 to 6-2 verified in Thailand and India (Kumar et al. 2007). This QTL co-localizes with *sd1* (semi dwarf) region and QTL for plant height and flowering under water deficit condition. Also, another main

QTL, *qDTY*$_{3.2}$, was recognized in the region of *HD9* that influences flowering in Vandana/Way Rarem population (Venuprasad et al. 2009). Analogous but close elucidate observation on coincidence of plant height and flowering QTLs with drought escape QTLs has been built by Xu et al. (2005). Dixit et al. (2012) fine-mapped four QTLs, namely, *qDTY*$_{2.1}$, *qDTY*$_{2.2}$, *qDTY*$_{9.1}$, and *qDTY*$_{12.1}$, for grain yield under drought stress in four dissimilar mapping populations evolved from Apo/Swarna (BC_1F_4), Adaysel/IR64 (BC_4F_3), and Vandana/Way Rarem (BC_2F_3 and BC_3F_3). Later on, the linkage between the plant height and drought tolerance was destroyed by fine-mapping of *qDTY*$_{1.1}$ region wherein the QTL region was obtained to be 200–400 kb distal to the *sd1* region (Vikram et al. 2016). They deliberate the pleiotropic and interaction effects of the yield under stress QTLs, namely, *qDTY*$_{1.1}$, *qDTY*$_{3.2}$, and *qDTY*$_{12.1}$, and obtained that all these three are not in fact pleiotropic but linked with QTLs for plant height and flowering. Additionally, *qDTY*$_{3.2}$ exhibited positive interaction with other QTLs by decreasing flowering duration, thus helping to circumvent drought and leading to rise in grain yield (Vikram et al. 2016). These attempts paved the way for introgression of yield under drought QTLs in cultivated varieties and development of superior drought-tolerant varieties. Each of these recognized QTLs has revealed a yield benefit of 300–500 kg/ha under reproductive stage (RS) drought stress relying upon the severity and timing of the drought existence (Kumar et al. 2014, 2018a, b).

14.3.1.3 Salinity Tolerance-Related QTLs in Rice

Over the past two decades, mapping of QTL/genes relevant with salt tolerance in rice is going on. Plenty of quantitative trait loci (QTL) linked to salinity tolerance have been discovered in rice. Salt-tolerant QTL on chromosome 1 (surrounding 40 Mb) was detected underlying several salt-related traits in diverse rice species including cultivated varieties, landraces, and wild rice (Tiwari et al. 2016; De-Leon et al. 2017; Chen et al. 2020).

Even though, QTLs showing significant effects have been successfully isolated or fine-mapped. Two major QTLs, qSNTQ-7 for shoot Na^+ concentration (SNC) and qSKC-1 for shoot K^+ concentration (SKC), were identified from a cross generated from salt-tolerant Nona Bokra and salt-susceptible Koshihikari (Lin et al. 2004). The qSKC-1 on chromosome 1 responded for 40.1% of total phenotypic variation and was fine-mapped for isolation. The primitive functional analysis suggested that the SKC1 gene encodes an HKT-type transporter (OsHKT1;5), which is preferentially expressed in the parenchyma cells surrounding the xylem vessels in rice and regulates K^+/Na^+ homeostasis under salt stress (Alnayef et al. 2020). QTL for Saltol trait associated with the shoot Na^+/K^+ ratio at the seedling stage was identified from a salt-tolerant landrace Pokkali (Bonilla et al. 2002). The SKC1 gene (OsHKT1;5) was assumed as functional gene underlying the Saltol segment (Thomson et al. 2010). The QTL qST1.1 for seedling salinity tolerance originated from Sea Rice 86 was located within the Saltol region with the amino acid sequence of SKC1 (OsHKT1;5) of Sea Rice 86 which was similar to that in Nona Bokra (Wu et al. 2020).

The qSE3 QTL identified in a Chinese landrace Jiucaiqing was found to perform as a high-affinity K^+ uptake transporter (OsHAK21), facilitating seed germination and seedling establishment under salinity stress (He et al. 2019).

Increasing both K^+ and Na^+ uptake and abscisic acid (ABA) levels and reducing ROS levels were suggested to be the main physiological mechanisms of qSE3. The genome-wide meta-analyses of QTL for various traits associated with rice salt tolerance (Islam et al. 2019) and integrative meta-analysis for rice salinity tolerance (Mansuri et al. 2020) were identified more than 20 candidate genes in Saltol and around 20 meta-QTL regions.

Reports on QTL mapping for salt tolerance during the reproductive phase are limited in comparison with the seedling stage. A QTL for Na^+ uptake and Na^+/K^+ ratio in the shoot at reproductive phase was identified on chromosome 1 at a different position to Saltol (Hossain et al. 2015). Three QTLs on chromosome 1 at positions 32.3, 35.0, and 39.5 Mb were found to response for grain yield per plant under salinity stress from a salinity-tolerant *indica* rice variety CSR11. Two QTLs designated as qDEG-S-2-2 for spikelet degeneration and qSSI-STE2-1 for spikelet sterility were mapped on chromosome 2, responsible for 34.4% and 38.8% phenotypic variances, respectively, with tolerance alleles from Pokkali (Chattopadhyay et al. 2021). The genome-wide association study (GWAS) technique could offer a powerful strategy for dissecting the genetic architecture of different complex traits and simultaneously pointing out allelic variations of candidate genes in rice (McCouch et al. 2016) and some other cereal crops. GWAS has also been applied on trait-associated loci for salt tolerance. The Saltol QTL region (affecting Na^+/K^+ ratio at rice seedling stage) was also responsible for balancing Na^+/K^+ ratio at reproductive stage (Kumar et al. 2015). Another major QTL, RNC4, which controls root Na^+/K^+ ratio and root Na^+ content in moderate salinity stress, was located at ~575 kb region on chromosome 4 (Campbell et al. 2017). The gene for sodium transport OsHKT1;1 is located in this region regulating root Na^+ content and underlying the variability between *indica* and *japonica* rice subspecies.

14.3.1.4 QTLs Towards Cold Tolerance in Rice

Cold injury is a common production constraint in rice that affects rice growth and ultimately decreases grain yield. Low temperature at booting and flowering stages reduced plant growth and caused a significant decline in spikelet fertility. The development of rice varieties with cold stress tolerance has been a severe challenge for rice breeders for a long time. QTLs and genes on the chromosomal location for cold tolerance are limited in different growth stage-specific traits in rice. In rice, seedling stage cold tolerance is regulated by multiple genes and several QTLs (Zhang et al. 2014). Xu and Cai (2014) reported on OsRAN1, the Ran gene required for the development of cold tolerance rice varieties. Pan et al. (2015) marked 22 QTLs for cold tolerance for germination stage through SSR markers in 174 Chinese accessions. Similarly, Fujino et al. (2015) mapped 117 markers originated from a Hokkaido rice core panel, comprising 63 Japanese landraces and breeding lines, and identified six QTLs for cold tolerance at heading stage and 17 QTLs for germinability in low temperature by GWAS approach. Nogoy et al. (2016) reported four putative QTLs for the cold tolerance trait, which were mapped on chromosomes 1, 3, 7, and 11. Among these QTL, qSCT-11 showed a large additive effect on the trait, explaining 26% to 30% of the phenotypic variation in the treatment of 13 days at 10 °C with a LOD of 16–19. Furthermore, Lv et al. (2016) identified 132 loci regulating 16 traits evaluated under natural cold shock stress from a large collection of 529 rice accessions with more than 4.35 million SNP markers. Sales et al. (2017) found 24 SNPs responsive to low-temperature germination and growth rate, while Shakiba et al. (2017) identified 42 QTLs regulating cold tolerance at seedling stage. Schläppi et al. (2017) reported on 48 QTLs for cold tolerance in 202 *O. sativa* accessions from the USDA mini-core collection. Recently, Li et al. (2018a, b) investigated a set of RILs using the germination rate under low-temperature stress as an indicator and detected seven QTLs for cold tolerance on chromosomes 4, 6, and 9 in rice.

14.3.1.5 QTLs Towards Heat Stress in Rice

Heat tolerance is the capacity of the plant to raise and proper development in heat stress. Plants differ within plant to plant and species to species in their response to tolerate heat stress (Camejo et al. 2005). Breeding with heat tolerance rice varieties (QTL incorporated) is necessary to cope with for sustainable food security and future climate change. The flowering stage is the furthermost sensitive stage to high temperature. Many high temperature stress tolerance rice germplasms and wild species have been identified through screening and utilized as a donor parent (Jagadish et al. 2007; Pradhan et al. 2016). Nagina 22 is the utmost heat-tolerant variety evaluated so far (Pradhan et al. 2016). Nevertheless, no genes have been categorized for increasing heat tolerance in rice from Nagina 22 until now; however, enough heat shock factors and proteins have been involved. Following, the putative QTLs for heat tolerance situated on different chromosomes were testified by several researchers. The doubled haploid population developed from IR64/Azucena cross was the first one to be used for QTL mapping of heat stress tolerance by percent spikelet fertility (Cao et al. 2003) similar to that of drought tolerance (Courtois et al. 2000) (Table 14.2). Subsequently, about dozen of aforementioned evidence using various parents and categories of mapping populations are available (Chang-Lan et al. 2005; Chen et al. 2008; Zhang et al. 2008; Jagadish et al. 2010a, b; Cheng et al. 2012; Poli et al. 2013; Xiao et al. 2011; Ye et al. 2012, 2015; Shanmugavadivel et al. 2017). Phenotyping under high temperature was evaluated in open field conditions by late planting in several lessons (Xiao et al. 2011; Zhao et al. 2016), whereas others used oversight conditions where control temperature and humidity are provided (Jagadish et al. 2010a, b; Shanmugavadivel et al. 2017). IR 64 and Nagina 22 are the utmost frequently used parents in these mapping attempts. IR 64 was utilized as one of the parents in four different studies (Cao et al. 2003; Ye et al. 2012, 2015; Shanmugavadivel et al. 2017), although either Nagina 22 or its developed mutant lines have been utilized as parents in generating mapping population in three different studies (Poli et al. 2013; Buu et al. 2014; Shanmugavadivel et al. 2017). There are yet three evidences on using IR 64/Nagina 22 developed populations for mapping heat tolerance QTLs (Ye et al. 2012, 2015; Shanmugavadivel et al. 2017).

Table 14.2 QTLs associated with heat stress in rice

Sl	Parentage	Heat stress	Chrom no.	Marker interval	Reference
1	IR64 × Azucena	7 days from heading	1	RG381-RZ19	Cao et al. (2003)
			11	RG118-Adh	
2	Nipponbare × Kasalath/Nipponbare	5 days after heading	4	C1100-R1783	Chang-Lan et al. (2005)
			7	C1226-R1440	
3	T219 × T226	7 days from heading	3	RM570-RM148	Chen et al. (2008)
			12	MRG0103-RM235	
			3	RM570-RM148	
			9	RM242-RM566	
4	Zhongyouzao8 × Toyoeishiki	8 days from heading	5	RM405-RM274	Zhang et al. (2008)
5	Bala × Azucena	Move everyday	4	C734-RG449	Jagadish et al. (2010a, b)
			1	B1065E10-RZ14	
			10	C16-C223	
			1	B106E10	
			3	R1618-G164	
			10	C16-C223	
			10	C16-C223	
6	996 × 4628	4 days from heading	4	RM5687-RM471	Xiao et al. (2011)
			10	RM6132-RM6100	
			4	RM5687-RM471	
			10	RM6132-RM6100	
7	IR64 × Nagina22	14 days from heading	1	id1023892	Ye et al. (2012)
			4	id4005120	
8	Xuishui 09 × IR2061	14 days from flowering	4	RM518-RM401	Cheng et al. (2012)
			6	RM589-RM588	
			5	RM430-RM440	

(continued)

Table 14.2 (continued)

Sl	Parentage	Heat stress	Chrom no.	Marker interval	Reference
9	IR64 × Giza 178	14 days from heading	1	id1013342	Ye et al. (2015)
	Milyang23 × Giza 178		2	id2006486	
	IR64 × Milyang23 × Giza 178		3	id3001137	
			4		
			6		
			11		
10	Nagina22 × IR64	From booting for 10 days	9	SNP12393-SNP12417	Shanmugavadivel et al. (2017)
			5	SNP8377-SNP8401	

The reliable QTLs for heat tolerance are from chromosome 4 (Chang-Lan et al. 2005; Zhang et al. 2009a; Jagadish et al. 2010a, b; Xiao et al. 2011; Ye et al. 2012; Buu et al. 2014) and chromosome 5 (Zhang et al. 2008; Shanmugavadivel et al. 2017). Numerous QTLs for heat tolerance have too been recognized using chromosome segment substitution lines developed from the genotypes, Sasanishiki and Habataki (Zhao et al. 2016). Also, the QTLs from nurtured germplasm, introgression lines (ILs) derived in the BG of Swarna and KMR3 with *Oryza nivara* and *Oryza rufipogon*, were examined for heat tolerance. Since this resource, three ILs with higher tolerance to heat (K-377-24, K-16-3, and S-148) were recognized (Prasanth et al. 2017). Due to the lack of fine-mapping of the recognized QTLs in cultivated rice, there are no varieties until now entirely breed for heat tolerance moreover by conventional or molecular means. As far as, the QTL on chromosome 5 evidenced in <400 kb interval by Shanmugavadivel et al. (2017) could be a good candidate for introgression.

14.3.1.6 Heavy Metal Toxicity-Related QTLs in Rice

Heavy metal (HM) toxicity is one of the major abiotic stresses leading to deleterious effects in rice plants. A common consequence of HM toxicity is the excessive accumulation of reactive oxygen species (ROS) and methylglyoxal (MG), both of which can cause peroxidation of lipids, oxidation of protein, inactivation of enzymes, and DNA damage and/or interact with other vital constituents of plant cells. Modern breeding approaches, namely, marker-assisted selection (MAS) and QTL mapping, also contributed tremendously in improving tolerance of plant to heavy metals. QTL mapping is a powerful genetic approach to identify the number, position, and effects of genetic factors, which control As concentration in rice tissues (Syed et al. 2016; Zhang et al. 2008). Firstly, Dasgupta et al. (2004) reported a major gene, *AsTol*, which was mapped from Bala × Azucena mapping population of rice between markers RZ516 and RG213 on chromosome 6. Zhang et al. (2008) have identified arsenic (As) accumulating four QTLs from a doubled haploid population originated from anther-culture of F_1 plants between CJ06, a *japonica* cultivar, and TN1 (*Oryza sativa*), an *indica* cultivar. One arsenic concentration-responsive QTL was mapped on chromosome 2 for seedling stage in shoots having 24.4% phenotypic variance, and another one QTL in roots was detected on chromosome 3. Two QTLs for As concentrations in grains were found on chromosomes 6 and 8, with 26.3% and 35.2% phenotypic variance, respectively. No common loci were found for the three traits. Furthermore, Norton et al. (2010) have identified six QTLs for arsenic accumulation in rice shoots: two on chromosome 1 (*qAs1* and *qAs2*), one on the lower part of chromosome 3 (*qAs3*), one on chromosome 5 (*qAs5*) which co-localizes with a phosphorus accumulation QTL, and two on chromosome 6 (*qAs6.1* and *qAs6.2*) both of which co-localize with other QTLs for toxic elements. Kuramata et al. (2013) reported two QTLs on chromosome 6 (*qDMAs6.1* and *qDMAs6.2*) and one QTL on chromosome 8 (*qDMAs8*) responsible for DMA concentration variations in the grain. A total of four main effect QTLs (*qAsTSL8*, *qAsTSL12*, *qAsTRL8* and *qAsTRSB8*) with significant LOD comprising two for relative growth in shoot length, one for each of root length and root-shoot biomass

Table 14.3 QTL/genes responsive to heavy metal tolerance in rice

QTL/gene	Trait	References
qAsR8.1, qAsR8.2	Arsenic content root	Murugaiyan et al. (2019)
qAsS2, qAsS5.1, qAsS5.2, qAsS6, qAsS9.1, qAsS9.2	Arsenic content shoot	Murugaiyan et al. (2019)
qAsTSL8, *qAsTSL12*	Arsenic phytotoxicity in shoot length	Syed et al. (2016)
qAsTRL8, and *qAsTRSB8*	Arsenic phytotoxicity in root length	
qDMAs6.1, *qDMAs6.2*, *qDMAs8*	Rice grain DMA concentration	Kuramata et al. (2013)
OsHMA3	Cd compartmentalization	Miyadate et al. (2011)
OsPDR5/ABCG43	ABC-type protein for cellular Cd tolerance	Oda et al. (2011)
LCD	Cd accumulation and tolerance	Shimo et al. (2011)
OsCDT1	Cd tolerance	Kuramata et al. (2009)
OsMTP1	Cd, Zn	Yuan et al. (2012)
Os08g01480	As, Cd, and Cr	Rai et al. (2015)
OsMTP11	Cd, Ni, Zn, and Mn	Zhang and Liu (2017)

under arsenic stress were identified on chromosomes 8 and 12 (Syed et al. 2016) (Table 14.3). A large effect QTL for arsenic phytotoxicity tolerance in root length contributing 24.9% phenotypic variation was identified on chromosome 8 with plausible pleiotropic effect on shoot length and root-shoot biomass. Zhang and Liu (2017) identified, characterized, and localized a metal stress-tolerant protein *OsMTP11* in rice. The expression of *OsMTP11* was significantly upregulated when rice was subjected to Cd, Ni, Zn, and Mn stress, suggesting the probable involvement of this protein in heavy metal stress tolerance. Recently, Murugaiyan et al. (2019) found quantitative trait locus (QTL) mapping to identify candidate genes involving As toxicity tolerance and accumulation in rice (*Oryza sativa* L.) seedlings. The experiment was conducted with an early backcross breeding population accommodating 194 lines derived from a cross between WTR1 (indica) and Hao-an-nong (japonica). Marker-trait association was determined for 7 As-related traits using 704 single nucleotide polymorphism (SNP) markers identified from a 6 K SNP-array. One QTL on chromosome 1 for relative chlorophyll content, two QTLs for As content in roots on chromosome 8, and six QTLs for As content in shoots were mapped on chromosomes 2, 5, 6, and 9, respectively.

14.3.2 Marker-Assisted Breeding (MAB) for Improving Tolerance of Rice Varieties Under Abiotic Stress Condition

Innovative molecular breeding tools are being capable for redesigning crop varieties that primarily utilize MAS and gene transformation. Marker-assisted selection (MAS) refers to such a breeding procedure in DNA marker detection, and selection is integrated into a traditional breeding program. It utilizes DNA-based markers that are directly linked with the targeted QTL to assist phenotypic evaluation and improvement of breeding efficiency by selecting the target genes within the germplasm, but not genetic engineering, which involves transfer of foreign gene sequences (Chukwu et al. 2019; Oladosu et al. 2018; Quibod et al. 2016). Single nucleotide polymorphisms are available throughout the entire genome of plants. These SNPs are a good source for MAS in breeding programs (Hayashi et al. 2004). Marker-assisted selection strategy includes genotype identification, diversity and purity analysis, selection of parent lines, and the study of hybrid vigor (Collard and Mackill 2008). Marker-assisted selection (MAS) can accumulate genes or QTLs with potential characteristics into a single genotype, which can accelerate the advancement of tolerant breeding lines in minimum succeeding generations with the utmost precision through the process of gene pyramiding. Selection strategy utilizing genomic marker technology has been effectively applied for stress resistance development and quality improvement in many important crops, such as rice (resistance to bacterial blight, blast, flood, and salinity and improvement in grain quality), wheat (resistance to leaf rust and powdery mildew), and cotton (resistance to insect pests), through precise transfer of genomic regions of interest and acceleration of the recovery of the recurrent parent genome (Das et al. 2017). Marker-assisted backcrossing (MABC), an easy and effective technique of marker-assisted selection, is currently being widely applied in molecular breeding (Pilet-Nayel et al. 2017). Marker-assisted backcrossing targets one or more genes or QTLs transferred from one donor parent into another elite breeding line to upgrade a targeted trait. Through MABC, the result can be visualized within a shorter period of time (Chukwu et al. 2019). So, QTL-based marker-assisted selection (MAS) and marker-assisted backcrossing (MABC) have been accepted as an optimistic tool in crop molecular breeding.

14.3.2.1 MAS of SUB 1 Gene for Submergence Tolerance in Rice

About 25% rice fields are submerged by flash floods in every year throughout the world that losses grain yields (Loo et al. 2015). With negligible QTLs, submergence tolerance in rice is regulated by a sole major QTL on chromosome 9 that delivers complete submergence tolerance for 14 days or more (Septiningsih et al. 2009). The highly submergence tolerance germplasm FR13A which contains submergence tolerance gene, SUB1, was inserted into a number of popular varieties by MABC, and BC_2 or BC_3 generation has selected the final product. They can withstand total inundation for 2–3 weeks. The phenotypic acceptability and growth habits were close to the original plants. Siangliw et al. (2003) was the first reporter and convey that chromosome 9 contains a submergence-tolerant gene, discovered by marker-assisted backcrossing (MABC). They include dis-absorbed endurance QTL in the genetic background of KDML-105 which is the jasmine type of Thai rice variety. They used four backcrosses to produce BC_4F_3 populations and found that the repeated parent genome (93% in the better plants) was not so high to select promising but the report of introgression size was ignored. The SUB1 QTL which is introgressed in many rice cultivars and used for the improvement of submergence-tolerant rice varieties that are extensively grown in south Asia subcontinents was reported by Neeraja et al. (2007).

To expedite this attempt, the SUB1 gene has been entered into two new widespread varieties from Indonesia and the Philippines, i.e., Ciherang and PSB RC18, utilizing a single backcross (BC1) through MABC as a previously developed IR64-Sub1 donor. Meanwhile newly developed Sub1 population is closely interrelated to both parents. The MABC technique can be followed more quickly due to similar genetic backup. Using this technique, the new submerged-tolerant breed Ciherang-sub1 and PSB RC18-sub1 evolved in less than 2 years, awarding an encouraging method to future over-popular breed conversion (Septiningsih et al. 2015). This Ciherang-sub1 was released as a submergence-tolerant variety in the name of Binadhan-11 for the flash flood ecosystem of Bangladesh. The SUB1 QTL was incorporated in BR11, BRRI dhan33, and BRRI dhan49 as a recipient parent through MABC by using foreground and background selection and phenotypically confirmed for submergence tolerance at the BRRI (Iftekharuddaula et al. 2015). In MABC, a backcross was created between SUB1 donor IR40931-33-1-3-2 and BR11 (a rain-fed lowland mega variety in Bangladesh) where a tightly connected two gene-based SSR markers and four neighboring SSR and 116 background SSR markers were intended for foreground, recombinant, and background selection, correspondingly. The BC_2F_2 plant of BR11-Sub1 was found in the population which occupied all the pieces of BR11 type SSR allele except SUB1 QTL (inward size amounted to 800 kb), about 99.8% B. This BR11-sub1 line was released in Bangladesh in the name of BRRI dhan52 as a submergence-tolerant variety which withstand flash flood for 2 weeks (Iftekharuddaula et al. 2016a). Furthermore, the SUB1 QTL was used through MABC to access short-term rice variety, BRRI dhan33. The BC_1F_1-BC_4F_1 stage performed a sequence of foreground and phenotypic selection, and the final stage of BC_4F_2 used a combination of foreground, recombinant, and background marker in backcross populations between a SUB1 donor, BRRI dhan52 (evolved from BR11 and the Sub1 line IR40931) and BRRI dhan33. A total of 15 lines with homozygous genetic background with BC_4F_3 generation-specific SUB1 QTL were selected. On the selected line, the proportion of recipient genome recovery ranges from 90.7% to 95.2%. The tolerance line BR9157-12-2-37-13-15-40 has been found to be the best, with a survival ratio of 87.7% and a prolonged ratio of 11.4%. The Sub1 genotype yielded significantly higher than the originate variety BRRI dhan33 in both stress and non-stress circumstances under experimental and farmer field. This evaluated Sub1 line could be released for early maturity and submergence-tolerant high-yielding variety for early flash flood-prone area in Bangladesh (Iftekharuddaula et al. 2016b). Laterly, the another submergence tolerant rice variety BRRI dhan79 (BRRI dhan49-Sub1) also developed in Bangladesh Rice Research Institute (BRRI) through MABC which has the potential to withstand about 18–21 days of comprehensive submergence ahead with 50–60 cm stagnated water tolerance later the flood-water receded.

14.3.2.2 Improving Drought Tolerance Rice Varieties Through MAS of Drought-Related QTLs

Meaningful developments have been occurred in mapping QTLs for drought tolerance characters in rice; nevertheless, some of them have been efficiently used in marker-assisted breeding (Prince et al. 2015). The first attempts towards marker-assisted selection for carrying QTLs in rice for drought tolerance were the cross between Azucena (deep-rooted *japonica* upland cultivar) along IR 64 (a popular lowland *indica* variety) which caused recognition of lines accompanying larger root mass under drought stress conditions (Courtois et al. 2000). Birsa Vikas Dhan 111, an enhanced drought-tolerant variety, was evolved in India by crossing Kalinga III alongside Azucena and applying MAS for root characters among root QTLs recognized on chromosomes 2, 9, and 11 (Steele et al. 2006, 2007). Swamy et al. (2013) revealed that upscaled lines with two to three *qDTYs* in an IR64 background provided a yield benefit of 1200–2000 kg/ha under reproductive stage drought situations and to that of IR64 under non-stress situations. In the middle of IR64 upscaled lines, IR87707-445-B-B-B was recognized for release in India, whereas IR87707-446-B-B-B was recognized for release in Nepal. The different drought related QTLs of grain yield were incorporated through MAS in seven well-known varieties, Swarna, IR64, Vandana, Sabitri, TDK 1, Anjali, and Sambha Mahsuri, which appeared in the advancement of upgraded Swarna ($qDTY_{1.1}$, $qDTY_{2.1}$, and $qDTY_{3.1}$), IR64 ($qDTY_{2.2}$ and $qDTY_{4.1}$), Vandana ($qDTY_{12.1}$), Sabitri ($qDTY_{3.2}$ and $qDTY_{12.1}$), TDK 1 ($qDTY_{3.1}$, $qDTY_{6.1}$, and $qDTY_{6.2}$), and Anjali ($qDTY_{12.1}$ and $qDTY_{3.1}$), including Sambha Mahsuri ($qDTY_{1.1}$, $qDTY_{2.1}$, $qDTY_{2.2}$, $qDTY_{3.1}$, and $qDTY_{3.2}$) (Mishra et al. 2013; Yadaw et al. 2013; Kumar et al. 2014; Singh et al. 2016). A Malaysian well-known variety, MR219, was incorporated with $qDTY_{2.2}$, $qDTY_{3.1}$, and $qDTY_{12.1}$, and the effects of single or multiple QTLs were likewise informed (Shamsudin et al. 2016). The drought and flood stresses have been conveyed to effect around 40 Mha of total rice area at diverse crop stages, generating negative effects on plant progress, advancement, and production (Neeraja et al. 2007). Both flood and drought may happen in the similar season at diverse crop developmental stages, occurring a lot of catastrophes to rice production. Several drought and submergence-tolerant high-yielding rice varieties utilizing marker-assisted incorporated of *qDTY1.1*, *qDTY2.1*, *qDTY3.1*, and *Sub1* were evolved to delivery in flood and drought affected areas of northern part of India and Nepal (Sandhu et al. 2019) (Table 14.4).

14.3.2.3 Marker-Assisted Breeding Towards Improving Salinity Tolerance in Rice

Sensitivity to salt stress changes throughout the life cycle in rice, but the effects are extremely vulnerable in the seedling and reproductive stages (Thitisaksakul et al. 2015). However, most of the QTL/genes were trait specific and growth stage

Table 14.4 Drought and flood tolerance variety released in India and Nepal through MAS approach

Released as variety	QTL name	NILs designation	Background	Trait	Country	Year
Bahuguni dhan-1	$qDTY_{1.1} + qDTY_{3.1} + Sub1$	IR96321-1447-651-B-1-1-2	Swarna-Sub1	Drought + flood	Nepal	2017
Bahuguni dhan-2	$qDTY_{3.1} + Sub1$	IR94391-131-358-19-B-1-1-1	Swarna-Sub1	Drought + flood	Nepal	2017
DRR dhan-42	$qDTY_{2.2} + qDTY_{4.1}$	IR87707-445-B-B-B	IR64	Drought	India	2014
CR dhan-801	$qDTY_{1.1} + qDTY_{2.1} + qDTY_{3.1} + Sub1$	IR96322-34-223-B-1-1-1	Swarna-Sub1	Drought + flood	India	2017

Source: Modified from Kumar et al. (2014)

CR, Rice from National Rice Research Institute (NRRI), Cuttack; DRR, Directorate of Rice Research, rice from Indian Institute of Rice Research (IIRR) specific; pyramiding multiple genes would be practical in improving salinity tolerance at different periods of growth cycle for various environments. Molecular breeding techniques have been proven as the most efficient tools for development of improved varieties tolerable to salt (Mondal et al. 2013). In the salinity-tolerant cultivar NonaBokra, SKC1 gene mapping on chromosome1 was a breakthrough that preserved K^+ ion homeostasis in salt stress (Ren et al. 2005). Designed QTL pyramiding has been successfully practiced for increasing rice salinity tolerance at IRRI and in China based on trait-specific introgression lines (ILs) in target backgrounds developed via MABC (Li and Xu 2007; Pang et al. 2017). This MABC technique is utilized to accumulate relevant QTL from diverse salinity-tolerant donors into single target background or cultivar. However, MAS and MABC have been regularly used in salinity tolerance rice breeding with the drawback of “linkage drag.” The Saltol QTL derived from Pokkali, which is responsive to shoot Na^+/K^+ homeostasis, has been successfully introgressed into commercial varieties via MAS and MABC (Qin et al. 2020). Similarly, the OsRR22 gene, isolated from the salinity-tolerant mutant hst1, was successfully introgressed into Hitomebore, as elite cultivar (Takagi et al. 2015) and a high-yielding cultivar Yukinko-mai (Rana et al. 2019) has developed salinity tolerance at both seedling and reproductive stages. Linh et al. (2012) reported on using marker-assisted backcrossing (MABC) to develop a new salt-tolerant rice cultivar. To improve rice salt tolerance in BT7 cultivar, FL478 was used as a donor parent for the Saltol QTL conferring salt tolerance into BT7. Transferring of positive alleles for Saltol from FL478 into BT7 was successful from three backcrosses. The plants numbered as IL-30 and IL-32 in BC_3F_1 population were expected for recurrent genome recovery up to 99.2% and 100%, respectively. Field screening for agronomic traits of the selected lines carrying the Saltol alleles was performed. All improved lines were found to possess Saltol allele similar to the donor parent FL478, whereas their agronomic performances were the same as the original BT7. To improve seedling level salinity tolerance, attempts have been made to introgress Saltol to a high-yielding but salinity-sensitive rice Pusa Basmati 1121 (Babu et al. 2017). The study showed upgraded tolerance for salinity in the recipient to generate high-yielding rice using marker-assisted selection.

14.4 Advances of Omic Approaches for Developing Abiotic Stress Tolerance in Rice

Abiotic stresses are an increasing threat to crop production and food security (Bita and Gerats 2013). Thus, the importance of understanding the key factors through which rice responds to stress will lead to production of proper strategies for keeping sufficient productivity under adverse conditions. For sustainable crop production, there are also abundant opportunities, especially with rapid developments in omics approaches (Li and Yan 2020). Generally, omics is termed as the sequence of sciences for assessing the functions and interactions of genetic information in different clusters of living organism. It includes the study of genes (genomics), transcripts (transcriptomics), proteins (proteomics), metabolites (metabolomics), ions (ionomics), and phenotype (phenomics) (Fahimirad and Ghorbanpour 2019; Langridge and Fleury 2011). During the last decades, an enormous amount of genomics research has been deployed in the field of rice research, including the identification of enormous QTLs for agronomically important traits, the generation gene expression data, and cloning and characterization of major genes (Collard et al. 2008). In present time, plant omics-based research have played crucial role in identifying metabolic pathways and their molecular factors, which regulate and control several plant developmental processes (Kumar et al. 2017; Razzaq et al. 2019).

14.4.1 Genomics Approach for Improving Abiotic Stress Tolerance in Rice

Sequencing of the primary DNA code and identifying the pathways of gene expressions under different levels are fundamental resources for genomic studies (Fahimirad and Ghorbanpour 2019). Genomics may be defined as the study of gene location, function, and expression (Collard et al. 2008). Genomics can support to produce climate resilience crops through the characterization of diverse cultivars and wild crop relatives, identifying genetic variation and genes and characterizing relevant pathways associated with different biotic and abiotic stresses (Marsh et al. 2021) (Table 14.5). The first step in plant adaptation to stress is sensing the stress and producing response against it. The initial recognition of stress induces a signal

Table 14.5 The most important genes and improved traits related to abiotic stress tolerance in rice

Stress type	Traits	Related genes	Reference
Cold	Cold tolerance at seedling stage	*qPSST-3*, *qPSST-7*, *qPSST-9*, *qSCT1*, *qSCT2*	Jena et al. (2010)
Drought	Improve drought tolerance and enhance yield	*Dro1*	Uga et al. (2011)
	Enhance yield under drought stress	*qDTY2.2*, *qDTY3.1*, and *qDTY12.1*	Shamsudin et al. (2016)
Heat	Increase spikelet fertility under high temperature	*qHTSF4.1*	Ye et al. (2015)
Salinity	Improve salinity tolerance	*Saltol*	Das and Rao (2015)
	Improve salinity tolerance	*SKC1*	Emon et al. (2015)
	Controlling relative shoot length (RSL) at the bud burst stage under salt stress	*qRSL7*	Lei et al. (2020)
Submergence	Improve submergence tolerance up to 15 days under flood	*Sub1*	Das and Rao (2015)

transduction pathway, and tolerance genes have codes for the production of responsible enzymes or proteins involved in osmolyte biosynthesis and the detoxification of reactive oxygen species (Fahimirad and Ghorbanpour 2019). These pathways are highly controlled, and the product of one gene can act as receptors or activators for other different genes and can also regulate the gene expression pattern of plants (Lexer and Fay 2005). It has been showed that different abiotic stresses can cause similar levels of signaling pathways leading to the activation of the same genes (Hasegawa et al. 2000). The fragments of DNA or small regions of DNA sequences revealing the polymorphism in sequences (base deletion, insertion, and substitution) are called DNA markers. DNA markers are able to detect polymorphisms between particular sequences of DNA in a population (Collard et al. 2005). In addition, marker-assisted selection transfers genomic regions of interest more accurately and precisely. Thus, marker-assisted selection has become a routine work in crop breeding (Das et al. 2017).

It has been reported that rice develops different mechanisms of salinity acclimation using two different Na^+ transport systems, activating regulation of Na^+ transport at the transcription level and improving salinity tolerance in rice (Sriskantharajah et al. 2020). The varieties with low Na^+ accumulation in the leaf blades highly induced the expression of the *OsHKT1;5* gene in the roots, which may contribute to Na^+ exclusion from the shoots. In contrast, the varieties with high Na^+ accumulation in the leaf blades showed higher induction of the *OsNHX1* gene, whose gene product participates in the partitioning of Na^+ ion into vacuoles. *Saltol* QTL derived from the landrace Pokkali plays an important role in the maintenance of salinity tolerance in rice. Dhar et al. (2011) have shown that both SNP markers and SSRs markers could be used to find salinity-tolerant rice varieties. Some SNP arrays have also been reported which are linked with the specific genes related to biotic stress tolerance and other desirable traits like high yield in rice (Kurokawa et al. 2016).

Furthermore, the early-morning flowering (EMF) strategy has been used to produce introgression lines with the EMF trait transferred from wild rice *O. officinalis*. EMF NILs carrying the QTL *qEMF3* had earlier flower opening time by 1.5–2.0 h than recurrent parents, which decreased heat-induced sterility at flowering at elevated temperature. Actually, the shift in flower opening time to early morning is effective for escaping from heat stress at flower stage (Hirabayashi et al. 2015; Ishimaru et al. 2010). Pyramiding lines with QTLs for heat tolerance (*qHTSF4.1*) and EMF (*qEMF3*) effectively improved heat tolerance at flowering in both controlled and field conditions. Besides, two heat tolerance QTLs with high genetic effect (qSTIPSS9.1 and qSTIY5.1/qSSIY5.2) were mapped in less than 400 kbp genomic regions which may contribute greatly in developing heat-tolerant rice (Shanmugavadivel et al. 2017).

In case of submergence tolerance, sequencing of *Sub1* genomic region identified three genes which encode ERFs (*Sub1A*, *Sub1B*, and *Sub1C*) in which *Sub1A* has been reported as a key component of submergence tolerance (Xu et al. 2006). Furthermore, in other studies, major QTLs, namely, *qAG9-2* and *qAG7-1*, were reported for anaerobic germination tolerance under submerged condition (Angaji et al. 2010; Septiningsih et al. 2013). Later on, *qAG9-2* QTL has been fine-mapped and found a candidate gene *OsTPP7* which encodes a trehalose-6-phosphate phosphatase which is responsible to regulate anaerobic germination (Kretzschmar et al. 2015).

14.4.2 Transcriptomics for Understanding Abiotic Stress Tolerance in Rice

The complete RNA molecules expressed by a particular cell or population of cells are described as a transcriptome. There are many species of RNA, for example, messenger RNA (mRNA), transfer RNA (tRNA), ribosomal ribonucleic acid (rRNA), microRNA (miRNA), RNA interference (InRNA), small RNA (sRNA), nRNA, small nuclear ribonucleic acid (snRNA), Piwi-interacting RNA (piRNA), etc. The study of transcriptome data is defined as transcriptomics (McGettigan 2013). The combination of a high-throughput sequencing methodology with computational methods is defined as RNA-Seq. This approach determines and quantifies transcripts present in an RNA extract. Microarray is a hybridization-based technology. Thus, this technique is limited to known genes and has a restricted range of quantification. RNA-Seq is capable of being used for the analysis of previously unidentified genes and splice variants (Lowe et al. 2017). The activation of certain particular genes is the first response of plant cells to stress signal transductions. Transcription factors are the main members of signal transduction proteins which activate or inhibit the RNA polymerase II activity. Thus, during stress conditions, the cell patterns of transcriptome components determine the tolerance against the damaging effects of stresses (Khong et al. 2008). Comparative analysis of the transcriptomic data of plants exposed to stresses reveals the important molecules involved in adaptation to a particular stress. Validation of transcriptomic information is concluded in the identification of major stress-responsive genes (Muthuramalingam et al. 2017) (Table 14.6).

Through transcriptome analysis, it was identified that overexpression of OsC3H10 elevated the expression levels of genes involved in stress responses, including late embryogenesis abundant proteins, pathogenesis-related genes, and germin-like proteins (Seong et al. 2020). The expression of *OsC3H10* was induced by drought, high salinity, and abscisic acid. It was also found that OsC3H10 is involved in the regulation of the drought tolerance pathway by modulating the expression of stress-related genes. In case of investigating drought response in rice, the function of one regulatory gene *OsMIOX* was thoroughly studied (Shi et al. 2020). A transcriptomic profile between *OsMIOX* overexpressing plants and wild type was performed by RNA sequencing. Under drought stress, a total of 23 million high-quality reads, assembled into 28,552 unigenes, were generated, and 743 genes (534 upregulated and 209 downregulated) were significantly differentially expressed between overexpressing plants and wild type. These identified genes were mainly relevant to plant hormones transduction and sugar metabolism. Interestingly, several transcription factors were prominently identified in overexpressing lines. A significant positive correlation between RNA-Seq data

Table 14.6 Recent progress of transcriptome analysis for improving tolerance to different abiotic stress in rice

Gene	Key regulatory processes	References
OsAP2 and *OsWRKY24*	Reduce plant and cell size. Downregulate genes encoding EXPs and XETs/XTHs	Jang and Li (2018)
OsTF1L	Promotes shoot lignin accumulation and effective photosynthetic rate; reduces water loss	Bang et al. (2019)
OsERF48	Causes longer and dense root phenotype; regulates the expression of cell wall-related genes such as OsXTH9, OsAGP24, and OsAGP3	Jung et al. (2017)
OsNAC9	Improves the root architecture and efficiency; upregulates OsCCR1 delays leaf rolling; photochemical and wall-associated kinase genes	Redillas et al. (2012)
OsNAC10	Enlarges roots; reduces chlorophyll loss, leaf rolling, and wilting; upregulates wall-associated kinase	Jeong et al. (2010)
OsCCR10	Decreases leaf rolling and wilting; increases survival rate, chlorophyll content, and photochemical efficiency	Choi (2019)
SLE1	Causes rolled and narrow leaf phenotype; reduction in plant height; failure of cell-plate formation	Yoshikawa et al. (2013)
OsBURP16	Decreases cell wall pectin content and plant survival; increases electrolyte leakage, H_2O_2 accumulation, and water loss of leaves	Liu et al. (2014a)
OsDSS1	Causes dwarf phenotype by reducing early seedling growth, plant height, and internode length. Alters expression of cell wall metabolism-related genes	Tamiru et al. (2015)
OsDIL	Protects the pollen and anther cell walls from drought	Guo et al. (2013)

and qRT-PCR results was found. These results may provide partially valuable resources in identifying candidate genes involved in drought stress resistance and elucidating basis theory underlying the role of OsMIOX in drought resistance. Through RNA-Seq studies, the expression of seven genes was dramatically changed, including *LOC_Os03g37290*, *LOC_Os06g31800*, *LOC_Os09g13440*, *LOC_Os09g19229*, *LOC_Os10g13430*, *LOC_Os10g41040*, and *LOC_Os12g28177* (Zhou et al. 2016). Among the differentially expressed genes, it was found that a number of genes have been proved to be involved in responding to salt stress in the previous studies and they are transcription factors, which depicts that transcription factors play critical roles in responding to salt stress via transcriptional regulation of the downstream genes responsible for plant tolerance to salt challenges.

In drought stress, it was found from sequence analysis that a 1 bp Indel difference in *Os07g0569700* (*OsSAP16*) between IR36 and Weiguo. *OsSAP16* encodes a stress-associated protein whose expression is increased under drought stress (Lei et al. 2020). *OsSAP16 also* was identified as the candidate gene of *qRSL7* which control relative shoot growth under salt stress. Furthermore, recently two genes have been identified as accelerator of internode elongation 1 (*ACE1*) and decelerator of internode elongation 1 (*DEC1*) which are responsible to control stem elongation (Nagai et al. 2020). ACCELERATOR OF INTERNODE ELONGATION 1 (*ACE1*) gene encodes an unknown function protein which is associated with internodes elongation via gibberellic acids (GA), whereas *DEC1* gene encodes a zinc finger TF, which suppresses internodes elongation. Both the genes influence gibberellin-activated cell division in stem nodes. Further gene *ACE1C9285* is controlled by *SUB1C*, a gibberellin-activated TF which is upregulated in response to submergence (Fukao and Bailey-Serres 2008). *SUB1C* expression level is seemingly low in cultivars that contain the *SUB1A-1* regulator gene, a homolog to *SUB1C*. In short rice cultivars expressing gene *SUB1A-1*, GAs responsiveness altered, subsequently use carbon pool for leaves elongation, and restrict overall plant development and enter to transient quiescent stage during flooding, an adaptation to overcome deep floods (Fukao et al. 2006; Xu et al. 2006).

However, transcriptome analysis is used to examine the changes at transcript level under different environmental or biological conditions (Thompson and Goggin 2006). The transcriptional response of expressed genes can be analyzed through microarrays, and this technique can be used as the standard tool for the comparison of the expression level of a particular gene or a group of genes in different varieties or under different biological conditions (He et al. 2007). Zhou et al. (2016) compared the transcript profile of leaves and roots of a wild-type rice variety Dongxiang, under salinity and control conditions by using Illumina HiSeq 2000 platform. Soda et al. (2013) showed the differential transcript level of genes encoding signaling-related proteins (SRPs) in IR64 and Pokkali under control and stressed conditions. Nutan et al. (2020) studied the transcript level of Saltol QTL localized transcription factors and their differential regulation in contrasting rice genotypes.

14.4.3 Proteomics Analysis in Rice Towards Abiotic Stresses

Proteomics includes study and characterization of complete series of proteins present in a cell. Actually, the same genes have different patterns of expression under various circumstances (Dhingra et al. 2005; Fahimirad et al. 2013). Generally, proteomic approaches can be applied to proteome profiling, their differential expression, identifying post-translational modifications, and profiling protein interaction networks (Chandramouli and Qian 2009). During stresses, cells make different sets of proteins, and their post-translational modifications of proteins create proteome complexity in plants (Fahimirad and Ghorbanpour 2019). Thus, specific proteins can be treated as special biomarkers for particular stress conditions (Barkla 2016).

A thermotolerance gene (*TT1*) was identified and cloned from African rice variety CG14 (Li et al. 2015). This gene encodes α-subunit of the 26S proteasome involved in the degradation of ubiquitinated proteins in rice. Subsequent analysis indicated that *OgTT1* protects cells from heat stress through elimination of cytotoxic denatured proteins. From proteomic analysis, temperature tolerance in rice was defined based on production of heat shock proteins (sHSP). The tolerant genotype (N22) had higher accumulation of sHSP than the sensitive genotype (Moroberekan). The moderately tolerant genotype (IR64) had intermediate sHSP accumulation. Thus, it was confirmed that the accumulation of sHSP regulates heat tolerance in N22 rice (Jagadish et al. 2010a).

Under salinity stress, protein expression level is different in rice leaves and roots (Liu et al. 2014b). *OsRMC* has been reported as a salinity responsive protein via 2DE which is secreted in rice roots (Zhang et al. 2009b). Transgenic plants with downregulation of *OsRMC* showed overall improved salinity tolerance than wild type. It was also confirmed that *OsRMC* acts as a negative regulator of salt tolerance in rice. Phenotypic analysis of one protein that was upregulated during salt-induced stress, cyclophilin 2 (*OsCYP2*), indicated that *OsCYP2* transgenic rice seedlings had better tolerance to salt stress than wild-type seedlings. Moreover, *OsCYP2* is also likely to act as a key component that is involved in signal pathways of other types of stresses such as heat, cold, etc. (Ruan et al. 2011). Overexpression of the downregulated gene encoding rice β subunit of glyceraldehyde-3-phosphate dehydrogenase (*OsGAPB*), an enzyme in Calvin cycle, significantly elevated the CO_2 assimilation rate, chlorophyll content, and fresh weight under low light conditions but has no obvious effect on rice growth and development under control light. Low light stress on vegetative stage of rice inhibits photosynthesis possibly by decreasing the photosynthetic proteins and *OsGAPB* gene which might be responsible for low light stress tolerance in rice (Liu et al. 2020a).

14.4.4 Metabolomics for Abiotic Stress in Rice

The comprehensive study of metabolites related chemical processes may be defined as metabolomics which includes the quantification of synthesized or degraded biological compounds. Furthermore, metabolite profiling provides a static picture of the physiological status of the cells (Fahimirad and Ghorbanpour 2019). To reveal a clear picture of the whole plant system, transcriptomic, proteomic, and metabolomic results should be integrated into a single pipeline (Arbona et al. 2013). Gas chromatography-mass spectrometry is a widely used technique for plant metabolomics research. This technique is used for volatiles and primary metabolites after derivatization. In contrast, thin layer chromatography can be applied to a huge array of compounds including secondary metabolites without prior derivatization (Arbona et al. 2013). The metabolic profiling through nuclear magnetic resonance for contrasting rice varieties has shown that the carbohydrates and aliphatic compounds are the key metabolites and their accumulation pattern affects the salinity tolerance. Furthermore, they showed that plant growth and salinity tolerance are positively correlated with metabolite changes in rice cultivars (Nam et al. 2015).

There are very few studies including genomics-transcriptomic-proteomics along with metabolomics, which is the most complicated phase in phenotype expression. The rapid innovation in modern high-throughput metabolome analyzing platforms has increased the discovery of a wide variety of biochemical types of metabolites and pathways and also helped in improving the understanding of known existing pathways. To understand the metabolic networks that regulate the complex developmental process, metabolomics-based quantitative trait locus (mQTL) studies are important for improving the quality and performance of elite cultivars (Sharma et al. 2021). Another mQTL study conducted in back-crossed inbred lines (BILs) of rice identified 700 different metabolic characteristics under 802 mQTLs which show an unusual range that could regulate various metabolic traits (Matsuda et al. 2012). Further metabolic genome-wide association study (mGWAS) in rice leaf and in vivo metabolic analysis of the transgenic plants identified *Os12g27220* and *Os12g27254* as two spermidine hydroxyl cinnamoyl transferases that might underlie the natural variation of levels of spermidine conjugates in rice. The result demonstrates that gene-to-metabolite analysis by mGWAS provides a useful tool for functional gene identification and omics-based crop genetic improvement (Dong et al. 2015).

Under salt stress, sedoheptulose-1,7-bisphosphatase (SBPase) maintained the activation of ribulose-1,5-bisphosphate carboxylase-oxygenase by providing more regeneration of the acceptor molecule ribulose-1,5-bisphosphate in the soluble stroma and by preventing the sequestration of Rubisco activase to the thylakoid membrane from the soluble stroma and, thus, enhanced the tolerance of photosynthesis to salt stress. This study revealed that overexpression of SBPase was an effective for enhancing salt tolerance in rice (Feng et al. 2007). Auxin signaling is a fundamental part of many plant growth processes and stress responses and operates through Aux/IAA protein degradation and the transmission of the signal via auxin response factors. A knockdown mutant of *OsIAA6* showed abnormal tiller outgrowth, apparently due to the regulation of the auxin transporter *OsPIN1* and the rice tillering inhibitor *OsTB1*. The results confirm that the *OsIAA6* gene is involved in drought stress responses and the control of tiller outgrowth (Jung et al. 2015).

14.4.5 Phenomics Studied for Abiotic Stress in Rice

Phenomics is described as the high-throughput analysis of phenotype through the evaluation of the morphological, physiological, and biochemical traits and the correlation of genetic, epigenetic, and environmental factors (Deshmukh et al. 2014; Zhao et al. 2019). Advances in DNA sequencing, and high-throughput phenotyping platforms, have accelerated genomics and phenomics into the era of big data, providing important resource of population-scale information for researchers (Bayer et al. 2020; Yang et al. 2020; Zhao et al. 2019). Climate resilient traits such as different abiotic stresses including drought, flood, extreme temperature, and salinity tolerance are important in plant phonemics to underpin adaptive genetic variants which can be isolated and utilized in breeding to secure crop production (Adhikari et al. 2015; Foyer et al. 2019; Ongoma et al. 2018; Sultan and Gaetani 2016).

Numerous technologies and platforms have been evolved to study and characterize phenotypic information with higher accuracy. Deep learning (DL) models have been effectively trained for phenotype extraction in rice (Xiong et al. 2017; Yu et al. 2020). As an emerging machine learning method, DL has been applied to advance automated image recognition and machine vision across a range of disciplines (Voulodimos et al. 2018). For phenomics, DL has experienced increasing implementation as a way to determine crop phenotypes from complex image datasets (Harfouche et al. 2019). It has been used to provide robust and accurate feature extraction, classifying the presence of morphological traits in both root and shoot, which enables association of traits with genomic variation (Pound et al. 2017). Furthermore, crop health assessment algorithms also offer an innovative solution using image capture and machine vision data to automate plant stress phenotyping (Bock et al. 2020) and have been applied to major crops such as rice to differentiate between drought-tolerant and drought-sensitive individuals in a non-intrusive manner (Duan et al. 2018). In addition, the open-source modeling software "OpenSimRoot" takes advantage of high-throughput phenomics to integrate phenotypic data with environmental data to estimate water and nutrient uptake in different environments and plant species (Postma et al. 2017).

14.5 Advances in Biotechnological and Genome Editing Tools for Enhancing Abiotic Stress Tolerance in Rice

Abiotic stresses, such as cold stress, drought, salinity, heat, flooding, ion toxicity, and radiation, are the major constrains that hinder agricultural production. Traditional breeding approaches have improved much of rice production, but the enhancing demand of world population and new environmental stresses makes more difficult to produce rice with old breeding strategies. With time, new breeding strategies and techniques like molecular breeding and mutational breeding have open new dimensions in rice breeding. Still the yield is a great concern for feeding the vast population. The newer technique genome editing tools are most recent advances which revolutionarily changed the field of plant science through overcome the limitations of traditional breeding methods (Hossain et al. 2022). Genome editing technique is the usage of engineered site-specific nucleases (SSNs) to modify specific genes at desired locations in the genome to introduce specific mutation, insertion, deletion, and also sequence modification. Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated endonuclease Cas9 (CRISPR/Cas9) are the SSNs which make double-strand break (DSB) in the target DNA repaired by cell's own natural repair mechanism of homologous recombination (HR) or non-homologous end joining (NHEJ) (Voytas 2013; Mahfouz et al. 2014; Kumar and Jain 2015; Miglani 2017). The error-prone pathway creating random insertions and deletions (indels) causing frame shift mutations and targeted gene knockouts is repaired by NHEJ (Feng et al. 2013; Bortesi and Fischer 2015); on the other way, much more precise in the exchange of homologous sequence leading to gene knock in or gene replacement is HR pathway (Voytas and Gao 2014; Baltes et al. 2014).

With advance of time, a simple modular DNA recognition code by transcription activator-like effector (TALE) proteins (Gupta et al. 2012; Boch et al. 2009) has been discovered, opening an alternative platform of engineering programmable DNA-binding proteins. TALENs are genome editing tools having specific DNA-binding proteins containing 33 or 34 amino acid repeats. These artificial designed restriction enzymes of the fusion of DNA cutting domain of a nuclease to TALE domains can be made to specifically recognize a unique DNA sequence. TALENs are "DNA scissors" which are used to edit genes and modify targeted sequences by performing insertion, deletion, repair, and replacement in sequence of living cells (Gaj et al. 2013). Different authors have been reported that genome editing is done in rice for abiotic stresses tolerance through TALENs (Shan et al. 2015; Endo et al. 2016).

The most accepted and advanced technology in genome editing tools in plant biology is CRISPR/Cas9 as an accurate, simple, and highly efficient technique (Belhaj et al. 2015; Weeks et al. 2016), a short RNA molecule associated with Cas9 which is a DNA endonuclease responsible for cutting DNA of invading phage and gets integrated to the CRISPR array. The guide RNA is consisted of crRNA (CRISPR-derived RNA) and tracrRNA (trans-activating RNA). The crRNA and tracrRNA can be formed into a single guide RNA chimera (sgRNA) which directs specific Cas9 dsDNA cleavage (Jinek et al. 2012).

The DNA strand moves along with protein/RNA complex (Cas9-sgRNA), makes a double-strand break for matching of sgRNA and specific DNA sequence (Jinek et al. 2014).

Genome editing in the agricultural research has strengthened with the identification of another class 2 CRISPR effector, Cpf1 (Zetsche et al. 2015). Overcoming the limitations of base editing technology of NHEJ and HR methods converting one target base into another without DSB or donor template is another success (Komor et al. 2016).

The CRISPR/Cas9 and its modified versions have been successfully used in major crops like rice, wheat, and maize (Bortesi and Fischer 2015; Ma et al. 2015; Xu et al. 2016; Zhong et al. 2017).

The small genome size of rice, availability of genetic resources, and high transformation efficiency have made rice an ideal crop for studying genome editing (Li et al. 2012; Feng et al. 2013) especially various gene function and observing potential improvement (Xu et al. 2014, 2016; Wang et al. 2016b).

Rice research has upscaled to new era with the application of genome editing tools which open new opportunities to develop improved and quality varieties (Table 14.7).

Herbicide-tolerant varieties are introduced by TALENs genome editing, in which ALS gene (Li et al. 2016a; Sun et al. 2016) has been introduced. TALEN-based HR gene replacement strategy in rice was used to produce double point mutations in rice OsALS (Li et al. 2016a) with the efficiency of 6.3% with the entire stable mutant. In another similar experiment, multiple discrete point mutations in the rice ALS gene were introduced through CRISPR/Cas9-mediated HR (Sun et al. 2016). Wild-type plant died after 36 days of spraying of bispyribac-sodium (BS), whereas genome-edited lines showed tolerance. This shows evidence that within one generation,

Table 14.7 Representative sensitivity (S) genes proposed as the potential targets for improving tolerance towards abiotic stresses through genome editing in rice

Gene name	Genome editing tool	Stress	References
OMTN2, OMTN3, OMTN4, OMTN6	CRISPR/Cas9	Drought	Fang et al. (2014)
OsDIS1	CRISPR/Cas9	Drought	Ning et al. (2011)
OsiSAP7	CRISPR/Cas9	Drought	Sharma et al. (2015)
MODD	CRISPR/Cas9	Drought	Tang et al. (2016)
OsERF922	CRISPR/Cas9	Salt	Liu et al. (2012)
OsRMC	CRISPR/Cas9	Salt	Serra et al. (2013)
OsMATE1 and OsMATE2	CRISPR/Cas9	Arsenic and disease	Tiwari et al. (2014)
OsGIRP1	CRISPR/Cas9	Radiation stress	Park et al. (2015)
BEL	CRISPR/Cas9	Herbicide resistant	Xu et al. (2014)
OsEPSPS	CRISPR/Cas9	Glyphosate resistant	Li et al. (2016a)
OsALS	TALENs	Herbicide resistant	Li et al. (2012)
ALS	CRISPR/Cas9	Herbicide resistant	Sun et al. (2016)
Os SAPK2	CRISPR/Cas9	Drought tolerance	Lou et al. (2017)
C287	Base editing	Herbicide resistant	Shimatani et al. (2017)

genome editing can be used for generation of homozygous herbicide-tolerant rice plant.

Improvement of cold tolerance in rice increases the productivity. One of the cold tolerances involving genes was transcription factor TIFY1b gene in rice. CRISPR/Cas9 edited TIFY1b and its homologous TIFY1a (Huang et al. 2017). Adaptation of rice to low temperature can be investigated by studying more about TIFY1 mutant lines. The OMTN genes were abiotic stress-responsive genes. Genome editing through CRISPR/Cas9 targeting OMTN improves drought stress. Overexpression of OMTN2, OMTN3, OMTN4, and OMTN6 in rice led to negative effects on drought resistance at the reproductive stage. In OMTN2-, OMTN3-, OMTN4-, and OMTN6-overexpressing plants, the expression of various genes of stress response, development, and metabolism was altered. The upregulated genes in the OMTN-overexpressing plants were downregulated by drought stress. This made a suggestion that the conserved miR164-targeted NAC genes may be negative regulators of drought tolerance in rice and also to their reported roles in development (Fang et al. 2014). CRISPR/Cas9-mediated genome editing in rice shows plants fine-tune their drought responses by elaborate regulatory mechanisms, including the coordination of activity and stability of key transcription factors. MODD negatively regulates OsbZIP46 activity and stability. MODD represses OsbZIP46 activity via interaction with the OsTPR3-HDA702 co-repressor complex and downregulation of the histone acetylation level at OsbZIP46 target genes. MODD promotes OsbZIP46 degradation via interaction with the U-box-type ubiquitin E3 ligase OsPUB70. Interestingly, the D domain is required for both deactivation and degradation of OsbZIP46 via its interaction with MODD and increase drought tolerance (Tang et al. 2016). In a study, CRISPR/Cas9-mediated genome editing in rice shows OsRMC, a negative regulator of salt stress response in rice, which is regulated by two AP2/ERF transcription factors (Serra et al. 2013)

CRISPR/Cas9-based base editing is the most advanced alternative way to conversion of one target base into another, and it does not need DSB or donor template (Komor et al. 2016; Nishida et al. 2016). Fusion of catalytically inactive Cas9 domain and a cytosine deaminase domain which converts G-C base pairs to A-T base pairs is called base editing (Li et al. 2017b; Lu and Zhu 2017; May 2017). Efficient optimization of base editing has been introduced in rice, wheat, and maize (Zhong et al. 2017). Similarly, herbicide resistance by editing C287 genes through base editors in rice was reported by Shimatani et al. (2017).

14.6 Transgenic Approaches for Improving Abiotic Stress Tolerance in Rice

Abiotic stress has negative impact on rice cultivation which causes of yield loss worldwide. Various techniques are being explored to develop efficient rice plants with higher productivity that meets the growing demand for food and to minimize the adverse effects of abiotic stress. Compared to different breeding approaches, gene transformation seems to be a more convenient and time, labor, and cost saving process for developing crop varieties tolerant to stress without affecting their genetic background (Ahmar et al. 2020). However, development of abiotic stress-tolerant plants has been intensively investigated through genetic engineering (Cui et al. 2018, 2020; Hossain et al. 2020). For developing transgenic rice plants, three methods are commonly used in rice protoplast as the recipient via electroporation-mediated or PEG-mediated (Todaka et al. 2015), particle bombardment (Gosal et al. 2009), and *agrobacterium*-mediated method (Chan et al. 1993). Hiei et al. (1994) reported *Agrobacterium*-mediated transformation method for *japonica* rice, and then Toki et al. (2006) shorten transformation procedure. Following the available genetic transformation techniques, the target gene was isolated from different sources and then cloned for the development of transgenic plants.

For the development of submergence-tolerant rice, Ronald began to isolate the gene from Sub1 locus (Xu et al. 2000), and then it is reported that Sub1 locus consisted a cluster of three genes, namely, Sub1A, Sub1B, and Sub1C. But Sub1A is playing major role in submergence tolerance in rice through ethylene formation (Xu et al. 2006). In this connection, *SNORKEL1* and *SNORKEL2* were induced in rice plants for accumulation of ethylene under deepwater condition (Hattori et al. 2009). Recently, overexpression of *OsARD1* transgenic lines exhibited enhanced water holding capacity due to release of endogenous ethylene and showed potential submergence tolerance in rice (Liang et al. 2019).

Drought is a major abiotic stress, where the drought-tolerant plants demonstrated different techniques that are mainly divided into three categories: improving water consumption ability from the soil, controlling water loss from the plant, and inhibiting internal water use efficiency. The regulatory methods are controlled by different genes which are mostly ABA-independent and related to ABA-independent regulatory systems (Du et al. 2018; Gupta et al. 2020). Three major groups such as genes controlled membrane transportation, signaling related genes and genes related with transcriptional factors are played major role under drought stress (Upadhyaya and Panda 2019; Kim et al. 2020). Similarly, ABA signaling *OsJAZ1* gene regulates plant growth and development of rice plant during drought stress condition (Fu et al. 2020).

Transgenic rice exhibited tolerance through varied expression of different genes like *DRO1*, *OsPYL/RCAR5*, and *EcNAC67* which develop deeper root system in drought condition (Uga et al. 2013; Kim et al. 2014; Rahman et al. 2016). Similarly, *OsDRAP1*, *OsDREB2B*, *CYP735A*, and *OsDREB1F* adapted under water deficit conditions by overexpression (Huang et al. 2018; Kim et al. 2020). Besides, some genes are supportive for the higher rice grain yield production in limited water condition, namely, *OsNAC5* (Hu et al. 2006), *OsLEA3-1* (Xiao et al. 2007), *OsbZIP71* (Liu et al. 2014b), *OsWRKY47* (Raineri et al. 2015), *OsbZIP46* (Tang et al. 2012), and *OsNAC10* (Jeong et al. 2010), through increased water use efficiency, and introducing *EDT1/HDG11* gene (Yu et al. 2013) enhanced photosynthesis in transgenic rice. In addition, transformation of *LEA* genes within rice cultivars showed better tolerance to drought stress (Babu et al. 2004; Duan and Cai 2012). Moreover, overexpression of *OsLEA3-1* gene enhanced grain yield in rice through delaying leaf wilting in drought condition (Xiao et al. 2007).

In rice, overexpressing of different genes *OsOAT* (You et al. 2012), *OsSGL* (Cui et al. 2016), *OsGRXS17* (Hu et al. 2017), *OsDSSR1* (Cui et al. 2018), *OsPM1* (Yao et al. 2018), and *OsSCE3* (Joo et al. 2019) acts a vital role in resistance to drought by accumulation of proline, osmolytes, ROS accumulation, stomatal closure, and altering the root systems. Recently, the transgenic rice plant was developed by overexpression of ABA synthesis genes such as *OsANN3*, *OsRab16A*, and *AtDREB1A* for drought condition (Li et al. 2019; Ganguly et al. 2020). Besides, the plant which carry *OsESG1* gene can withstand in drought stress and maintain crown root development in rice (Pan et al. 2020), and *OsNADK1* showed increased drought tolerance in rice by maintaining intracellular redox homeostasis, enhanced water holding capacity, and also enhanced accumulation of proline (Wang et al. 2020). Furthermore, *OsHAK1*, a high-affinity K transporter, showed improve drought tolerance at the reproductive stage in rice plant (Chen et al. 2017). However, overexpression of a mutated *OsNAC2* resulted in improved drought tolerance (Jiang et al. 2019).

Genetic engineering for salinity tolerance is a complex issue that encodes organic solutes, proteins, ROS, ion transport, osmoregulation, cell death, signal transduction, and transcription factor (Sahi et al. 2006) because of their dependency on various mechanisms and pathways. Besides, transgenic approaches to salt tolerance are gained across the overexpression of different genes that involved in Na^+ extrusion or Na^+ compartmentalization from the root vacuoles (Wang et al. 2004b; Bhatnagar-Mathur et al. 2008; Uddin et al. 2008). Earlier reports suggest that transgenic rice plants *PgNHX1* gene from *Pennisetum glaucum* (L.) showed an extensive root system (Verma et al. 2007) and overexpression of the *SOD2* gene (Zhao et al. 2006) performed better in saline conditions. The salt tolerance in rice has been enhanced due to overexpressed *P5CS* gene from moth bean (*Vigna aconitifolia*) (Su and Wu 2004). Similarly, transgenic rice plants developed with *mtlD* and *gutD* genes also exhibited improve salt tolerance through accumulation of mannitol and sorbitol (Wang et al. 2000), and the adverse effects of salinity can be reduced by transferring *OPBP1* gene from tobacco into rice plant (Chen and Guo 2008). Mohanty et al. (2002) also proposed that *coda* gene is highly responsible to salinity tolerant in transgenic rice. In addition, some salt-related genes *OsRR22* (Huang et al. 2009), P5CS (Karthikeyan et al. 2011), and SNAC2 (Chen et al. 2014) have been successfully cloned to salinity tolerance plants. Besides, various genes like *OsDREB1A*, *OsDREB1F*, OsDREB2A (Mallikarjuna et al. 2011), *SERF1* (Schmidt et al. 2013), *OsMYB48-1* (Xiong et al. 2014), and *SaVHAc1* (Baisakh et al. 2012) containing transgenic rice plant exhibited increased salt tolerance under salt stress condition. *PDH45* also promotes salt tolerance by controlling Na^+ level, ROS production, Ca^{2+} cyt homeostasis, and cation transporters in roots of transgenic rice (Nath et al. 2016).

Scientists have been contributed much to develop genetically engineered salinity tolerance rice plants (Pental 2019). Recently, overexpressing *OsIF* gene from wild rice has been widely used to introduce salt-tolerant transgenic rice in Bangladesh, India, Sri Lanka, and Myanmar (Soda et al. 2018). Practically, there is no single transgene that was discovered that secure salinity tolerance in the entire rice growth period (Hoang et al. 2016). Overexpression of *OsCYP2* gene accelerated salt tolerance in rice seedlings (Ruan et al. 2011). Rice bearing *OsCPK12* gene strengthens salinity tolerance by ROS accumulation (Asano et al. 2012). Similarly, Xu et al. (2013) reported calmodulin-like gene *OsMSR2* promoted salt tolerance of rice plant. Besides, overexpression of the *OsNHX1* (Amin et al. 2016), *SIDP361* (Li et al. 2016b), and OsPP1a (Liao et al. 2016) gene displayed significant level of salt tolerance in transgenic rice plants.

Cold stress-responsive genes have been reported for the improvement of rice plant through overexpressing gene like *OsRLK1*, *OsDMKT1*, and *OsGSK1* (Lee et al. 2004; Koh et al. 2007). Besides, *OsDREB1F* gene from maize (Xu et al. 2011) and *OsNAC5* (Song et al. 2011) increased cold tolerance of constructed transgenic rice. The overexpression of *CBF1* (Jing et al. 2005; Wu et al. 2006) and *ICE1* (Xiang et al. 2008) from *Arabidopsis* improved the cold tolerance in transgenic rice. Kim et al. (2009) depicted ectopic expression of a cold-responsive cDNA gives extended cold tolerance in transgenic rice. Besides, overexpression of heterologous *CBF* genes (Byun et al. 2015) and OsAsr1 (Kim et al. 2009) displayed enhanced cold tolerance in transgenic rice plants.

Heat-tolerant transgenic rice plants showed more heat tolerance after introduction of *Athsp101* gene in basmati rice from *Arabidopsis thaliana* (Katiyar-Agarwal et al. 2003). Heat-tolerant rice overexpressing the *sHSP17.7* was developed through deploying transgenic approaches (Murakami et al. 2004). Transgenic rice plant containing *FAD7* gene showed heat tolerance by increasing the growth rate and chlorophyll content (Sohn and Back 2007). Wu et al. (2009) reported that the HSP101 promoter controlling gene *OsWRY11* exhibited more heat tolerance in transgenic rice seedling. Moreover, Qi et al. (2011) illustrated that overexpressing mitochondrial gene for *mtHsp70* displayed higher heat tolerance in transgenic rice. Recently, Liu et al. (2020b) exhibited expression of heat stress-responsive AtPLC9 exhibited higher heat tolerance in transgenic rice plants.

The heavy metal tolerance transgenic lines can withstand in the metal-contaminated soils through regulation of specific cation transporter genes like encoded tonoplast-localized Cd transporters (Koren'kov et al. 2007; Guo-ming et al. 2012) and plasma membrane-based HM transporter (Ishimaru et al. 2012; Ovecka and Takac 2014; Sasaki et al. 2014). Ueno et al. (2010) reported that the overexpression of *OsHMA3* gene showed increased movement of cadmium in rice. The *OsIRT1* and *OsLCT1* genes were used to develop low cadmium-accumulating transgenic rice which involved in cd transportation (Lee and An 2009; Takahashi et al. 2011). Likely, the transgenic rice plant containing *OsNAS3* gene showed enhanced tolerance to Zn, Cu, Ni, and Cd stress (Lee et al. 2009; Sasaki et al. 2014). Yuan et al. (2012) developed transgenic rice with *OsMTP1* gene which exhibited heavy metal tolerance in rice. Furthermore, the transgenic rice plant carrying gene which showed multiple stress tolerances likely *TPS1* from yeast exhibited tolerance to salinity, drought, and cold stress (Garg et al. 2002). Similarly, expression of barley *HVA1* gene in basmati rice significantly contributed to drought and salinity tolerance (Rohila et al. 2002). Overexpressing *OsMYB2* gene in transgenic rice plants showed more tolerant to salt, cold, and dehydration (Yang et al. 2012); *OsCPK* increased tolerance to salt and drought (Campo et al. 2014); and *OsLEA4* provided drought, high salt, and heavy metal tolerance (Hu et al. 2015). Indeed, it is need to develop *improve* transgenic approaches as well as require to field evaluate those transgenic plants for higher yield production underspecific abiotic stress condition.

14.7 Conclusion

Abiotic stress tolerance of rice is a complex parameter that regulates numerous genes related with multiple physiological and biochemical mechanisms. Thus, the different strategies like molecular, physiological, and metabolic aspects should be combined, and the genes representing distinctive approaches must be incorporated to significantly increase rice abiotic stress tolerance. For developing tolerant rice genotypes, the plant breeder and plant biotechnologist should work together, and they should have knowledge regarding the injury and plant symptoms and tolerance mechanism under specific stress. Under such stress conditions, rice plants modify themselves to quickly adjust to the existing circumstances by changing the expression pattern of genes, proteins, and metabolites. To discover those changes, various tools and techniques such as genomics, transcriptomics, metabolomics, ionomics, and phenomics have been utilized to better understand the genetic makeup of rice plants and their adaptability towards stress conditions. These techniques also help to understand the nature of rice domestication as well as trait improvement. These omics approaches could be combined with the potential to map different agronomical trait and stress-related QTLs and also identify linked molecular markers. Furthermore, through application of marker-assisted breeding programs utilizing either tightly linked markers or an allelic form of stress-related genes and QTLs, stress tolerant with high-yielding rice varieties could be developed to meet the demand for cereal food. However, genome sequencing of rice plant was completed a decade back, but the function of a large group of genes is not known yet. Besides rice genome, many genes from other crop plants can convey multiple stress tolerance that can be used for developing stress-tolerant transgenic rice. In recent years, genetic engineering has been proved to be a potential approach to the development of stress-tolerant rice plants utilizing rice or other crop genomes. So, development of transgenic rice plants with stress-tolerant gene is the direct solution to combat with stress conditions and also increase crop productivity. Moreover, combining the transgenic techniques with traditional and molecular breeding approaches will be a potential method to develop abiotic stress-tolerant rice cultivar.

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Genomics and Transcriptomics Approaches to Understand Abiotic Stress Response in Rice

15

S. Kamali and Amarjeet Singh

Abstract

Rice is a staple food crop for over half the world's population. Due to its cultivation in semi-aquatic, tropical, or subtropical habitats, rice is susceptible to abiotic stresses like, drought, cold, and salinity. From the earliest of rice cultivation, various agronomical strategies were employed for imparting abiotic stress tolerance. In the pre-genomic era, forward genetic approaches were used for the identification of abiotic stress-related genes, and conventional breeding approaches were employed for the development of abiotic stress-tolerant lines. In the post-genomic era, sequencing technologies have facilitated genetic studies via availability of genome and transcriptome sequences. Genome assemblies provide information about gene function and gene structure. The reference genome sequences of other plant species enable comparative finding of gene families in rice, i.e., genome-wide identification. Genomic approaches are complemented by transcriptomics via gene expression profiling, dominated by RNA-Seq in recent times. All these genomic and transcriptomic approaches had led to the identification of various key QTLs, genes, promoter elements, transcription factors, and miRNAs involved in abiotic stress response in rice. This information could be put forward for the development of elite varieties. Also, this information is made available publicly through various databases. In this chapter, utilization of various genomics and transcriptomic methods for understanding abiotic stress tolerance in rice has been highlighted.

Keywords

Rice · Abiotic stress · Genomics · Transcriptomics

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_15

15.1 Introduction

Rice is a staple food for most of the world's population, and it has been an important crop from agriculture point of view (Deepika and Singh 2021). In this century, the world population has reached over eight billion, and rice is inevitable for global food security (Sasaki and Ashikari 2018). Like any other crop, rice is affected by adverse environmental conditions, including both biotic and abiotic stresses. Biotic stress involves pathogens and herbivore attacks, while abiotic stress includes heat, cold, drought, osmotic, salinity, and heavy metal toxicity (Grennan 2006; Zhang et al. 2021). Among abiotic stresses, salinity, temperature fluctuations, and drought affect cultivation of plants worldwide, thus affecting the productivity and in turn food security. This adversity is exaggerated by anthropogenic climate change, which causes increased frequency of extreme weather (Zhu 2016). Rice being evolved in semi-aquatic and partial-radiation environment exhibits a unique pattern of tolerance and susceptibility to abiotic stresses. Rice thrives well under waterlogged conditions and is tolerant to submergence, where other cereal crops may die. However, rice is highly sensitive to salinity among cereals and susceptible to drought and cold (Gregorio et al. 2013). Also, being cultivated majorly in tropical and subtropical zones, rice is highly sensitive to chilling stress. Therefore, in temperate areas, rice cultivation is severely hampered by cold stress, especially at the reproductive stage (Paul and Roychoudhury 2019). Thus, the quality of rice cultivated in high altitude and high latitude is poor (Zhang et al. 2014). Rice is very sensitive to heat stress during reproduction and grain development (Aghamolki et al. 2014). It has been estimated that rice yields are going to be reduced to 41% by the end of the twenty-first century due to temperature stress (Aghamolki et al. 2014). To combat these environmental challenges, improvements in two key aspects have to be addressed: (1) crop management strategies and (2) development of elite cultivars. The development of elite cultivars was traditionally achieved by breeding (Lei et al. 2020). In modern times, since the development of various sequencing and bioinformatic tools, multi-omics approaches are employed for the development of elite cultivars (Roychoudhury et al. 2011). Multi-omics approaches involve genomics, transcriptomics, proteomics, and metabolomics which helps in the identification and functional analysis of genes, expression analysis, protein-protein interaction, and their regulatory networks (Rashid et al. 2014). In this chapter, genomic and transcriptomic approaches employed in identification of various genes and their regulatory networks involved in abiotic stress responses in rice are extensively discussed (Roychoudhury and Banerjee 2015).

15.2 Genomics Approaches for Understanding Abiotic Stress Response in Rice

Genomics is the science of studying genomes. Various genomics approaches aim to decode the genome to understand the plant molecular regulations, and this, in turn, provides information for the development of novel crop improvement strategies

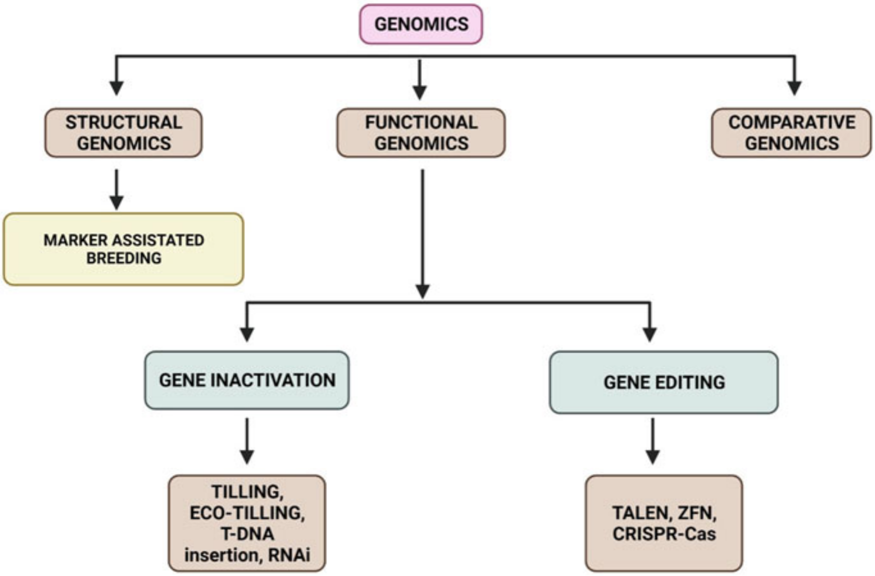

Fig. 15.1 Genomic approaches for crop improvement. Genomic approaches are grouped into three major categories: (1) structural genomics which involves general gene structure and helps in development of physical maps; (2) functional genomics which aims at identifying specific function of a gene and could be achieved by gene inactivation and gene editing approaches; (3) comparative genomics which employs reference genome sequence of a species for identification of gene families in the species of interest

(Rashid et al. 2014). Genomics has been broadly classified into functional, structural, and comparative genomics, based on the methodologies and outcomes aimed (Akpinar et al. 2013). In this section, the three classes of genomics, their methodologies, and their application in rice for abiotic stress tolerance will be discussed. Various genomic approaches involved in crop improvement are shown in Fig. 15.1.

15.2.1 Functional Genomics

Functional studies of genomes are found to be readily producing information that is applicable for crop improvement. Functional genomics involves the functional characterization of genes and their interactions with other genes in regulatory networks (Joshi et al. 2019). Recent advancements in biotechnological techniques have led to the discovery of novel genes and their functions. Functional genomics approaches involve sequence or hybridization-based methodologies and gene inactivation or editing-based approaches (Joshi et al. 2019). Sequence-based approaches involve EST (expressed sequence tags), SAGE (serial analysis of gene expression), and 5′RACE (rapid amplification of cDNA ends). Hybridization-based approaches include DNA microarray. These approaches will be discussed under transcriptomics since they deal with RNA transcripts (Rashid et al. 2014).

Two main gene inactivation-based approaches include TILLING (Targeting Induced Local Lesions In Genomes) and T-DNA insertion mutation (Ma et al. 2012). TILLING is a high-throughput technique to identify single nucleotide mutations in a specific region of a gene of interest which resulted from chemical-induced mutagenesis. TILLING populations can be employed for screening of both phenotypic and genotypic variations under abiotic stresses (Casella et al. 2013). Another updated method, called EcoTILLING, was developed for the identification of natural polymorphisms (Negrão et al. 2011). Insertion of T-DNA fragments randomly in coding or non-coding region results in the gene inactivation of targeted genes. *Agrobacterium*-mediated T-DNA transformation has proven to be an efficient way of targeting candidate genes in plants. In addition, RNAi technology can be used for gene inactivation and functional studies (Rashid et al. 2014).

For functional characterization of plant genes, genome editing by targeted mutation, INDEL, and sequence modifications within the genome can be employed (Rashid et al. 2014). The most commonly used genome editing tools are zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 (CRISPR-associated nuclease9) (Rashid et al. 2014). ZFNs are nucleases designed to create double-strand breaks (DSB) at specific genomic loci, leading to targeted mutagenesis like chromosomal deletions, transgene removal, and targeted DNA integration (Ran et al. 2018). ZFNs consist of a nonspecific cleavage domain from the type IIS restriction endonuclease FoKI and fused to custom-design Cys2-His2 zinc finger proteins. These nucleases create a DSB. Plant system employs a DNA repair mechanism, i.e., error-prone non-homologous end joining (NHEJ) (Tsutsui et al. 2011). TALENs entered the picture as an alternative to ZFNs. TALEN employs recombinant adeno-associated viruses to create targeted double-strand breaks. TALEN has a very high success rate (Bi and Yang 2017). Nowadays, the most widely used approach to genome editing is CRISPR-Cas9. In prokaryotes, CRISPR-Cas9 is an adaptive immune system that combats DNA viral infections. This natural immune system CRISPR-Cas9 has been manipulated to create an efficient genome editing technology. It consists of a customizable Cas9 nuclease and a synthetic short guide RNA (sgRNA). sgRNA provides target specificity (Bi and Yang 2017). Hence, the CRISPR-Cas9 system is much easier than ZFNs or TALENs and allows the targeting of multiple genes at once (Zhang et al. 2021).

15.2.2 Structural Genomics

While functional genomics focuses on the gene functional characterization, structural genomics focuses on the physical genome structure. Knowledge of the structure of an individual genome can be useful in manipulating genes and DNA segments (Rashid et al. 2014). Structural genomics involves the construction of high-resolution genetic and physical maps. Genome sequencing and molecular mapping are an important approach in structural genomics for crop improvement (Chaudhary et al. 2019). Molecular markers are based on the polymorphism identified in any given DNA and are useful in characterizing genetic diversity in the germplasm (Nogoy et al. 2016). DNA markers are widely useful in plant breading through mapping of genes, quantitative trait loci (QTL), germplasm evaluation, and marker-assisted breeding (MAB). MAS has been accelerated with the recent development of genotyping methods based on single nucleotide polymorphisms. Due to advancement in next-generation sequencing (NGS), genome resequencing and comparison of various genotypes help in identification of thousands of SNPs (Lei et al. 2020). A recent development in molecular marker is insertion site-based polymorphisms (ISBPs). This makes use of the polymorphism created by insertional regions in the repeat junctions (Nogoy et al. 2016).

15.2.3 Comparative Genomics

The comparative genomics approach compares two or more genomes to discover the similarities and differences between the genomes. The gene annotations from model plants can be transferred to newly sequenced crop species, for which functional studies are yet to be done. The information about the orthologs that evolved from the common ancestor is required for comparative genomics, because these execute the same function among species evolved from the same ancestor (Wei et al. 2002). Comparative genomics can also be employed to analyze the expression profiles of less examined plants under various stresses to identify stress-related genes and compare the expression profiles between the species. Both intraspecific and interspecific sequence comparisons are based on a variety of computational methods like multiple sequence alignment and phylogenetic tree construction (Haubold and Wiehe 2004).

15.2.4 Gene Inactivation and Gene Editing for Functional Analysis

Using TILLING approach lines with SNPs in membrane transport genes and changes in responses to salt stress were identified in chemically mutated lines. Out of 2961 M_2 mutant lines, 41 mutant lines containing SNPs in the nine target membrane transporters were identified. Among these nine were found to have altered sequences in the exon region of the genes. Seven of them were tolerant to salt stress. Furthermore, five mutant lines having SNPs in the exon region of *OsAKT1*, *OsHKT6*, *OsNSCC2*, *OsHAK11*, and *OsSOS1* showed altered expression levels for each gene. These mutants can be employed for the development of salt-tolerant lines (Hwang et al. 2020). Similarly, the screening of TLLING mutant population of Donganbyeo rice cultivar had chilling-tolerant lines. Comparative transcriptome studies of these chilling-tolerant lines with wild-type plants showed that chilling stress tolerance was associated with monosaccharide catabolic processes. This shows the energy requirements necessary for adaptation to cold temperatures in rice (Cho et al. 2012). High-temperature stress during grain filling delays the endosperm formation and causes grain chalkiness. Multi-omics investigation showed the downregulation of starch synthesis enzymes and upregulation of α-amylases. It is been suggested that TILLING mutants of α-amylases genes may lead to the reduction of chalkiness in grains developed under heat stress and this may avoid the need for transgenic lines (Mitsui et al. 2016). TILLING M_2 populations of ethyl methane sulfonate (EMS)-mutagenized lines were validated for four target genes (*SD1*, *HD1*, *SNAC1*, and *BADH2*, involved in determining plant height, flowering time, drought tolerance, and aroma, respectively). Two independent mutations identified in the *SD1* gene caused 21% height reduction, and this may help in drought tolerance since the reduction of height reduces the growth cycle and causes drought escaping (Casella et al. 2013). M_7 TILLING population generated from gamma ray-induced mutants was evaluated in six abiotic stress treatments, and then principal component analysis was done via genome-wide association study (GWAS). Two SNPs at *Os02g0528900* loci were found to be heat tolerant. These SNPs could be useful in future mutational breeding (Hwang et al. 2020). Among 375 accessions of domesticated rice, EcoTILLING technique targeting genes involved in salt stress signal transduction (*OsCPK17*) or tolerance mechanisms (*SalT*) identified 15 and 23 SNPs or indels, respectively. These allelic variants are found to be located in the 3′-untranslated region (3′-UTR), and this could be potentially explored for salt-tolerant breeding (Negrão et al. 2011).

The group A bZIP transcription factors were found to play important roles in ABA signaling pathways (Banerjee and Roychoudhury 2017). To understand its role in rice, a homozygous T-DNA insertional mutant of *OsABF2* was developed. The mutant was more sensitive to abiotic stresses like drought, salinity, and oxidative stress compared to wild type, and also, the mutant was having decreased sensitivity for ABA. Hence, it was concluded that *OsABF2* was found to be stress-responsive gene in rice (Hossain et al. 2010). Members of OsPP2CA group were identified as stress-responsive genes in rice. OsPP108 overexpressing lines showed higher insensitivity to ABA and increased tolerance to drought stress. Also, expression profiling using various stress marker genes in these lines showed interplay between ABA-dependent and ABA-independent signaling pathways for abiotic stress tolerance (Singh et al. 2015). *OsLEA3-2*, a late embryogenesis abundant protein, was found in rice. The overexpression line of *OsLEA3-2* rice generated using binary pHB vector showed better performance under salinity stress compared to wild type and was able to regain growth after 20 days of abiotic stress treatments (Duan and Cai 2012). Stress-associated proteins (SAPs), containing A20/AN1 zinc finger domains, are found to confer abiotic stress tolerance in different plants. OsSAP1 in rice was found to interact with its close homolog OsSAP11 and a rice receptor-like cytoplasmic kinase OsRLCK235 via the A20 domain. Overexpression of *OsSAP11* and *OsRLCK253* in rice showed an increased abiotic stress tolerance in rice (Giri et al. 2011). CYP5, an immunophilin protein, was identified to interact with members of ARF guanine nucleotide exchange factors and is required for localization of PIN1 (auxin efflux carrier). In rice, $OsCYP_{19\text{-}4}$ was found to be regulated by stress and upregulated significantly under cold stress. Further, functional analysis through overexpression in rice confirmed that this protein increases tolerance to cold stress and enhances grain yield in rice (Yoon et al. 2016).

TALEN- and ZFN-based genome editing for abiotic stress tolerance is not done extensively in rice, yet. But CRISPR-Cas9 has been extensively used in rice for genome editing in this decade. Using CRISPR-Cas9 knockout mutation of miRNAs *OsMIR408* and *OsMIR528* lines was developed. These lines were salt-sensitive and were found that these genes were positive regulators of salt stress tolerance (Yan et al. 2011). In the past decade, many salt-related genes were identified. *OsRR22* was one of them; it encodes a 696-amino acid B-type response regulator transcription factor that is involved in cytokinin signaling. CRISPR-Cas9 mutagenesis of *OsRR22* gene showed increased salt tolerance. This shows that loss of function of *OsRR22* leads to significantly increase salt tolerance (Zhang et al. 2019). Plant annexins are calcium-dependent phospholipid-binding proteins, which exist as multigene families in plants. Knockout mutation of *OsAnn3* using CRISPR-Cas9 showed cold tolerance phenotype compared to wild type (Shen et al. 2017). Many genes were identified using mutation studies, and among them, the drought and salt tolerance (*DST*) gene was identified as non-desirable genes and is present in the genome due to linkage. Genome editing via CRISPR-Cas9 technology caused 366 bp deletion and led to the loss-of-function mutation of the *DST* gene. This mutant line was having enhanced leaf water retention under dehydration stress. These are very few mentions of gene analysis done using functional genomic approaches for abiotic stress in rice. But many more have been done over these years. All this information would help in establishing the development of transgenic or targeted mutant lines for abiotic stress tolerance. Various abiotic stress-responsive genes identified in rice using functional genomic approaches are listed in Table 15.1.

15.2.5 Molecular Mapping and Marker-Assisted Breeding for Abiotic Stress Tolerance

Molecular marker techniques in traditional breeding are useful for developing elite lines. Mapping studies have led to the identification of genotypes having extensive variation at the structural level of the genome and the association of this variation with stress conditions. These variations are useful to confirm target genes for abiotic stress response (Rashid et al. 2014). Several stress-related QTLs have been located. Development of precise NGS technologies and DNA polymorphism detection techniques and map-based cloning is helpful in more QTL identification and marker development (Nogoy et al. 2016). The salinity tolerance of 235 temperate japonica rice accessions was evaluated. These accessions were already been marked with 30,000 SNP markers. GWAS study had led to the identification of 27 QTLs. The positions of these QTLs were compared with 300 genes already known for rice salt tolerance. The positions of several QTLs were found close to genes involved in calcium signaling and kinases, hence shows the importance of calcium signaling in salt stress response (Frouin et al. 2018). Using bulked segregant analysis-NGS (BSA-Seq), four candidate regions for thousand grain weight (TGW) under alkali

Table 15.1 Summary of abiotic stress-responsive genes in rice identified using genomic approaches

S. No.	Genes	Function	Associated stress response	Method of validation	References
1	*Os05g30750*	Anthranilate phosphoribosyl	Chilling stress	TILLING	Cho et al. (2012)
	Os12g39210	Transferase			
	Os07g36630	Cyclin			
	Os01g61160	CSLF8-cellulose synthase-like family			
		Laccase precursor protein			
2	*Os02g0528900*	ABC transporter	Heat stress	TILLING	Hwang et al. (2020)
3	*OsAKT1*	Ion transporters	Salt tress	TILLING	Hwang et al. (2016)
	OsHKT6				
	OsNSCC2				
	OsHAK11				
	OsSOS1				
4	*SD1*	Plant height	Drought stress	TILLING	Casella et al. (2013)
	Hd1	Flowering time			
	SNAC1	Drought tolerance			
	BADH2	Aroma			
5	*OsCPK17*	Stress signal transduction	Salt stress	ECOTILLING	Negrão et al. (2011)
6	*VP14*	Carotenoid dioxygenase	Drought stress	ECOTILLING	Wang et al. (2004)
7	*OsGSK1*	Glycogen synthase kinase	Cold and salt stress	T-DNA insertional mutation	Koh et al. (2007)
8	*OsTP1*	Trehalose-6-phosphate synthase	Drought stress	T-DNA insertional mutation	Kim et al. (2005)
9	*OsHSP40*	Heat shock protein	Salt stress	T-DNA insertional mutation	Wang et al. (2019b)
10	*OsTEF1*	Transcription elongation factor	Drought	T-DNA insertional mutation	Paul et al. (2012)

(continued)

Table 15.1 (continued)

S. No.	Genes	Function	Associated stress response	Method of validation	References
11	*OsSAP1*	Stress-associated protein	Drought	T-DNA insertional mutation	Giri et al. (2011)
	OsRLCK253	Receptor-like cytoplasmic kinase			
12	*OsLEA3-2*	Late embryogenesis abundant proteins	Salt and drought stress	T-DNA insertional mutation	Duan and Cai (2012)
13	*OsABF2*	ABA-responsive element-binding factor 2	Drought, salinity, and oxidative stress	T-DNA insertional mutation	Hossain et al. (2010)
14	*OsCYP19-4*	Cytochrome protein	Cold stress	T-DNA insertional mutation	Yoon et al. (2016)
15	*OsRR22*	Transcription factor	Salinity stress	CRISPR-Cas9 mutagenesis	Zhang et al. (2019)
16	*OsMIR528*	miRNA	Salt stress	CRISPR-Cas9 mutagenesis	Zhou et al. (2017)
17	*OsDST*	Drought and salt tolerance	Osmotic and salt stress	CRISPR-Cas9 mutagenesis	Santosh Kumar et al. (2020)
18	*OsPIN5*	Auxin transporter	Cold stress	CRISPR-Cas9 mutagenesis	Zeng et al. (2020)
	OsMYB30	MYB transcription factor			
	OsGS3	Grain size gene			

stress were identified. QTL-qATGW$_{2\text{-}2}$ was mapped within the range of 116 kb between two molecular markers RM13592 and Indel3 of Chr.2, which contained 18 predictive genes. The BSA sequencing showed that *Os02g39884* is the candidate gene for QTL-qATGW$_{2\text{-}2}$, an alkali-tolerant gene locus in rice (Sun et al. 2021). Muthu et al. (2020) aimed at pyramiding QTLs in improved white ponni (IWP) for developing triple stress tolerance against drought, salinity, and submergence through MAS. The QTLs used were drought (qDTY$_{1.1}$; qDTY$_{2.1}$), salinity (Saltol), and submergence (Sub1) (Muthu et al. 2020). Over this decade, many molecular markers, QTLs, have been developed, and this would help in finding rice accession with desired traits and faster breeding for the development of new hybrid lines with multiple abiotic stress tolerance.

15.2.6 Genome-Wide Analysis of Gene Families Involved in Abiotic Stress Response

Genome-wide identification and analysis of gene families are usually done by sequence homology of already known genes but in a new crop genome. This leads to the identification of nearly all the members of that particular gene family. Further expression analysis helps in the identification of a strong member of the gene family which is involved in a particular function and is also helpful in the identification of pseudogenes (Haubold and Wiehe 2004). Using this approach, many gene families have been identified in the rice genome. Pentatricopeptide repeat proteins (PPRs) are 35-amino acid motifs; these could bind RNA strands after transcription and could participate in RNA metabolism. In rice, by genome-wide analysis, a total of 491 *PPR* genes were found, of which 246 PPR genes belong to subclass P and 245 genes belong to subclass PLS. Expression analysis showed that many PPR genes were induced under biotic and abiotic stresses, while seven of these were strongly induced under salinity and drought (Chen et al. 2018). Dehydrins are known to be involved in dehydration stress tolerance. To study the evolutionary and conservation pattern of dehydrins in rice, a comparative analysis among 11 rice species was carried out. Sixty-five *DHNs* were identified and three *DHNs* were found to be highly conserved. The correlation pattern was analyzed between the dehydrin gene conservation and pattern of domestication and diversification. This showed that *Oryza rufipogon* and *Oryza sativa* ssp. *japonica* being wild species follow an adaptive evolutionary pattern (beneficial genes getting selected against deleterious alleles) suggesting diversification, whereas *Oryza nivara* and *Oryza sativa* spp. *indica* being domesticated species show a conserved evolutionary pattern (Verma et al. 2017). DUF221 domain-containing genes (*DDP* genes) have been previously recorded to have important roles in plant development, hormone signaling pathways, and stress responses. In rice, through comparative genomics, at least nine members of *DDP* genes are found in domesticated and wild rice. Various expression analyses showed that they are upregulated by salt stress, yet *OsDDP6* was upregulated at all the developmental stages in FL478, a salt-tolerant rice genotype (Ganie et al. 2017). Ca^{2+} is a major secondary messenger involved in various signaling pathways triggered by stress. Using genomic tools, repertoire of Ca^{2+} transport genes was identified in rice. Their expression pattern was studied under various vegetative and reproductive developmental stages like seedling, mature leaf, panicle, and seed developments using microarray and qRT-PCR techniques (Singh et al. 2014). Ribosomal proteins (RPs) are involved in translation by participating in ribosome biogenesis. The ribosomal protein large subunit (RPL) was already found to be involved in both biotic and abiotic stress responses. Genome-wide analyses showed that the rice genome has 56 ribosomal protein small subunits (RPS). All these 56 *RPS* are found to distributed among all the 12 chromosomes. Expression studies showed that *RPS* genes are also involved in biotic and abiotic stress responses. *RPS4*, *13a*, *18a*, and *4a* are shown to have higher transcript levels under most of the abiotic stresses (Saha et al. 2017). Dirigent (DIR) and DIR-like family proteins are found to be involved in lignification. In rice, the genome-wide analysis resulted in the identification of 49 *DIR* or *DIR-like* genes. Among 49, 23 *DIR* or *DIR-like* genes are found to be involved in abiotic stress response (Liao et al. 2017). Members of other gene families, including phospholipase A (Singh et al. 2012a); MADS-box family (Arora et al. 2007); phytocyanin (Ma et al. 2011); BURP (Ding et al. 2009); armadillo (Sharma et al. 2014); arabinogalactan (Ma and Zhao 2010); nuclear factor Y (Yang et al. 2017); ABA repressor (ABR1) (Mishra et al. 2013); transcription factors like NAC, ZF-HD, WRKY, EREBP, and bHLH (Muthuramalingam et al. 2018); phospholipase C (Singh et al. 2013); autophagy-associated genes (ATG) (Xia et al. 2011); protein phosphatase (Singh et al. 2010); malectin-like domain (Jing et al. 2020); C_4-related genes (Muthusamy et al. 2019); sulfate transporter (SULTR) (Yuan et al. 2021); and phospholipase D (Singh et al. 2012b), were also identified as abiotic stress-responsive genes in rice using comparative genomics approach.

Plant response to stress includes transcription regulation of gene expression, which in turn depends on several transcription factors and their interaction with the promoter region of the genes. It is important to identify stress-inducible promoter regions (Roychoudhury et al. 2008). This information could be useful to deploy transgenes with specific stress-inducible promoters (Cohen et al. 2017). Rai et al. (2009) selected promoter elements of three stress-responsive genes, viz., *OsABA2* coding for zeaxanthin epoxidase, *rab16A* coding for dehydrin, and *HP1* coding for a hypothetical protein. These were fused to the gusA reporter and introduced in the rice plant system and found *OsABA2* promoter as the most effective promoter. Since, it showed low constitutive expression of a transgene under normal conditions and strong expression under abiotic stress (Rai et al. 2009). In *Arabidopsis*, cis-acting promoter elements involved in cold- and dehydration-responsive gene expression have been extensively analyzed. However, the same was not done in rice. In order to do the same, transcription profiles of three different rice species for cold and dehydration were done. And the responsive genes were similar for all three species. The conserved sequences in cold and dehydration inducible promoters from these genes showed to have the abscisic acid-responsive element (ABRE) and were the most inducible under dehydration in all three species. Also, CGTACG and GTAGTA sequences were identified as novel cold-inducible rice promoters (Maruyama et al. 2012). A transcription factor AL RESISTANCE TRANSCRIPTION FACTOR1 (ART1) was identified to be involved in the detoxification of aluminum in rice. ART1 is C2H2-type zinc finger transcription factor and regulates the 31 downtown genes. While analyzing the promoter region of these genes, it was found that GGN(T/g/a/C)V(C/A/g)S(C/G) as a cis-acting element is responsible for aluminum toxicity response (Tsutsui et al. 2011). Genome-wide occurrence and positional arrangements of two cis-regulatory elements, ABRE and CE3, were studied in rice and *Arabidopsis*. It was found that ABRE is equally abundant in both rice and *Arabidopsis* (Roychoudhury et al. 2008), while CE3 was absent in *Arabidopsis*. The ABRE element was arranged as ABRE-ABRE pair and forms ABA-responsive complexes. Also, many other distinct combinations of ABRE and CE3 were present in rice gene promoters (Gómez-Porras et al. 2007). Besides these genes and promoter sequences, using comparative genomic methods, many other gene families have been characterized at genome-wide level in rice. All this information would help in further functional and structural genomic approaches for developing new varieties.

15.3 Transcriptomics Approaches for Abiotic Stress Tolerance in Rice

The transcriptome refers to the capture of all RNAs transcribed by the cell or tissue at a certain state of function. This includes both coding and non-coding RNA, while transcriptomics is the term which refers to the study of type, structure, function, and regulation of gene transcription (Nejat et al. 2018). Under stress conditions, transcriptomics can be used to quantitatively analyze the changes in plant gene expression at a specific time point and state; this helps in deciphering the regulatory network and expression at the whole genome level and pinpoints new genes related to stress tolerance and adaptability (Wang et al. 2020). Transcriptomics has attained zenith since development in transcript sequencing and analysis technologies. Traditional transcriptomic approaches such as northern blotting and the RT-PCR allow only single transcripts or small groups of transcripts to be analyzed at a given instance (Lowe et al. 2017). The entrance of microarrays in the practice during the mid-90s made a revolution in the field of transcript profiling. This allows large-scale analysis of thousands of genes simultaneously (Wang et al. 2020). Later the real-time RT-PCR or qRT-PCR method came to be a very sensitive technique for the detection of low-abundance transcripts. It has been widely used for absolute quantification of a particular gene or relative quantification of different gene expressions (Nejat et al. 2018). Recently NGS has essentially revolutionized gene expression profiling for the second time. Through high-throughput sequencing, it improved our knowledge of gene regulatory networks and epigenetics. NGS-based RNA sequencing (RNA-Seq) allows detection and quantification of already known novel and less abundant transcripts, both coding and non-coding RNA (Lowe et al. 2017).

15.3.1 Transcriptomics Technologies

ESTs are random individual transcripts sequenced from cDNA libraries. Sequencing is done by one time, low-throughput Sanger sequencing ESTs and was considered to be a proficient method to determine the gene content of an organism without sequencing the entire genome (Nejat et al. 2018). After ESTs, another sequencing-based gene expression analysis came to existence during 1995, i.e., serial analysis of gene expression (SAGE). This includes sanger sequencing of concatenated random transcript fragments. The quantification was done by matching the transcripts with known genes. A variant of SAGE using high-throughput sequencing techniques, called digital gene expression analysis, was also briefly used (Lowe et al. 2017). These techniques are overtaken by contemporary technique microarrays and RNA-Seq. Each transcriptomics technology has been briefly discussed below

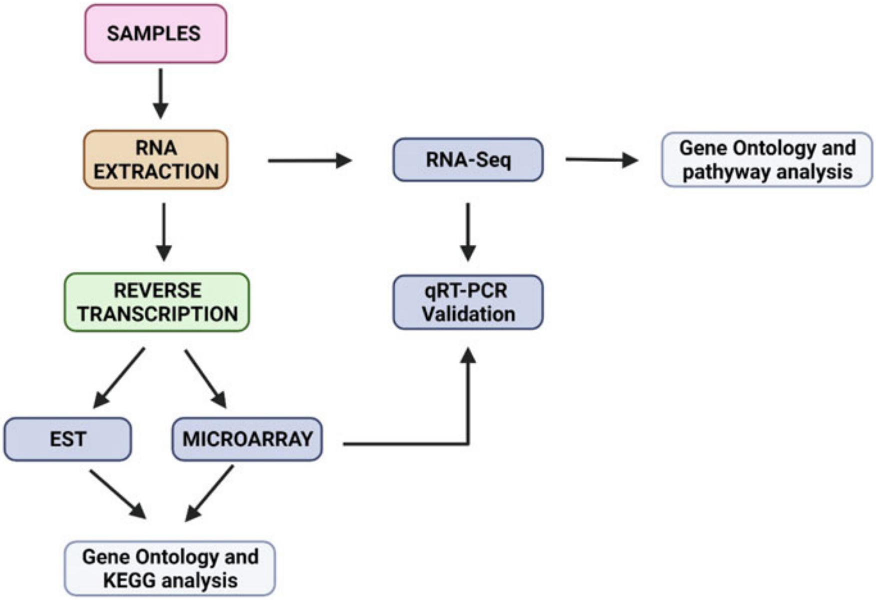

Fig. 15.2 Transcriptomic methodologies and workflow for crop improvement: Transcriptomics involves extraction of total RNA of an organism at particular time point under particular environmental condition and is further processed variably for expression analysis. In EST and microarray analysis, cDNAs are synthesized using reverse transcriptase, and later cDNA libraries are prepared. This could be sequenced for development of ESTs and microarray probe for expression profiling. Otherwise, the total RNA could be normalized and sequenced for identification of all the differentially expressed genes. qRT-PCR provides further validation. Gene ontology and KEGG analysis help in functional association of identified genes

(Wang et al. 2020). The workflow of transcriptomic approaches for the identification of genes is shown in Fig. 15.2.

EST is useful to identify what are the genes an organism expresses at a given time point. An EST results from one-shot sequencing of a cloned cDNA. EST generation starts with the mRNA extraction from a whole organism or specific tissues (Lowe et al. 2017). mRNAs are reverse transcribed to create libraries of cDNA. Using universal primers, random colonies are subjected to a single sequencing reaction. After sequencing, bioinformatics procedures are used to process the raw sequence reads to remove low-quality sequences and vector sequence contamination. The resultant sequences are then deposited in the dbEST database (Parkinson and Blaxter 2009). EST has its application in addition support for full genome sequencing and gene discovery. Like microarray, ESTs are not biased by probe selection and hybridization intensities. However, ESTs can provide sequence information for designing and constructing novel microarray platforms (Parkinson and Blaxter 2009). Microarrays consist of short nucleotide oligomers; these are known as "probes," and these are bound in an arrayed manner on a solid substrate. The expression analysis is done using the determination of transcript abundance of the transcripts. It is through hybridization of the fluorescently labeled transcript to these probes. The fluorescence intensity at each probe loci on the array indicates the transcript abundance for that probe sequence (Lowe et al. 2017). RNA-Seq refers to high-throughput sequencing combined with computational methods to quantify transcripts in an RNA pool. The nucleotide sequences generated are around 100 bp length and can vary based on the sequencing method employed. The RNA transcripts are aligned to a reference genome or to each other. RNA-Seq is useful in identifying genes within a genome or identifying which genes are active at a particular point in time and could find the relative gene expression level accurately (Nejat et al. 2018).

15.3.2 EST and Microarrays for Identification of Abiotic Stress-Related Genes in Rice

Gorantla et al. (2007) identified genes associated with water-stress response in rice. Analysis of 7794 cDNA sequences led to the identification of 5815 rice ESTs. Of these, 334 showed no sequence homology with any rice ESTs or full-length cDNAs in public databases of rice grown under normal conditions; this indicates that these transcripts are enriched during drought stress. Analysis of these ESTs showed that 1677 out of these were unique sequences. These sequences were compared with abiotic stress-induced sequences from expression profiling of *Arabidopsis*, barley, maize, and rice. This analysis led to the identification of 589 putative stress-responsive genes. These were again compared with the expression profile of drought-stressed panicle library, and this showed 125 genes among 589 genes are expressed both in leaf and panicle tissues during drought. The gene ontology of these 125 genes showed that most of them are associated with cellular metabolism, signal transduction, and transcriptional regulation (Gorantla et al. 2007).

Likewise, using ESTs and microarray techniques, many individual gene families responsible for abiotic stress in rice were identified over the years. A few of them are discussed here. Glutathione S-transferases (*GSTs*) were found to be involved in biotic, abiotic, and arsenate stress tolerance in rice. Transcript profiling using microarray and ESTs suggested that *GSTs* are having similar and specific roles during various stages of development in rice, also mediating cross-talk between various stress and hormone response pathways (Jain et al. 2010). Heat shock proteins are involved in plant response to heat stress and get activated through heat shock factors (HSFs). Plant HSFs are grouped into three classes A, B, and C (Andrási et al. 2021). Expression profiling through ESTs, microarray, and qRT-PCR showed that eight *OsHSTs* are upregulated during seed development and six HSFs during abiotic stress both root and shoot. *OsHSFA2a* and *OsHSFA3* are upregulated during cold and drought stress. *OsHSFB4a* showed no or little change in expression during expression profiling through FL-cDNA/ESTs and qRT-PCR (Chauhan et al. 2011).

Metallothioneins (MT) are low molecular weight, cysteine-rich metal-binding proteins. Bioinformatics analysis of the rice genome sequence revealed that there are 13 genes and 15 protein products in rice (Banerjee and Roychoudhury 2021).

OsMT1e-P protein member had conserved cysteine amino acid. And this was found to be involved in salinity stress. EST database and publicly available microarray data suggested that *OsMT1e-P* was abundant during seedling and reproductive stages, which are stress-sensitive stages (Gautam et al. 2012). Half-size adenosine triphosphate-binding cassette transporter subgroup G (ABCG) genes were known to have a role in abiotic stress in dicot model plants *Arabidopsis thaliana*. But in rice, this was not established. Thirty half-size ABCGs in rice were found, and their preliminary evidence of gene expression under abiotic stress was established using FL-cDNA and EST databases. Evidence of expression was not found for *OsABCG2*, *6*, *10*, *24*, and *29*. For *OsABCG6* and *OsABCG10*, the expressions were not detected under semi-quantitative RT-PCR. This suggested that these two genes are pseudogenes (Matsuda et al. 2012).

Rice is a C3 plant, and being a model plant and important agricultural crop, it is a potential target for genetic engineering of the C4 pathway. It is already known that genes encoding C4 enzymes are present in C3 plants. Fifteen genes belonging to the rice C4 gene families in the rice genome were found through sequence homology using maize C4 gene sequence as query (Muthusamy et al. 2019). The expression analysis using EST and FL-cDNA database showed at least one EST or FL-cDNA was present for all the identified genes. Abiotic stress and heavy metal-regulated expression analysis using microarray datasets showed that *Osnadp-me2* and *Osnadp-me3* were upregulated during salt and drought stress. *Osppdk1* was upregulated during salt, drought, and anoxic conditions (Muthusamy et al. 2019). Ubiquitin-conjugating enzyme E2s (UBCs) are involved in the ubiquitination of proteins and are involved in the protein degradation pathway. Thirty-nine UBC genes were found in rice. Expression profiling of OsUBC genes using EST, microarray, and qRT-PCR showed that many *OsUBC* genes exhibited abundant and tissue-specific expression patterns. Moreover, 14 *OsUBCs* were found to be differentially expressed under treatments with drought or salt stresses (Zhiguo et al. 2015). Through a microarray-based investigation on salt-induced genes in rice seedlings, 1834 genes were found to be upregulated by salt stress. Among these, an EST showed a 23.3-fold induction. Database searches showed that this EST encodes a previously unknown type 2C protein phosphatase. This was named *Oryza sativa* salt-induced PP2C protein 1 (*OsSIPP2C1*) (Li et al. 2013). Analysis of cold-induced transcriptome led to the discovery of a putative MYB transcription factor, designated as *Cold induced MYB1 (CMYB1)*. qRT-PCR assay showed that the expression of *CMYB1* increased by >100-folds during cold stress. And it was found that *CMYB1* is also involved in circadian rhythm. Taken together, *CMYB1* probably functions as a transcriptional activator in mediating stress- and rhythm-responsive gene expression in rice (Duan et al. 2014). Meprin and TRAF homology (MATH) domain-containing protein (MDCP) has been known to be involved in biotic stress response. Expression analysis using qRT-PCR and microarray showed that out of 11 *MDCP* in rice, *OsM4* and *OsMB11* are involved in drought and salinity stress (Kushwaha et al. 2016). Likewise, numerous abiotic stress-responsive genes have been identified in rice using EST and microarray methods. A list of such genes is given in Table 15.2.

Table 15.2 Summary of abiotic stress-responsive genes in rice identified using transcriptomic approaches

S. No.	Genes	Function	Associated stress response	Method of validation	References
1	*OsDHOD1*	Dihydroorotate dehydrogenase	Salt, drought	EST, microarray	Liu et al. (2009)
2	*OsHsfA3*	Heat shock factor	Heat stress	EST, microarray	Chauhan et al. (2011)
	OsHsfA7				
	OsHsfA9				
	OsHsfA1				
3	*Os01g47050*, *Os01g59690*, *Os02g15950*, *Os02g51350*, *Os04g33820*, *Os05g43490*, *Os06g39370*, *Os07g09710*, *Os10g30280*	F-box proteins	Osmotic stress	EST, microarray	Jain et al. (2007)
4	*OsABCG26*	Half-ABC proteins	Cold stress	EST	Matsuda et al. (2012)
	OsABCG27		Salt stress		
5	*OsDhn1*	Dehydrins	Drought stress	EST	Lee et al. (2005)
6	*Os02g47744*, *Os12g41920* *Os06g19980*	MYB transcription factors	Drought stress	EST, microarray	Katiyar et al. (2012)
7	*OsZFP177*, *OsZFP181*, *OsZFP176*	A20/AN1-type zinc finger protein	Drought, cold, and osmotic stress	EST, microarray	Huang et al. (2008)
	OsZFP173				
	OsZFP181				
	OsZFP176				
	OsZFP157				
8	*OsMT1e-P*	Metallothioneins	Drought and salinity stress	Microarray	Gautam et al. (2012)
9	*OsHPPD*	Vitamin E biosynthetic enzymes	Dehydration, cold, and salt stress	EST, microarray	Chaudhary and Khurana (2009)
	OsγTMT				
	OsHPT				
	OsMPBQ MT2				
10	*Osnadp-me2*	C4 photosynthetic enzymes	Salt and drought stress	EST, microarray	Muthusamy et al. (2019)
	Osnadp-me3				
11	*OsUBCs*	Ubiquitin-conjugating enzymes	Salt and drought stress	EST, microarray	Zhiguo et al. (2015)

(continued)

Table 15.2 (continued)

S. No.	Genes	Function	Associated stress response	Method of validation	References
12	*OsFKBP20* *OsSce1*	SUMO-conjugating enzyme and peptidyl prolyl cis-trans isomerase	Heat stress	EST	Zhang et al. (2018)
13	*OsSIPP2C1*	Protein phosphatase 2C	Salt and drought stress	EST, microarray	Li et al. (2013)
14	*OsGLYI6*, *OsGLYI11*	Glyoxalase enzymes	Drought stress	EST, microarray	Mustafiz et al. (2011)
15	*OsM4* *OsMB11*	Meprin and TRAF homology (MATH) domain-containing protein	Salinity and drought	Microarray	Kushwaha et al. (2016)
16	*OsPP2C* *OsPP2A*	Protein phosphatase	Salt, cold, and drought	Microarray and Q-PCR	Singh et al. (2010)
17	*OsMADS4*, *OsMADS5*, *OsMADS6*, *OsMADS7*, *OsMADS15*	Transcription factors	Aerobic adaptation	RNA-Seq	Phule et al. (2019)
18	*OsSWEET3A*	Sugar transporters	Aerobic adaptation	RNA-Seq	Phule et al. (2019)
19	*OsLEA3*	Late embryogenesis abundant proteins	Drought stress	RNA-Seq	Mangrauthia et al. (2016)
	OsDREB1A	Dehydration-responsive element binding			
	OsRAB16B	Responsive to ABA			
20	*OsPRX*	Peroxidase precursor	Cold stress	RNA-Seq	Dametto et al. (2015)
	OsKET	3-Ketoacyl-CoA synthase			
	OsAQU	Aquaporin protein			
	OsCSLE1	Cellulose synthase-like family E			
	OsCDKB2;1	Cyclin-dependent kinase B2-1			

(continued)

Table 15.2 (continued)

S. No.	Genes	Function	Associated stress response	Method of validation	References
21	*OsSulfT2.1*, *OsPotT2*	Sulfate transporter Potassium transporter	Fluoride susceptibility	RNA-Seq	Banerjee et al. (2020)
22	*OsSweet11*	Sugar transporter	Fluoride tolerance	RNA-Seq	Banerjee et al. (2020)
23	*OsPhyB*	Phytochrome B	Drought stress	RNA-Seq	Yoo et al. (2017)
24	*OsMYB-R1*	Transcription factor	Drought stress	RNA-Seq	Tiwari et al. (2020)
25	*OsSAP16*	Stress-associated protein	Drought stress	RNA-Seq	Lei et al. (2020)

15.3.3 EST and Microarray-Based Rice Databases

EST libraries are reposited into the web for future and public use. There are two large EST repositories, i.e., dbEST and UniGene, both are from NCBI, and these include EST data from a variety of organisms (Singh et al. 2019). Many rice-specific EST databases are also available. Databases which include rice EST datasets are OryGenesDB (Droc et al. 2006), Rice Genome Annotation Project (Tanaka et al. 2008), and the Rice Genome Knowledgebase (RGKbase) (Wang et al. 2013). The Rice Expression Profile Database (RiceXPro) is a repository of gene expression profiles derived from microarray analysis and includes information from possibly all the growth stages and tissues and from different stress treatments (Sato et al. 2013). Other rice microarray databases are Rice Oligonucleotide Array Database (ROAD) (Cao et al. 2012), RiceArrayNet (Lee et al. 2009), RiceMetaSys (Sandhu et al. 2017), and OryzaExpress (Hamada et al. 2011).

15.3.4 RNA-Seq for Identification of Abiotic Stress-Related Genes in Rice

In the past 5–10 years, expression analysis using RNA-Seq is at peak. Few abiotic stress-responsive genes identified using RNA-Seq are discussed here and are listed in Table 15.2.

RNA-Seq analysis of two inbred indica lines, IR64, a drought-sensitive line, and Apo, a moderate drought-sensitive line, showed that IR64 under drought stress had transcriptional changes related to signal transduction, protein binding, and receptor activity (Ereful et al. 2020). Apo showed increased expression of genes associated with an oxygen-binding function and peroxisome pathway. In conclusion, the scientists suggested that drought-sensitive line employs energy-consuming pathway to cope with drought hence fails to survive, while drought-tolerant cultivar employs energy-efficient pathways as response and succeeds being tolerant plants (Ereful et al. 2020). Seeds activate a series of genes to avoid stress during imbibition and germination. RNA-Seq analysis between 8 h of imbibed seeds and dry seeds of rice showed that genes related to the cell wall, abiotic stress, and antioxidant-related DEGs were associated with stress response in initial imbibition of rice seed germination (Zhao et al. 2020). Among signaling-related genes, receptor kinase was significant, and among cell wall-related genes, pectinesterase and polygalacturonase were significant. Among abiotic stress-related genes, cupin domain protein, methyltransferases, SPX domain, GSTs, and peroxidase were significant. qRT-PCR analysis showed that GST showed the highest activity at that stage. GSTs might prevent the burst of H_2O_2 accumulation at the initial imbibition stage that contributes to the following successful seed germination (Zhao et al. 2020).

Adaptation of rice to the aerobic condition is needed to cope with the water scarcity as well as to ensure sustainable yield in the future. RNA-Seq analysis of root and shoot between aerobic adapted (drought tolerant) and anaerobic cultivars (drought intolerant) showed that the number of differentially expressed transcripts was higher in the root than that in the shoot under both aerobic and anaerobic conditions (Phule et al. 2019). And it was found that MADS transcription factors and transporters involved in sugar (*SWEET3A*) and nutrient uptake were highly expressed in aerobic adapted cultivar but not in anaerobic cultivar. This suggests that these genes are important for drought tolerance (Phule et al. 2019). RNA-Seq analysis of Aus, drought, and heat-tolerant cultivar found 56 differentially expressed genes in developing seeds under combined drought and heat stresses. One of the significantly induced genes, *B12288*, is annotated as *RAB21*. This gene has homologs in different *O. sativa* subspecies and in various wild species of *Oryza*. It belongs to the dehydrin family of LEA proteins (Roychoudhury et al. 2007). *RAB21* was also found in other rice cultivars. Sequence differences are not large, but the functions were not the same though (Schaarschmidt et al. 2020). It is still unclear how dehydrin exhibits positive stress regulation responses in plants. However, it has recently been shown that in *Arabidopsis*, four amino acids change in LEA protein led to increased membrane stability under cold stress. Hence, it was concluded that minor differences among the amino acid sequence of RAB21 from different rice species have significant functional effects (Schaarschmidt et al. 2020).

Comparative analysis of drought-tolerant (N22) and salt-tolerant (Pokkali) with susceptible cultivar (IR64) using RNA-Seq showed that members of NAC and DBP transcription factors in N22 and members of bHLH and C2H2 transcription factors in Pokkali show differential regulation under dehydration and salt stress (Shankar et al. 2016). Transcripts encoding for thioredoxin and involved in phenylpropanoid metabolism were upregulated in N22, whereas transcripts involved in wax and terpenoid metabolism were upregulated in Pokkali. This shows that different and multiple kinds of responses via various transcription factors and metabolic pathways are exhibited by rice plants under varying abiotic stress (Shankar et al. 2016). QTL identification using two rice cultivars, IR36 (salt-sensitive) and Weiguo (salt-tolerant), led to the identification of qRSL7 related to relative shoot length (*RSL*) located on chromosome 7 (Lei et al. 2020). RNA sequencing for IR36 and Weiguo after 36 h of salt stress at the budburst stage revealed five differentially expressed genes in this candidate region. qRT-PCR analysis and further deep RNA-Seq found 1 bp indel difference in *Os0790569700* (*OsSAP16*), a stress-associated protein upregulated during drought stress (Lei et al. 2020). Among chloroplast genes involved in photosynthesis, RNA-Seq was aimed at identifying genes differentially expressed during cold, iron, and salt stress. Of all genes expressed in each stress, cold had the highest number of differentially expressed genes (DEGs) related to light and chloroplast reactions (do Amaral et al. 2016). Comparative transcriptome analyses using RNA-Seq of cold-sensitive and cold-tolerant variety showed 13,930 and 10,599 DEGs, respectively. Functional classification of these DEGs showed that in cold-tolerant variety lipid-binding activity, catalytic and hydrolase activities, photosynthesis, energy and carbohydrate metabolism were enhanced during cold stress, while in susceptible variety absence of photosynthesis related genes, storage products like starch and fatty acids were noticed (Pradhan et al. 2019). These two studies suggest that photosynthetic genes are directly involved in cold stress response.

MicroRNAs (miRNAs) are small RNA molecules that play important regulatory roles in plant development and stress responses (Barrera-figueroa et al. 2012). RNA sequencing using Illumina deep sequencing for four small RNA libraries from the inflorescence of rice plants grown under control and abiotic stress conditions such as drought, cold and salt stress lead to identification of 227 miRNAs belonging to 127 families. The expression levels of these miRNAs were compared, which revealed that 18, 15, and 10 miRNAs were involved in drought, cold, and salt stress responses in rice respectively (Barrera-figueroa et al. 2012). RNA-Seq analysis of fluoride-sensitive cultivar IR64 after NaF treatment showed upregulation of 1303 transcripts and downregulation of 93 transcripts. Expression analysis revealed that increased fluoride level leads to suppression of ABA signaling and biosynthetic pathways (Banerjee et al. 2020). However, the gibberellic acid pathway and signaling via ABA-independent transcription factors were activated. Comparative analysis of DEGs in IR-64 and fluoride-tolerant variety, Khitish showed that fluoride sensitive variety had escalated levels of autophagy (Banerjee et al. 2020). Further expression analysis revealed that fluoride susceptibility was associated with high expression of genes responsible for amino acid transport, monosaccharide transport, and nutrient transport while fluoride tolerance with high expression of *Sweet11* (Banerjee et al. 2020). Root transcriptome analysis of rice treated with Cd and As showed that the genes in common to Cd and As stress were associated with redox control, stress response, transcriptional regulation, transmembrane transport, signal transduction, as well as biosynthesis and metabolism of macromolecules and sulfur compounds (Huang et al. 2019).

15.3.5 RNA-Seq-Based Rice Databases

Various databases that encompass mRNA sequences obtained from rice have been developed over the year. TENOR (Transcriptome ENcyclopedia Of Rice) is a database for comprehensive mRNA-Seq experiments in rice. mRNA sequences obtained from various abiotic and hormone treatments are provided along with novel genes identified from mRNA-Seq data, expression profiles, co-expressed genes, and cis-regulatory elements. This is a publicly available database (Kawahara et al. 2016). Rice Expression Database (RED) is an integrated RNA-Seq-derived gene expression database for rice. RED integrates gene expression patterns from all growth stages and various abiotic stresses (Xia et al. 2017). RECoN: Rice Environment Coexpression Network for Systems Level Analysis of Abiotic-Stress Response. It is useful in identifying clusters of both functionally characterized and uncharacterized genes that tightly co-express during abiotic stress from the given new differential expression profile. (Krishnan et al. 2017). In the year 2019, Wang et al. developed CropCircDB, a comprehensive circular RNA resource for crops in response to abiotic stress. CropCircDB was designed mainly for rice and maize, to detect circular RNA responsible for stress conditions. This database is aimed to incorporate other crops in the future (Wang et al. 2019a, b).

15.4 Conclusion

Rice is a staple crop in the world. Agricultural challenges like biotic and abiotic stresses affect rice production. Due to the increasing world population, rice productivity has to be increased while combating these stresses. Abiotic stresses like drought, cold, salinity, and heavy metal stress adversely affect rice productivity. To overcome the negative effect of these adverse effects, two major strategies are adopted: (1) crop management strategies and (2) crop breeding. Crop breeding technologies are now been supported by multi-omics approaches like genomics, transcriptomics, proteomics, and metabolomics. These help in the functional analysis and identification of new genes and protein regulatory networks. Among these omics approaches, genomics and transcriptomics help majorly by identification of abiotic stress-responsive genes, genome-wide analysis of various rice accession for development of various markers, and QTLs for marker-assisted breeding. Transcriptome analysis combined with NGS helps in the identification of novel genes, transcription factors, and non-coding RNAs involved in abiotic stress response. Over these decades, many genes have been identified in rice, and many elite cultivars by gene pyramiding, transgenic, and gene editing have been developed in rice. Yet, these have not led to complete stress tolerance in rice. Many functional genes are to be studied in order to decipher abiotic stress responses. Studying many orphan crops, halophytes, thermophytes, and other plants belonging to environmental extremities would contribute to novel target genes to be identified in rice. Successful breeding would be one with a lesser number of non-desirable genes, and during natural selection, many non-desirable traits are carried forward in evolution along with desirable traits. Identification and structural analysis of these genes are necessary for their elimination. All these could be applied by integrated omics approaches.

Acknowledgment The financial support from Science and Engineering Research Board (SERB), Department of Science and Technology (DST), Govt. of India (Grant No: EEQ/2018/000106), and core research grant from the National Institute of Plant Genome Research (NIPGR) in AS lab is acknowledged. KS is thankful to Council of Scientific and Industrial Research (CSIR), India, for research fellowships. Authors are thankful to DBT (Department of Biotechnology), eLibrary Consortium (DeLCON), for providing access to e-resources.

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Biochemical and Molecular Mechanism of Wheat to Diverse Environmental Stresses

16

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Abstract

Wheat (*Triticum aestivum* L.) is the most important cereal crop next to rice for meeting the global food needs. Abiotic stressors such as drought and salinity are widespread in wheat. However its production is faced by multiple biotic and abiotic stresses that seem a challenging task in the future climate change scenario. Drought and salinity are the most limiting factors among wheat stresses. Hence, understanding of wheat physiology, biochemistry, and molecular responses is most urging. Wheat physiology is highly affected by drought and salinity stresses including seed germination, number of tillers, grains yield, grain quality, etc. Similarly, plant biochemical parameters like photosynthesis, nutrient uptake, carbon assimilation, reactive oxygen species (ROS) synthesis, and ROS neutralizing antioxidant enzymes activity are considered as important biomarkers. Besides this, molecular responses at various stages including genomics, transcriptomics, proteomics, and metabolomics also play vital role in plant sustenance during drought stress. Thus, the chapter is mainly focused to elaborate the various mechanisms involved in drought and salinity stresses tolerance of wheat crop.

Keywords

Wheat · Drought · Salinity · Molecular mechanism · Environmental stresses

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_16

16.1 Introduction

Wheat (*Triticum aestivum*) is the first domesticated cereal crop on Earth. It is an important staple food crop of greater civilizations like Europe, West Asia, and North Africa during Fertile Crescent 8000–10,000 years. Wheat is the second widely grown cereal crop after rice that nourishes 35% of world population (FAO 2011; Shiferaw et al. 2013). Global wheat production is estimated at 750 million tons during 2016–2018 (OECD/FAO 2019). Two-third of this produce is consumed as food and one-fifth as livestock feed. Europe and North America are the largest producers in developed world whereas China and India in the developing world. Wheat belongs to the family Poaceae. Commercially two species of wheat are important including hexaploid bread wheat (*Triticum aestivum*; $2n = 6x = 42$) and tetraploid durum wheat (*Triticum durum*; $2n = 4x = 28$). The chapter focuses on the most widely consumed bread wheat (Kulkarni et al. 2017). Hence, the hexaploid wheat forms 21 pairs of chromosomes during meiosis that are further classified as three types/groups of homologous chromosomes like A, B, and D. Each of these groups contains seven pairs of chromosomes with homology of the hexaploid and tetraploid wheat species. The current bread wheat resulted from the hybridization of A, B, and D genome progenitors. In the context of global population growth, it is predicted to raise the wheat demand up to 60% by 2050, but coincidentally, the produce might be reduced by 29% due to various constraints in wheat production. The constraints in wheat production include several biotic stresses like root rot, rusts, seed born, and viral diseases and abiotic stresses like waterlogging, drought stress, nutrient/mineral stress, cold stress, heat stress, etc. Among these hazards or constraints of wheat production, drought stress is one the most important factors which is spontaneously increasing as result of climate change (Zahoor et al. 2020). The world is predicted to face absolute water shortage by 2025 (Nezhadahmadi et al. 2013; Zahoor et al. 2020). Thus, it is imperative to cope with such exacerbated situation, understand, devise, and adopt strategies that enhance the drought stress tolerance in wheat for food security in near future. Many factors determine how plants respond to environmental stress (Amanullah et al. 2019; Amanullah et al. 2021). Environmental stresses affect various physiological processes and cause alterations in plant growth and development (AL-Issa et al. 2020).

16.2 Biochemical and Molecular Mechanism of Wheat to Drought Stress

16.2.1 Drought Stress in Wheat

From agricultural context, drought stress can be defined as the prolonged period of limited water supply or shortage of water either natural due to lack of precipitation or programmed water deficit (Sallam et al. 2019). This drought stress may induce several types of physiological, biochemical, and molecular changes during the plant life cycle, adversely affecting crop growth, development, and yield (Abid et al. 2018; Rabara et al. 2021). Thus, reduced water supply to plants cause damaging effects like reduced seed germination, weak root system, small leaf area, low plant water content, decreased photosynthetic activity, limited plant nutrient uptake, low transpiration, low protein content, reduced cell division, poor antioxidant activity, CO_2 assimilation, weak cell turgor, oxidative damages to cell and cellular components and inhibited enzymatic activities, reduced hormone signaling, downregulation in primary metabolism and degeneration of metabolites, etc. (Roychoudhury et al. 2013). All these growth markers are either individually or exclusively/mutually coincide with drought stress in wheat crop thereby limiting quality, yield, and productivity (Hasanuzzaman et al. 2018; Sallam et al. 2019).

16.2.2 Biochemical Mechanism of Wheat to Drought Stress

Drought stress is a vital abiotic stress to plants that exclusively inhibit various physiological, biochemical, and molecular activities/traits which are regarded essential for normal growth, development, yield, and quality of crop plants. Many biochemical traits in plants, which are regarded as biochemical indicators/markers to any kind of stress and their optimal activity, may change in case of stress to plants. These biochemical features/traits include photosynthetic activity and gaseous exchange, relative water content (RWC), nutrient uptake, reactive oxygen species (ROS) synthesis, antioxidant system, osmotic/osmolyte balance, and hormonal effect (Basu et al. 2010). Each of these is described as follows.

16.2.3 Photosynthesis and Drought Stress

Plants adopt a series of mechanisms to cope with drought stress by limiting water losses and gaseous exchange through stomatal pores. Plant roots when subject to drought induce stress signals that are carried by abscisic acid (ABA) to plant leaves, whereby plant stomata and mesophyll conductance are reduced as result of ABA accumulation. These early stomatal closures enable plants to decrease transpiration and thus withstand limited water supply. Consequently, decrease in gaseous exchange and photosynthetic activity occurs by limited CO_2 assimilation and chlorophyll biosynthesis; however, imbalanced level of electron utilization and excitation from low photosynthesis ultimately results in reactive oxygen species generation (Das and Roychoudhury 2014). These ROS molecules are quite toxic as they react with lipids and protein, causing damages at cellular and ultrastructural levels leading to lipid peroxidation of cell membrane (Liu et al. 2015a, b, Singh et al. 2016). Hasanuzzaman et al. reported that decrease in plant photosynthetic activity results from reduced soluble protein, RuBisCo, and RuBisCo-binding proteins. Limiting photosynthetic activity of plants results from low leaf area expansion, injured photosynthetic machinery (thylakoid lamellae and stroma), and premature leaf senescence.

16.2.4 Compatible Solutes, Osmolytes, and Drought Stress

Like photosynthesis, plants can also change water relation and adjust osmotic balance to maintain their cellular function. This is mainly achieved by synthesis of compatible solutes (low molecular weight and highly soluble compounds) that are nontoxic to plants. These solutes may be free amino acids, sugars, and proline (Izanloo et al. 2008; Roychoudhury et al. 2015). The compatible solutes protect the plants by osmotic balance (maintain cell turgor and volume) (Fang and Xiong 2015), ROS detoxification/scavenging, membrane structure stability, and native enzyme and protein rehabilitations (Blum 2016).

16.2.5 ROS Synthesis and Drought Stress

ROS (reactive oxygen species) are the important molecules produced abnormally upon the exposure of plants to different biotic and abiotic stresses. ROS molecules are very much toxic to plants since they react with lipids, proteins, cell membrane, etc. and thus disturbing normal cellular, organelles, and enzymatic function (Banerjee and Roychoudhury 2017a, b; Zahoor et al. 2020). ROS are either generated in enzymatic or non-enzymatic reactions. Xanthine oxidase is an important enzyme responsible for the production of ROS molecules. Non-enzymatic ROS are normally synthesized as by-products during the processes of electron transport chain (ETCs) at various sites like mitochondria, chloroplasts, and plasma membranes (Sairam et al. 2005). Increased ROS generation is regarded as oxidative stress markers while exposed to some kind of stress (Scandalios 1993a, b; Nezhadahmadi et al. 2013).

16.2.6 Antioxidants and Drought Stress

Antioxidants are a group of both enzymatic and non-enzymatic molecules that are regarded as plant defense components. Such enzymes include SOD, CAT, peroxidase, ascorbic acid peroxidase, and glutathione reductase, and non-enzymatic components include cysteine, reduced glutathione, and ascorbic acid (Matsumoto et al. 2001). Such antioxidants lower the toxic effects of ROS and safeguard plant cellular machinery. Superoxide dismutase (SOD) converts the O_2^- into H_2O_2 molecule which is further detoxified to H_2O and O_2 by catalase (CAT) and ascorbate peroxidase (APX). Similarly, glutathione (GSH) and carotenoids belong to non-enzymatic antioxidants (Sairam et al. 2005). Glutathione (GSH) mostly occurs in chloroplasts where it maintains the balance of reduced glutathione to oxidized glutathione, hence protecting the chloroplast from oxidative damages. However, carotenoids control the dissipation of excessive excitation energy and heat to safeguard the photosynthetic apparatus (Abid et al. 2018). The concerted action of all enzymatic and non-enzymatic antioxidants alleviates the oxidative stress in plants and help in tolerance to stress.

16.2.7 Molecular Mechanism of Wheat to Drought Stress

16.2.7.1 Drought Stress Tolerance and Gene Identification

Drought tolerance in wheat is a polygenic trait which is regulated by interlinked genes and synchronized by some key transcription factors (TFs). With the development of recent laboratory techniques and computational biology analyses like transcriptomics, proteomics, and metabolomics, it has become possible to locate and identify genes and their up- or downregulation in response to drought stress (Roychoudhury and Banerjee 2015). Similarly, marker-assisted selection (MAS) and quantitative trait locus have enabled the scientists to determine the relative stress-responsive genes (Budak et al. 2013). Molecular biology techniques like blotting, PCR, gel electrophoresis, AFLP, 2D gels, chromatography, and mass spectrometry are mainly used for transcriptomics, proteomics, and metabolomics analysis (Nezhadahmadi et al. 2013).

16.2.7.2 Transcription Regulation in Wheat

The process of transcription is regulated by some transcription factors (TFs) that control the transcription of certain drought-responsive genes. Transcription regulation is either ABA (abscisic acid)-dependent, constituting osmotic pressure-responsive genes that defend the plant from dehydration and stabilizing cell structure (Roychoudhury et al. 2013), or ABA-independent (Budak et al. 2013; Nakashima et al. 2014). ABA is also called as stress hormone which is synthesized in plants and involved in several cell processes like leaf abscission, seed development and maturation, dormancy, etc. (Vishwakarma et al. 2017). During drought situation, ABA synthesis occurs to initiate signals for downstream pathways for managing drought tolerance. ABA is known as the first line of defense against stress in plants. 9-*cis*-epoxycarotenoid dioxygenase (NCED) is considered as the main enzyme for ABA biosynthesis during stress (Finkelstein et al. 2002; Agnes et al. 2014). The de novo biosynthesis of ABA is due to *ZEP* gene expression and related to basal transcription level (Xiong and Zhu 2003). ABA biosynthesis level has also been used as selection index for screening drought-tolerant parents during hybridization of wheat.

16.2.7.3 ABA (Abscisic Acid)-Dependent Transcription Factors (TFs)

The basic leucine zipper (bZIP), i.e., AREB (ABA-responsive element binding protein)/ABF (ABA-binding factor) (Banerjee and Roychoudhury 2017b) and MYC (myelocytomatosis oncogene)/MYB (myeloblastosis oncogene), constitutes the ABA-dependent TFs (Cramer et al. 2011). Fujii and Zhu (2012) reported that sucrose non-fermenting responsible protein kinase 2 family (SnRK2) is plant-specific Ser/Thr kinases that also regulate ABA-mediated signaling. Similarly, AP2/EREBP (ERF) play role mostly via ABA-independent signaling pathways. Rong et al. (2014) reported that AP2/EREBP (ERF) family contains some ethylene-responsive TFs that induce drought tolerance in wheat, viz., TaERF increases drought tolerance in wheat by enhancing proline level and chlorophyll content. ABA-induced genes, viz., *Wdhn13*, *Wrab17*, *Wrab18*, *Wrab19*, and *PKABA1*, are responsible for drought tolerance in wheat (Kobayashi et al. 2008;

Table 16.1 ABA-dependent TFs and related genes in wheat

Transcription factor family	Genes	References
bZIP	*WLIP19*, *TaOBF1*, *TaABF1*	Wang et al. (2015)
MYC/MYB	*TaMYBsdu1*,*TaMYB33*, *TaMYB3R1*	Okay et al. (2014)

Quellet et al. 1998; Budak et al. 2015; Aurelio et al. 1999). Table 16.1 represents the TFs found in wheat along with the related genes.

16.2.7.4 Abscisic Acid (ABA)-Independent TFs

ABA-independent TFs include CBF/DREB (cold-responsive element-binding factor/dehydration-responsive element binding) and ZF-HD (zinc finger homeodomain) (Lata and Prasad 2011). DREBs constitute a large class of TFs related to AP2/ERF family. DREB shows an immediate response upon plant exposure to any biotic or abiotic stress (He et al. 2011; Zahoor et al. 2020). DREBs are further classified as DREB1/CBF and DREB2. The DREB1/CBF comprise of genes expressed during cold-induced dehydration, while DREB2 is expressed during drought-induced dehydration in plants. The DREB-associated genes include *rd29A*, *kin1*, and *erd10* that work potentially during abiotic stress (Kulkarni et al. 2017). WRKY class of TFs is regarded as the superfamily involved in regulation of many developmental and physiological processes of plants (Phukan et al. 2016). This group of TFs functions efficiently during plant exposure to different biotic and abiotic stress conditions (Banerjee and Roychoudhury 2015; He et al. 2016). Several studies reported the overexpression of *WRKY* genes (*WRKY1* and *WRKY2*) during stressed conditions (Proietti et al. 2011). Wang et al. (2015) reported upregulation of *TaWRKY44* and *TaWRKY93* genes in wheat during heat stress. Similarly, some genes for TaWRKYs (*TaWRKY16*, *24*, *59*, *61*, and *82*) showed differential expression in root and leaf of wheat exposed to drought stress (Okay et al. 2014). NAC is another TF that plays a vital role in gene regulation not only in plant development but also in stressed conditions. NAC TFs consist of three types of proteins, i.e., NAM, ATAF, and CUC, all of which exhibit a highly conserved DNA-binding domain (David et al. 2016). NAC has strong role in plant development and stress tolerance mechanisms in wheat. Several genes, *TaNAC8*, *TaNAC2*, *TaNAC4*, and *TaNAC69* were reported that were involved in plant drought tolerance mechanism and pathways with 265 genes at junction stage and 146 at seedling stage, involved in drought tolerance mechanisms of wheat. However, more than half of these genes also function in other biotic and abiotic stresses as well (Shi et al. 2010).

16.3 Biochemical and Molecular Mechanism of Wheat to Salinity Stress

Environmental stresses are a major constraint to crop production over the world (Al-Rifaee et al. 2004). Salinity stress or hyper ionic stress is the major stress that limits crop production. Plants growing in high salt concentration suffer negative impacts like high sodium accumulation often leading to inhibition of uptake of essential macronutrients like K^+ and Ca^{+2}. This inhibition causes halting in growth and development of plants (Roychoudhury and Chakraborty 2013). Wheat is considered as staple crop across the globe, as it provides calories to around 30% population. However, the growth and development of wheat have been declined due to increasing salt concentration in the soil. The major impact of salinity stress was exhibited in inhibited photosynthesis in wheat plants by energy deficiency (Dadshani et al. 2019). Therefore, breeders and researchers are engaged in understanding response mechanisms of plants and their adaptation or tolerance at biochemical and molecular level of the plant.

16.3.1 Biochemical Mechanism

Water potential plays a crucial role in uptake of water from plant roots via osmosis. Plant cell has more negative internal water potential than pure water which causes the uptake of water by roots. This very potential can be utilized for the tolerance of wheat plant against salinity stress by decreasing the water potential. This decrease leads to increased solute concentration inside the plant cell, just building up the turgor pressure to withstand the stress, via lowering of osmotic pressure across the cell and the environment. The osmotic potential of the cell is more abruptly affected by salinity stress due to energy requirement to maintain the potential, often leading to poor growth. Cumulatively, increase in salinity in the environment decreases the water and osmotic potential which lead to increase in turgor pressure (Mudgal et al. 2010). Wheat plants exclude excess of Na^+ by mechanism of exclusion. This minimizes accumulation of Na^+ in leaf by pumping large amount of Na^+ ions into vacuoles to maintain the osmotic potential and thus conferring salinity tolerance (Dadshani et al. 2019). Specific ions during salinity stress inhibit the growth and development of wheat plant. Chloride ions along with Na^+ affect the osmotic potential. The protection against such specific ions is achieved via exclusion by xylem tissues. Such ions first enter the older leaves and then are excluded by xylem of younger leaves. The older leaves protect the younger leaves, and in return the younger ones exclude the ions. Another way to combat salinity stress is by vacuolar compartmentalization of excessive toxic Na^+. The movement of excess Na^+ is via apoplast movement through membrane or involving cellular processes via plasma membrane to tonoplast. Several osmolytes are also involved in tolerance, viz., simple to complex sugar, quaternary amino acids, and sulfonium compounds that confer tolerance against salinity stress. Proline accumulation increases with increasing salt concentration which is necessary for adaptive changes of the wheat plant

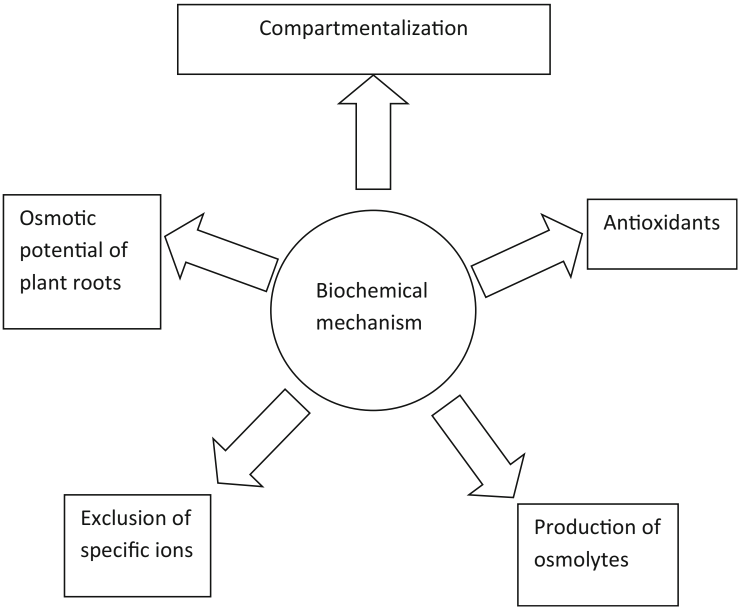

Fig. 16.1 Various biochemical mechanism involved in wheat during salinity stress

(El Moukhtari et al. 2020). Salinity stress often leads to oxidative stress by generation of ROS such as hydrogen peroxide, superoxide radical, and hydroxyl ion radicals. Such free radicals disrupt various biomolecules like lipids, proteins, and nucleic acids through oxidation (Roychoudhury et al. 2008). However, this oxidative stress is eliminated by the production of antioxidants like glutathione, ascorbate, and carotenoid. Various radicals generated through oxidative stress are also scavenged through enzymes like SOD (superoxide dismutase), catalase, ascorbate peroxidase, and glutathione reductase (Mudgal et al. 2010) (Fig. 16.1).

16.3.2 Molecular Mechanism

HKT transporter constitutes a class of protein involved in transport of Na^+ across the cell. Such genes like *HKT 1;1/2-, 1;3-, 1;4-, 1;5* are present on homologous chromosome groups 2, 6, 2, and 4, respectively. These genes are targeted for conferring tolerance against salinity stress conditions by Na^+ exclusion. Till now, two processes like DNA methylation and DNA reprogramming are utilized to tackle against salinity stress. Reactive oxygen species homeostasis in wheat has been achieved through somatic hybrid introgression lines. The hybrids between beneficial species and common wheat plant lead to production of more stable and tolerant varieties. For instance, hybrid of tall wheatgrass and common bread wheat produced improved wheat that can even grow in barren areas with tolerance to several abiotic stresses. Using somatic hybridization, Shanrong No. 3 (SRE 3) was cultivated

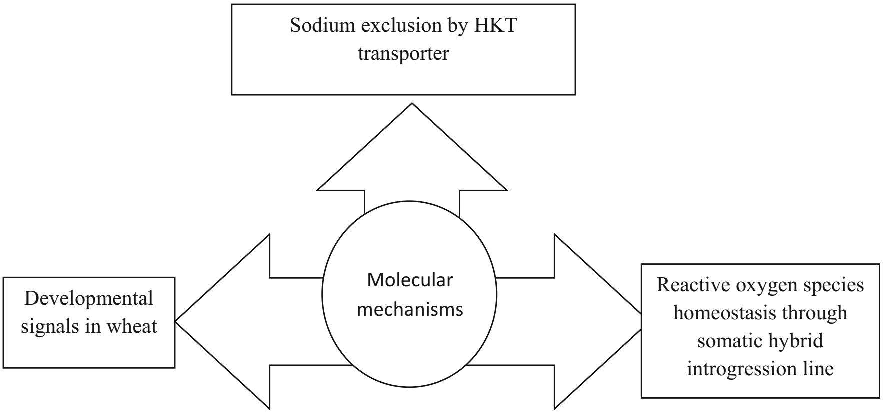

Fig. 16.2 Various molecular mechanisms in wheat against salinity stress

showing tolerance against salinity stress (Wang and Xia 2018). Developmental signals along with phytohormone signal pathway plays an additional role in tolerance against salinity stress (Javid et al. 2011). The best studied phytohormone genes in wheat were *TaAOC1* and *TaGBF1*. *TaAOC1* encodes allene oxide cyclase, which is involved in jasmonic acid (JA) biosynthesis, and the gene was induced by high salt concentration. *TaGBF1*, another gene involved in phytohormone synthesis, is responsible for blue-light photomorphogenesis; this gene was also induced during salinity stress through G-box-binding factor. Much lesser information are available in these areas, which can be further exploited using omics tools and enriched genomic data such as omics networking (Fig. 16.2).

16.4 Conclusion

Drought and salinity stresses profoundly degrade the optimal environment needed for wheat growth, development, yield, and quality. Drought stress adversely affects the plant physiological, biochemical, and molecular pathways. The increased accumulation of malondialdehyde and H_2O_2 is considered as damaged response to stress. Antioxidants like SOD, POD, CAT, GSH, and proline nullify the effect of oxidative stress. Besides, there are other metabolites such as osmolytes and phytohormones that reduce the deteriorating effects of stress inside the plants. Similarly, molecular pathways and associated TFs, i.e., ABA-dependent and ABA-independent TFs, regulate the up- and downregulations of several genes during drought stress, thereby ensuring plant adaptation and survival to harsh environments. Thus it is essential to understand the behavior of plant physiology, biochemistry, and underlying omics platforms for drought stress tolerance. This will indeed be helpful in future planning and management of environmental stresses that are predicted to rise with climate change.

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How microRNAs Regulate Abiotic Stress Tolerance in Wheat? A Snapshot

17

Deepu Pandita

Abstract

Wheat (*Triticum aestivum* L.) belongs to family Poaceae and is one of the critical cereals worldwide. Plant microRNAs (miRNAs) are noncoding regulatory RNAs, 20–24 nucleotides (nt) in length, and small master regulators of plant gene expression belonging to small RNAs class. microRNAs act as key regulator/s of miRNA-directed post-transcriptional gene regulation pathways of huge set of biological processes, for instance, development, mediating defense responses, and effective adaptive abiotic stress tolerance. In this chapter, the contemporary status of abiotic stresses triggered by various miRNAs in wheat plant will be discussed. Understanding of functions of miRNA families under stress will provide prospects to improve plant performance or engineered abiotic stress resistance through targeting the molecular modification in the upcoming global climate changes.

Keywords

Wheat (*Triticum aestivum* L.) · miRNAs · Abiotic stress · Stress response · Global climate change

17.1 Introduction

Wheat (*Triticum aestivum*) is a vital, allohexaploid (AABBDD) and widely preferred staple food crop for human consumption which provides about 20% of calories in human food (UNDP 2007; Shewry 2009). Wheat of family Poaceae is one of the critical cereals planted universally, nurtures above 1/3 of world's

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_17

population, and provides approximately 55% of levels of carbohydrate (Samad et al. 2017; Boucas et al. 2012). Different abiotic stress conditions like water deficiency, low and high temperatures, heavy metals, salinity, and nutritional insufficiencies are key restraining factors in the production of agriculture (Akpinar et al. 2013; Budak et al. 2013, 2015). Abiotic stress factors are foremost precincts in wheat production. Mostly wheat is grown in hostile environs, specifically heat and drought areas, in that way limit yield potential. It is anticipated that a worldwide temperature upsurge of 2 °C could reduce yield of wheat by 50% through changes at the physiological and biochemical levels (Holoch and Moazed 2015). Climate change which has fast-tracked in current periods induces abiotic stresses leading to changes in the homeostasis at cellular, molecular, and physiological levels and causes cellular damage with devastating impact on yield, growth, and development of crops and threatens food security (Mickelbart et al. 2015; Raza et al. 2019; Nadarajah 2020). To alleviate this hazard, it is indispensable to improve the adaptableness of wheat at genetic levels to handle the changing climate drifts. Consequently, designing of abiotic stress-tolerant and climate-resilient/smart wheat genotypes is imperative for worldwide food protection (Powell et al. 2012; Raza et al. 2019).

microRNAs (miRNAs) are hairpin-derived, small, 20–24 nucleotides long, non-coding regulatory RNA transcripts, and chief regulators of gene expression that regulate genes at post-transcriptional levels and also cause epigenetic changes (Khraiwesh et al. 2010; Budak et al. 2015a, b, c; Wu et al. 2010; Alptekin and Budak 2016). The microRNAs influence all facets of growth and development and physiology and mediate defense from biotic pathogens and tolerance to ecological stresses in plants (Kantar et al. 2011; Kouhi et al. 2020). The miRNA-guided post-transcriptional gene silencing (PTGS) of stress-responsive genes is deliberated to be a specific profit in abiotic stress environments (Palatnik et al. 2003). PTGS machinery regulates expression of developmental and stress-linked genes (Guan et al. 2013; Ravichandran et al. 2019a, b). Quite a lot of investigations determine the active function of miRNAs in regulating developmental stages under diverse abiotic stress conditions (Palatnik et al. 2003). Wheat miRNAs (16 in number, of 9 conserved miRNA families) were predicted initially bioinformatically from Expressed Sequence Tag (EST) database of wheat in year 2005 (Zhang et al. 2005). Then in year 2007, 58 wheat miRNAs from 43 miRNA families were recognized via small RNA cloning and sequencing of small RNAs (Yao et al. 2007). To date, an enormous number of wheat miRNAs have been released in the microRNA database named www.mirbase.org. The stress-responsive miRNAs and miRNA targets have been documented from *Oryza sativa*, *Zea mays*, *Triticum aestivum*, and barley (Liu et al. 2016). Differences were observed in expression profiles of six extremely conserved miRNAs across numerous species under water deficiency or salinity stresses (Kantar et al. 2011). "Positive" miR396 expression profile under drought stress is uncommon in plants. Microarray analysis of emmer wheat subjected to drought confirmed a negative response for miR396 to drought with decrease in miR396 abundance (Kantar et al. 2011). Some 202 miRNAs were identified in wheat, out of which 36 showing differential expression patterns after heat stress and their target genes were identified (Ravichandran et al. 2019a, b). In plants, knock-in/out of miRNAs improves stress tolerance (Sanz-Carbonell et al. 2019) and same holds true for wheat.

17.2 Biogenesis of miRNA

The microRNA biogenesis originates with transcription of single-stranded primary microRNA or pri-miRNA from microRNA (MIR) genes in the nucleus. MIR gene loci reside in intergenic regions, or microRNAs originate from intragenic regions of introns, few from exons of genes or transposable elements (TEs). TE processed microRNAs are named B transposable element-related miRNAs (TE-miRs) (Kim and Kim 2007; Li et al. 2011; Colaiacovo et al. 2012; Liu 2012; Lucas and Budak 2012; Kurtoglu et al. 2014; de Rie et al. 2017). Primary miRNA transcript is biosynthesized by RNA polymerase (Pol II or III) and folds into an imperfect stem-loop. DAWDLE (DDL) stabilizes pri-miRNAs (Ha and Kim 2014). The stem-loop structure of primary transcript is accepted by the microprocessor complex members including DiGeorge Syndrome Critical Region 8 (DGCR8) and ribonuclease III enzyme called Drosha in D-bodies and processed into stem-loop of precursor miRNAs (pre-miRNAs) (Yu et al. 2008; Lee et al. 2004; Kurihara et al. 2006; Kim 2005; Voinnet 2009; Denli et al. 2004). DGCR8 recognizes *N*6-methyladenylated GGAC of pri-miRNA (Alarcon et al. 2015). DCL1 (Drosha)-facilitated pri-miRNA duplex cleavage is supported by Serrate (SE), Hyponastic Leaves 1 (HYL1), and nuclear cap-binding complex (CBC) which are DICER-LIKE 1 (DCL1) interacting proteins and forms 2 nt 3′ overhang in pre-miRNA (Han et al. 2004). DCL1 and Hyponastic Leaves 1 are specific in biogenesis of miRNA. CBC and SE have wider roles in metabolism of transcripts of mRNA (Kurihara et al. 2006; Fang and Spector 2007; Laubinger et al. 2008a, b; Voinnet 2009). Mutations in se, dcl1, and hyl1 showed that the formation of nuclear complex by their functional proteins is vital for specific process of pri-miRNA into the pre-miRNA (Liu et al. 2011; Jones-Rhoades et al. 2006). CBP20 and CBP80 elements and SE take place in processing of the pri-miRNA (Kim et al. 2008; Laubinger et al. 2008a, b). The pre-microRNA processed from transcripts of pri-microRNA lacks conservation (size and/or structure) which allows scheme of non-generalized mode for maturation of pre-miRNA into the functional miRNA (Naqvi et al. 2012).

Two models named stem-to-loop and loop-to-base processing have been recommended for cleavage of miRNA/miRNA* duplex from pre-microRNA via activity of DLC1 and other proteins (Werner et al. 2010; Bologna et al. 2009). In the stem-to-loop model, cleavage of the pre-miRNA structure into mature miRNA/miRNA*duplex takes place at 15th nucleotide via DCL1 (Werner et al. 2010; Naqvi et al. 2012). Stem-to-loop model in plants resembles maturation of pre-miRNA in animals and denotes common mode for processing of pre-miRNA. MiR159 and miR319 show different cleavage patterns. In these, primary pre-miRNA cleavage is done adjoining pre-miRNA terminal loop. That is why this model is known as loop-to-base processing. Manifold cleavages are indispensable for the production of functional miRNA/miRNA* duplexes through this model.

The bulge in structure of pre-miRNA stabilizes valid microRNA formation (Bologna et al. 2009). MiRNA/miRNA* duplex undergoes biochemical modifications, preceding nuclear export to cytoplasm for stability, function, and amount. The miRNA/miRNA* duplex 3′ overhangs are uridylated by uridyl-transferases for degradation by small RNA-degrading nucleases (SDN) (Song et al. 2015). Adenylation of miRNA/miRNA* duplex in *Arabidopsis thaliana* is indefinable (Li et al. 2005). miRNA/miRNA* duplex 2′-O-methylation of all plant miRNAs by Hua Enhancer 1 (HEN1) before export from nucleus protects it from exonucleases (Ren et al. 2014). These biochemical modifications may also take place after transfer of miRNA/miRNA* duplex to cytoplasm. AGO1 prefers alteration of 5′ uridine residues, whereas AGO2 and AGO4 associate with residues of 5′ adenosine (Voinnet 2009). The miRNA/miRNA*-generated duplexes are then transferred to cytoplasm by homolog of mammalian Exportin-5 known as (XPO5)/RanGTP (HASTY) complex. In the cytoplasm, terminal loop of pre-miRNA is removed by RNase III endonuclease dicer to form mature duplex of miRNA (Denli et al. 2004; Zhang et al. 2004). Inside cytoplasm, guide strand (with lower 5′ stability or 5′ uracil) of miRNA/miRNA* duplex is loaded into ARGONAUTE (AGO) proteins containing PAZ domain for sRNA binding and PIWI domain for endonucleolytic target cleavage. The AGO proteins and guide strand assemble into RNA-induced silencing complex (RISC) for cleavage of mRNA or mRNA translational repression. The graphic depiction of miRNA biogenesis is displayed in Fig. 17.1. Thermodynamic stability of 5′ of both strands partly decides the choice of guide strand. HYL1 accessory proteins also assist in it (Park et al. 2005; Rogers and Chen 2013; Sun et al. 2012; Iwakawa and Tomari 2013; Ha and Kim 2014). The comprehensive outline of biogenesis of miRNAs in plants is deliberated in various chapters (Pandita 2019; Pandita and Wani 2019).

17.3 The miRNAs in Regulation of Plant Abiotic Stress Responses

Wheat is main staple crop for 35% of world population with wide-ranging adaptability to different ecological conditions (IDRC 2010). This adaptableness includes quite a lot of stresses and development-responsive genes. The miRNAs have appeared as significant regulatory agents. Presently, approaches conditioned to characterize miRNAs in wheat focus on conserved and greatly expressed miRNAs and avoid lineage- or condition-specific, or recently evolved. Moreover, several ecological and biological factors which affect miRNA expression were not taken into consideration. This resulted in a partial repertoire of wheat miRNAs (Agharbaoui et al. 2015). In plants, miRNA-based regulatory functions of numerous plant stress genes under biotic and environmental stress factors are well understood (Kantar et al. 2011a; Kumar et al. 2014; Hackenberg et al. 2015; Wang et al. 2014a, b; Pandita 2022; Šečić et al. 2021). During this, gene expression alterations were attained by directly targeting gene transcripts. In contrast, indirect miRNA-based regulation takes place through targeting of transcription factors (TFs) or

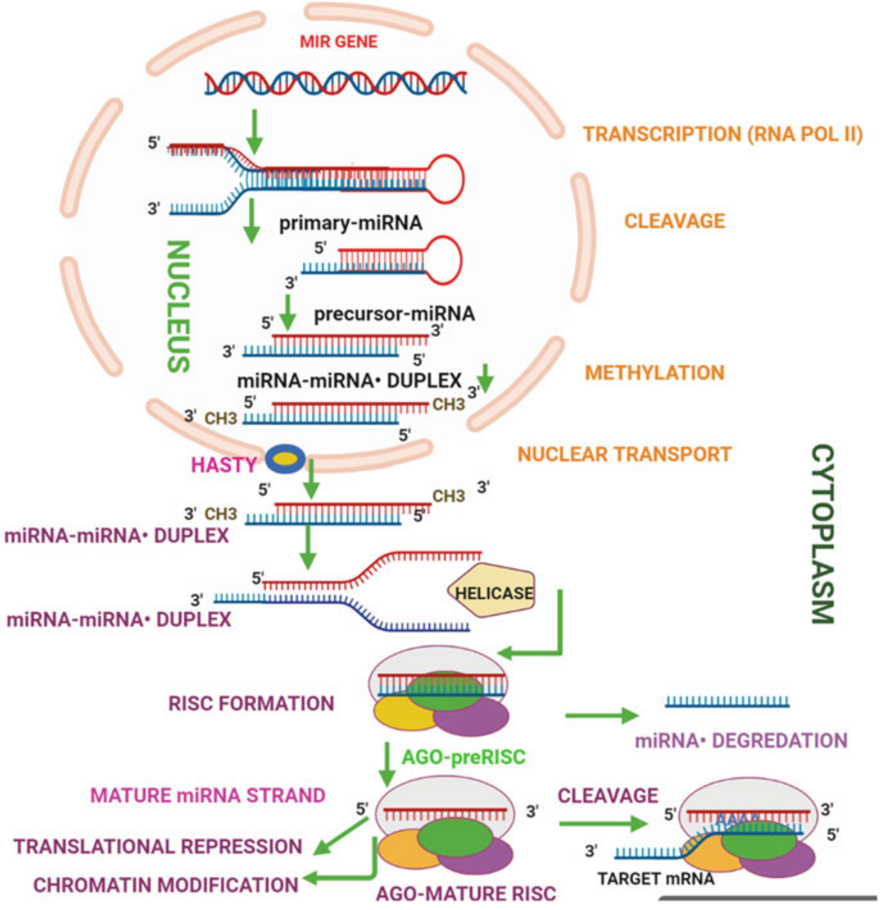

Fig. 17.1 Model depicting mechanism of miRNA biogenesis

phytohormones which sequentially control expression of particular stress-linked genes in plants (Jones-rhoades et al. 2011). Role of miRNA targets involves development of organs, floral organ identity, morphogenesis of leaves, development of roots, and stress responses in *Arabidopsis thaliana* (Aukerman and Sakai 2003; Palatnik et al. 2003).

Most of miRNAs mainly target metabolic transporters, transcription factors (TFs), and signal transduction pathways (Bonnet et al. 2004). Ta-miR855 targets transcription factor known as MYB. MYB transcription factors mainly regulate development of plant leaves and possibly genes involved in development of other organs. This is compatible with function of microRNA, i.e., miR159 (Palatnik et al. 2003). The miR5653 target ubiquitin carrier proteins and serine/threonine protein kinases. Ubiquitin carrier proteins have key role in regulation of various cellular processes, for instance, cell cycle control, activation of several transcription factors, metabolic regulation, and reprocessing of abnormal proteins (Ciechanover 1994). Serine/threonine protein kinases have important roles in regulating diverse signal transduction pathways which lead to plant defense under various stresses (Hardie 1999). Ta-miR5156 targets 40S ribosomal protein which forms a structural component of ribosome. Ta-miR3708 targets F-box proteins which facilitate plant hormone signaling, protein-protein interaction in transcription elongation, polyubiquitination, centromere binding, and translation repression (Zheng et al. 2002). MiR398 targets mRNA of B copper superoxide dismutase. It was overexpressed in leaves of barley and *Triticum* (Kantar et al. 2011; Hackenberg et al. 2015; Liu et al. 2015).

Besides, a line of studies on miRNAs in wheat has focused on prediction of target genes and expression profile analyses (Wang et al. 2014a, b; Sun et al. 2014; Bakhshi et al. 2017). Wheat miR159 induced expression in leaves and downregulated expression in the plant roots (Gupta et al. 2014; Ma et al. 2015). Five new abiotic stress-responsive miRNAs named Ta-miR3708, Ta-miR5653, Ta-miR819k, Ta-miR855, and Ta-miR5156 were predicted in wheat by mining of Expressed Sequence Tags (EST) database with 4677 mature miRNAs which belong to 50 miRNA families of different plant species. Ta-miR1122, Ta-miR1133, Ta-miR1134, and Ta-miR1117 and 14 potential targets were also predicted including serine/threonine protein kinase, ubiquitin carrier protein, F-box/kelch-repeat protein, 40S ribosomal protein, transcription factors, and BTB/POZ domain-containing proteins involved in growth and development, metabolism, and stresses (Pandey et al. 2013). Ta-miR819k targets BTB/POZ domain protein and Rho GTPase. Rho GTPases are chief regulators of cellular functions, for instance, organization of cytoskeleton, cell death, stress-induced signal transduction, and plant cell growth and differentiation (Winge et al. 2000), whereas BTB/POZ domain proteins mediate leaf morphogenesis in *Arabidopsis thaliana* (Ha et al. 2004). Thus, miRNAs not only target genes of plant development but also take part in various biochemical and physiological processes which initiate tolerance to abiotic stresses in plants. A conservation-independent technique allowed potential identification of broad repertoire of novel wheat miRNAs common to numerous species or specific to species or clade with more confidence. Identification of some 199 miRNAs linked with diverse abiotic stress (cold, salt, and aluminum (Al)) responses, tolerance, and development stages was predicted. These investigations will help to elucidate regulatory mechanisms of freezing and Al responses and tolerance along with development and flowering in plants. In longer time periods, it may benefit in breeding of stress-resilient crops (Agharbaoui et al. 2015). Sequencing technologies of Illumina, SOLiD, and Roche 454 besides bioinformatics analysis are presently the most potent tool for the detection and prediction of small RNA (Zhang et al. 2009; Gonzalez-Ibeas et al. 2011; Kurtoglu et al. 2014). The miRNAs from wheat growing in standard conditions (Wei et al. 2009) were discovered with Illumina-Solexa (Kenan-Eichler et al. 2011; Yao et al. 2007; Xin et al. 2010; Tang et al. 2012) and 454 Roche sequencing (Yao et al. 2007; Li et al. 2013), besides wheat plants subjected to diverse abiotic stresses, for instance, drought (Kantar et al. 2011), extreme high temperatures (Xin et al. 2010), low temperatures (Tang et al. 2012), salinity (Feng et al. 2013), and deficiency of phosphorus (Oono et al. 2013). Possible targets of tae-miR477b, tae-miR1438, tae-miR827, tae-miR1435a, tae-miR1522, taemiR1861b, tae-miR3476, tae-miR5641, tae-miR8135, and tae-miR8154 include a number of genes responsible for protein kinase, protein amino acid phosphorylation, signal transduction, ATP binding, Rab GTPase, Rab GTPase activator, phosphoprotein phosphatase inhibitor, and phosphoprotein phosphatase. Thus, miRNA cell signaling players can manage growth and development and stress tolerance in wheat (Achakzai et al. 2018). Tae-miR858 targets MYB-transcription factor. This can act as a potential reserve to cope biotic and abiotic stresses for improved wheat crop production (Achakzai et al. 2018).

Heat stress, water deficiency, and their blend harshly and antagonistically limit quantity and quality in wheat (Mittler 2006; Chen 2009; Zhang 2015). Some examples of wheat stress-responsive miRNAs and their targets under stress conditions are enlisted in Table 17.1.

17.3.1 Drought Stress

Sequencing of small RNA in drought-tolerant *Triticum aestivum* L. cv. XF 20 confirmed expression of 199 miRNAs and identification of 32 novel miRNAs. The expression of 37 miRNAs including five novel miRNAs (27 upregulated, 10 downregulated) was considerably affected by PEG6000-induced water deficiency stress. Degradome sequencing displayed cleavage of 222 target genes by these miRNAs. miRNA targets related to water deficiency and/or stress response include epoxide hydrolase, 6-phosphogluconate dehydrogenase, sucrose synthase, phytochrome, and flowering time regulatory proteins. Drought-responsive miRNAs and their targets were identified in drought-resistant variety (Hua et al. 2019). After drought stress treatment, about 14 miRNAs showed differential expression pattern in bread wheat (Akdogan et al. 2016), which varied between roots and leaves (Eldem et al. 2012). The miR896 from wild emmer wheat was downregulated after 4 h of water deficiency and upregulated after 8 h of water deficiency (Kantar et al. 2011). This recommends dynamic expression array of water deficiency-responsive microRNAs. Diverse miRNA families were identified in drought-tolerant or drought-susceptible wheat. The miR5387 was identified only in TTD-22 (drought susceptible), and miR1435 and miR5024 were identified only in TR39477 (drought tolerant) (Akpinar et al. 2015). The miRNA-facilitated response was studied in C-306 and NI-5439 (drought-tolerant) and HUW-468 and WL-711 (drought-sensitive) genotypes of wheat on exposure to 25% PEG6000 for 1 h, 12 h, and 24 h. In C-306 and NI-5439, miR393a and miR397a showed most accumulation at 1 h of stress (Kaur et al. 2017). Quantitative real-time PCR of 12 drought-responsive miRNAs and their analogous nine targets in C-306, NI-5439, HUW-468, and WL-711 wheat genotypes showed their differential expression patterns of upregulation and downregulation (Kaur et al. 2017). Negative association was detected in miR164, miR168a, miR398, and miR159a and their respective targets named NAC, AGO, SOD, and MYB. MiR529a and miR1029 were validated for the first time in wheat (Kaur et al. 2017). The miRNA target duos, namely, miR164

Table 17.1 Wheat stress-responsive miRNAs and their targets under stress conditions

microRNAs	Potential gene targets	References
Drought stress-responsive miRNAs		
miR1029		Gupta et al. (2014)
miR1117	Receptor-like protein kinase	Pandey et al. (2013)
miR1137		Ma et al. (2015)
miR1318		Ma et al. (2015)
miR1432	Phenylalanyl-tRNA synthetase (PheRS)	Ma et al. (2015)
miR156	Squamosa-promoter binding protein-like (Spl) proteins	Ma et al. (2015)
miR159	MYB transcription factor	Ma et al. (2015)
miR166	Homeodomain leucine zipper (HD-zip) proteins	Ma et al. (2015)
miR167	Auxin response factor	Alptekin and Budak (2016), Ma et al. (2015)
miR168		Ma et al. (2015), Gupta et al. (2014)
miR169	Nuclear transcription factor Y subunit	Zhou et al. (2010)
miR171	Scarecrow-like transcription factor (SCL-6)	Ma et al. (2015)
miR172		Gupta et al. (2014)
miR3708	F-box/kelch-repeat protein, TP53-regulating kinase	Pandey et al. (2013)
miR393	Transport inhibitor response 1 (TIR1)	Gupta et al. (2014), Ganie et al. (2016)
miR395	ATP sulfurylase (sulfate adenylyltransferase)	Zhou et al. (2010)
miR398	Copper-zinc superoxide dismutase (CuZnSOD, SOD1 protein)	Kantar et al. (2011), Liu et al. (2015)
miR5156	40S ribosomal protein	Pandey et al. (2013)
miR528	Superoxide dismutase [Cu-Zn], L-ascorbate oxidase	Wu et al. (2017)
miR5368		Ma et al. (2015)
miR819k	BTB/POZ domain-containing protein, Rho GTPase	Pandey et al. (2013)
miR827		Ma et al. (2015)
miR829		Ma et al. 2015
miR916		Ma et al. (2015)
Cold stress-responsive miRNAs		
miR1122	Maf-like protein, single-strand DNA-binding protein	Lu et al. (2011)
miR5653	Wheat ubiquitin carrier protein	Pandey et al. (2013)
Salinity stress-responsive miRNAs		
miR1124	Serine carboxypeptidase family protein, ribulose-1,5-bisphosphate carboxylase/oxygenase activase precursor, ribulose 1,5-bisphosphate carboxylase activase isoform 2, chloroplast nucleoid DNA-binding protein	Lu et al. (2011)

(continued)

Table 17.1 (continued)

microRNAs	Potential gene targets	References
miR1133	Fca-like protein, SnRK3.23, ATCIPK23, CIPK23 (CBL-interacting protein kinase 23), kinase, pentatricopeptide (PPR) repeat-containing protein, NB-ARC domain	Pandey et al. (2013), Lu et al. (2011)
miR14769	Putative RSH disease tolerance-related protein, T-complex protein 1	Agharbaoui et al. (2015)
miR167	Auxin response transcription factor (ARF6)	Lu et al. (2011)
miR174	RLK4, CRK10 (CYSTEINE-RICH RLK10), scarecrow-like transcription factor 6 (SCL6), kinase	Lu et al. (2011)
miR19980	Putative membrane-associated protein	Agharbaoui et al. (2015)
miR408	Putative basic blue copper protein, putative blue copper protein precursor, ORF1, plastid division regulator MinD	Lu et al. (2011)
miR1029	Downregulation	Gupta et al. (2014)
miR159	Phosphoinositide-specific phospholipase C, pyruvate decarboxylase, putative disease resistance protein RPS2	Wang et al. (2014a, b), Gupta et al. (2014), Lu et al. (2011)
miR160 under cold, salt, Al	Auxin response factor, auxin-responsive protein, plastid omega-3 fatty acid desaturase	Agharbaoui et al. (2015), Lu et al. (2011)
miR164		Gupta et al. (2014)
miR165		Wang et al. (2014a, b)
miR171		Wang et al. (2014a, b)
Mir20602	Glutathione peroxidase, putative phosphatase phospho1	Agharbaoui et al. (2015)
miR319		Wang et al. (2014a, b)
miR393		Gupta et al. (2014)
miR395-21 under cold, salinity, Al	Bifunctional 3′-phosphoadenosine 5′-phosphosulfate synthase, ATP sulfurylase	Agharbaoui et al. (2015)
miR855	Transcriptional activator Myb; transmembrane protein	Gupta et al. (2014), Pandey et al. (2013)
Nutrient deficiency (N)-responsive miRNAs		
miR1117		Sinha et al. (2015)
miR1120		Sinha et al. (2015)
miR159	MYB3	Sinha et al. (2015)
miR160		Sinha et al. (2015)
miR164	NAM (no apical meristem), ATAF1–2 (*Arabidopsis thaliana*-activating factor), and CUC2 (cup-shaped cotyledon)	Sinha et al. (2015)
miR399		Sinha et al. (2015)
miR444		Gao et al. (2016)
Nutrient deficiency (P)-responsive miRNAs		
miR1122		Zhao et al. (2013)
miR1125	Annexin-like proteins	Zhao et al. (2013)

(continued)

Table 17.1 (continued)

microRNAs	Potential gene targets	References
miR1135	Auxin-induced proteins	Zhao et al. (2013)
miR1136	PWWP domains of TFs	Zhao et al. (2013)
miR1139	Nicotinamide adenine dinucleotide (NAD) + hydrogen (H) (NADH) dehydrogenase subunit 6 (ND6)	Zhao et al. (2013)
miR159	MYB3	Zhao et al. (2013)
miR167	Auxin-responsive factor	Zhao et al. (2013)
miR399		Zhao et al. (2013)
miR408	Plantacyanin	Zhao et al. (2013)
Heavy metal stress (Cd)-responsive miRNAs		
miR156	SQUAMOSA-PROMOTER BINDING PROTEIN-LIKE (SPL) family of transcription factors	Qiu et al. (2016)
miR159	MYB3	Qiu et al. (2016)
miR164	NAM (no apical meristem), ATAF1–2 (*Arabidopsis thaliana*-activating factor), and CUC2 (cup-shaped cotyledon)	Qiu et al. (2016)
miR398	CuZnSOD, SOD1 protein	Qiu et al. (2016)
miR408	Plantacyanin	Qiu et al. (2016)
miR408	Plantacyanin	Feng et al. (2013)

(targeted by NAC), miR168a (targeted by AGO), miR171 (targeted by SCL), miR172a (targeted by AP2), and miR398 (targeted by SOD), displayed analogous expression patterns in HUW-468 and WL-711 (Kaur et al. 2017). Only miR168a and miR393a were substantially upregulated in C-306, whereas miR168a and miR397a were upregulated in NI-5439 (Kaur et al. 2017). The miR159a, miR397a, miR398, miR408, and miR529a were upregulated with downregulated targets in HUW-468 and WL-711, respectively (Kaur et al. 2017). TaMIR1119 regulates drought response in plants. The Ta-miR1119 mediates drought tolerance in wheat by regulation of six target genes categorized into osmotic stress-associated biological processes including transcriptional regulation, RNA and biochemical metabolism, osmolyte accumulation, photosynthetic functions, trafficking, and homeostasis of cellular reactive oxygen species in plants. TaMIR1119 responds to drought and upregulated in roots steadily within 48 h of drought. TaMIR1119 target genes downregulate by a cleavage mechanism (Gui-qing et al. 2018).

17.3.2 Heat Stress

Heat-responsive microRNAs have been described from numerous susceptible and resilient cultivars of wheat. The functions of isomiRs and differential expression of mature miRNAs among families were mainly ignored (Qin et al. 2008; Xin et al. 2010; Kumar et al. 2015). Additionally, miRNA and target identifications (Qin et al.

2008; Xin et al. 2010; Kumar et al. 2015; Ragupathy et al. 2016) depend on prediction models. Sequencing of sRNAs and degradome of leaf tissues lead to identification and validation of 202 heat stress-responsive miRNAs. Out of these, 104 were mature miRNA and 36 showed differential expressions after heat stress. PARE sequence analysis enabled characterization of 589 targets of 84 miRNAs. Squamosa promoter-binding-like, transport inhibitor-responsive proteins, and homeobox leucine zipper were targets of miRNA156, miR393, and miR166, respectively. Heat-responsive miRNAs target superoxide dismutase, F-box proteins, homeobox leucine zipper proteins, and protein kinases. Precise identification and authentication of miRNAs and their targets will help to develop novel regulatory gene-based breeding approaches (Ravichandran et al. 2019a, b). Nine putatively heat-responsive microRNAs were recognized in leaves of heat-tolerant wheat line. Out of which, miR172 reduced and miR156, miR168, miR169, miR159, miR160, miR827, miR166, and miR2005 enhanced in response to 1 h heat (Xin et al. 2010). Differential miRNA expression profiling in susceptible wheat line after 0.5–1.2 h heat stress leads to identification of miR159, miR166, miR393, and miR2002 (Xin et al. 2010).

17.3.3 Cold Stress

Cold-related Tae-miR398 precursor sequence is highly conserved in *Triticum* and other plants and regulates cold tolerance by cleavage and downregulation of Cu-Zn-type superoxide dismutase (CSD1). Tae-miR398 expression reduced under cold, while CSD1 displayed an opposed expression pattern (Huang et al. 2014; Zeng et al. 2018; Lu et al. 2020). Small RNA sequencing of stressed spikes of TGMS wheat lines identified putative cold-responsive miRNAs. The qRT-PCR verified cold-responsive miR396a, miR167d, miR444c.1, miR167c, miR172a, miR393, and tasiRNA-ARF (Tang et al. 2012).

17.3.4 Salinity Stress

Salt-responsive wheat Ta-miR159a, Ta-miR174, Ta-miR160, Ta-miR399, Ta-miR167, Ta-miR11124, Ta-miR408, and Ta-miR1133 showed an upregulation during salt stress. A subsection of miRNAs is involved in facilitation of salinity signaling responses in wheat plants through target genes regulation at post-transcriptional and translational levels (Lu et al. 2011). Growth and development, survival and sustainability of plants, and various stresses are also controlled by MAP kinase phosphatases (MKPs) of MAPK signaling pathways. Numerous phosphorylation and kinase associated genes are noticeable players of MAPK signaling pathways. Tae-miR435, tae-miR827, tae-miR5490, taemiR5167a, tae-miR1522, tae-miR6180, tae-miR6275, tae-miR6191b, tae-miR7714, and tae-miR7768b target two antiporter genes such as with antiporter activity and solute: hydrogen antiporter activity. These miRNAs could be employed for enhanced salinity tolerance in wheat

(Ghorbel et al. 2017). TaemiR408 targets six genes of biochemical metabolism, organization of microtubules, and signaling transduction and has a role in mediation of plant responses to starvation of Pi and salinity stress. TaemiR408 is upregulated, and its target genes were downregulated by cleavage mechanism under starvation of Pi and salinity stress. NtPT2 upregulated during Pi-deprived. TaemiR408 knockdown of NtPT2 decreases acquisition of Pi in low Pi stress. NtPYL2 encoding abscisic acid (ABA) receptor and NtSAPK3 encoding SnRK2 protein showed upregulation in salt-confronted conditions. TaemiR408-mediated knockdown of NtPYL2 and NtSAPK3 declined growth and dropped osmolytes quantities of plants during salinity stress. Consequently, TaemiR408 is decisive for plant adjustment under Pi starvation and salinity (Bai et al. 2018).

17.4 Conclusion

Thus to conclude, wheat miRNAs find a role in development of plant and stress response and resilience (Wei et al. 2009; Kurtoglu et al. 2013, 2014). Current investigations on miRNAs of wheat plant and their response to plant under hostile ecological dynamics will prove positive in refining this plant (Edwards and Batley 2010).

Acknowledgments DP acknowledges Dr. Saurabh Raghuvanshi, Associate Professor, Department of Plant Molecular Biology, South Campus University of Delhi, New Delhi, India, for introducing her to the domain of plant microRNAs.

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18 Molecular Genetic Studies and Breeding and Genomics-Based Approaches to Develop Abiotic Stress Tolerance in Sorghum

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Abstract

Sorghum is a semi-arid crop acting as a nutritious food source for a large proportion of the low-income population. Abiotic stresses like salinity, drought, temperature extremes and heavy metal toxicity are known to detrimentally affect the growth and productivity of susceptible sorghum varieties. Recent advances in sorghum research show that a large number of breeding programmes and few genomics-based studies have been undertaken to develop abiotic stress tolerance in the crop cultivars. Breeding programmes in association with quantitative trait loci studies have helped in identifying genetic information that is linked to stress tolerance in sorghum genotypes. Furthermore, identification of differentially expressed genes using transcriptomic and transgenic studies overexpressing stress-responsive genes has also helped to understand the mechanism of stress adaptation in sorghum. This chapter concisely integrates the advancements in developing abiotic stress tolerance in the cereal crop sorghum using breeding, genetic and genomics-based strategies.

Keywords

Sorghum · Abiotic stress · Tolerance · Breeding · Quantitative trait loci · Genetics · Genomics

18.1 Introduction

Sorghum bicolor L. Moench (sorghum) is an important cereal food crop which is mainly cultivated across arid and semi-arid areas where the minimum temperature should not fall below 25 °C. It ranks fifth in the global list of the most cultivated

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_18

cereal crops after rice, wheat, corn and barley. Sorghum serves as the staple food across Latin America, Africa and semi-arid tropical landscapes of Asia (Djanaguiraman et al. 2021). Sorghum is a nutrition-rich cereal, containing appreciable reserves of proteins, vitamins, minerals and energy. These cereals are also processed and consumed as pasta and other types of beverages (Ramatoulaye et al. 2016). Due to developing political scenario and increasing population, the demand for sorghum is gradually increasing, especially in the countries of Western Africa like Nigeria, Ghana, Burkina Faso, etc. which have aimed to focus on growing sorghum rather than barley for the food industry (Ba et al. 2010). Among other cereals, sorghum seeds are also the richest in secondary metabolites like flavonoids, phenolics and tannins and about 85% of the total phosphorus in sorghum seeds is stored as phytic phosphorus, i.e., phosphorus remains as a complex with phytic acid (Evers and Millar 2002).

Due to its ability to grow in drier regions, *Sorghum* was previously hypothesized to be inherently drought-tolerant in nature. In response to brief periods of drought, the seedlings roll their leaves to avoid transpiration, whereas in case of prolonged drought, the plants continue to remain dormant and do not dry due to the presence of waxy cuticles on leaf surfaces which reduce unwanted water loss (Ramatoulaye et al. 2016). However, recent evidence shows that drought, temperature, flooding and salinity significantly limit the yield and production of sorghum seedlings (Djanaguiraman et al. 2021). It has also been observed that exposure to such environmentally challenging conditions at critical reproductive stages further increases the severity of the stress. Lowered photosynthetic efficiency due to reduced leaf area along with developmental inhibition during abiotic stress has been correlated with increased loss of gamete viability, accelerated embryo abortion and abnormal embryo development (Banerjee and Roychoudhury 2021a, b; Banerjee et al. 2021a, b). As a result, the overall seed setting and grain filling in sorghum are greatly compromised in response to multiple abiotic stresses (Djanaguiraman et al. 2021; Almodares and Sharif 2005; Almodares et al. 2007; Devi et al. 2018a, b, 2019). Recent technologies including molecular genetic studies, new age breeding and genomic studies have immense potential to generate abiotic stress tolerance in crops and vegetation. These techniques have been widely regarded as cutting-edge strategies to enhance genotypic as well as phenotypic adaptations to multiple abiotic stresses like salinity, drought, temperature extremes and light and heavy metal toxicity. Therefore, this chapter provides a concise idea on the recent uses and success stories of these technologies in generating stress-tolerant sorghum genotypes and phenotypes.

18.2 Breeding Approaches to Generate Abiotic Stress Tolerance in Sorghum

18.2.1 Breeding for Drought Tolerance in Sorghum

Heterosis breeding, breeding for efficient drought escape and breeding for enhancing developmental stage-specific drought tolerance in sorghum cultivars have been undertaken (Reddy 2019). Mapping studies using quantitative trait loci (QTLs) has been verified as an effective strategy to develop abiotic stress tolerance in elite sorghum cultivars (Harris et al. 2007; Srinivas et al. 2009; Habyarimana et al. 2010; Vadez et al. 2011; Sabadin et al. 2012; Wang et al. 2014a, b; Kiranmayee et al. 2015). Rakshit et al. (2016) correlated the preservation of green leaves in sorghum during seed setting with post-reproductive drought adaptation. As a result, this trait is referred to as 'stay-green' phenotype and is utilized to screen drought-tolerant sorghum genotypes (Borrell and Hammer 2000; Borrell et al. 2001; Harris et al. 2007). The QTLs for 'stay-green' phenotypic variants have been mainly identified from seven sources, viz., B35, E36-1, QL41, SC56, 296B, SC283 and SDS 1948-3 (Deshpande et al. 2016). The durra sorghum B35 has been mostly utilized for identifying QTLs necessary for drought acclimation. Important 'stay-green' QTLs that have been detected are *stgC* (SBI-01), *stg3A* and *stg3B* (on SBI-02), *stg1* and *stg2* (on SBI-03) and *stg4* (on SBI-05) (Deshpande et al. 2016). These QTLs have also been used in marker-assisted backcrossing to generate sorghum varieties which could resist drought stress (Kassahun et al. 2010; Jordan et al. 2012; Vadez et al. 2013). Potential of generating sorghum genotypes, capable of efficiently absorbing water and nutrients due to effective root angling, was observed from rapid root angle screening strategies which led to the identification of four QTLs which regulated the nodal root angling in sorghum and were also co-localized with the seven 'stay-green' QTLs (Mace et al. 2012). In another study, 39 polymorphic simple sequence repeats (SSRs) and 2,35,009 single nucleotide polymorphisms (SNPs) were identified throughout the genome of 258 sorghum genotypes, out of which 1439 SNPs were observed to lie in the co-ordinates of QTLs that have been found to mediate drought tolerance and post-drought recovery (Rakshit et al. 2014).

Introgression and breeding experiments have revealed the roles of 'stay-green' QTLs like *stg3A* and *stg3B* to regulate transpiration and responses associated with deficit in vapour pressure (Deshpande et al. 2016). Vadez et al. (2011) reported that another 'stay-green' QTL *stg1* controlled the water absorption physiology in mildly senescent *caudatum* line S35. However, similar regulation by *stg1* was not observed in the more senescent R16 durra lines, and hence specific QTLs need to be identified, which can govern drought-adaptable responses in varying genetic backgrounds (Vadez et al. 2011). A large number of simple sequence repeats (SSRs) have been identified in regions adjoining the identified QTLs, and this has been validated using QTL analysis and bioinformatic investigations (Srinivas et al. 2008, 2009; Ramu et al. 2010). Large-scale marker-assisted backcrossing also helped to reduce the genetic distances between *stg3A* and *stg3B* QTLs, and the presence of almost 70 SNPs has been observed in this region (Deshpande et al. 2016). The retention of the mesocarp colour during drought stress is also regulated by 'stay-green' QTLs. Furthermore, co-localization of the root-angling QTL and *stg3B* has been reported (Vadez et al. 2013). Lysimeter-based predictions, regarding the mode of functioning of the 'stay-green' QTLs, *stg3A* and *stg3B* in regulating water uptake and retention during water-deficient conditions, have been made in spite of the laborious efforts required to make such estimation for a large number of bred and segregating lines (Vadez et al. 2011). The use of phenomics has also been put forward to facilitate high-throughput phenotypic screening of the sorghum seedlings after large-scale breeding experiments. Vadez et al. (2015) used 'LeasyScan' to estimate leaf area, dynamics of foliar growth and stomatal conductance in segregating sorghum lines. The technology was based on three-dimensional (3D) scanning and continuously monitored leaf development using analytical calculations which also included measurements regarding gravimetric transpiration (Vadez et al. 2015). The use of such phenotypic platforms would inevitably accelerate the screening for drought-tolerant lines of sorghum among a large population of segregating lines that have been scientifically bred for gaining beneficial QTLs, associated with desiccation tolerance and enhanced water-use efficiency (WUE) (Deshpande et al. 2016).

18.2.2 Breeding for Low-Temperature Tolerance in Sorghum

Sorghum plants have been regarded to be susceptible to temperatures below 15 °C, since it detrimentally affects reproduction, flowering and seed setting (Reddy 2019). In line with this report, Prasad et al. (2008) reported that midseason chilling stress reduced panicle development and associated yield-related attributes in sorghum seedlings. Chilling temperatures in regions of the Northern United States have also been reported to affect the growth of sorghum during the early developmental phases (Lu et al. 2007). In contrary to this report, Franks et al. (2006) inferred the enhanced growth vigour of Chinese landraces of sorghum (kaoliangs) during low temperatures. Knoll et al. (2008) identified multiple QTLs regulating cold tolerance in a kaoliang cultivar, Shan Qui Red. Fourteen QTLs ranging across chromosomes 1, 2, 4, 7 and 9 related to germination and improved seedling vigour during cold were identified from another kaoliang cultivar, PI610727 (Burow et al. 2011). However, Wu et al. (2012) reported that these QTLs could not be used for breeding purposes as these were co-localized with QTLs associated with tannin metabolism.

It was also found that the cold-tolerant sorghum cultivars were able to generate more fertile pollens than the sensitive varieties exposed to chilling stress (Salem et al. 2007). As a result, increased cold tolerance of the reproductive machinery could be recorded using in vitro pollen growth assays, leading to efficient screening of a large germplasm (Kakani et al. 2002, 2005; Singh et al. 2008). Increased selection of cold-adaptable and stress-fit pollens through breeding of sorghum genotypes has been attributed as an advantageous strategy for generating stress tolerance (Hedhly et al. 2008). Decelerated anthesis and stigma growth, resulting in reduced fertilization of gametes and seed formation, has also been observed to be influenced by cold stress in the sensitive genotypes of sorghum (Downes and

Marshall 1971). Thakur et al. (2010) reported that along with these anomalies, chilling temperature also initiated flower abscission, abnormal growth of pollen tube, formation of pollens with distorted structures and premature abortion of ovules. Sorghum hybrids with tolerance against such low-temperature stress have been suggested to be developed from parental genotypes where both male and female plants are moderately tolerant to cold or low temperature (Rao et al. 1986). Furthermore, Reddy et al. (2014) also verified the importance of the female parent to have increased pollen growth and receptivity of stigma during cold stress in order to efficiently generate sorghum hybrids that would inherently adapt to chilling temperature and exhibit normal growth physiology and yield during low-temperature stress. Overall, the proper identification of parental genotypes with specific genetic traits has been observed to be essential to generate cold-tolerant hybrids of sorghum.

18.2.3 Breeding for Salt Tolerance in Sorghum

Yield loss due to high soil salinity is one of the most widely reported agricultural problems in recent times. Wang et al. (2014b) reported that salt-mediated oxidative stress induced the expression of *high-affinity* K^+*-transporter 1;4* (*HKT1;4*) which helped to maintain higher K^+/Na^+ ratio in the salt-adaptive sorghum cultivars. Furthermore, Wang et al. (2014a) observed that breeding between Shihong137 and L-Tian sorghum genotypes generated 181 recombinant inbred lines (RIL). Thorough genetic screening in this RIL population led to the identification of 38 QTLs associated with salt adaptation in sorghum (Wang et al. 2014a). Six among the identified QTLs were observed to be responsible for 10% differences in the phenotypes (Deshpande et al. 2016).

18.2.4 Breeding for Stimulating Nitrogen (N) Metabolism in Sorghum

Variations in N use efficiency (NUE) have been noted across sorghum genotypes, especially between the cultivars cultivated in less fertile regions of underdeveloped and developing countries and the varieties grown across highly fertile areas of the developed countries (Maranville et al. 1980; Youngquist et al. 1992). Transcriptomic studies involving sorghum genotypes adaptive to lower N availability (San Chi San, China17, KS78 and high-NUE bulk) and cultivars susceptible to the same conditions (CK60, BTx623 and low-NUE bulk) were performed under low and normal levels of N (Gelli et al. 2014). This experiment revealed that the differentially expressed genes (DEGs) associated with tackling oxidative stress were induced in the susceptible varieties under low N stress, whereas root biomass development was accelerated in the tolerant genotypes during the same conditions in order to facilitate higher translocation of N and crucial growth nutrients (Gelli et al. 2014). Upregulated expression of *high-affinity nitrate transporters*, viz., *NRT2.2*, *NRT2.3*, *NRT2.5* and *NRT2.6*, along with induction of *lysine histidine transporter 1* (*LHT1*) was also reported in the tolerant sorghum cultivars, so that they could efficiently accelerate the absorption of soil-bound forms of N during N-deficient conditions (Gelli et al. 2014). About 6.2–50.8% of difference in phenotypes of a RIL population, comprising of 131 lines derived by breeding the low N assimilating sorghum genotype, i.e. CK60, and the high N metabolizing variety, i.e. China17, were due to 38 QTLs that were identified by composite interval mapping of 642 polymorphic SNPs (Gelli et al. 2016). Transcriptomic studies also verified that among the identified 726 DEGs, almost 108 could be mapped near to the genomic positions which were occupied by the QTLs. These were associated with N assimilation, sugar metabolism, phytohormone signalling and growth responses, thus revealing that the QTLs conferred pleiotropic effects on the DEGs which could be the future molecular targets for developing improved NUE upon successful genetic manipulation (Gelli et al. 2016).

18.3 Molecular Genetic and Genomics-Based Approaches to Generate Abiotic Stress Tolerance in Sorghum

18.3.1 For Tolerance Against Aluminium (Al) Toxicity

Aluminium is a heavy metal which promotes phytotoxicity by inhibiting mitosis, biomass growth and root elongation (Marschner 1991; Kochian et al. 2004). Furthermore, excess Al level in acidic soils results in Al-mediated replacement of other beneficial elements like calcium (Ca), magnesium (Mg) and potassium (K) which are crucial for the physiological growth and development of plants (Rao et al. 1993). Free phosphorus (P) present in the soil is also chelated in the presence of Al^{3+} ions and stably conserved as aluminium phosphate. Phosphates present in this chemical form are sparingly soluble, and hence plant roots cannot absorb P. As a result, plant growth under excess Al pollution also leads to P deficiency-mediated growth inhibition and drooping (Rao et al. 1993; Delhaize et al. 2004, 2009; Magalhaes et al. 2004). Marschner (1991) reported that plant tolerance against Al toxicity could be via the release of root exudates containing Al chelators like citrate or malate.

It has been observed that the gene *Al tolerance/multidrug and toxic compound extrusion* (*Alt/MALT*) located on chromosome 3 regulates systemic adaptation of Al toxicity in the susceptible sorghum cultivar BR007 (Magalhaes et al. 2004). Magalhaes et al. (2007) used positional cloning to analyse the functioning of ALT. It was observed that *Alt* encoded an Al-activated citrate transporter, which probably mediated the release of citrate for efficient scavenging of Al^{3+} ions via formation of coordination complexes (Magalhaes et al. 2007). Anami et al. (2015) reported the requirement of proper introgression of *Alt* alleles into sensitive cultivars for effectively generating tolerance against Al stress. Though the sequence of the cDNA of *Alt* is similar in the sensitive and tolerant genotypes of sorghum, appreciable polymorphism was observed in the sequence of the second intron (Magalhaes et al. 2007). Furthermore, the presence of a tourist-like miniature inverted repeat transposable element (MITE) has been detected across the promoter sequence of *Alt/MATE* in sorghum. Interestingly, positive correlation was observed between a number of MITEs present in the promoter region of these genes with the extent of Al tolerance exerted by the sorghum genotype (Magalhaes et al. 2007). This data helped to infer that occurrence of mutations in the cis-acting elements of the regulatory genes could promote crop adaptation against sub-optimal conditions and dictate the stress-tolerant behaviour of a genotype (Anami et al. 2015).

18.3.2 Non-coding RNAs as Genetic Regulators of Abiotic Stress Tolerance in Sorghum

Non-coding RNAs like microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been found to regulate post-transcriptional gene regulation in several plant species, exposed to environmental stresses (Banerjee et al. 2016). Desiccation stress activated miRNA-dependent signalling cascades and metabolic pathways in the sorghum cultivar IS1945 during early vegetative stages (Pasini et al. 2014). Zhao et al. (2007) identified five homologues of the rice miRNA169g (responsive to drought) across sorghum genotypes. This illustrated the potential of miRNAs to mediate drought tolerance in sorghum seedlings (Ram and Sharma 2013). Furthermore, drought-inducible lncRNAs in foxtail millet were found to have similar conserved sequence stretches in corresponding lncRNAs of sorghum cultivars (Qi et al. 2013).

18.3.3 Genetic Regulation of Phytohormone Signalling Mediates Abiotic Stress Tolerance in Sorghum

Abscisic acid (ABA) is the chief stress hormone in plants which mediates downstream responses for promoting abiotic stress tolerance (Banerjee and Roychoudhury 2016). Multiple ABA-responsive elements (ABREs) have been detected across more than 50 drought-inducible genes in sorghum (Dugas et al. 2011). Wang et al. (2010) showed that the genes, viz., *Gretchen Hagen 3* (*GH3*), *lateral organ boundaries domain* (*LBD*), *auxin-responsive protein 1* (*IAA1*), *GH3-13* and *LBD32*, belonging to the auxin-dependent signalling pathways were upregulated in sorghum seedlings, subjected to salinity or water-deficit conditions. Shen et al. (2010) also reported the induction or suppression of specific auxin transporters in stressed seedlings of sorghum. Several stress-inducible ethylene response factors (ERFs) have also been detected across sorghum genotypes (Yan et al. 2013). Transcriptomic studies verified the upregulation of *drought response element binding* (*DREB*), *C-repeat binding factors* (*CBF*s) and *ERF* genes encoding corresponding transcription factors during low-temperature stress in the salt-tolerant sorghum cultivar HongkeZi (Chopra et al. 2015).

Phytohormone signalling usually triggers downstream *osmotic responsive* genes involved in abiotic stress protection and mitigation. Overexpression of the *dehydrin1* (*Dhn1*) gene isolated from sorghum triggered heat stress tolerance in transgenic tobacco lines by efficiently scavenging excess ROS and also preventing stress-induced protein aggregation and ubiquitinylation (Halder et al. 2017). Halder et al. (2016) isolated another novel dehydrin gene (nomenclatured as *Dhn2*) from sorghum which efficiently scavenged oxidative radicals due to the presence of multiple glycine and histidine residues. Furthermore, the protein could protect lactate dehydrogenase enzyme during in vitro studies, thus highlighting its ability to protect protein macrostructures from oxidative aggregation and stress-induced misfolding.

18.3.4 Genetic Regulation of Osmolytes Imparts Abiotic Stress Tolerance in Sorghum

Drought stress induced the expression of glycine betaine biosynthetic gene, *betaine aldehyde dehydrogenase 1* (*BADH1*), in sorghum seedlings, because glycine betaine behaved as a compatible solute and maintained the cellular osmoticum during water deficit (Wood et al. 1996). Increased transcription of *glycine-rich RNA-binding protein* (*GRRNP*) was observed in salt-stressed and ABA-supplemented sorghum seedlings (Aneeta et al. 2002). Transgenic sorghum lines accumulating high levels of mannitol could exhibit high degree of salt tolerance (Maheswari et al. 2010). Su et al. (2011) reported the induction of proline biosynthetic genes, viz., *pyrroline-5-carboxylate synthetase 1* (*P5CS1*) and *P5CS2* in sorghum genotypes exposed to salinity and drought and also when treated with jasmonic acid.

18.4 Conclusion

Sorghum is a popular cereal crop which provides nutrition to a large population of the world. It also acts as source of ‘food, feed, fodder and fuel’ for farmers dwelling in semi-dry lands and suffering from poverty. Abiotic stresses like salinity, drought, temperature extremes and heavy metal toxicity severely impede the growth and development of sorghum genotypes, thus threatening the food security of associated consumers. Breeding approaches supported by identification of QTLs which are linked to stress-tolerant traits have been beneficial for generating stress-adaptable genotypes which can produce optimal yields even under suboptimal conditions like salinity, drought, chilling and Al toxicity. Genomics-based studies, associated with identification of DEGs and potential signalling genes induced by salinity or drought, have made it possible to create strategies, utilizing which the key genes can be overexpressed to trigger stress-adaptable responses in sorghum. These genes have been linked with phytohormone signalling and osmolyte biosynthesis. Furthermore, desiccation stress also modulated the expression of crucial miRNAs and lncRNAs which post-transcriptionally regulated downstream signalling pathways to generate tolerance in sorghum cultivars.

18.5 Future Perspectives

In spite of access to a large number of germplasms, molecular advances in sorghum research for generating environmental stress tolerance have not been exhaustively undertaken by the scientific community. Due to the available genetic and genomic information on sorghum genotypes, this species should be considered as a model plant for C_4 crops. Furthermore, the marker-associated traits should also be independently tested across generations of sorghum seedlings. New-generation breeding approaches, supported by high-throughput genetic screenings using next-generation sequencing (NGS) platforms, need to be designed for rapidly identifying genotypes with appreciable tolerance to a particular type of or multiple abiotic stresses. Transgenic studies can also be performed where overexpression of osmolyte or antioxidant biosynthetic genes would efficiently produce multiple stress tolerance in the genetically engineered sorghum lines.

Acknowledgements Financial assistance from Science and Engineering Research Board, Government of India, through the grant [EMR/2016/004799] and the Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal, through the grant [264 (Sanc.)/ST/P/S&T/1G-80/2017] to Dr. Aryadeep Roychoudhury is gratefully acknowledged. Mr. Aditya Banerjee is thankful to the University Grants Commission, Government of India, for providing Senior Research Fellowship in course of this work.

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MicroRNAs Shape the Tolerance Mechanisms Against Abiotic Stress in Maize

19

Deepu Pandita

Abstract

Maize (*Zea mays* L.) is the third most significant cereal crop in Asia. Abiotic stress reduces the productivity of highly sensitive maize crop which has many mechanisms to fight against their stern negative influences. One of the mechanisms is gene expression regulation through microRNA machinery. MicroRNAs (miRNAs) are small, ubiquitous, non-coding 20–24 non-coding RNAs present in plants that have emerged as vital regulators of gene expression by sequence-specific interactions with their target mRNAs at post-transcriptional levels. Spatiotemporal upregulated or downregulated miRNA expressions regulate target genes contributing positively or negatively to tolerance, consequential to multifarious responses of physiological and biochemical pathways relative to tolerance response mechanism of maize. The miRNAs and their targets help in understanding the interwoven regulatory networks of physiological, molecular and biochemical changes triggered during stress for adaptation and survival. These can potentially be used in the engineering of new stress-tolerant and efficient defence machinery in maize plants.

Keywords

Maize · Abiotic stress tolerance · Stress-responsive microRNA · Potential targets · Post-transcriptional gene regulation

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_19

19.1 Introduction

Maize (*Zea mays* L.) is one of the oldest and most significant cultivated and dietary cereal crops all over the world. Maize is one of the most vital agricultural sources for human food supply, livestock and animal feed and has economic relevance as key plant resource for the industry as biofuels worldwide (Huang et al. 2002; Schnable et al. 2009; Kim et al. 2019; Kong et al. 2011). Maize has been a widely used key model system for the research in plant genetics and genomics, biological research and improvements (Huang et al. 2002; Schnable et al. 2009; Bennetzen and Hake 2009). Studies on the maize include mechanisms of crop development and environmental adaptation to the abiotic stress (Farooq et al. 2015; Zurek et al. 2015).

MicroRNAs (miRNAs) are a class of endogenous, ubiquitous, small (~20–24 nucleotides in length), non-coding regulatory small RNAs (sRNAs) that downregulate target genes through sequence-specific interaction of mRNA cleavage or translational inhibition (Carrington and Ambros 2003; Bartel 2004; He and Hannon 2004; Wei et al. 2009; Chen et al. 2012; Li et al. 2013b; Kong et al. 2014; Kaur et al. 2020). The miRNAs play crucial regulatory roles in different biological processes, for instance, plant development, differentiation, metabolism, signal transduction and broad variety of abiotic and biotic stress conditions in plants, and show negative control/regulation of target gene expression at post-transcriptional (mRNAs) levels by targeting the primary transcripts for cleavage/degradation or translation inhibition during stress conditions in plants and animals (Carrington and Ambros 2003; Bartel 2004; He and Hannon 2004; Zhao et al. 2007; Zhang et al. 2009a; Covarrubias and Reyes 2010; Sunkar et al. 2012; Zhou et al. 2013; Li et al. 2013b; Rogers and Chen 2013; Panda et al. 2014; Kaur et al. 2020). Identification of miRNAs which are prospective targets for plant abiotic stress tolerance and miRNA targets is of colossal worth in comprehending the molecular regulatory networks activated during stress conditions (Shriram et al. 2016; Kaur et al. 2020). There is scanty evidence available on the role of miRNAs in maize during stress responses. Consequently, understanding of changes at molecular levels during stress requires unstitching of interlinked regulatory circuits and regulatory nodes of miRNA for efficient maize defence machinery (Banerjee et al. 2016). High-throughput approaches have enabled identification of some novel miRNAs and characterized miRNA functions in ecological signals in *Zea mays*, such as short-term waterlogging stress and long-term waterlogging stress, salinity, low nitrate and low phosphorus (Ding et al. 2009; Xu et al. 2011; Liu et al. 2012; Zhang et al. 2012; Trevisan et al. 2012; Zhao et al. 2013; Zhai et al. 2013; Pei et al. 2013).

19.2 Maize miRNAs and Abiotic Stress Response

Small RNA molecules of miRNAs act as promising and pivotal targets for designing plants with enhanced tolerance to manifold abiotic environmental stresses in plants (Shriram et al. 2016). Plant miRNAs negatively regulate gene expression at mRNA levels through degradation and/or translation inhibition (Song et al. 2019). In maize

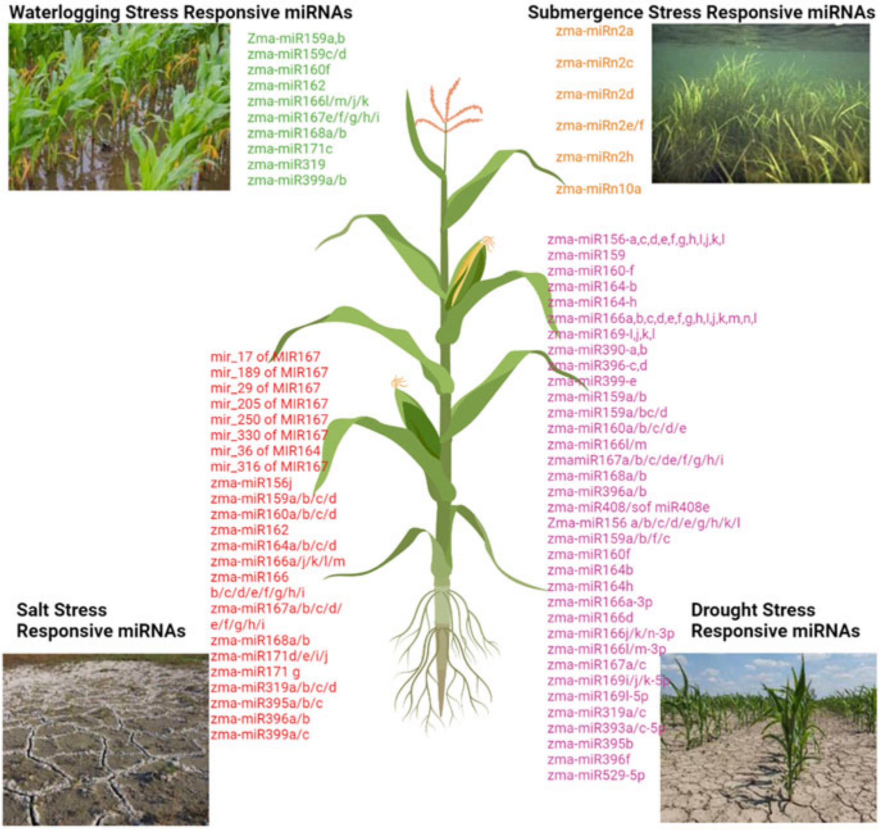

Fig. 19.1 Abiotic stress-responsive miRNAs in maize

genome, 325 mature miRNAs from 174 miRNA precursors subdivided into 29 families have been identified and reported in miRBase (Zhang et al. 2009b; Kozomara and Griffiths-Jones 2014; Kozomara et al. 2019). Some 34 novel miRNAs were identified and subjected to miRNA annotations in plants (Axtell and Meyers 2018). These miRNAs are responsive to drought (Li et al. 2013a; Seeve et al. 2019; Upadhyay et al. 2019), salinity (Ding et al. 2009; Fu et al. 2017), submergence (Zhang et al. 2008) or waterlogging (Liu et al. 2012; Zhai et al. 2013). With high-throughput genome-wide studies in maize, identification of some novel miRNAs and their roles in waterlogging stress (Liu et al. 2012; Zhai et al. 2013), salinity (Ding et al. 2009), low nitrate (Xu et al. 2011; Trevisan et al. 2012; Zhao et al. 2013) and low phosphorus (P) (Zhang et al. 2012; Pei et al. 2013) was reported. The miRNAs regulate expression of target genes through upregulation or downregulation, resulting in several physiological and biochemical responses in *Zea mays* (Upadhyay et al. 2019). Various miRNAs responsive to drought, salinity, waterlogging and submergence are reported in maize (Fig. 19.1).

19.2.1 Drought Stress

A lot of miRNAs have been described to respond to drought stress in maize (Ferdous et al. 2015). The miRNA166 and miRNA169 drought-responsive miRNAs are downregulated in maize, and miRNA169 overexpression reduces stomatal opening and drought tolerance as in tomato plants (Zhang et al. 2011). The miRNA166 controls class III homeodomain leucine zipper (HD-Zip III) transcription factors at post-transcriptional levels involved in development of lateral roots, leaf polarity and initiation of axillary meristem (Boualem et al. 2008). After drought treatment in *Zea mays*, 34 drought-responsive miRNAs from 13 plant miRNA families with changed expression were analysed with microarray. Majority of miRNA-mediated target genes have a cis-acting element responding to ABA phytohormone stimuli during water deficiency. The miR474 was significantly upregulated during drought which in turn downregulated its predicted target of negative regulators of proline accumulation known as PDH. miR474-mediated cleavage of mRNAs of PDH leads to osmoprotective responses by proline accumulation. The induced expression of miR474 under water deficiency can thus initiate improved osmoprotection mechanism in maize. The downregulation of drought stress-responsive miR168, miR528 and miR167 caused upregulation of their respective predicted targets MAPK, POD and PLD. MAPK, POD and PLD were accumulated to induce regulation of movement of stomata and antioxidant defence in maize under water deficiency. Accumulation of POD and MAPK eliminates reactive oxygen species, whereas accumulation of PLD signals stomatal closure and antioxidant defence (Wei et al. 2009). Thus, differentially expressed miRNAs facilitate post-transcriptional regulation of drought defence in seedlings of maize (Wei et al. 2009). The miRNA529 was downregulated. Differentially expressed novel miRNAs and their target genes involved in water deficiency response and/or tolerance (Table 19.1) were identified in maize (Zhou et al. 2010). The miRNA164 is downregulated during drought stress. miR164 cuts NAC mRNAs which modulate plant development and response to abiotic stresses. The 14 microRNAs in seven miRNA families were differentially expressed at post-transcriptional levels on encountering drought. The expression pattern of microRNA families including miRNA160, miRNA164, miRNA166, miRNA169, miRNA2275 and miRNA529 was more significant in root samples and plays important roles in regulating drought stress in maize. The novel miRNA2275 responded well to drought in maize. The miRNA targets take part in various biological processes, transcriptional regulation and response to stimulus (Upadhyay et al. 2019). Target genes of four miRNAs displayed differential expression patterns between contrasting inbreds for waterlogging stress. The target genes of Zm-miR24 [taemiR164] and Zm-miR28 [zmamiR444b] encode ferredoxin 3, showing downregulation in susceptible inbred line (I110) and upregulation in tolerant inbred line (I172) after waterlogging. Zm-miR25 [zmamiR159g5p] target gene encodes zinc finger type family protein in both I110 and I172 inbred lines. Zm-miR30 [zmamiR164b5p] targets cytochrome c1 gene which displayed higher expression in waterlogging stress and displayed downregulation in I172 (Kaur et al. 2020). With genome-wide small RNA sequencing, 34 drought stress-responsive miRNAs from

Table 19.1 Maize stress-responsive miRNAs and their targets

miRNAs	miRNA targets	References
Drought stress-responsive miRNAs		
zma-miR156-a,c,d, e,f,g,h,I,j,k,l	tpa: *aquamosa* promoter-binding (SBP domain) transcription factor family protein, *squamosa* promoter-binding-like protein 13-like	Aravind et al. (2017)
zma-miR159	Transcription factor gamyb	Aravind et al. (2017)
zma-miR160-f	gdsl esterase lipase at5g45910-like	Aravind et al. (2017)
zma-miR164-b	PsbP domain-containing protein chloroplastic-like	Aravind et al. (2017)
zma-miR164-h	Wound-responsive protein	Aravind et al. (2017)
zma-miR166a,b,c,d, e,f,g,h,I,j,k,m,n,l	Tpa: Homeobox lipid-binding domain family protein, homeobox-leucine zipper protein athb-15-like	Aravind et al. (2017)
zma-miR169-I,j,k,l	Nuclear transcription factor y subunit a-3	Aravind et al. (2017)
zma-miR390-a,b	Activator of 90 kDa heat-shock protein ATPase	Aravind et al. (2017)
zma-miR396-c,d	Growth-regulating factor 1, growth-regulating factor 9, growth-regulating factor 8	Aravind et al. (2017)
zma-miR399-e	60s ribosomal protein l7a-like, heavy metal-associated domain-containing expressed	Aravind et al. (2017)
zma-miR159a/b	Serine/threonine protein phosphatase	Wei et al. (2009)
zma-miR159a/bc/d	GAMyb transcription factor (Myb)	Wei et al. (2009)
zma-miR160a/b/c/d/e	40S ribosomal protein S16	Wei et al. (2009)
zma-miR166l/m	Homeodomain leucine zipper protein	Wei et al. (2009)
zmamiR167a/b/c/de/f/g/h/i	Auxin response factor 12	Wei et al. (2009)
zma-miR168a/b	Serine/threonine protein phosphatase	Wei et al. (2009)
zma-miR396a/b	DEAD-box ATP-dependent RNA helicase 3	Wei et al. (2009)
zma-miR408/sof miR408e	Leucine-rich repeat family protein	Wei et al. (2009)
Zma-miR156 a/b/c/d/e/g/h/k/l	DNA-binding protein, transcription factor, homoserine kinase, serine/threonine protein kinase	Li et al. (2013a)
zma-miR159a/b/f/c	DNA-binding protein, transcription factor	Li et al. (2013a)
zma-miR160f	gdsl esterase lipase at5g45910-like	Upadhyay et al. (2019)
zma-miR164b	psbp domain-containing protein chloroplastic-like	Upadhyay et al. (2019)

(continued)

Table 19.1 (continued)

miRNAs	miRNA targets	References
zma-miR164h	Wound-responsive protein	Upadhyay et al. (2019)
zma-miR166a-3p	tpa: Homeobox lipid-binding domain family protein	Upadhyay et al. (2019)
zma-miR166d	Transcription factor, protein methyltransferase	Li et al. (2013a)
zma-miR166j/k/n-3p	Homeobox-leucine zipper protein athb-15-like	Upadhyay et al. (2019)
zma-miR166l/m-3p	tpa: Homeobox lipid-binding domain family protein	Upadhyay et al. (2019)
zma-miR167a/c	Hormone stimulus	Li et al. (2013a)
zma-miR169i/j/k-5p	Nuclear transcription factor y subunit a-3	Upadhyay et al. (2019)
zma-miR169l-5p	Nuclear transcription factor y subunit a-3	Upadhyay et al. (2019)
zma-miR319a/c	TCP family transcription factor	Li et al. (2013a)
zma-miR393a/c-5p	Protein transport inhibitor response 1-like	Upadhyay et al. (2019)
zma-miR395b	Secondary active sulphate transmembrane transporter, ATP sulphurylase	Li et al. (2013a)
zma-miR396f	Actin-binding protein	Li et al. (2013a)
zma-miR529-5p	tpa: *squamosa* promoter-binding (SBP domain) transcription factor family protein isoform 1	Upadhyay et al. (2019)
Salt stress-responsive miRNAs		
mir_17 of MIR167	Translation initiation factor IF-1	Fu et al. (2017)
mir_189 of MIR167	2-isopropylmalate synthase B	Fu et al. (2017)
mir_29 of MIR167	Transport protein SEC24, PHD finger family protein, phospholipase D	Fu et al. (2017)
mir_205 of MIR167	p5cs isoform 1	Fu et al. (2017)
mir_250 of MIR167	Glutathione peroxidase, DNA (cytosine-5-)-methyltransferase	Fu et al. (2017)
mir_330 of MIR167	Casein kinase II subunit alpha, photosystem I reaction centre 6, glycine-rich RNA-binding protein 8, cysteine proteinase inhibitor	Fu et al. (2017)
mir_36 of MIR164	Sucrose-phosphatase 1 (SPP1)	Fu et al. (2017)
mir_316 of MIR167	Gibberellin receptor GID1, pyruvate, orthophosphate dikinase	Fu et al. (2017)
zma-miR156j	SBP domain protein 6, P-type R2R3 Myb protein, zinc finger protein-like	Ding et al. (2009)

(continued)

Table 19.1 (continued)

miRNAs	miRNA targets	References
zma-miR159a/b/c/d	MADS box interactor, ZAG1, protein phosphatase PP2A-3, transcription factor GAMyb	Ding et al. (2009)
zma-miR160a/b/c/d	40S ribosomal protein S16, two-component response regulator ARR11	Ding et al. (2009)
zma-miR162	Endoribonuclease Dicer, cytochrome P450	Ding et al. (2009)
zma-miR164a/b/c/d	NAC domain protein NAC1	Ding et al. (2009)
zma-miR166a/j/k/l/m	HD-ZIP rolled leaf1	Ding et al. (2009)
zma-miR166 b/c/d/e/f/g/h/i	Homeodomain leucine zipper protein	Ding et al. (2009)
zma-miR167a/b/c/d/e/f/g/h/i	Auxin response factor 8	Ding et al. (2009)
zma-miR168a/b	PZE40 protein, cytoplasmic aldolase, AGO1-1, A-winged helix TF MFH-1	Ding et al. (2009)
zma-miR171d/e/i/j	Sprouty homologue 2 (Spry-2), Scl1 protein	Ding et al. (2009)
zma-miR171 g	GPI-anchored protein	Ding et al. (2009)
zma-miR319a/b/c/d	Transcription factor GAMyb, ZAG1 protein, deoxycytidine deaminase	Ding et al. (2009)
zma-miR395a/b/c	ATP sulphurylase, L-isoaspartyl methyltransferase, beta-D-xylosidase, NADP-dependent malic protein	Ding et al. (2009)
zma-miR396a/b	Cytochrome oxidase subunit 1	Ding et al. (2009)
zma-miR399a/c	HASTY, granule-bound starch synthase	Ding et al. (2009)
Waterlogging stress-responsive miRNAs		
zma-miRn2a	Zinc/iron permease, zinc/iron permease, sodium: dicarboxylate symporter, lumazine-binding protein, riboflavin synthase-like beta-barrel, heat-shock protein Dna J, chaperonin Cpn60, chaperonin Cpn60/TCP-1, chaperone, tailless complex polypeptide 1, chaperonin Cpn60	Liu et al. (2012)
zma-miRn2c	DNA-binding WRKY	Liu et al. (2012)
zma-miRn2d	Osteopontin, myeloid transforming gene on chromosome 16 (MTG16)	Liu et al. (2012)
zma-miRn2e/f	Heat-shock protein DnaJ, N-terminal	Liu et al. (2012)
zma-miRn2h	Zinc/iron permease, zinc/iron permease	Liu et al. (2012)
zma-miRn10a	CCAAT-binding transcription factor	Liu et al. (2012)
Submergence stress-responsive miRNAs		

(continued)

Table 19.1 (continued)

miRNAs	miRNA targets	References
Zma-miR159a,b	Serine/threonine protein phosphatase, GAMYB	Zhang et al. (2008)
zma-miR159c/d	Ubiquitin isopeptidase, V-ATPase subunit D, ZAG1, AGAMOUS	Zhang et al. (2008)
zma-miR160f	Alpha/beta fold family protein	Zhang et al. (2008)
zma-miR162	DICER-LIKE1	Zhang et al. (2008)
zma-miR166l/m/j/k	Rolled leaf1, HD-ZIP	Zhang et al. (2008)
zma-miR167e/f/g/h/i	Auxin response factor (12,17,25), neutral invertase	Zhang et al. (2008)
zma-miR168a/b	Serine/threonine protein phosphatase, leucine-rich repeat family protein, AGO1	Zhang et al. (2008)
zma-miR171c	Scl1, WRKY transcription factor	Zhang et al. (2008)
zma-miR319	Peptide transporter	Zhang et al. (2008)
zma-miR399a/b	Aminotransferase, granule-bound starch synthase	Zhang et al. (2008)

17 miRNA families were recognized from the growth zones of primary roots. Majority of miRNAs displayed differential regulation under severe abiotic stress, whereas only zma-miR169r-3p, zma-miR168a-3p, zma-miR159c and d-3p exhibited differential regulation under mild stress (Seeve et al. 2019). The miR399e,i,j-3p regulates phosphate acquisition under nutrient deficiencies and responds to primary root growth zone in both mild and severe drought responses (Seeve et al. 2019). Aravind et al. (2017) identified and functionally characterized 13 water deficiency-responsive miRNA families comprising 65 members which regulate 42 unique target mRNAs, for instance, HD-Zip III, GAMYB and NAC in the tolerant HKI-1532 and the sensitive V-372 maize seedling genotypes by microarray analysis. Around 35% were upregulated in HKI-1532, and 31% of miRNAs showed upregulation in V-372. The zmamiR166 and zma-miR395 families had large number of members. The miRNAs and miRNA targets of drought stress pathways will be beneficial in the designing of drought-tolerant maize (Aravind et al. 2017). In both studies of Seeve et al. (2019) and Aravind et al. (2017), miR156, miR396, miR169, miR159, miR390, miR393, miR166 and miR399 were drought-responsive. The miR399, miR159, miR319, miR169, miR396, miR167, miR168 and miR408 were differentially regulated under mild drought stress conditions (Seeve et al. 2019). Differential miRNome expression analysis in H082183 (drought-tolerant) and Lv28 (drought-sensitive) maize inbred lines displayed 11 miRNAs exclusively responding to water deficiency in H082183 and 34 miRNAs in Lv28 leaves. In leaves, miR398, miR397, miR1432 and miR399s and miR827s were downregulated and upregulated under water deficiency in both inbred lines of

H082183 and Lv28, respectively. miR164, miR528s, miR169, miR398 and miR408s and miR395 and miR399s were completely downregulated and upregulated, respectively, in the leaves of H082183. miR156s, miR160s, miR162, miR164s and miR171s were downregulated, and miR399s and miR156, miR159, miR166s, miR168s, miR172s, miR171 and miR444s were upregulated in Lv28 leaves. In roots, 19 and 23 miRNAs uniquely responded to water deficiency in H082183 and Lv28, respectively. In roots, miR156s and miR168 and miR166s and miR399s were commonly downregulated and upregulated in both H082183 and Lv28, respectively. The miR156e-3p showed upregulation in H082183 but downregulation in roots of Lv28. Drought-responsive miRNA-mRNA modules showed miR159-MYB-, miR164-MYB-, miR156-SPL-, miR160-ARF- and miR164-NAC-negative regulatory relationships. Two maize inbred lines showed downregulation of miR164 and upregulation of miR159, miR390 and miR398 in the tolerant line and sensitive line, respectively. Two miR164-MYB and miR164-NAC modules modulated stress response in the tolerant line, whereas miR156-SPL and miR160-ARF modules lead to inhibition of metabolism in the sensitive line (Liu et al. 2019). At two time points of drought stress, 68 microRNAs of 29 families exhibited differential expression in the seedlings of drought-tolerant inbred line (R09) (Li et al. 2013a). The differential expression of microRNAs results in manifold responses at physiological and biochemical levels corresponding to drought tolerance in maize. Under drought stress, miR159a, predicted to target two MYB gene family members, was upregulated in early stage. All zma-miR159 family members were downregulated at later stages of drought (Li et al. 2013a, b). The drought-responsive miR827 exhibited upregulation, and other microRNAs showed downregulation at 24 h of drought stress. Downregulation of drought-responsive microRNAs in the drought-tolerant inbred line (R09) has the basis of induced target gene expression, which can improve maize tolerance. Drought-responsive microRNA targets (Table 19.1) encompassed serine/threonine protein kinase, secondary active sulphate transmembrane transporter, homoserine kinase and ATP sulphurylase. These microRNAs target proteins for increased tolerance of plants to water deficiency (Conley et al. 1997; Kobayashi et al. 2004; Buchner et al. 2004; Lee et al. 2005; Cohen et al. 2010; Li et al. 2013a).

19.2.2 Salt Stress

Salt-responsive miRNAs (98) belonging to 27 miRNA families showed changed expression profiles after salinity stress in salinity-tolerant maize inbred line (NC286) and salinity-sensitive maize line (Huangzao 4). These miRNAs perform different functions in response to salinity, and miRNAs of the same miRNA families exhibited different expression profiles. Only 77 miRNAs aligned with 55 members of 10 maize miRNA families. The 18 miRNAs were expressed only in NC286, and 25 miRNAs exhibited delayed regulation patterns in Huangzao4 (Ding et al. 2009). The miRNA genotype-specific expression elucidates distinctive sensitivity to salt between maize lines. Salt-responsive miR168 (AGO1), miR162 (DCL1) and miR395 (NADP-dependent malic protein ATP sulphurylase) were upregulated, whereas miR164, miR167 (NAC1, ARF8), miR156 (R2R3 Myb SBP domain protein) and miR396 (cytochrome oxidase) were downregulated in the roots of NC286 (Ding et al. 2009). Several miRNAs (1040) were recognized from four maize libraries which included 762 miRNAs (from leaves) and 726 miRNAs (from roots). Some 448 miRNAs displayed overlap between maize roots and leaves. miR167 and miR164 were already known miRNAs, whereas miR167 and miR164 were novel putative miRNAs (Fu et al. 2017). Mature miRNA sequences of zma-miR167a, a-5p, zma-miR167e3p and zma-miR164a-3p were detected. In miR167 family, mir-29 was alone significantly altered. In miR164s, mir36 exhibited upregulation in leaves. The mir-29 of zma-miR167 and mir-36 of zma-miR164 families play chief roles in response to high salinity. During salinity stress in maize, expression profile of novel mir-330, mir-250, mir-205 and mir-17 was repressed in maize leaves or roots. Their targets, for instance, GPX, P5CS, IF-1, casein kinase II and other genes, were upregulated, and several salinity-tolerant pathways were induced for survival under salinity stress (Fu et al. 2017) (Table 19.1).

19.2.3 Submergence

Thirty-nine potential miRNAs were identified as submergence-responsive miRNAs in maize roots. Out of these miRNAs, 30 aligned with nine maize miRNA families. Other miRNAs matched to ath-miR395, ptc-miR474, ath-miR854, osa-miR396 and osa-miR528 (Zhang et al. 2008). Upregulated stress-responsive miR399 directly targeted aminotransferase and starch synthase. This prevented synthesis of amino acids and starch. Repression of miR159, ath-miR395-like and ptc-miR474-like improves accumulation of carbohydrate and energy metabolism. Upregulation of SOD and ALDH, because of downregulation of osa-miR528, eliminates reactive oxygen species (ROS) and acetaldehyde, and in turn subsistence of root cells becomes possible. Upregulation of miR166 and miRNA167 and miR159 downregulation modulate hormonal homeostasis through regulation of HD-ZIP, ARF and GAMYB. This initiates morphological changes involving formation of adventitious roots and development of lateral roots for adapting to low-oxygen environment (Zhang et al. 2008). Differential expression of 164 miRNA sequences was studied in maize and/or teosinte under submergence stress. Maize showed ten upregulated miRNAs and 72 downregulated miRNAs. In maize exclusively (Table 19.1), 21 miRNAs exhibited upregulation, and 49 miRNAs exhibited downregulation during drought stress. In the overlap between submergence and drought responses in *Zea mays*, most of the miRNAs (68) were downregulated (Sepúlveda-García et al. 2020). During submergence in maize, miR159a, miR167c, miR166b and miR169c were downregulated, and miR156k and miR164e were downregulated by water deficiency (Sepúlveda-García et al. 2020).

19.2.4 Waterlogging

Quantitative real-time PCR expression profiles of 24 miRNA signatures (22 known and 2 novel) and their predicted targets (92) in waterlogging-tolerant Hz32, waterlogging-mid-tolerant B73 and waterlogging-sensitive Mo17 inbred lines of *Zea mays* were performed. In short-term waterlogging circumstances, miR159, miR393, miR164, miR408, miR167 and miR528 were upregulated and recognized as key regulators of post-transcriptional regulation in three inbred lines mediated by hormonal regulation (Liu et al. 2012). Several miRNAs (Table 19.1), for instance, miR166 and miR159, were upregulated in roots of maize plant during waterlogging (Zhang et al. 2008; Liu et al. 2019). miR164 develops lateral roots and responds to waterlogging in maize roots (Guo et al. 2005; Liu et al. 2012). Similarly, miR167 also responds to waterlogging (Zhang et al. 2008; Liu et al. 2012). In B73 line, miR159, miR408 and miR528 (as in Hz32) were upregulated, whereas miR164, miR167 and miR393 (as in Mo17) were downregulated. miR408 and miR528 target *cupredoxin* involved in oxidative stress response signal and early signal of waterlogging prevent damage to cellular structures and respond to submergence in maize roots with negative correlation to its target (Liu et al. 2012). This leads to suppression of ABA and *cupredoxin* signals and stimulation of auxin signals at the initial stages of waterlogging (Liu et al. 2012). RNA sequencing identified expressed miRNAs and miRNA targets in crown roots of two inbred lines (Hz32 and Mo17) of maize seedlings under waterlogged conditions. Sixty-one mature miRNAs included 36 known and 25 novel miRNAs. Waterlogged and normal crown roots showed 32 waterlogging-responsive miRNAs, mostly downregulated during waterlogging in 2 inbred lines (Zhai et al. 2013). Identification and characterization of differentially expressed novel miRNAs were performed in the in bred lines of drought-tolerant and drought-sensitive maize (Sheng et al. 2015). Through integrative sequence analysis of early developmental stage of primary roots (PRY) and later developmental stage of maize primary roots (PRO), seminal roots (SR) and crown roots (CR), 278 miRNAs (246 conserved and 32 novel) were identified with different expression patterns in diverse maize roots (Kong et al. 2014). On the basis of expression patterns under $AlCl_3$ stress, miRNAs respond differentially in different root types. The levels of zma-miR390 and zma-miR393 were upregulated and showed highest expression in CR. The accumulation of zma-miR156, zma-miR172 and zmamiR396 increased in SR but declined in PR and CR. The expression profiles of zma-miR395, zma-miR399 and zma-miR528 showed an opposite pattern between PR, CR and SR. Expression of zma-miR171 was reduced in PR and CR. The miRNAs mediate differential growth responses to Al stress as well as root development (Kong et al. 2014). During flood stress conditions, cytochrome c interacts with caspase cascades and initiates programmed cell death (Qi et al. 2018). Zm-miR30 [zmamiR164b5p] targets cytochrome c. Cytochrome c1 expression profile was downregulated in the tolerant inbred line I172. A membrane-bound, cyanide-insensitive and metalloprotein alternative oxidase (AOXs) is involved in mitochondrial electron transport chain bypassed complex III and IV of cytochrome pathway. AOX maintains metabolic homeostasis and signalling dynamics by regulation of superoxide, reactive oxygen species (ROS), cellular nitric oxide (NO) and reactive nitrogen species (RNS) (Kumari et al. 2019; Kaur et al. 2020). Zm-miR25 [zmamiR159g5p] targets transcriptional activators of zinc finger C3H-type proteins. The zinc finger proteins control gene expression by binding zinc (Zn) and iron (Fe) centres to DNA and modulate growth and development. Zinc finger protein expression pattern in I110 (S) exhibited constant downregulation upon initiation of waterlogging and higher expression in I172 (T) after 24 h of stress. Higher expression of zinc finger protein modulates metabolic pathway for anaerobic adaptations to deal with waterlogging stress (Kaur et al. 2020).

19.3 Conclusion

This chapter highlights the fact that quite a substantial number of abiotic stress-responsive miRNAs are available in the maize plant. Environmental adaptation to abiotic stress is the need of the hour in maize. Maize being oldest and important dietary cereal crop can be designed as abiotic stress-tolerant by the new, yet powerful, tool of miRNA engineering.

Acknowledgements DP acknowledges Dr. Saurabh Raghuvanshi, Associate Professor, Department of Plant Molecular Biology, South Campus University of Delhi, New Delhi, India, for introducing her to the domain of plant microRNAs.

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Transcriptome, Proteome and Metabolome Profiling for Abiotic Stress Tolerance in Maize and Barley

20

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Abstract

In the field of biology and agricultural sciences, the term omics is used for the knowledge which aims at the qualitative and quantitative analysis of biomolecules including DNA, RNA, protein and metabolites. These biomolecules are responsible for the structure, function and dynamics of an organism. Through the use of omics, a number of plant agronomic traits, physiological traits and reproductive traits have been improved for enhanced crop productivity and resistance to biotic and abiotic stresses and exhibit high nutritional value. Moreover, this field of science strengthens the systematic approach to explore the complex interactions between genes, proteins and metabolites within the resulting phenotype. This scientifically integrated

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_20

approach for crop protection and improvement mainly depends on the biology of an organism, chemical analytical approach, bioinformatic tools and computational analysis. Among the important agricultural cereal crops, maize and barley are used as a staple source of nutrients for human being as well as animal feed and beer production. The global population is expanding at a high rate, and food demand is increasing with the passage of time. To meet this demand, improvement in major crop production including maize has been made; however, barley production has stagnated over the past two decades. Several approaches are adopted by scientists to improve the productivity of maize and barley crops, namely, genetic and genome analysis and genomic research. This study is focused on reviewing the approaches of omics in the form of transcriptomics (RNA), proteomics (protein) and metabolomics (metabolites) for the improvement of maize and barley crop productivity.

Keywords

Maize · Barley · Abiotic stress · Omics · Crop improvement

20.1 Introduction

For the survival of every living organism, the supply of food is one of the fundamental components. Different leguminous plants, cereal crops and pulses are consumed by human beings as sources of food and energy. Among these sources of food, cereals are the potential one, which is cultivated as a crop since ancient times. Moreover, it is also used directly in the form of meat because major livestock feed consists of cereal crops (Das et al. 2012). Cereals also known as grains belong to grass family Poaceae formerly called Gramineae. Important cereals are wheat (*Triticum*), rye (*Secale*), barley (*Hordeum*), oat (*Avena*), rice (*Oryza*), millet (*Pennisetum*), maize (*Zea*), sorghum (*Sorghum*) and triticale (hybrid of wheat and rye) (Gallagher 2013). They produce starchy seeds and are used as a daily diet at the global level. Comparatively, their grains are easy to process and stockpile due to their low water content and adequate amount of nutritional value. Moreover, they can be cultivated in diverse environmental conditions in a vast area.

Globally, cereals are cultivated over an area of 700 million hectares, and in 2020–2021, the expected production of cereals set up by the Food and Agriculture Organization of the United Nations is 2769.4 million tonnes, while their current supply is 3633.0 million tonnes. However, its consumption is 2810.5 million tonnes, and this rate is on surge especially in developing countries (Food and Agriculture Organization of the United Nations 2021). The major dietary portion of grains is comprised of carbohydrates with a low amount of protein. Moreover, they contain small quantities of micronutrients (iron), vitamins (vitamin B complex, vitamin E, niacin, riboflavin and thiamine), different minerals and fibres. Being a potential diet, cereals are helpful in different disease prevention, e.g. cardiovascular disease, digestive disorders, diabetes and cancer. Moreover, the milled dry cereal products contain a high concentration of bioactive compounds, while the wet-milled products are comprised of starch and gluten. Similarly, through the malting process, beer and alcoholic beverages are produced (Kushi et al. 1999; McIntosh 2001; McKevith 2004; W. H. Organization 2003).

Cereal crops play an important role in the nutritional requirement and economy of a country. However, the productions of these crops are affected by a number of factors including biotic and abiotic factors. These factors affect the cereal crop cultivation and production from their germination to harvesting and storage (Roychoudhury et al. 2011a). This chapter is focused on the omic analysis of maize and barley crops under abiotic stresses posed by different environmental conditions.

20.2 Crop Improvement Through Modern Technologies

To meet the global food demands, researchers are developing and adopting modern biotechnological tools to improve the production of the crops and their sustainability in the environment. Conventional agricultural practices are facing challenges to fulfil the current and future food needs due to an increase in world population with an expected 9.7 billion people as estimated by the United Nations (2015). However, fortunately, in the current era of biotechnology and molecular biology, there are several alternative approaches in the field of crop production and improvement. With the help of these techniques, improvement in the genetic make-up of crops and resistance development to the environmental biotic and abiotic stress has been achieved. Advancements in the field of omics (genomics, transcriptomics, proteomics and metabolomics), computational biology and stress biology can be used to improve the agronomic traits, tolerant to biotic and abiotic stress. New and improved varieties can be developed by transferring the stress tolerance gene (Paul and Roychoudhury 2018; Rasool et al. 2020), gene pyramiding, gene mapping and mutagenesis (random and site-directed) to change the expression of a gene or inhibit the pathway of the desired trait (Pedersen and Leath 1988). Nowadays, in genomics, DNA microarray, genome-wide selection and next-generation sequencing have made exploring and improvement of traits easier (Roychoudhury 2020). Moreover, proteome, transcriptome and metabolome profiling of stress-tolerant crops enables best trait selections, and thus these approaches will be discussed in scenario of abiotic stress tolerance in maize and barley.

20.3 Omics

Enhancing the yield by improving the quantitative and qualitative traits of crops with the help of high-throughput omic approach is now a reality. In omics, quantification and characterization of the desired traits of an organism are done mainly through universal detection of their genome (genomics); transcription products, i.e. RNA (transcriptomics); functional product of a gene, i.e. protein (proteomics); and products of biochemical reactions i.e. metabolites (metabolomics) (Roychoudhury et al. 2011b; Vailati-Riboni et al. 2017). Through such technologies, researchers can easily understand the more complex pathways by using organism's whole genetic, RNA, protein and metabolite contents living in different environmental conditions, e.g. an organism living in suitable condition and stress conditions. Transcriptome gives a picture of total RNA contents expressed by an organism under specific conditions (Roychoudhury and Banerjee 2015). The expression of the inducible genes keeps on changing with the changing environmental condition, and sometimes one gene may generate more than one transcript due to alternative splicing in higher organisms. The transcriptome could help researchers to understand the gene nature of a particular trait, its RNA expression level and its involvement in biochemical processes. Assembly of transcriptome can be generated through the NGS approach, which is a useful procedure to annotate the gene of a particular trait. Moreover, it gives the understanding of functional markers, such as simple sequence repeat (SSR) and single-nucleotide polymorphism (SNP), of susceptible and resistant crop varieties. Moreover, the level of the transcription level can be determined with the help of a DNA microarray chip. In this approach, the RNA is converted into cDNA, and then the synthesized cDNA is tested on the chip for upregulation and downregulation of a particular trait under stress conditions. Furthermore, they can be used in the form of expressed sequence tag (EST); in this method, the short nucleotide sequence generated from a single RNA transcript is used for sequence information of pre-microarray design. Other options in transcriptomics include serial analysis of gene expression (SAGE); this approach uses the sequence expression profiling of an organism, and this is utilized for the expression analysis of an organism with a less characterized genome. Similarly, the identification and quantification of an RNA transcript are done through massively parallel signature sequencing (MPSS); this process involves the generation of RNA sequences through complex series of hybridization (Lowe et al. 2017; Mochida and Shinozaki 2010). The transcriptome profile of organisms can be created based on cell type, species, developmental stage and physiological conditions. Moreover, transcriptome analysis of the mutant varieties together with phenotype is helpful in the identification of genes involved in a metabolic pathway and functional annotations of uncharacterized genes.

Another advanced approach for crop improvement is proteomics. This scientific approach of omics provides the knowledge of the total protein contents of an organism. It involves the identification and characterization of protein expression and presence under a particular physiological condition. The proteins which are decoded from DNA with mRNA transit phase are involved in the structural and functional sustainability of an organism, regulate the different biochemical processes and involve in cell signalling pathways. There is a great diversity in protein contents that can be observed as compared to genomics due to the translation and post-translational modifications of the mRNA and protein processing, respectively (Kav et al. 2007; Renaut et al. 2006; Wilkins et al. 1996). A number of biological techniques are involved in proteomic analysis, namely, protein isolation from cell and tissue followed by separation of isolated protein mixture through 2D polyacrylamide gel electrophoresis (2D-PAGE) or chromatography techniques. The purified protein components are then subjected to mass spectroscopy either GS-MS (gas chromatography mass spectroscopy) or LC-MS (liquid chromatography mass spectroscopy), for the analysis of peptide sequence of proteins. The two commonly used approaches in MS are electrospray ionization (ESI) and MALDI-TOF (matrix-assisted laser desorption/ionization time of flight). During these processes of MS, the peptides are converted into ions due to their passage through high-voltage columns (Halket et al. 2005; Klose 1975). A number of studies have been done through a proteomic approach in plants and crops during abiotic stresses. For example, the proteomic profile of C4 and C3 plants was compared for light fixation and energy conversion. Similarly, heat stress condition affects the growth and productivity of crops (Hossain and Komatsu 2013; Zhao et al. 2013).

The fourth approach of the omics is metabolomics; this approach is used for the identification and quantification of the metabolites of a cell or an organism. The metabolites are the products of the biochemical process of a cell in the form of primary metabolites and secondary metabolites. The productions of these metabolites depend on the particular developmental stage, physiological conditions and environmental stresses of an organism (Memelink 2005). The metabolites are analysed by advanced biological techniques known as mass spectrometry (MS), nuclear magnetic resonance (NMR) and Fourier transform infrared spectroscopy (FTIR). Through metabolomics, researchers can understand the complex metabolic control and diversity of biological reactions. Moreover, it is helpful in determining the susceptibility and resistance of plants or crops to heat via antioxidants, etc. (Roychoudhury et al. 2012).

20.4 Maize

The world's population is increasing tremendously which is estimated to rise up to 9000 million by the year 2050. Moreover, more than 50% of the world's fertile land will be adversely affected by increasing soil stress parameters which would be a major factor in land scarcity and ultimately crop production (Shelden and Roessner 2013). Essentially, the huge increase in the population is becoming the main reason for the depletion of land resources, especially food and feed. Approximately 80% of human food is dependent on agricultural crops so that these are the major sources of food for the inhabitants of the land. Moreover, about half of the global food production is made up of cereals (Jain et al. 2019; Tardieu et al. 2017).

To ensure adequate food production for the growing population in the current era as well as in the future, a dire need exists for getting resistant crops (maize, barley, wheat, etc.) that can cope with the environmental trough and crest (Gong et al. 2014). Nevertheless, an efficient indulgence into the mechanisms of stress tolerance in such crops is imperative. According to the Food and Drug Administration, maize is becoming more popular than other staple foods (e.g. rice, wheat, etc.) due to its valuable nutritious profile for humans (http://www.fao.org/home/en/).

Maize, which is one of the important staple and nutritious foods for humans, is also a key component of animal fodder and feed. Moreover, it is also becoming a popular source of biofuel production. Growing demands along with the production shortfalls in global maize supplies have contributed to escalating maize prices worldwide. The major factor in the loss of maize production has been the climatic variations and the enhanced abiotic and biotic stress factors associated with the climatic changes. Abiotic stresses are usually found to be the main hindrance in the successful plant production; therefore, attention has been paid to the molecular and breeding aspects of plant research which can introduce the excellent tolerant qualities to the important crops for enhanced production for sustaining the ever increasing population. Nevertheless, it is worth mentioning here that maize harbours highly diverse genetics. This genetic diversity combined with the advent of newly established omic technologies has provided a wide opportunity for maize to be employed as a model plant for the sake of understanding the stress tolerance mechanisms and developing improved transgenic crops. The research applied on maize plants for the improvement of characters can be successfully implemented on other major plant species as well. This advantage is the result of a greater variety in the allelic pool of the maize genome which confers better stability to the plant under a number of different stress factors (Beadle 1972; Liu et al. 2020).

The main goal of plant breeding scientists and geneticists is the establishment of maize varieties with improved quality and production/quantity. In this perspective, the mutual application of computational biology and different omic technologies for fishing out the genes or biomolecules of interest has received far more attention. An attempt was made to compensate for the loss of maize yield caused by insect pests (such as European corn borer (ECB)), through the bioengineering of a recombinant gene *Bacillus thuringiensis* (*Bt*), from a soil bacterium, which conferred a definite resistance in maize plant to ECB. This attempt revolutionized crop production and established a high-yielding maize/corn variety known as “Bt-corn hybrids” to fight ECB invasion (Raybould et al. 2007). Similarly, Jiang et al. (2019) analysed the transcription factors and final fused network which played a key role in maize improvement.

In fact, the hybrid maize is not a new entity, but for about 10,000 years, man has been exploiting the rich genetic variability of the maize genome as the basis for domestication and consequent crop improvement. Moreover, current efforts in crop improvement research have been made to further introduce genetic diversity, yielding greater insights into the detailed information of genes responsible for maize improvement and domestication. Similarly, the functional diversity experiments help in fishing out the alleles for future crop improvement (Buckler et al. 2006; Duvick 2001). The yield improvements of the maize plants can be attributed to genetic improvement, changes in the cultural management and climatic changes and interactions among different factors. In the United States, Europe and Ontario, Canada, the average maize grain per hectare was increased at a rate of about 1.5% per year when maize hybrids were introduced in the 1940s, while before this period no appreciable yields of maize were seen in these regions (Tollenaar et al. 1994;

Tollenaar and Wu 1999). Furthermore, the development of genome sequence and germplasm resources is likely to further accelerate this progress.

20.4.1 Abiotic Stresses in Maize

Attention needs to be focused on the proper alliance of conventional and molecular breeding approaches which can lead to the development of proficiently stress-tolerant, broadly adapted and high-yielding maize varieties. The use of improved germplasm will not alone be enough to achieve the tasks and will need to be complemented by improved crop and agronomic practices (Shiferaw et al. 2011). This section will focus on constraints to high productivity of maize faced by abiotic factors and the proper solution to deal with their negative impacts. Abiotic stresses including drought, waterlogging, imbalanced moisture levels, etc. are adversely affecting the production of maize just like other crops. Moreover, the combinations of abiotic stresses coupled with emerging microbial attacks (diseases) and different kinds of pests enhance the possibility of crop yield reduction and affect its quality as well (Mittler 2006).

Drought is one of the major factors which significantly reduces maize production and lowers its quality. Usually, maize grown in semi-arid and/or tropical/humid environments encounter various abiotic stresses especially high temperature and drought (Cairns et al. 2012). Drought is one of the most important abiotic stress factors for maize production in areas with temperate and tropical climates. A drought stress scenario of sub-Sahara in Africa has been illustrated by Heisey and Edmeades (1999). They quoted that maximum parts of 10.4 million hectares of maize in the lowland tropics and 7.6 million hectares in the mid-altitude/subtropics were under drought stress. Additionally, globally 15% of maize yield are lost due to drought on an annual average basis (Edmeades 2008).

Maize losses are further supplemented by the rise in temperature which is also one of the important parameters controlling plant growth. It has been observed frequently that usually the dry areas are vulnerable to rise in temperature and, therefore, heat is a critical stress factor which adversely affects maize alone as well as in combination with drought (Phillips 2010; Thornton et al. 2015). Edmeades (2008) quotes that higher losses of maize yield may reach an increase as high as ten million tonnes/year with the rise in temperature level and changes in the rainfall patterns under climate change. A similar impact of temperature on maize growth and productivity has been documented by Tesfaye et al. (2018) and Byjesh et al. (2010).

As the temperature of the Earth is getting higher and higher with each subsequent year due to increasing deforestation and extensive use of fossil fuels, so the concentration of CO_2 has escalated to more than 390 μmol^{-1} in the atmosphere which is estimated to increase twofold, till the end of this century. The rise in the CO_2 level and other toxic gases is the main cause of roaring warmer global temperature (Vaughan et al. 2018).

Soil fertility is another even more serious limitation reducing maize production. This factor actually comes into play in the regions with very low use of fertilizer and the lack of appropriate use of agricultural land and proper practices for replenishment of soil fertility (Smale et al. 2013; Wood 2013). The soil fertility problems combined with aluminium toxicity, acidity and low accessibility of phosphorous reduce the maize yields on about four million hectares of the maize growing area globally (Mike et al. 2001; Shiferaw et al. 2011). Further, as maize crop is primarily grown as a rain-fed crop, so increased/decreased rate of rainfall owing to climate change will lead to an escalated reduction in the crop production and price changes as well (Jones and Thornton 2003).

20.4.2 Stress Tolerance in Maize

Variation in the environment has a long-lasting influence on agriculture and global food security which are severely threatened by the extreme/fluctuating weather conditions. Although the problem has existed for a long time, unfortunately, no definite solution to the problem was available at that time. Auspiciously, technology and science are well elaborated in recent years that they can be exploited successfully to track such issues. In this regard, appropriate approaches are required for our crops to adapt them to the changing environmental stresses (Raza et al. 2019).

Currently, biotechnology opens magnificent ways to enhancing the ability of plants to stay healthy and grow in stressed conditions. In other words, it offers the technology which can increase the speed and efficiency of plant breeding in environmental stress period (Masuka et al. 2012). Molecular breeding is a tool of biotechnology which employs the modern breeding approaches which in turn exploit the genotypic markers as a substitute for the phenotypic selection of a trait to be improved (Gazal et al. 2016). Recent developments in the field of molecular biology in the form of identification of genetic markers, construction of molecular maps and complete genome sequencing facilities have enabled researchers to explore and understand the living organism at the molecular level. A large number of different approaches have been used for enhancing the abiotic stress tolerance in maize including gene expression studies using QTL analysis to locate the putative genes and alleles involved in conferring the tolerance under different abiotic stresses (Prasanna et al. 2013). Moreover, transgenic plant development is also important among other techniques (Swamy et al. 2011).

The marker-assisted selection (MAS) was first coined by Beckmann and Soller (1986). Since then, MAS has been considered a major molecular breeding strategy in plants. The MAS is used for plants carrying appropriate genomic regions involved in the expression of a concerned peculiarity. Therefore, it is very effective in improving tolerance to complex abiotic stresses. The MAS is very fast compared to conventional phenotypic selection because the plants with required characters are selected during the sapling phase, thus effectively saving time, effort and resources (Morris et al. 2003).

The molecular marker strategy has progressed greatly over the past few years when the hybridization markers including RFLP (restriction fragment length polymorphisms) and PCR-based markers RAPD (random amplified polymorphic

DNA) and AFLP (amplified fragment length polymorphism) were introduced. Currently the advanced form, the molecular markers are implemented including SNPs (single-nucleotide polymorphism) and SSR (simple sequence repeat) genetic improvement or transgenic studies (Mir et al. 2012).

Furthermore, genomic selection (GS) has been found as an innovative, high-throughput and potent tool for establishing the plant varieties with resilience to stressing environmental parameters. In plants, GS was first established in *Zea mays*, *Hordeum vulgare* and *Arabidopsis thaliana* and demonstrated a high exactitude compared to the studies based on sole pedigree information (Lorenzana and Bernardo 2009). However, lack of knowledge of statistics (required for the GS) and simulation studies in plant breeding programs has been the main hindrance for this program to be popularized (Jannink et al. 2010).

More recently, a genome-wide association study (GWAS) and next-generation sequencing (NGS) applications have greatly revolutionized the decoding of genotype-phenotype associations in many plant species. Maize has been dealt as an ideal crop for GWAS which has shown significant progress in the field of plant biotechnology and especially for plant tolerance improvement. With the release of the B73 reference genome (Schnable et al. 2009), GWAS has been applied substantially, and a number of agriculturally important different traits have been explored.

Significant understanding of tolerance mechanisms of maize to abiotic stress factors can be achieved by omic approaches which can help to bioengineer stress-tolerant maize varieties in response to different stress factors. The maize responses to abiotic stresses (such as salinity, drought and heat) have been found very helpful for bioengineering as well as for breeding of novel and tolerant maize varieties (Uddin et al. 2018). These responses include all aspects such as physiological, morphological or molecular.

In recent years, Prohens et al. (2017) introduced a new term, “introgressiomics”, which is an all-encompassing, efficient and well-organized establishment of plant varieties and subsequent populations which consist of introgressed genomes. This engineering is conducted by transfer of genes of wild crop variety into the genetic make-up of other crop varieties for developing new cultivars/varieties with desired characters and properties (D’Agostino and Tripodi 2017). This engineering strategy has provided high success level with the sacrifice of oil yield, quality and plant grain quality or quantity (Grewal et al. 2020).

20.5 Barley

Barley or *Hordeum vulgare* is one of the most primeval crops of the world, which were cultivated about 17,000 years ago in the Nile River Valley, Egypt. Because of its high nutritious value, barley was used as a special training diet by Roman gladiators or *hordearii* (barley men) (Giraldo et al. 2019; Purugganan and Fuller 2009). Recently, barley has become the fourth most abundantly growing cereal crop which is being cultivated in more than 100 countries throughout the world, and in the last decade, the highest percentage of barley has been produced by Europe (60%), followed by Asia (15%) and the United States (13%) (Giraldo et al. 2019). In addition to be used as food for humans, barley grains have multiple end uses such as animal fodders and alcoholic beverage production (Horsley et al. 2009). Importantly, the barley crop harvested for malting is far more valuable for farmers while selling their barley fruitages to the malting market (Baker et al. 2020; Giraldo et al. 2019).

20.5.1 Abiotic Stresses in Barley

The productivity of barley varies depending on the seasonal variability in the level of heat, temperature range, precipitation rate and other environmental stress factors. They affect the life cycle and alter the plant's expression and its genetic potential. For example, a number of factors are responsible for grain loss including variation in seasonal temperature, drought and salinity. The barely respond to these varying stress factors by altering its metabolic pathways in the form of regulating the gene expression and by evolving its genetic make-up. Thus the possible evolving genetic variations making the barely variety resistant/tolerant to these stresses (Cattivelli et al. 2002). A drought condition exists when the water availability is less than the required amount of water for the maximum crop production. Drought with the addition of increasing temperature is responsible for valuable productivity losses. However, to overcome the shortage of water, irrigation system is used, but it contributes to the augmentation of salt concentration in the soil. Thus, the excessive quantity of salt in the soil affects the normal growth and productivity of crops (Cattivelli et al. 2017).

In response to different climate conditions, a dire need exists to introduce new variants of new barley which could potentially tolerate the changing environment (Condon et al. 2004; Rajpal et al. 2019). For example, in dry environments, breeders usually develop such programs which are basically based on direct selection for crop/grain yield. Nevertheless, many traits other than the grain yield have been targeted that include growth habit, time/duration of flowering, maturity rate, early growth vigour and plant height (Baum et al. 2007). However, unlike other cereals, barley has a simple genetic make-up comprising of only seven chromosomes with a haploid genome of about 5.3 GB. The simplicity of genome makes it a perfect experimental model for inbreeding diploid genetics. Due to the genetic simplicity, barley offers a flexible platform for genetic modifications and mutations, and hence, over the last decade, 180 new cultivars or breeding lines of barley have been tested in the official variety trials, out of which 80 new varieties have been approved for domestication (Hakala et al. 2020). Furthermore, barley shows an excellent capability to adapt to diverse environmental ups and downs, such as cold, drought or poor soils, and is considered more tolerant than wheat and other crops to harsh growing conditions (Gürel et al. 2016). Its diverse metabolic profile makes it thrive in diverse climatic territories with a wider range of environmental conditions (Britannica; Mahdi et al. 2008). Another important feature of barley is that it is able to grow and ripen in a shorter season than any other cereal (Peltonen-Sainio and Jauhiainen 2014).

It is worth mentioning that barley holds a remarkable efficiency regarding different nutrients and water than any other cereal crop (Kishor et al. 2014). Additionally, barley offers a number of health benefits to humans such as high β-glucan content which is important to maintain the cholesterol levels of the body (El Khoury et al. 2012; Theuwissen and Mensink 2008). More recently, Khan et al. (2021) presented a meta-analysis on the biofortification of zinc in barley. They further demonstrated that different cultivars of barley were very specific to accumulate and translocate specific concentrations of zinc which could efficiently solve the problem of zinc deficiency in humans. Similarly, moreover, barley genotypes have been reported to play an excellent role in the remediation of soil contaminated with heavy metals (Ali et al. 2017; Andrey et al. 2019). The phytoremediation capability of barley is indirectly associated with enhancing the beneficial effect on human health as well as elevating the nutrient profile of the plant itself (Astley and Finglas 2016; Bohn et al. 2008). Therefore, it is not wrong to say that barley is still a significant food for humans especially in extreme or unfriendly climates, e.g. in Ethiopia regions, Bhutan, Tibet (China), Morocco, Nepal, etc. (Baik and Ullrich 2008; Day 2013).

Barley plant has been found to be an important tool in plant breeding and genetics as it naturally contains resistant genes in its genetic profile that can be successfully employed for crop engineering techniques. A significant step towards the successful exploitation of barley genes would be the complete knowledge of the functional annotation of the barley genome. More recently, Lee et al. (2020) have established a “BarleyNet”, a network (co-functional in nature) of more than 26,000 barley genes as well as a web server for network-based predictions. They further demonstrated the significance of BarleyNet for understanding the stress tolerance by means of proteomic and transcriptomic studies from barley roots or leaves under drought or heat conditions. As the barley genome presents a greater diversity than other cereal crops and is often categorized as spring or winter variety, it shows a range of trait variants. Therefore, breeding programs, which usually rely on the high level of genetic variation in plants, provide a significant opportunity for the maximum level of success by employing the barley. Specific traits of the barley can be exploited in backcross experiments by hybridizations between high-yielding and resilient cultivars and original or wild-type barley crop in conventional breeding platforms (Badr et al. 2000; Nevo 1992; Zohary et al. 2012).

Plant yield, growth and productivity are affected by abiotic stress factors such as drought, salinity, temperature fluctuations and the presence or absence of pollutants in the soil. As a consequence, physiological and biochemical responses in plants show a discrepancy at different conditions. Also, hundreds of genes and their products respond to these stresses at the transcriptional and translational level (Sreenivasulu et al. 2007; Umezawa et al. 2006). Therefore, molecular mechanisms of stress tolerance must be revealed to understand the naturally conferred tolerance capabilities of plants that would be significant in the arena of transgenic plants/crop generation with elaborated tolerance competencies (Uçarlı et al. 2016).

Barley has long been considered as an excellent model for understanding the physiological, breeding and genetic facets of stress tolerance. This capability of barley can be attributed to amazing natural tolerance to drought, heat and many other stress factors than any other crop, and therefore, it is remarkably suitable to grow in areas with a high rate of weather fluctuations (Cattivelli et al. 2008; Munns and Tester 2008; Nevo et al. 2012). Owing to its marvellous natural tolerance to environmental stresses, barley has been employed in the generation of many sophisticated genetic resources and databanks/databases, which were created with the help of many latest techniques used in omics such as sequence data analysis programs, genetic maps, NGS (next-generation sequencing), microarray technology, etc. (Mochida and Shinozaki 2013; Riaz et al. 2021).

20.5.2 Stress Tolerance in Barley

Barley genome has been assembled in terms of physical, genetic and functional sequence (Mayer et al. 2012). It is found that relatively meek diploid genetic pattern of barley and its close affiliation with the family Triticeae have assisted the breeders and plant geneticists in that they can employ the expertise and insights gained from barley research to other cereals as well (Wiegmann et al. 2019). In this regard, many reports depict the applications of barley gene understandings to wheat, rye, maize and others (Schulte et al. 2009; Nese Sreenivasulu et al. 2008; Varshney et al. 2010). Due to the natural or inherent abiotic stress tolerance capacity of barley, the scientific community is interested to employ this plant for the studies of omic, comparative genomics and to fish out the stress-tolerant genes from this incredible plant species (Al Abdallat et al. 2014). Furthermore, drought, salinity and heat sensitive and tolerant varieties of barley crop have also been studied under abiotic stresses and subjected to proteomic or metabolomic analyses (Ahmed et al. 2020; Rollins et al. 2013).

Barley bears a well-equipped genetic make-up to resist and withstand the severe and versatile abiotic factors. Research has revealed that early flowering followed by an optimum duration of seed development and maturation plays a major role in boosting up the natural stress tolerance in barley (Tamang et al. 2015). The genes which principally affect the flowering duration in barley have been documented to be very closely related to photoperiod (vernalization and circadian/24-h clock). (Saade et al. 2018; Turner et al. 2005).

Abiotic stress-tolerant crops can work as an important tool for food security by presenting the brilliant potential of fighting climatic fluctuations. In this regard, the barley plant can serve as a pool of stress-tolerant genes, which can encode adaptability to a number of abiotic factors that are usually not resisted by other crops. However, Visioni et al. (2019) narrated that harvesting these genes and exploiting them for improving food security are not possible without the complementation of traditional breeding tactics with new analytical selection techniques. They further added that such accompaniment will definitely pave the way to the ultimate grain/food increase which can be resulted in fulfilling the global food and industrial demands. Therefore, it has become necessary to take the advantage of new molecular-based breeding and omic strategies for enhanced agricultural yield and the development of novel, human-friendly crop varieties with improved adaptation to abiotic stresses (Cavanagh et al. 2008; Visioni et al. 2019).

Different transcription factors (TFs) have been reported to date which is responsible for tolerance to various stress factors (abiotic/biotic) in plants. Among these transcription factors (TFs), some, such as CBFs (C-repeat binding factors) and DREB (dehydration-responsive element-binding) proteins, have been successfully transformed into other plants through genetic engineering.

Both CBF and DREB are generally associated with the regulation of transcription of drought- or cold-related genes (Kizis and Pagès 2002; Sakuma et al. 2002; Stockinger et al. 1997). These TFs have been reported to be present in numerous plant species where they control or regulate the abiotic stress responses. Moreover, many reports are available which depict the successful exploitation of CBF/DREB genes in plant engineering to boost up the tolerance level of plants in extreme/unfavourable environments (Yang et al. 2020).

The proteins that are principally involved in stress tolerance are usually the "transcription factors" (TFs), late embryogenesis abundant (LEA) proteins, osmolytes, transporter proteins and antioxidant enzymes. TFs are mainly concerned with the reprogramming of the metabolism in trauma or stressed environment (Gürel et al. 2016). These TFs have been extensively reported to present more pronounced results in the stress-tolerant transgenic plants compared to that produced by the single gene expressions (Cominelli and Tonelli 2010). Furthermore, Na+/H+ antiporters, channel proteins as well as lipid transporter entities can also be strong candidates for engineering the tolerant or resistant plants for drought, low temperatures and high salt conditions (Khan et al. 2015).

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Omics Tools to Understand Abiotic Stress Response and Adaptation in Rye, Oat and Barley

21

Ankur Singh and Aryadeep Roychoudhury

Abstract

Rye, oat and barley are one of the most highly consumed cereals in the world after wheat and rice. Their demand in the food industries has enormously increased; however, despite high productivity, continuous supply as per the market demand is hard to achieve, mostly because of periodic crop losses occurring due to abiotic stresses such as drought, salinity, heavy metal toxicity and non-uniform temperature. Generation of abiotic stress-tolerant crops is one of the most serious challenges that need to be addressed by the scientific communities. In this regard, efforts are being made to understand the stress tolerance mechanism, gene discovery and interaction of genetic and environmental factors. Several omics tools and approaches have recently been used for the development of stress-tolerant crops having better grain quality. Modern sequencing technologies have greatly accelerated the genomics and transcriptomics studies in the above-mentioned species. In contrast, limited efforts have been made in other omics branches like proteomics and metabolomics. Extensive cataloguing of omics resources has highlighted the need for integration of omics approaches for efficient utilization of resources and a better understanding of the molecular mechanism. The information provided in this chapter will be helpful to understand the plant responses and genetic regulatory networks involved in abiotic stress tolerance and efficient utilization of omics resources for improvement in performances of rye, oat and barley.

Keywords

Omics · *Secale cereale* · *Avena sativa* · *Hordeum vulgare* · Microarray · Mass spectroscopy · Abiotic stresses

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_21

21.1 Introduction

“Omics” approach is primarily adopted to decipher the biological changes occurring in plant components such as gene, mRNA, metabolites and proteins in response to abiotic stress, being described as genomics, transcriptomics, metabolomics and proteomics, respectively (Kumar et al. 2015). These studies mainly help to understand the vast changes that occur in plants upon being exposed to unfavourable environmental conditions. In recent year, omics investigations are increasingly used by various research groups to study the genes, proteins and other molecular responses occurring in plants such as rye (*Secale cereale*), oat (*Avena sativa*) and barley (*Hordeum vulgare*) in response to abiotic stresses.

Rye belongs to the biennial graminaceous forage grass family which is extremely adapted to growth under warm and humid climate and can survive in cold climate. Rye is mostly cultivated in the northern parts of Central and Eastern Europe, Belarus and the Russian Federation with an approximate yield of about 4.1 million ha and has similar feature as that of wheat and barley (Rabanus-Wallace et al. 2021). It is commonly used in bread- and whiskey-making industries. Rye is a rich source of alkylresorcinols, a phenolic lipid. Alkylresorcinols commonly inhibit the formation of oxidized low-density lipoprotein (Parikka et al. 2006) and protect the cells from reactive oxygen species (ROS) by scavenging excess ROS (Korycinska et al. 2009). Along with this, other health benefits of consuming rye include weight loss, control of diabetes, improvement of digestion, prevention of gallstone formation and asthma, lowering of blood pressure and induction in the metabolic rate of organism (Liu and Saneoka 2019). Crespo-Herrera et al. (2017) has reported that rye has been used as a source of several genes for improved resistance to pathogens and pests in wheat for more than 50 years. Thus, rye plants are generally grown for animal feed and are slightly consumed by humans. The unique stress (biotic and abiotic) tolerance capacity of rye, along with high-yielding capacity, makes it a widely important crop for regions facing unfavourable environmental condition. The importance of rye is considered in terms of being one of the parents for a man-made cereal species, Triticale (Bartoš et al. 2008). Because of such important features, rye is an important crop for human being, and a wide array of work has been done to decipher the molecular response (which comprises of genes, proteins and other metabolites) during abiotic stresses.

Another important crop that is widely cultivated all over the world is oat. Oat is an annual graminaceous forage grass. It has an average annual yield of around 700,000 tonnes which makes it the fifth most widespread cultivated grain worldwide (Kong et al. 2015). Oat is an important crop widely cultivated in North America, South America, Canada, Australia, China, Russia, Western Eurasia and the Mediterranean Basin. In recent years, consumption of oat has vastly gained popularity due to its high content of soluble fibre with hypocholesterolemic properties (Oraby and Ahmad 2012). Oats are also a good source of antioxidants such as tocotrienols, tocopherols, avenanthramides, β-glucan and phenolic compounds (Lee et al. 2015). β-glucan present in oats is mostly linked with the reduced risk of heart diseases (Othman et al. 2011). Avenanthramides are also important components of oats and have high anti-inflammatory property along with antioxidative nature (Sur et al. 2008). Oats are considered to be moderately salt-tolerant and can easily grow in moderate salt and alkaline soil (Bai et al. 2016a). According to Han et al. (2014), oats have higher salt-tolerant capacity than that of wheat, rice or any other forage crops. High tolerant property of oats against salt and alkaline stress makes it an excellent phytoremediation crop. In addition, its high nutritional value has greatly attracted the attention of various research groups, and an extensive study based on omics approach has been done to extensively elaborate the molecular mechanisms that help oat plants to survive under such marginalized habitats.

Among all the cereals, barley is one of the most economically important crops and is considered as an excellent model for studying agronomy, abiotic stress tolerance and plant physiology due to its high growth rate and adaptability to harsh environmental conditions. Barley is cultivated throughout the temperate regions of the world (Zhou et al. 2019). It is essentially used as food for malt and livestock, and in some parts of the world, it is also consumed by humans. In recent times, barley has drawn a huge attention of various companies due to its medicinal importance. It is a rich source of antioxidants such as carotenoids, tocopherol, flavonoids and phenolics (Kamiyama and Shibamoto 2012). Various recent works have shown the anti-inflammatory and antioxidative nature of barley. In addition, barley also helps to manage blood glucose levels and reduces the risk of cardiovascular diseases (Kale et al. 2013). According to Gulvady et al. (2013), oats contain 12.4–24.5% protein and 3–11% oil which is primarily triglycerides and oleic, palmitic and linolenic acids and are also a good source of vitamins and minerals. Thus, barley is an important crop for regions challenged with drought, low temperature and salinity, thus serving as major necessity of humans residing in those places.

Environmental stress can be defined as any harsh condition that hamper the normal homeostasis of plants, thus affecting their growth and yield. According to the reports of the Food and Agricultural Organization (2011), the world population is estimated to cross a mark of 9.1 billion by 2050. To feed this enormous population, crop production should be raised by 70% (FAO 2011), but abiotic stresses such as drought, salinity, unfavourable temperature and heavy metal toxicity drastically affect the production of major cereals which led to food insecurity. In order to survive under such harsh environmental conditions, plants have acclimatized themselves by regulating the formation of protective metabolites by altering their molecular responses according to external stimuli. A wide range of omics approaches have been undertaken to elaborately decipher such molecular changes occurring in plants, so that in the near future these can be artificially integrated or enhanced in other crops that will help them to survive under challenged situations. In this chapter, a detailed overview of omics approaches undertaken so far has been presented, which will appear fruitful to better understand the changes occurring in rye, oats and barley plants in response to abiotic stress, enabling them to counter the detrimental effects and maintain normal growth and yield.

21.2 Genomics Modulation in Rye, Oat and Barley

Rye belongs to Triticeae tribe of the grass family Poaceae and is closely related to wheat and barley. It has the largest genome (~7.9 Gbp) as compared to that of all other members of diploid Triticeae family, most of which, i.e., around 90%, comprise of repetitive sequences (Bartoš et al. 2008; Flavell et al. 1974). Due to such enormous size, the information about the rye genome is limited, and only 9298 expressed sequence tags (ESTs) are submitted in public domain [http://www.ncbi.nlm.nih.gov/dbEST/dbEST_summary.html (release 070111)]. For studying rye genome, BAC library is only publicly available (Shi et al. 2007). However, with recent advancement in sequencing technology and introduction of next-generation sequencing technologies such as Roche 454 sequencing and Illumina's Genome Analyzer, sequencing of large genomes can be comfortably done. Haseneyer et al. (2011) performed Roche 454 GS FLX and identified more than 2.5 million reads which were further assembled into 115,400 contigs, thus representing a comprehensive rye EST resource. Along with these, they also identified 1385 simple sequence repeats and more than 5000 single-nucleotide polymorphisms that further improved the genomics research and genome-based breeding for developing stress tolerance in rye. In another study, Brunetti et al. (2018) reported the presence of *Esi3* gene family in rye. They reported the presence of ten members of *Esi3* gene family in rye. Earlier, Wang et al. (2016) characterized the DNA of *H^+-pyrophosphatase* gene in rye. They stated that *ScHP1*cDNA included a 2289 bp open reading frame (ORF) encoding 762 amino acid residues with 14 transmembrane domains. The genomic *ScHP1* DNA was 4354 bp and contained 8 exons and 7 introns. They further reported that ScHP1 protein was present in plasma membrane and the expression of *ScHP1* gene was higher in leaves, as compared to that of roots when plants were subjected to salinity, cold and low nitrogen stress, whereas the converse result was noted when plants were exposed to water, potassium and phosphorus deprivation. However, under all the above-mentioned conditions, the gene was least expressed in the stem. Milla et al. (2002) generated 1194 ESTs in aluminium-stressed rye plants and identified 13 novel genes such as ubiquitin-like protein SMT3 and tonoplast aquaporin (involved in cell elongation and cell division); ascorbate peroxidase; glucose-6-phosphate dehydrogenase and glutathione peroxidase (oxidative stress); *S*-adenosyl methionine synthase; methionine synthase; iron deficiency-specific proteins IDS3a, IDS3b and IDS1 (iron metabolism); pathogenesis-related protein 1.2, heme oxygenase; and epoxide hydrolase (cellular mechanism) that were responsible for inducing aluminium tolerance.

Genome of oats is not well characterized, as compared to that of cereals, and only limited information is available in the public domain. Jinqiu et al. (2021) sequenced the genome of oats by using Illumina HiSeq 2000, using six cDNA from oat leaves and obtained an average of 6.66 Gb reads for each sample. They also demonstrated that upon exposing the oat plants to high-altitude stress, 5203 differentially expressed genes were upregulated, whereas 6436 genes were downregulated which indicated that in response to high-altitude stress, the number of genes downregulated was higher than that of the number of genes upregulated. Earlier, Sapre et al. (2018) demonstrated that inoculation of oats seedling with *Klebsiella* sp. during 100 mM NaCl stress showed higher expression of *rbcL* gene, whereas its expression was downregulated in seedlings exposed only to salt. Contrasting result was obtained in the case of *WRKY1* gene which was significantly upregulated in response to salt stress and was downregulated in the presence of *Klebsiella* sp. Another interesting work was reported by Wu et al. (2018) where they analysed the gene expression profile in two different cultivars of oats, i.e. Hanyou-5 (salt-tolerant) and Huazao-2 (salt-susceptible), using Illumina HiSeq 4000 platform. The de novo assembly of the high-quality cleaned reads from Huazao-2 generated 465,630 transcripts with an average length of 922 bp. For Hanyou-5, the assembly generated 460,234 transcripts with an average length of 931 bp. Using other bioinformatics tools, they further classified the genes under different heads [mitogen-activated protein kinase (MAPK) signalling pathway, plant hormone signal transduction, oxidative phosphorylation, metabolic process and plant-pathogen interaction pathway] based on the function controlled by them in stressed plants. Oraby and Ahmad (2012) demonstrated that integration of *CBF3* gene controlled by *rd29A* stress-inducible promoter in oats induced their leaf area, chlorophyll content, relative water content, photosynthesis, transpiration, proline and soluble sugar levels under high salt (100 mM NaCl) stress, whereas leaf wilting was delayed, as compared to that of non-transgenic plants. Integration of *CBF3* gene also maintained the yield of the plants which was around 4–11% loss in transgenic plants as compared to that of >56% loss in wild-type plants.

Among the Poaceae species, barley has been well studied genetically. The genome size of barley is 5.1 Gbp, having more than 80% repetitive elements (IBSC 2012). Due to large genome size, development of ESTs from various cDNA libraries was initiated on a large scale which ultimately resulted in the identification of 4,37,713 ESTs from different plant tissues at various life stages and challenged with several abiotic and biotic stress. A clear knowledge about the genome of barley has opened up new opportunities to focus on the various adaptive characters of the plants that can be further introgressed in other plants. Barley is one of the most Al-sensitive plant of all the cereal crops. Ma et al. (2001) reported that Al tolerance in barley is mostly attributed to the formation of organic acids like citrate and malate from root apices which further releases certain anions that can bind to Al^{3+} and reduces its availability and toxicity (Zhou et al. 2013). Earlier, Minella and Sorrells (1992) observed that the presence of *Alp* locus on the long arm of chromosome 4H controls Al tolerance in plants. They further showed that the same locus is also responsible for controlling the pH (*Pht*) tolerance in plants (Minella and Sorrells 1997). Another major stress faced by the plants is high salt concentration in soil. Saade et al. (2016) reported a wild allele on 2H chromosome that increased the yield of salt-stressed plants by 30%. Along with reducing the Na^+ uptake and compartmentalizing the same, the tolerance of barley towards salt stress is also contributed by its ability to retain K^+ ions in cells under saline condition. Ligaba and Katsuhara (2010) reported higher expression of high-affinity K^+ transporter gene (*HvHAK1*) in the root and shoot of salt-tolerant barley cultivar (K305) as compared to that of salt-sensitive variety (I743). Introgression of barley genes into other plants also induces the salt tolerance capacity of recipient plants. *HvNHX2* gene (vacuolar Na^+/H^+ antiporter) was isolated from barley and was further overexpressed in *Arabidopsis* and potato plants which resulted in higher salt tolerance level of the transgenic plants (Bayat et al. 2010, 2011). In addition, Fan et al. (2015) examined a mapping population derived from a cross between TX9425 (a drought-tolerant Chinese landrace) and Franklin (a drought-sensitive genotype). They used wilting as the trait to evaluate drought response. This work led to the identification of a quantitative trait loci (QTL) on chromosome 5H that was independent of two other developmental traits, awn length and heading date. The gene underlying this QTL was suggested to be 9-*cis*-epoxycarotenoid dioxygenase2 (*HvNCED2*), which is involved in the synthesis of abscisic acid. In addition, genetic mapping of barley identified the loci associated with freezing tolerance on chromosomes 1H, 2H, 5H and 6H (Saade et al. 2018).

21.3 Advancement in Transcriptomics

Modulation of gene expression plays a major role in the transfer of genetic information. Understanding about the level of expression and location of genes gives an insight into the functional regulation of plants at different life stages, exposed to various stressed conditions. Various tools such as gene-by-gene and global methods allow generating and mining the transcriptomics of plant species. Global methods allow for a nearly comprehensive analysis of the transcriptome, which comprise of hybridization-based (microarrays and GeneChips), sequence tag-based [EST sequencing, cDNA deep sequencing, serial analysis of gene expression (SAGE) and massively parallel signature sequencing (MPSS)] and RNA sequencing (RNA-seq) approaches. Chip-based technologies involving microarrays have become a dominant platform after EST sequencing and genome sequencing in several plant species. In spite of such large number of resources, knowledge about rye transcriptomics is highly fragmented, and more research work is required in this field to completely decipher the tolerance mechanism of rye under various abiotic stressed conditions. Sánchez-Parra et al. (2015) characterized two *superoxide dismutase* (*SOD*) genes, i.e., *ScCu/ZnSOD* and *ScMnSOD*, which are located on the 2RS and 3RL arms of the chromosome, respectively, and play a major role in inducing tolerance of plants against Al stress. Further studies demonstrated that the identified genes were orthologous to the corresponding genes of other Poaceae species. They also reported that on being exposed to Al stress, the level of above-mentioned genes was found to be more induced in Al-tolerant cultivar (Petkus), as compared to that of Al-sensitive cultivar (Riodeva). Earlier, Fontecha et al. (2007) used PCR primers designed from wheat Al-tolerant gene encoding an Al-activated malate transporter (TaALMT1) to amplify, sequence and clone an orthologous gene *ScALMT1* that was found to be localized on 7RS arm of chromosome in rye. In an another study, two isoforms of malate dehydrogenase genes (*ScMDH1* and *ScMDH2*) from three Al-tolerant rye cultivars (Petkus, Imperial and Ailes) and one sensitive variety (Riodeva) were isolated. They further demonstrated that these genes, which have seven exons and six introns, were located on the 1R (*ScMDH1*) and 3RL (*ScMDH2*) chromosomes. Exon 1 of *ScMDH1* and exon 7 of *ScMDH2* were most variable among the different ryes, and the phylogenetic relationships obtained using both cDNA and protein sequences indicated that the ScMDH1 and ScMDH2 proteins are orthologous to mitochondrial MDH1 and MDH2 proteins of different Poaceae species. Along with this, expression studies demonstrated that the amount of their corresponding mRNAs in roots from plants treated and not treated with Al was higher in the tolerant cultivar Petkus than in the sensitive inbred line Riodeva. In addition, *ScMDH1* and *ScMDH2* mRNA levels decreased in response to Al stress (repressive behaviour) in the roots of both the tolerant cultivar Petkus and the sensitive cultivar Riodeva.

Transcriptome study of oat has started very recently, and only fragmented information is available till date. Wu et al. (2017) prepared six cDNA libraries from whole plants of cultivated hulless oat under normal and salt-stressed conditions. Formed libraries were subjected to Illumina HiSeq 4000 platform, generating a total of 72,291,032 and 356,891,432 raw reads in the control and salt-treated samples, respectively. Of the entire transcript obtained, 77,246 were assigned to molecular function, 181,717 in the cellular components category and 197,926 in the biological process category. They further showed that the expression levels of 65,009 putative unigenes were significantly altered at various salt stress stages. These genes include salt sensor or signal transduction genes, salt-responsive transcription factor (TF) genes, plasma membrane stabilization-related genes, osmosensing-responsive genes and genes encoding detoxification enzymes. In a similar work, Xu et al. (2021) analysed the transcriptome profile of root sample in salt-stressed oat cultivars (BY2 and BY5). Their data showed that under 150 and 300 mM NaCl, the differentially expressed genes in BY2 were higher by 3915 and 16,076 and lower by 13,492 and 23,707, respectively, whereas the differentially expressed genes of BY5 under 150 and 300 mM NaCl were higher by 4898 and 34,040 and lower by 3414 and 14,757, respectively. Based on their observations, they concluded that most of the *BY2* genes enhanced energy consumption (e.g. glycolysis) and biosynthesis (for instance, starch and sugar metabolism) under salt stress, whereas *BY5* was found to be downregulated, leading to the inhibition of energy consumption and biosynthesis, which may also be attributed to salt sensitivity in BY5.

As compared to that of the two above-mentioned cereals, the transcriptome of barley is very well understood, and a wide range of work has been performed that elaborately decipher the adaptive nature of plants on being challenged with various abiotic stresses. In a comparative transcriptomics study, Barrero et al. (2009) showed that after ripening, embryos enhance the catabolism of abscisic acid that ultimately reduces the sensitivity level of abscisic acid in plants as compared to that of dormant embryos. They also showed that two members of lipid phosphate phosphatase family were differentially expressed in ripened embryo as compared to that of dormant one. Transcript profile of barley is significantly affected by abiotic stress. Osthoff et al. (2019) reported that on being exposed to drought or salinity or combination of both stresses, the seminal root length of the plants was reduced.

They demonstrated that the number of stress-regulated genes was significantly higher after 24 h of stress application, when compared to that of 4 h stress-treated plants (953 at 6 h and 1802 at 24 h). The most severe impact on gene expression was observed in the combined water deficit and salt treatment with 4845 differentially expressed genes at 6 h and 8105 differentially expressed genes after 24 h. In an interesting study, Janiak et al. (2018) compared drought-tolerant (CamB) and drought-susceptible (Maresi) cultivar of barley and reported that 170 and 979 genes were differentially expressed, respectively, in the roots of the above-mentioned cultivars that regulate major process such as small molecule and lipid metabolism, signal transduction, cell cycle and regulation of DNA replication and metabolism. Five genes involved in fructose metabolism and seven genes that regulate the process of myo-inositol hexakisphosphate biosynthesis were exclusively expressed in roots of Maresi cultivars. Similar to that of roots, 239 genes were found in CamB genotype only, and 908 genes were differentially expressed in Maresi genotype only in case of leaves. Gene ontology enrichment of these 1628 genes showed that they belong to several processes related to pigment metabolism, including chlorophyll metabolic processes, in response to light and energy generation, ROS metabolism, transport (ion or monovalent inorganic cation transport, mitochondrial transport or protein localization in organelle), reproduction, morphogenesis and organelle organization, RNA metabolism and other metabolic processes, such as carboxylic acid metabolism, secondary metabolic process or lipid and small molecule metabolic processes. Thus, based on their results, they hypothesized that CamB exhibited a “stressed-like” transcriptome that was active already under optimal water conditions, and this genotype does not need to initiate expression changes to such a degree as the drought-sensitive cultivar, when stress occurred. Earlier, Tommasini et al. (2008) reported the alteration in the transcriptome of barley on being exposed to drought and low temperature. Three thousand three hundred thirty and 3757 genes were differentially expressed in drought and low-temperature stressed barley seedlings, respectively, as compared to that control seedlings. In total, 2207 genes were upregulated, and 1497 genes were downregulated by drought, whereas 1268 genes were induced and 1075 genes were downregulated in response to chilling stress. Thus, based on these studies, it can be inferred that barley transcriptome is one of the widely studied and well-characterized transcripts of all the cereals which provide a great insight into the stress tolerance mechanism of plants.

21.4 Advancements in Proteomics

In recent time, proteomics have gained popularity over genomics and transcriptomics due to their direct correlation with biochemical processes, facilitating the study of post-translational modification in plants. Proteomics refer to a vast range of study comprising of structural and functional features of a set of protein present in organism. In spite of advancement of technology, research work focusing the proteomics of rye is very limited, and no such advancement has been done in this field. Masojc and Kosmala (2012) showed the qualitative and quantitative difference between two-dimensional electrophoresis spectra of 546 proteins isolated from resistant preharvesting sprouting and susceptible preharvesting sprouting cultivars of rye. Their analysis of mass spectrometry of resolved proteins showed that four spots, specific for post-harvesting sprouting susceptibility, represented high molecular weight glutenin subunit, glutathione transferase, 16.9 kDa heat-shock protein and monomeric α-amylase inhibitor. Two spots, specific for post-harvesting sprouting resistance, contained cytosolic malate dehydrogenase and functionally unrecognized protein with sequence homology to rubber elongation factor protein. Majority of 14 proteins with at least twofold higher accumulation level in preharvest sprouting-susceptible lines, relative to that found in sprouting-resistant lines, showed sequence homology to proteins involved in defence mechanisms against biotic and abiotic stresses including oxidative stress. Two spots were identified as regulatory proteins from the 14-3-3 family with one molecular form prevailing in sprouting-susceptible and another form highly accumulated in sprouting-resistant lines. Leaving this aside, no significant work has been done in this regard, and in the coming future, more research works need to be conducted in this field to decipher the correlation between the proteome changes that occur in plants in response to abiotic stresses.

Proteomics study on oats is very scanty and has started only in this decade. There is only handful of reports which elaborately shows the alteration in the proteome of oats on being exposed to abiotic stressed condition. Chen et al. (2016) recently performed a two-dimensional gel electrophoresis and detected 94 and 32 different protein spots from oat seeds on being exposed to 10% and 16% moisture content, respectively, in the presence of heat stress. They further reported that most of the proteins detected are weakly acidic and their size ranges from 10 to 20 kDa that plays a major role in deteriorated seeds. Protein turnover, chaperones, post-translational modification, carbohydrate and nucleotide metabolism and transport, and translational and ribosomal structure and biogenesis were assigned as the possible functions controlled by the isolated protein in the seeds exposed to 10% moisture content, whereas for the proteins isolated from seeds exposed to 16% moisture content, the major functions assigned were those involved in amino acid metabolism and transport, chaperone activity, protein turnover, energy conversion and production and post-translational modification. Similarly, Bai et al. (2016a, b) reported the differential expression of 23 proteins in salt-stressed oat seedlings. Of all the proteins identified, the level of nine proteins (one lipase, one choline monooxygenase, one enolase, two fructose-bisphosphate aldolases and four alcohol dehydrogenase) was enhanced, whereas the level of four proteins (one polyubiquitin, one F1-ATPase alpha subunit, one 14-3-3 protein and one UDP-glucuronic acid decarboxylase) was found to be downregulated in response to salt stress. They also analysed the proteome of the leaves of salt-stressed (150 mmol L^{-1} NaCl in Hoagland solution for 16 days) oat seedlings and determined 30 differentially expressed proteins that range from 20.1 to 66.2 kDa and were located in a pI range of 3–10 (Bai et al. 2016b). Of all the identified proteins, only the level of one protein was upregulated, whereas the level of other 29 proteins was downregulated. In a recent report, Zhao et al. (2019), using tandem mass tag-based proteome analysis, elucidated that 164 and 93 proteins were upregulated and 241 and 139 proteins were downregulated in the roots and shoots of alkali stress-exposed oat seedlings. Using Kyoto Encyclopedia of Genes and Genome pathway analysis, they showed that the proteins in roots were mostly involved in carbon metabolism and fixation, phenylalanine and glutathione metabolism, and photosynthesis and phenylpropanoid biosynthesis after 16 h of treatment, whereas on being exposed for 96 h, the induced proteins were shown to be involved in amino acid, starch and sucrose biosynthesis and metabolism, nitrogen metabolism, processing of protein in the endoplasmic reticulum, mannose and fructose metabolism and oxidative phosphorylation in roots.

The proteome of barley is one of the most widely and elaborately studied proteomes of all the cereals belonging to Poaceae family. Various studies have widely demonstrated the alteration in the proteome of barley on being exposed to abiotic stressed conditions like cold, drought, salinity and hypoxia. Hlaváčková et al. (2013) reported that the expression level of 90 and 63 proteins were changed in crown and leaves, on being exposed to cold stress. Of all the proteins identified, only glutamine synthetase was identified in both the tissues whose expression was downregulated in response to cold stress. In another study, Wendelboe-Nelson and Morris (2012) analysed the proteome of drought-tolerant (Basrah) and drought-sensitive (Golden Promise) cultivar of barley using DIGE. They identified 295 and 323 differentially expressed proteins in leaf and root samples, respectively. However, in comparison to that of control plants, only 66 and 77 proteins were differentially expressed in the leaves and roots. Similarly, Ghabooli et al. (2013) reported that inoculation of barley plants with *Piriformospora indica* resulted in significant difference in the expression patterns of 62 proteins, as compared to that of non-inoculated stressed plants. The identified proteins regulated some major pathways in plants like energy metabolism, stress or defence response, protein synthesis and maturation, signalling and photosynthesis. Witzel et al. (2014) exposed barley seedlings to various levels of salt stress and analysed the proteome of stressed plants using two-dimensional gel electrophoresis. After mass spectrometry, they identified 74 proteins that were differentially expressed in two contrasting cultivars of barley. Earlier, Fatehi et al. (2012) compared the proteomes of two barley cultivars, i.e., salt-tolerant (Afzal) and salt-sensitive (Line 527), and reported differential expression of 44 spots, out of which 43 spots were upregulated, whereas 1 spot was downregulated. Of the 44 spots, 18 different proteins were identified which were further classified into seven different groups. Another interesting work was elucidated by Luan et al. (2018) where they compared the proteomics profile of two contrasting varieties, i.e., waterlogging-sensitive (TF57) and waterlogging-tolerant (TF58) cultivars of barley. Stressed conditions led to 20, 20, 30 and 30 differential expressed proteins in nodal roots, seminal roots, leaves and adventitious roots, respectively. Of the entire expressed proteins, photosynthesis and energy-related proteins were differentially expressed in the leaves.

21.5 Advancement in Metabolomics

Metabolomics studies provide a vast aspect of all the metabolites that are present in target organism or any specific tissue. Metabolomics along with various statistical and computational methods can provide elaborate information about the low molecular weight metabolites formed in plants in response to stressed environment. Metabolomics is an emerging field, which has been used to assist in the biochemical analysis of complex mixtures and considered a robust, sensitive and powerful technology (Nakabayashi and Saito 2013). Fukusaki and Kobayashi (2005) explained the technical elements, statistical analysis and practical applications, while Putri et al. (2013) elaborated on the latest developments in analytical methods and data analysis in the metabolomics area. Being an emerging technology, lesser information is available about the metabolomics profile of plants. Few reports have demonstrated the metabolomics profile of rye. In a study conducted by Busko et al. (2010), the volatile metabolites in cereals like durum wheat, spring wheat, rye, barley, oats and triticale were analysed. Of all the mentioned species, the level of volatile metabolites, i.e., 10 ketones and aldehydes, 6 terpenes, 11 benzene derivatives, 6 alcohols and 7 hydrocarbons, was the lowest in rye, whereas the level of linolenic acid was the highest in rye. They further showed that dimethyl sulfone can be regarded as the characteristic compound of rye. Apart from this, no reports are available which demonstrate the metabolites present in rye plants, and in the coming future, more research work needs to be conducted in this area to identify the quality and quantity of small metabolites generated by plants in response to abiotic stress.

As compared to that of rye, more research works have been conducted on the metabolome profile of oat; however, the work done till date is mostly in initial phase, and more work is required to fill the vast gap of knowledge. Henson et al. (2014) investigated the metabolic changes in oat plants in response to freezing stress. They reported that freezing stress induces the formation of most of the amino acids (significant increment was noted in the levels of proline, arginine and 5-oxoproline) in the crown of stressed plants, as compared to that of control plants. Contrasting results were observed in case of sugar and related metabolites where no significant change was noted; rather, the level of sucrose, fructose, citrate and malate was drastically reduced which can be linked with cold-susceptible nature of the plants. Earlier, Sánchez-Martín et al. (2015) compared the metabolomics profile of drought-tolerant (Patones) and drought-susceptible (Flega) cultivars of oat on being exposed to limited water conditions. They showed that accumulation of salicylic acid in the plants regulated the stomatal opening, antioxidative defences and photorespiration which maintained the water status of the drought-tolerant variety. Recently, Xu et al. (2021) reported that salt stress induces the level of sucrose, sophorose and isomaltose in the tolerant cultivar (Baiyan 2), whereas the level of these protective metabolites was reduced in the susceptible variety (Baiyan 5). The level of most of the amino acid and saccharides was induced in both the cultivars on being exposed to 300 mM NaCl stress. Another important stress most commonly encountered by the plants is the deficiency of macronutrient. One such macronutrient is phosphorus. Wang et al. (2018) showed that deficiency of phosphorus in oat plants enhances the release of citrate and malate from the roots of the plants. The level of phosphorylated metabolites such as myo-inositol phosphate and glucose-6-phosphate was drastically reduced in plants, whereas the level of malate, citrate, sugar and amino acid was slightly induced in the roots of phosphorus-stressed plants. An interesting work was conducted by Pretorius et al. (2021) where they differentiated five different cultivars (Magnifico, Dunnart, Pallinup, Overberg and SWK001) based on their key signatory metabolic marker which included carboxylic acids, amino acids, fatty acids, phenolic compounds (hydroxycinnamic and hydroxybenzoic acids and associated derivatives) and flavonoids, inferring that metabolomics can be used as a rapid tool for differentiating different phenotypes of oat cultivars.

As compared to that of other cereals, the metabolome profile of barley is widely studied, and a large number of works have been done in the past focusing on this area. Recently, Zhao et al. (2021) analysed the primary metabolites present in barley at the seedling stage in response to seven abiotic stresses, i.e., drought, salinity, Al toxicity, Cd toxicity and N, P and K deficiency. Based on their observation, they reported that metabolites like raffinose, polyamines and pipecolic acid showed a significant change in their level in response to all abiotic stresses, whereas certain metabolites such as proline were prominently increased during drought and salt stress. The levels of P-containing metabolites and amino acids (threonine, arginine, lysine, glutamine and ornithine) were reduced in P- and N-deficient plants, respectively. Earlier, Yuan et al. (2018) analysed the time course metabolomics profile of osmotic stress-tolerant and stress-susceptible varieties of barley seedlings. They reported that osmotic stress induces the formation of several metabolites (adenosine 3′-monophosphate, iP7G, adenosine 5′-monophosphate, uridine 5′-diphospho-D-glucose, A-nicotinamide mononucleotide, uridine 5′-diphosphate and 2-′-deoxyadenosine-5′-monophosphate) at a time course of 8 h in the tolerant cultivar, as compared to that of sensitive variety. Thus, 8 h was considered as a critical time for stress endurance in plants. The level of several other metabolites such as *C*-hexosyl-apigenin *O*-pentoside, gentisic acid, methyl jasmonate, 4-hydroxy-7-methoxycoumarin-beta-rhamnoside, di-*C*,*C*-hexosyl-apigenin, afzelechin, 6-*C*-hexosyl luteolin *O*-pentoside, 2,3-dihydroxybenzoic acid, hesperetin *O*-malonylhexoside, *N*-*p*-coumaroylhydroxydehydroagmatine, eudesmic acid and luteolin *O*-hexosyl-*O*-gluconic acid were downregulated. The levels of metabolites such as hesperetin 5-*O*-glucoside, delphinidin 3-*O*-glucoside, *N*-feruloyltryptamine, tricin 7-*O*-feruloylhexoside, isotrifolin, *N*-acetyl tryptamine, tricetin, selgin 5-*O*-hexoside, *O*-feruloylcoumarin and spiraeoside were upregulated in response to osmotic stress in the tolerant variety in due time course. Earlier, Cao et al. (2017) reported that in response to salt stress, the level of seven amines and amino acids, i.e. arginine, citrulline, glutamine, proline, phenylalanine, 4-hydroxyproline and asparagine, was increased in all the barley cultivars. On being exposed to low potassium level, 57 metabolites were identified in the leaves and roots of two wild and one domesticated barley cultivars. In general, the level of sugars and amino acids and the level of organic acids were upregulated and downregulated, respectively, in the stressed plants, as compared to that of control plants (Zeng et al. 2018). An interesting result was published by Shelden et al. (2016) where they analysed the metabolomics profile from the elongation zone, maturation zone and root cap/cell division zone of roots of salt-stressed barley. They isolated 76 metabolites which comprise 29 amino acids, 19 sugar and sugar phosphates and 20 fatty acids and organic acids. They further observed that the enhanced level of amino acids (proline), organic acids (shikimate and gluconate) and sugars (sucrose, xylose and maltose) contributes towards the higher tolerance level of the tolerant cultivar (Clipper), as compared to that of the susceptible variety (Sahara). Thus, a large number of works have been done in recent times regarding the metabolome profile of barley, and the knowledge obtained from these reports can be further utilized in future for development of more robust plants that can survive under harsh environmental conditions.

21.6 Conclusion

The advancement of next-generation sequencing technologies has opened up a wide scope of research which would allow researchers to develop improved quality and quantity of seeds. In recent times, large amount of unique and stress-related traits has been identified in cereals such as rye, oat and barley, but very few of them have been incorporated in the breeding programmes. The negative correlation, marginal improvement and low stability across different geographical locations have undermined the development of cultivars with high meal quality, oil and protein content and yield. As evident from the above-mentioned works, advancements in the "omics" technologies such as genomics, transcriptomics, proteomics and metabolomics need to be integrated in the coming future for betterment of cereals that will ultimately result in higher yield. To identify the changes occurring in plants in response to abiotic stress, various omics tools have been devised that help us to understand in details the genetic make-up, signalling cascade and adaptability of plants under stress conditions. In barley, rye and oat, mostly genomics and transcriptomics have been developed for abiotic stress, but researches in other major branches like proteomics and metabolomics are as of yet lingering behind. Diverse studies of omics tools and integrated approaches discussed in this chapter point out regarding the current situations of abiotic stress tolerance in rye, oat and barley cultivars, and such aspects need to be exploited in susceptible species for crop improvement.

Acknowledgements Financial assistance from Science and Engineering Research Board, Government of India, through the grant [EMR/2016/004799] and the Department of Higher Education, Science and Technology and Biotechnology, Government of West Bengal, through the grant [264 (Sanc.)/ST/P/S&T/1G-80/2017] to Dr. Aryadeep Roychoudhury is gratefully acknowledged.

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22

Genomic Tools and Proteomic Determinants for Abiotic Stress Tolerance in Pearl Millet (*Pennisetum glaucum*) and Foxtail Millet (*Setaria italica* L.)

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and Manoj Prasad

Abstract

Global warming and its subsequent consequences are continuously alarming us to explore alternative food sources and swift the pace of designing climate smart existing staple food crops. Being an underutilized crop, pearl millet (*Pennisetum glaucum*) and foxtail millet (*Setaria italica* L.) are well known for their exceptional environmental stress tolerance and high nutritive value. These crops are grown for food and fodder in arid and semi-arid lands with very low water and nutrient availability where other major cereals fail to grow due to their luxurious requirements. High-throughput genomic and proteomic approaches are gaining more attention to understand the molecular mechanisms conferring stress tolerance in the present scenario. Due to the availability of reference genome, structural and functional genomic studies have made significant progress in both pearl and foxtail millets. Development of genomic tools including DNA-based markers and establishment of genomics-based databases, genome-wide association studies (GWAS) and global transcriptomic and proteomic studies are a prerequisite to identify genetic determinants of abiotic stress tolerance. Once identified, the genetic determinants controlling abiotic stress tolerance can be either introgressed into the susceptible elite cultivar through genomic-assisted breeding or modified through genome editing and overexpression/silencing approach. Apart from this, they can be served as an excellent source of natural gene banks and superior alleles for creating climate-resilient stable crops like rice and wheat. Considering this, the present chapter discusses the available genomic tools and proteomic advancements in pearl and foxtail millet concerning their abiotic stress tolerance.

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_22

Keywords

Foxtail millet · Pearl millet · Abiotic stress · Genomic resources · Crop improvement · Proteomic analysis · Genomic database

22.1 Introduction

Increasing global temperatures, limited availability of irrigation water and increasing soil salinity are unfavourable climatic conditions in which major staple food crops like wheat, rice and maize fail to grow resulting in compromised crop yields. Ray et al. (2019) evaluated the impact of climate change on ten consumable crops and showed that the average reduction on consumable calories is 3.5×10^{13} kcal/year, which clearly indicates the adverse effect of climate change on crop production. To combat the adverse climatic conditions and achieve the food demands of constantly increasing global population, an urgent need arises for designing climate smart crops with increased productivity (Roychoudhury et al. 2008).

Millets, being an underutilized crop plant, become much important in this scenario. Millets are climate-resilient C_4 panicoid nutricereals, require very less water and almost negligible fertilizer input and are grown for food, fodder and biofuel production in arid-semi-arid regions. Millets exhibit various morphological, physiological, biochemical and molecular properties which help them to withstand various environmental stresses such as heat, drought, salinity and intense light compared to other cereals (Li and Brutnell 2011). Presence of relatively short life cycle (12–14 weeks), short plant height, thickened cell wall and profuse adventitious root systems are important physiological adaptations of millets. Presence of Kranz type of anatomy which is a prerequisite for C_4 photosynthesis makes these crop species efficient CO_2 assimilators even at high temperature and low CO_2 concentration (Li and Brutnell 2011). It is also well established that C_4 plants have improved water and nitrogen use efficiency (WUE and NUE) than C_3 plants (Sage and Zhu 2011). These properties make millets important model crops for exploring the climate-resilient attributes and imply the knowledge for the improvement of cereal crops.

Foxtail millet (*Setaria italica*) and pearl millet (*Pennisetum glaucum*) are two major millets grown and are used as C_4 model crops for abiotic stress-related researches (Lata 2015). Studies have been done to elucidate the abiotic stress tolerance of these millets, for example, increase in antioxidant levels, osmolytes and stress-related proteins has been observed during abiotic stress response in foxtail millet (Lata et al. 2011). Several molecular studies including identification and characterization of novel genes, alleles and QTLs have become possible recently due to the development of genomic resources for these millets. The availability of whole-genome sequence and the comparisons of gene families with another plant species revealed unique genes found in these millets only and are involved in providing abiotic stress tolerance (Bennetzen et al. 2012; Zhang et al. 2012; Varshney et al. 2017). Genome-wide, transcriptomic analysis and proteomic studies help in identification of novel genes and proteins of interest (Yue et al. 2014; Chanwala et al. 2020; Sun et al. 2020; Yu et al. 2020). Establishment of public databases related to foxtail and pearl millet helped scientific community and shifted the pace of genomic research in these two model crops (Muthamilarasan and Prasad 2015). Keeping this in mind, in the present chapter, we will discuss about genomic and proteomic resources developed in foxtail and pearl millet in response to abiotic stress. It is also discussed how we can apply this knowledge in crop improvement in other millets and non-millets against adverse climatic conditions.

22.2 Origin of Genomic Diversity in Pearl and Foxtail Millet

Origin of millet domestication from northern China is generally accepted; however, there is no clear record whether foxtail or pearl millet was the first millet species to be domesticated and diverged (Lu et al. 2009). The husk phytoliths and molecular components of pearl millet have been reported from Neolithic fossils of Cishan site of north China and were claimed to be older as ca. 10,300 and ca. 8700 cal year BP (calibrated years before present) (Lu et al. 2009). Foxtail millet seems to appear a bit late as its domestication is reported back to ca. 7400 and ca. 6000 in Yellow River Valley and some parts of northern China from its wild ancestor green foxtail (*Setaria viridis*) (Doust et al. 2009; Wang et al. 2018a). According to the most recent report, mixed agriculture of millets with rice from Zhuzhai site Yellow River Valley of northern China was practiced around 8000 years ago (Wang et al. 2018a). Subsequently, pearl millet and foxtail millet spread as major millets across semi-dry and dry lands of India and China and in entire Asian, African and American subcontinents as the two most cultivated millets (Muthamilarasan and Prasad 2015). Both pearl and foxtail millets are classified in the family Panicoideae under the tribe Paniceae with 140 and 125 species, respectively (Muthamilarasan and Prasad 2015; Shivhare and Lata 2017). The species are diverse in reference to their reproductive behaviour and ploidy level in both the genera *P. purpureum* Schumach (Napier grass) ($2n = 4x = 28$), *P. americanum* (syn. *P. glaucum*) ($2n = 2x = 14$), *S. viridis* ($2n = 2x = 18$), *S. faberi* ($2n = 4x = 36$) and *S. pallide-fusca* ($2n = 8x = 72$) (Brunken et al. 1977; Kawano and Miyake 1983). While most of the genera are annual in nature, some are known to exhibit perennial life cycle, for example, *P. purpureum* Schumach and *S. parviflora*. The green foxtail millet [*S. viridis* (L.) P. Beauv.] has been predicted to be a wild ancestor of foxtail millet, *S. italica*. Both green foxtail (*S. viridis*) and foxtail millets (*S. italica*) are native to Eurasia, whereas green foxtail has been globally distributed. Single-nucleotide polymorphisms (SNPs)-based population structure and genetic diversity studies of green foxtail collected from North America reveal their several invasions into North America from different gene pools of China, Central Asia and Europe. The origin of genetic diversity in foxtail millets still needs to be more enlightened through further research.

22.3 Status of Germplasm Collection for Pearl and Foxtail Millet

Germplasm refers to the overall genetic material transmitted to the offspring and serves as the basic material to conduct crop improvement programmes. Germplasm collection aims to preserve the genetic diversity that can be harnessed in the future for crop improvement programmes. A large number of wild and cultivated germplasm accessions of pearl and foxtail millet are stored in various gene banks across the world. The Rockefeller Foundation of the USA was the pioneer in the area of collecting germplasms of minor millets which dates back to the early 1970s. Currently, more than 12,000 pearl millet and 47,500 foxtail millet accessions are being preserved in different countries and made available for breeders (Singh et al. 2021). With a total of 11,243 wild-type and cultivated accessions, India is the largest repository of pearl millet accession followed by 7225 accessions in Brazil and 3764 accessions in Canada. The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) together with the National Bureau of Plant Genetic Resources (NBPGR), National Agricultural Research Systems (NARS) and All India Coordinated Pearl Millet Improvement Project (AICPMIP) has taken the initiatives of repositioning indigenous and global accessions of pearl millet in India. Preservation of wild relatives of any crop species is crucial as it can serve an important source of gene pools that might have lost following breeding cycle and artificial selection. The ICRISAT has also maintained the wild relatives of pearl millets available from 51 different countries (Upadhyaya et al. 2017). In case of foxtail millet, with more than 26,000 accessions, China has the largest collection followed by India (8506), France (3500) and Japan (2531). The germplasms of the genus *Setaria* deposited in various institutes of India represent the following species: *S. italica*, *S. viridis*, *S. chevalieri*, *S. pumila*, *S. incrassata*, *S. australiensis*, *S. neglecta*, *S. verticillate*, *S. sphacelate*, *S. parviflora*, *S. lachnea* and *S. sphacelate*. Majority of the foxtail millet accessions (approximately 90%) are either traditional cultivars or landraces of *S. italica*, while the rest are wild varieties. There is a lack of collection of improved, biofortified and genetically modified varieties. Table 22.1 summarizes the status of pearl and foxtail millet germplasm collections around the world.

22.4 Genomic Resources

22.4.1 Genome Sequencing

Genome research in millets started in parallel to other cereals but then lagged behind because the emphasis of most genomic studies was shifted to model plants and major crops. However, last few years witnessed acceleration in the genomic insights in these millets because of availability of their whole-genome sequence. Whole genome of foxtail millet has been sequenced by Beijing Genomics Institute, China (Zhang et al. 2012), and the US Department of Energy Joint Genome Institute (Bennetzen et al. 2012) under two independent projects. In their study, Zhang et al. (2012) showed that the 423 Mb of foxtail genome is anchored within

Table 22.1 Status of pearl and foxtail millet germplasm collection across the globe

Country	Institute	Number of germplasm accessions
Pearl millet		
India	ICRISAT, National Bureau of Plant Genetic Resources, AICRP on Small Millets	11,243
Brazil	Embrapa Milho e Sorgo, Sete Lagoas	7225
Canada	Plant Gene Resources of Canada, Saskatoon Research and Development Centre	3764
Foxtail millet		
China	Institute of Crop Science, Chinese Academy of Agricultural Sciences	26,233
India	National Bureau Plant Genetic Resources (NBPGR), AICRP on Small Millets, ICRISAT	8506
France	Laboratoire des Ressources Génétiques et Amélioration des Plantes Tropicales (ORSTOM-MONTP)	3500
Japan	Department of Genetic Resources I, National Institute of Agrobiological Sciences	2531

Source: http://www.fao.org/wiews-archive/germplasm_query.htm

9 chromosomes of foxtail and majorly consists of transposable elements (196.6 Mb), 1344 copies of noncoding genes and 38,801 protein encoding genes. Out of the 38,801 protein encoding genes, 32,701 genes were showing homology with another millet, viz., *Sorghum*. They further demonstrated that major chromosome reshuffling events are cause of genomic relatedness of rice and sorghum with foxtail millet. By using this genome sequence, they identified rearrangements in carbonic anhydrase gene family, which plays a crucial role in the CO_2-concentrating mechanism of C4 photosynthetic plants. Along with *S. italica*, Bennetzen et al. (2012) also sequenced its wild relative, *S. viridis*, and defined the SNP regions, transposable elements, small RNAs and their chromosomal distribution. They compared foxtail with switchgrass and depicted their close phylogenetic relationship.

The whole-genome sequence of pearl millet became available very recently due to the efforts of scientists of ICRISAT India, China and the USA (Varshney et al. 2017). The draft genome sequence of pearl millet reference genotype Tift 23D2B1-P1-P5 was found to be ~1.79 Gb long and exhibit 2079 noncoding genes and 38,579 protein encoding genes. Additionally, they highlighted the importance of genes involved in wax biosynthesis as they are considered as potential heat and drought tolerance regulators in this crop. So, the availability of reference genome sequence for foxtail and pearl millet provided a base for molecular studies to define the stress tolerance mechanisms involved in these crops.

22.4.2 Transcriptomic Analysis

Transcriptomic analysis, also known as RNA-seq analysis, is done for quantification of a set of protein-coding RNAs (mRNA) present in a cell at a given condition. Transcriptomic studies are widely performed to compare changes in mRNA levels in stressed and control plants which in turn give an idea about proteomic changes and hence developmental and physiological adjustments of plants to overcome stress conditions. In transcriptomic studies, the starting material is a gene transcript that is mRNA. The total RNA is isolated from control and stressed tissue and then reverse transcribed to form cDNA. cDNAs are then sheared to small fragments and ligated with adapters to create the sequencing library. Further, cDNA fragments are sequenced by synthesis with reversible terminators with the use of next-generation sequencing platforms. The raw sequencing data obtained is processed and analysed systematically with the help of a pipeline as discussed in Fig. 22.1.

Since the pearl and foxtail millets are best known for their abiotic stress tolerance, transcriptomic studies are carried out to undermine the same. Zhang et al. (2021a, b) tried to explain the drought tolerance mechanism of pearl millet by performing the transcriptomic analysis of drought treatment of roots at 3 different time points and showed that transcript abundance of 12 genes were higher at stress condition, as compared with control plants. Further, by using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) resource database, they showed that some of these differentially expressed genes (DEGs) belong to mitogen-activated protein kinase (MAPK) and abscisic acid (ABA) signalling pathway. Another study in pearl millet during heat and drought stress suggests differential expression of transcription factors (TFs) and transcriptional regulators (TRs) and more than 6000 genes related to photosynthetic and glycerophospholipid metabolic pathway; about 1881 genes were found to be involved in both heat and drought stresses (Sun et al. 2020). Transcriptomic analysis of PRLT2/89-33, a drought tolerant pearl millet genotype at both early vegetative and late flowering stages, showed its drought sensing ability at both developmental stages. GO and MapMan pathway analysis showed enrichment DEGs to lignin and secondary metabolite synthesis pathways (Shivhare et al. 2020).

There are few transcriptomic study reports in foxtail millet regarding abiotic stress. Tang et al. (2017) identified two foxtail millet cultivars, ‘Yugu 1’ and ‘An04’; ‘Yugu 1’ showing drought tolerance and ‘An04’ were identified as drought-sensitive. The transcriptomic analysis of these two cultivars under control and drought stress revealed that there were 9652 genes showing differential expression of genes that can be associated with altered biological functions in response to drought such as transport of ions, synthesis of secondary plant metabolites, vesicular transport, formation of intrinsic plant proteins and major carbohydrates, photosynthesis, ATP synthesis and transcriptional regulations through transcription factors (Tang et al. 2017). Transcriptomic studies in two Indian origin foxtail cultivars, cv. Prasad (tolerant) and cv. Lepakshi (sensitive), under control and polyethylene glycol (PEG)-induced dehydration stress revealed 327 uniquely expressed sequence tags (ESTs). On the basis of their putative functions, majority of these ESTs were

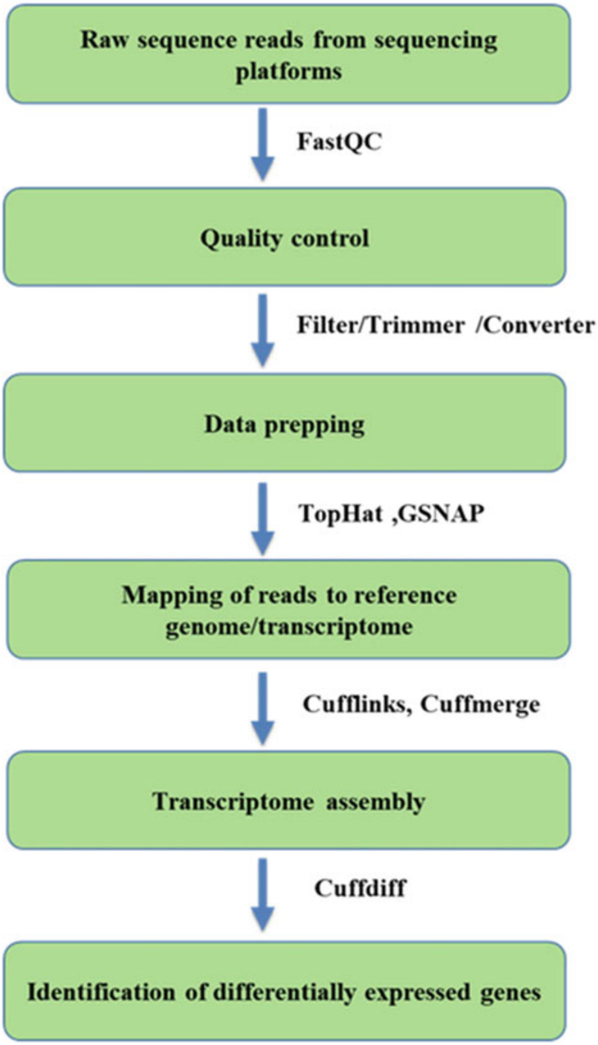

Fig. 22.1 Represents the flow diagram of transcriptomic study for identification of DEGs. After getting the reads from sequencing server, raw sequences are converted into FastQC format for assessment of read quality and quality filtering. After quality control, reads are mapped to reference genome with the help of softwares like TopHat and GSNAP followed by assembly of transcriptome and identification of DEGs using cufflinks, cuffmerge and cuffdiff

found to be in relation with metabolic process, stress signalling and transcriptional and translational regulation (Lata et al. 2010). Comparative transcriptomic analysis of another transcriptomic study demonstrated the involvement of phenylpropanoid biosynthetic pathways during drought and dehydration stress in foxtail millet seed germination process (Yu et al. 2020). Transcriptomic response of salt-tolerant cultivar ‘Chigu 10’ and salt-sensitive cultivar ‘Fenghong 3’ suggested 4090 genes involved in salinity response (Zhang et al. 2021b). They have also elucidated that two of the salinity-responsive genes, *SiDRM2* and *SiKOR1*, have significant effect upon seed germination during salinity stress (Zhang et al. 2021b). Hence, the transcriptomic studies in these millets help in the identification of different abiotic stress-responsive gene families and also suggest some critically important genes which can be further characterized to elucidate their contribution in abiotic stress tolerance.

22.4.3 Genome-Wide Analysis of Abiotic Stress-Responsive Gene Families

Genome-wide analysis is a method for identification and elucidation of gene family members in an agronomically important crop species. Unlike the transcriptomic studies, where the starting material is RNA which is reverse transcribed to cDNA and then sequenced and mapped to reference genome, it is performed through an entire genomic survey of a species by using different publicly available genomic databases and bioinformatics tools. Using in silico approaches for the identification of genes, genome-wide studies are less expensive when compared to transcriptomic studies. It gives an information about the members of a gene family, their classification, chromosomal distribution, structure and phylogenetic relationship with different plant lineages (Muthamilarasan et al. 2015; Singh et al. 2016). A researcher retrieves the protein (domain) sequence of a gene family from different plant species through publicly available databases such as The Arabidopsis Information Resource (TAIR), pearl millet genome database, Oryzabase, Phytozome, National Center for Biotechnology Information (NCBI), etc. which is followed by analysis of gene structure, chromosomal location and genomic distribution of a gene family through physical mapping, phylogenetic analysis and Gene Ontology annotations. Differential expression profiling of identified genes, for example, tissue-specific and under varying stress conditions, is done by using publicly available RNA-seq data to further narrow down the hunt for candidate gene. RNA-seq and q-PCR are in vitro studies performed for experimental validation of deferential expression of genes (Chanwala et al. 2020; Yu et al. 2021). Further, genes which are found to be differentially regulated are chosen to dissect their role and applicability against different stress conditions. As discussed earlier, these two millets are an excellent source of genomic variation; the genome-wide analysis of different stress-responsive gene families becomes more promising towards the identification of crucial candidate genes which can be further validated (Feng et al. 2015; Singh et al. 2019). Table 22.2 summarizes the available reports about genome-wide study of major environmental stress-responsive gene families in pearl and foxtail millet. As the reports claim, identified genes through genome-wide studies have shown differential expression when subjected to abiotic stress conditions which suggests their potential role in providing abiotic stress tolerance. Despite this, very few of them are functionally characterized using molecular approaches. Further, identification of more abiotic stress-responsive gene families will help us to elucidate potential candidates of abiotic stress responses in these two millet species.

Table 22.2 List of major stress-responsive gene families described in pearl and foxtail millet through genome-wide studies

Gene family	No. of members identified	Crop	Nature of abiotic stress	Differential expression during stress	References
WRKY	97	*Pennisetum glaucum*	Dehydration, salinity	Validated through qRT-PCR	Chanwala et al. (2020)
	105 and 45	*S. italica* and *S. viridis*	Dehydration, salinity, hormonal stress (ABA, SA, JA)	Validated through qRT-PCR	Muthamilarasan et al. (2015)
NAC	151	*Pennisetum glaucum*	Drought, salinity	Validated through qRT-PCR	Dudhate et al. (2021)
	147	*S. italica*	Dehydration, salinity, hormonal stress (ABA, SA, JA)	Validated through qRT-PCR	Puranik et al. (2013)
SQUAMOSA promoter binding (SPB) protein-like gene family	18	*Pennisetum glaucum*	Dehydration, salinity, ABA	Validated through qRT-PCR	Yu et al. (2021)
MADS-box genes	10	*S. italica*	Dehydration, salinity, hormonal stress (ABA, GA)	Validated through qRT-PCR, overexpression of SiMADS51gene in *Arabidopsis* and rice	Zhao et al. (2021)
bHLH	149	*S. italica*	Drought	Validated through qRT-PCR	Wang et al. (2018b)
Heat shock proteins (HSP)	113	*S. italica*	Heat, salinity, dehydration	Validated through qRT-PCR	Singh et al. (2016)
ATG gene family	37	*S. italica*	Drought, salinity, cold, light, hormone treatment, nitrogen starvation	Validated through qRT-PCR	Li et al. (2016)

(continued)

Table 22.2 (continued)

Gene family	No. of members identified	Crop	Nature of abiotic stress	Differential expression during stress	References
Calcium-induced protein kinases (CIPKs)	35	*S. italica*	Dehydration, cold, ABA	Validated through qRT-PCR	Zhao et al. (2019)
WD40 gene family	225	*S. italica*	Dehydration, salinity, cold, ABA	Validated through qRT-PCR	Mishra et al. (2014)
C_2H_2 zinc finger domain containing gene family	124	*S. italica*	Dehydration, salinity, cold	Validated through qRT-PCR	Muthamilarasan et al. (2014a)
MYB genes	209	*S. italica*	Dehydration, salinity, hormonal stress (ABA, SA, JA)	Validated through qRT-PCR	Muthamilarasan et al. (2014b)
SET domain-containing genes	53	*S. italica*	Dehydration, cold, salinity, hormonal stress (ABA, SA, JA)	Validated through qRT-PCR	Yadav et al. (2016b)
Aquaporins	49	*S. italica*	Dehydration, heat, salinity, hormonal stress (ABA, SA, JA)	Validated through qRT-PCR and yeast overexpression of *SiPIP3;1* and *SiSIP1;1* genes	Singh et al. (2019)
AP2/ERF	171	*S. italica*	Drought, salinity, ABA	Validated through qRT-PCR	Lata et al. (2014)
Superoxide dismutase (SOD)	8	*S. italica*	Drought, salinity, cold	Validated through qRT-PCR	Wang et al. (2018c)
LIM family genes	10	*S. italica*	Dehydration, salinity, pH, hormone treatment (ABA, SA, JA, GA)	Validated through qRT-PCR and transgenic overexpression of *SiWLIM2b* gene in rice	Yang et al. (2019)
NF-Y	39	*S. italica*	Drought, salinity, ABA, osmotic and oxidative stress	Validated through qRT-PCR and overexpression of *SiNF-YA1* and *SiNF-YB8* genes in tobacco	Feng et al. (2015)

HD-Zip	47	*S. italica*	Dehydration, salinity, ABA	Validated through qRT-PCR	Chai et al. (2018)
DNA binding with one finger motif (Dof) proteins	35	*S. italica*	Dehydration	Validated through in silico expression analysis	Zhang et al. (2017)

ABA abscisic acid, *JA* jasmonic acid, *SA* salicylic acid

22.4.4 Identification and Characterization of Small RNAs and lncRNAs

Small RNAs (s-RNAs), as the name suggests, are small (21–24 nucleotide), single-stranded (ss) RNA sequences. On the basis of their biogenesis mechanisms, small RNAs are categorized into micro-RNAs (miRNA) and small interfering RNAs (si-RNA), and are not able to code for any protein. Instead, they are known to regulate the expression pattern of a gene at transcriptional, post-transcriptional and epigenetic levels (Kumar et al. 2021). s-RNAs are synthesized by DNA polymerases and then processed via different dicer-like (DCL) proteins to form a functional RNA-induced silencing complex (RISC) which is responsible for complementary target mRNA recognition and its translational inhibition (Prasad et al. 2019). Besides s-RNAs, long noncoding RNAs (lncRNAs) are greater than 200 nucleotides, and they also lack coding sequences or any open reading frames (ORFs) as in the case of s-RNAs. Based on their effect on target DNA sequences, lncRNAs are divided as *cis*-acting and *trans*-acting lncRNAs. *Cis*-acting lncRNAs modulate expression of their adjacent genes, while *trans*-acting lncRNAs are known to control the expression of genes present at distant locations (Ma et al. 2013). Similar to s-RNAs, they regulate the expression of their target gene at both transcriptional and post-transcriptional levels. In addition to this, lncRNAs can also regulate s-RNA-mediated functions, translation, protein localization and telomere replication (Zhao et al. 2020).

Identification of s-RNA and lncRNA is done through both mining of publicly available databases such as NCBI, plant noncoding RNA database (http://structuralbiology.cau.edu.cn/PNRD) and degradome sequencing (Tang et al. 2012). Sequence homology search of filtered noncoding sequences against known noncoding sequences/whole reference genome sequence using BLASTN leads to the identification of s-RNA (Wang et al. 2016; Kumar et al. 2018). Further classification and functional annotations are performed through Gene Ontology (GO) and Blast2GO softwares. Degradome sequencing is a modified RNA sequencing technique used to sequence the target mRNAs of s-RNAs, as they are known to degrade their targets. For this, s-RNA library is prepared and is sequenced by deep sequencing methods. High-quality sequences are analysed to filter out protein-coding sequences, rRNA and tRNA. Functional validation and characterization of candidate s-RNA are done through northern blot and stem-loop q-PCR (SL-q RT PCR). In northern blot, s-RNA is hybridized against labelled complementary probes (Pall and Hamilton 2008). In stem-loop q-PCR technique, candidate s-RNA(s) are reverse transcribed through an adapter to increase their length and then quantified with the help of q-PCR (Kramer 2011). To validate the s-RNA targets found in the libraries of degradome sequencing, RNA ligase-mediated rapid amplification of cDNA ends is performed. By using this method, RNA sequences that are lacking 5′ cap are ligated to an RNA adapter, reverse transcribed and quantified through PCR (Sharma and Prasad 2020). Another approach for s-RNA target validation is short tandem target mimicry (STTM) which uses target mimics of s-RNAs against targets to be validated.

There are several reports suggesting the role of s-RNA and lncRNA in plant abiotic stresses (Ben Amor et al. 2009; Liu et al. 2015; Wang et al. 2015; Liu and He 2020). Although limited studies are available regarding identification and characterization of s-RNAs and lncRNAs in pearl and foxtail millet, most of them are restricted to identifying miRNAs only (Qi et al. 2013; Yi et al. 2013; Wang et al. 2016; Kumar et al. 2018; Chakraborty et al. 2020; Palakolanu et al. 2021).

In pearl millet, 14 miRNAs were identified through harnessing the publicly available databases and homology search (Kumar et al. 2018). Further, differential expression pattern of 5 out of 14 predicted miRNAs in contrasting genotypes, J2290 and 7042S, were validated through qRT-PCR. They were mainly expressed in root and leaf/shoot tissues of pearl millet seedlings. Using an s-RNA target predicting tool ps-RNA target server, they also identified 33 target genes of these miRNAs which are found to be involved in plant stress responses, transcriptional regulation, signal transduction, plant growth and development. In another study, two pearl millet genotypes, ICMR 1122 and ICMR 1152, showed low and high transpiration rate against vapour-pressure deficit (VPD) conditions investigated through deep sequencing method for differentially expressed miRNAs, and this led to identification of 116 known and 61 novel miRNAs from ICMR 1152 genotypes and 26 known and 6 novel miRNAs from ICMR 1122 genotypes (Palakolanu et al. 2021).

Genome-wide analysis studies of miRNA in foxtail millet led to prediction of 215 miRNAs and 447 miRNA targets, and the target validation of randomly selected miRNA targets has been done by using RNA ligase-mediated rapid amplification of cDNA ends (Yi et al. 2013). Another study was performed in drought-responsive foxtail millet inbred line An04-4783 to identify the drought-induced miRNAs and their targets by combined s-RNA and degradome sequencing method. Among 163 miRNA identified, 17 of them were found to be differentially expressed under drought conditions, and these miRNAs target 82 genes as suggested by degradome sequencing (Wang et al. 2016). Identification of 55 known and 156 novel miRNAs by using genome-wide studies has been done in 2 foxtail millet cultivars showing contrasting response towards dehydration stress. qRT-PCR analysis showed differential expression of 54 miRNAs, and the differential expression of selected miRNAs has been functionally validated through northern blot and SL-q RT PCR (Yadav et al. 2016a). In an effort to elucidate the role of si-RNA and lncRNA in response to drought in foxtail millet, Qi et al. (2013) found reduced expression of genes which were flanked by identified si-RNAs. They also identified 584 lncRNAs in foxtail millet, and among them, 19 were found to be differentially expressed under drought stress (Qi et al. 2013).

Above discussion concludes the reports about identification and characterization of s-RNAs in abiotic stress response in pearl and foxtail millet. It also suggests that most of the available studies under abiotic stress conditions are restricted up to identifying and validating miRNAs only. The molecular pathway studies how these miRNAs regulate their gene expression needs to be deciphered in these millet crops. Further identification and characterization of miRNAs and si-RNAs and lncRNAs including their targets will help to improve our understanding about s-RNA-mediated abiotic stress tolerance regulation of these millet crops.

22.5 Abiotic Stress-Responsive Proteomic Analysis

Proteins are direct effectors of abiotic stress responses in plants. Different proteomic strategies are applied to understand the stress acclimatization procedure in plants as discussed in Table 22.3. The main objective of stress-responsive proteomic analysis is to identify novel regulators of stress responses in stress-tolerant crop plants, for example, millets and implementation of these strategies in climate-sensitive crop plant species to stabilize crop productivity irrespective of climatic condition (Singh et al. 2021). Figure 22.2 represents a schematic methodological diagram for plant proteomic studies.

22.5.1 Present Status of Proteomic Study in Foxtail and Pearl Millet

Comparative proteomic analysis was executed in foxtail millet by imposing NaCl (100, 150 and 200 mM) gradients in dark condition. Reduction of growth rate and dry mass was observed due to salt stress. In 2D gel electrophoresis, 175 protein spots were detected, and mass spectrometry helped to identify 29 differentially expressed proteins including important salt-responsive proteins. Identified proteins were participated in major physiological processes like photosynthesis (enzymes of photosynthesis cytochrome P450, photosystem I reaction centre subunit IVB chloroplast precursors, etc.), nitrogen metabolism (urease, glutamine synthetase, etc.), signal transduction (several kinases), cell wall biogenesis and stress-related several metabolisms (Veeranagamallaiah et al. 2008).

As foxtail millet has outstanding drought stress tolerance capabilities, comparative proteomic investigation was carried out by Pan et al. (2018) to dissect the adaptation mechanism. During drought condition, 2474 proteins expressed differentially; among them, expression of 321 proteins was changed significantly, and 252 proteins were upregulated, while 69 proteins were downregulated. 43.6% of the total differentially expressed proteins, i.e. 140 drought-responsive proteins, were localized in chloroplast in relation with light reaction of photosynthesis, chlorophyll *a*/*b* binding proteins, oxygen-evolving enhancer protein, RuBisCo small chain, quinone reductase, etc. Expression of proteins involved in carbon assimilation was also enhanced including pyruvate phosphate dikinase (PPDK), pyruvate dehydrogenase, phosphoenolpyruvate carboxylase (PEPC) and NADP-dependent malic enzyme (NADP-ME). Other than photosynthesis, proteins related to carbohydrate metabolism, ATP metabolism and ROS scavenging enzymes play pivotal role in drought responses. Among 13 downregulated protein receptor-like protein kinase, pentatricopeptide repeat-containing (PPR) protein and aquaporins were reported. Cumulative effect of several protein synthesis and processing-related protein, transcription-related protein, stress- and defence-related protein and cytoskeleton- and signalling-related protein helps to maintain the homeostasis in plant cell under drought condition (Pan et al. 2018).

Another proteomic study was performed between two pearl millet genotypes to explore the proteomic signature behind stay-green phenotype in drought-tolerant

Table 22.3 List of techniques widely used in proteomic study

Techniques	Principle	References
Blue native PAGE	Use mild detergent and Coomassie blue for observing the proteins in native forms present in cell and biological membranes, provide information about protein mass, oligomeric state and protein-protein interaction	Eubel et al. (2005)
Two-dimensional polyacrylamide gel electrophoresis (2D PAGE)	Separation of protein based on their isoelectric point along a pH gradient in the first dimension, and then denatured proteins are separated according to their molecular weight in SDS-PAGE in second dimensions	Meleady (2018)
Two-dimensional differential gel electrophoresis (2D-DIGE)	Different protein samples (control vs. treated) are labelled with spectrally resolvable different fluorescent dyes (Cy3, Cy5, Cy2: all these three dyes impart +1 charge and 500 Da mass on protein samples) and then resolved in a single 2D gel for comparative protein quantitation	Meleady (2018)
Liquid chromatography-mass spectrometry (LC-MS)	Protein mixtures are separated through the column based on their affinity to the stationary phase. The peptides are transferred into gas phases by electrospray ionization (ESI) methods, and then separate peptides according to their mass to charge (*m*/*z*) ratio	Zhang et al. (2014)
Matrix-assisted laser desorption/ ionization time-of-flight mass spectrometry (MALDI-TOF MS)	Protein samples are mixed with large matrix which absorbs ultraviolet light to produce charged sample ions. The sample ion with smaller *m*/*z* value and high charge moves faster to reach the detector. Sample ions are differentiated according to their time of flight	Caruso et al. (2009)
Shotgun liquid chromatography tandem mass spectrometry (LC-MS/ MS) using iTRAQ tag	iTRAQ reagents are amine-specific isobaric reagents used for multiplexed protein profiling up to four different samples and labelled with four reagents in MS/MS, it gives four reporter ions to quantify proteins. It is used in global peptide labelling and provides information about post-translational modifications of protein samples	Zieske (2006)
Biomolecular interaction analysis mass spectrometry (BIA-MS)	Femtomole amount of proteins and peptides is characterized using surface plasmon resonance detection method. A combined approach was made along with MALDI mass spectrometry analysis of sensor chips during BIA	Krone et al. (1997)

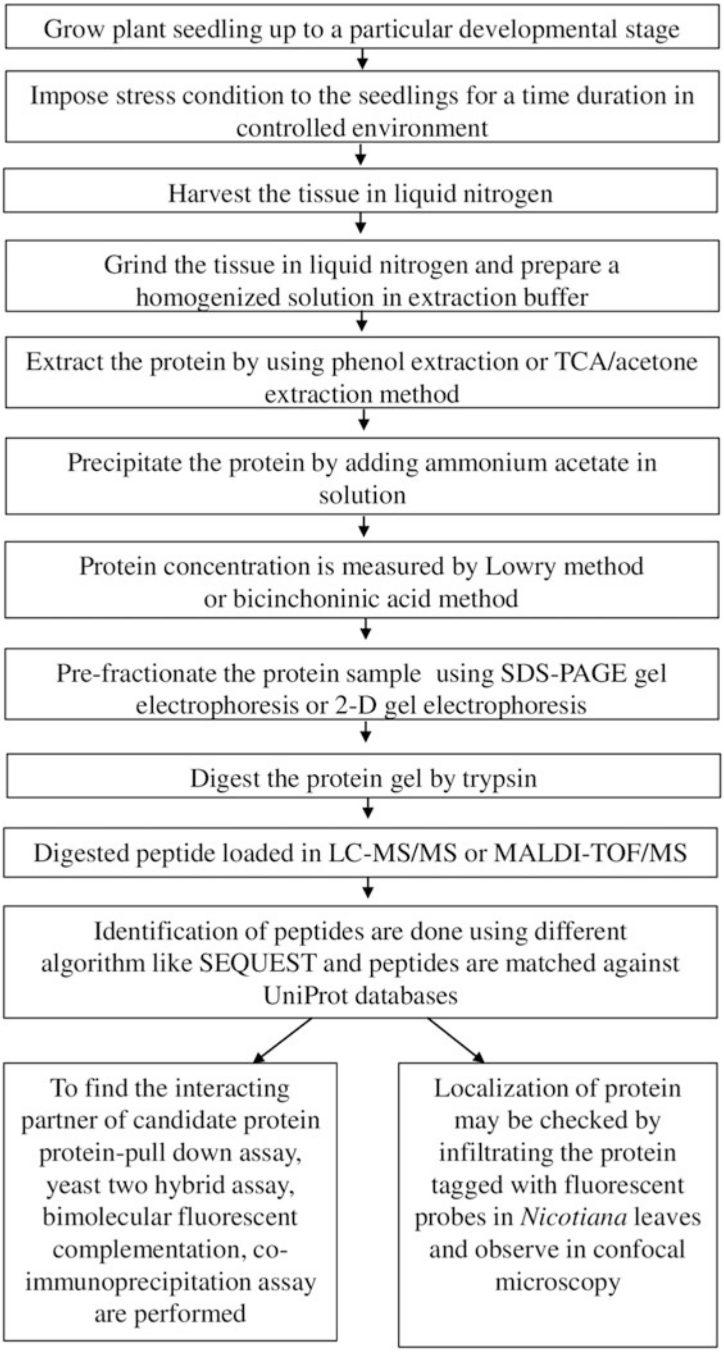

Fig. 22.2 Schematic representation of proteomic study from plant sample under stress condition

pearl millet (Ghatak et al. 2021). Analysis revealed that higher accumulation of proteins associated with photosynthesis, i.e. chlorophyll *a-b* binding protein, thylakoid luminal protein, protein kinase, ferredoxin-NADP reductase, 14-3-3 protein, chlorophyll synthesis protein and RuBisCo small chain protein, were expressed more than the susceptible variety and leads to stay-green phenotype. Reduced expression of reactive oxygen species (ROS) proteins, i.e. peroxidases, glutathione reductase and glutathione synthetase, helps to improve the photosynthetic efficiency instead of drought responses (Ghatak et al. 2021).

A shotgun proteomic study was performed in pearl millet to characterize drought-responsive protein (DRP) expression in various tissues in plants like leaf, root and seed. High abundance of peroxidases, heat-shock protein 70 (Hsp 70), germin-like protein (GLP) and annexin in root proteome along with significant expression of LEA seed maturation protein, threonine synthase and other 21 proteins in seed provide a reference to investigate the effect of drought stress response on proteome. Among all the tissues, leaf proteome was varied mostly in response to drought stress. Increased expression of chlorophyll *a*/*b* binding protein, thioredoxin, NAD(P)H subunit protein and photorespiration-associated proteins was observed suggesting that leaf photosynthesis machinery is severely affected due to water deficiency. Different signalling molecules like GTP-binding protein, leucine-rich transmembrane protein kinase, calnexin, calreticulin, 14-3-3 protein and phosphoinositide-specific phospholipase were expressed significantly to sense the water stress condition rapidly (Ghatak et al. 2016).

Few genes are identified for stress responsiveness in foxtail millet, and their overexpression in transgenic lines is also studied, but we need further distinct proteomic analysis to understand the mechanism behind the stress response. Wang et al. (2014) characterized a novel gene, *SiLEA14*, in foxtail millet which provides resistance to the plants under salt and drought stress conditions. These LEA proteins act as osmotic protectant enabling enhanced growth and germination of transgenic millet plants by lowering the electrolyte leakage caused by salinity stress. During drought stress, *SiLEA14* transgenic line showed significant increase in free proline and soluble sugar content. *Cis*-acting element search reported that *SiLEA14* promoter consists of four ABA and dehydration-responsive ACGT box motifs which are involved in drought and cold stress responsiveness in plants (Wang et al. 2014). However, the underlying mechanism of LEA protein is not yet deciphered. Another gene family was identified against high salt stress, i.e. remorin from foxtail millet transcriptome. *SiREM6* overexpression study in transgenic *Arabidopsis* depicts that this protein plays a significant role in high salt stress by increasing the germination rate and survival rate (Yue et al. 2014). Promoter analysis and expression analysis are done, but these are not sufficient evidence to elucidate the functional activity of remorin protein in salt stress.

22.5.2 Post-translational Modification: Effectors of Abiotic Stress

Abiotic stress has a great impact on post-translational modifications of protein that cause altered subcellular localizations, affect protein activity, stability and modulate protein-protein interactions (Wu et al. 2016). Salt stress and heat stress are well known to change the phosphorylation status of plant proteome (Chitteti and Peng 2007; Hu et al. 2015). Thus, differential analysis of phosphoproteome is an emerging method to know more about salt-responsive proteins. There are several examples about post-translational protein modifications under different abiotic stresses. Salt treatment causes ubiquitination of plasma membrane ATPase (Liu et al. 2012). Heat wave causes phosphorylation of serine/threonine protein kinase, protein phosphatases and phospholipase in maize plant (Hu et al. 2015). Cold stress causes rapid accumulation of SUMOylated proteins in rice (Chaikam and Karlson 2010). Knowledge about post-translational modifications of proteins in millets caused by the abiotic stress till now remains unexplored. Investigations should be done in this direction to find out unknown stress-resistant mechanisms.

22.6 Database Resources for Pearl and Foxtail Millet

Foxtail millet and pearl millet are treated as model crop plants for studying genomic complexity in C4 grasses. Thus, comprehensive database information in foxtail millet and pearl millet bridges the gap between research communities and provides core compendium for future crop breeders to enhance the crop productivity. Database creations of pearl millet and foxtail millet are flagship initiatives taken by different institutes to broaden the horizon of crop improvement. Till now, only few databases are created in pearl and foxtail millets. Here, we summarize the information about those databases.

22.6.1 FmTFDb

Single transcription factor regulates the expression of several genes and modulates different pathways. Thus, comprehensive knowledge about transcription factors gives us ample opportunity to investigate novel pathways leading to crop improvement. Bonthala et al. (2014) created a dedicated database for foxtail millet (http://59.163.192.91/FmTFDb/ index.html) comprising of 2297 predicted transcription factors classified into 55 families with its sequence features, location in chromosome, phylogeny analysis, tissue-wise expression profile as well as Gene Ontology assignment. The complete data of candidate transcription factor can be searched according to the families or physical map on chromosomes. This database allows users to execute BLAST search against protein sequence of transcription factor (Bonthala et al. 2014).

22.6.2 FmMiRNADb

Knowledge about miRNA is prerequisite to understand the molecular regulation of several abiotic stress-related genes. Numerous studies are performed to identify stress-responsive miRNA along with their targets. Khan et al. (2014) at the 'National Institute of Plant Genome Research' created foxtail millet miRNA database (FmMiRNADb: http://59.163.192.91/FmMiRNADb/index.html) to provide detailed information about 355 mature miRNAs in addition to their secondary structure and respective targets. In silico analysis revealed that predicted targets include transcription factors, DNA binding proteins and important enzymes that regulate different abiotic stress responses. This database also provided tissue-wise expression pattern of miRNAs during stress condition along with their physical map on nine chromosome of foxtail millet and comparative mapping between the genomes of sorghum, maize, rice and *Brachypodium distachyon* (Khan et al. 2014).

22.6.3 FmMDb

Molecular marker acts as a major tool in genotype as well as phenotype selection during marker-assisted selection breeding programme. Bonthana et al. 2013 at the 'National Institute of Plant Genome Research' developed a user-friendly interactive genomic marker dataset (http://www.nipgr.res.in/foxtail.html) encompassing 3 types of molecular markers such as 21,315 genomic simple sequence repeats (SSRs), 447 genic SSRs and 96 ILP markers. Another additional feature 'Microsatellite Repeat Finder' in this database provides search option for microsatellite present in query DNA sequences. Users can navigate the required marker according to motif sequence of marker, repeat length along with minimum number of repeats, NCBI probe ID or position in chromosome and functions associated with marker in case of genic SRs and IPLs. There are several hyperlinks present in search window like physical map hyperlink for searching the marker locations in the genome, info hyperlink for primer details, etc. (Bonthana et al. 2013).

22.6.4 PMDTDb

Pearl Millet Drought Transcriptome Database (http://webtom.cabgrid.res.in/pmdtdb/) was prepared by Jaiswal et al. (2018) in collaboration with Junagadh Agricultural University, Gujrat, and ICAR-Indian Agricultural Statistics Research Institute. This database provides information about 19,983 differentially expressed genes in pearl millet during drought stress along with 7595 transcription factors. Drought-responsive gene regulatory network (GRN) composed of 45 hub genes gives an excellent understanding about signalling pathways. PMDTDb database holds 34,652 putative markers such as 4192 simple sequence repeats (SSRs), 12,111 gene-based single-nucleotide polymorphisms (SNPs) and 6249 insertion-deletion (In-Del) markers. A list of predicted mature miRNA which targeted differentially expressed gene was also included. This database serves as a central source for pearl millet breeders and researchers for studying candidate gene-based SNP mining and trait-based association studies (Jaiswal et al. 2018).

22.7 Status of Genetic Engineering in Pearl and Foxtail Millet

Genetic engineering in millets started in the early 1990s, but the progress made so far is inadequate compared to other cereals including rice, wheat and maize. The major factor behind the slow progress of millet genetic engineering is its highly recalcitrance to plant regeneration through tissue culture (Singh and Prasad, 2016). Pearl millet genetic transformation was firstly attempted by Taylor and Vasil (1991) through particle bombardment method using immature embryos. *Agrobacterium*-mediated stable transgenic generation of pearl millet was initiated by Jha et al. (2011). Shoot apical meristem was infected by *Agrobacterium tumefaciens* harbouring pCAMBIA130:hptII:gus construct and resulted in the transformation frequency of 5.7%. Like pearl millet, foxtail millet genetic engineering also utilizes both biolistic and *Agrobacterium*-mediated transformation techniques (Sood et al. 2019). *Agrobacterium*-mediated gene transfer method in foxtail millet was initiated much earlier than the pearl millet. Liu et al. (2005) was the first to demonstrate stable transformation of foxtail millet with an efficiency of 6.6%. Later, Wang et al. (2011) utilized immature inflorescence as explant for embryogenic callus generation that resulted in 5.5% transformation efficiency of foxtail millet. Overexpression of *SiLEA14* in foxtail millet employing the method developed by Wang et al. (2011) resulted in tolerance of salt and drought stress (Wang et al. 2014). Transgenic studies have also revealed the abiotic stress-responsive functional characterization of *SiARDP* and *SiASR4* in foxtail millet (Li et al. 2014, 2017). Other than immature inflorescence, shoot apical meristem was also employed as starting material for foxtail millet transformation. RNAi lines of *SiPHT1:2*, *SiPHT1:3* and *SiPHT1:4* were developed with a transformation efficiency of ~10% (Ceasar et al. 2017). Recently, a highly efficient *Agrobacterium*-mediated genetic transformation system in foxtail millet was developed with ~27% transgene integration frequency (Sood et al. 2020). The method utilized mature seeds as explant for callus generation and primary calli was further proliferated into several secondary embryogenic calli following subculturing under dark conditions. The method is expected to hasten the foxtail millet functional genomic and trait improvement studies in the coming years. In foxtail millet, a virus-based DNA delivery system is also being utilized for further genetic studies. Foxtail mosaic virus-based gene silencing approach is adopted as an alternative to the RNAi in several monocots, especially cereals (Liu et al. 2016; Mei et al. 2016; Bouton et al. 2018; Yuan et al. 2020). Targeted genome editing through site-specific endonucleases including meganucleases (MegaN), zinc finger nuclease (ZFN) and transcription activator-like effector nucleases (TALENs) has already demonstrated their potential in several crops. In recent times, clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) technology has gained much attention because of its multidimensional application. Till date, no reports are there for genome-edited pearl and foxtail millet lines. However, the current progressions in structural and functional genomics of millets would prompt the employment of these tools for further trait enhancement.

22.8 Roadmap to Expedite Genomic and Proteomic Resources in Further Crop Improvement

Plant breeders promote two different approaches to imply the knowledge of proteomics and genomics in crop improvement. First one is stable introgression of genes conferring abiotic stress tolerance, and another is through targeted genome editing to achieve stress tolerance. Various millet genes providing tolerance are introduced using *Agrobacterium tumefaciens* to develop stable transgenics of maize, rice and wheat (Singh and Prasad 2016). Numerous transcription factors and genes related to cellular processes were introduced to enhance the stress tolerance (Singh et al. 2021). *SiATG8a*, an autophagy-associated gene, responsible for nitrogen remobilization provides higher tolerance in rice than the untransformed plant in low nitrogen stress (Li et al. 2015). Transcription factor *SiMYB3* confers resistance in nitrogen deficiency, and responsible for TAR2, an auxin biosynthesis gene synthesis was successfully introduced in *Arabidopsis* and rice (Ge et al. 2019). Transgenic *Arabidopsis* with overexpression of *PgNAC21* is another example of stable transformation carried out for generating climate-resilient plants tolerant to salinity stress (Shinde et al. 2019). Till date, there is no report available regarding targeted genome editing for stress tolerance in millets; it is a matter of exploration. Figure 22.3

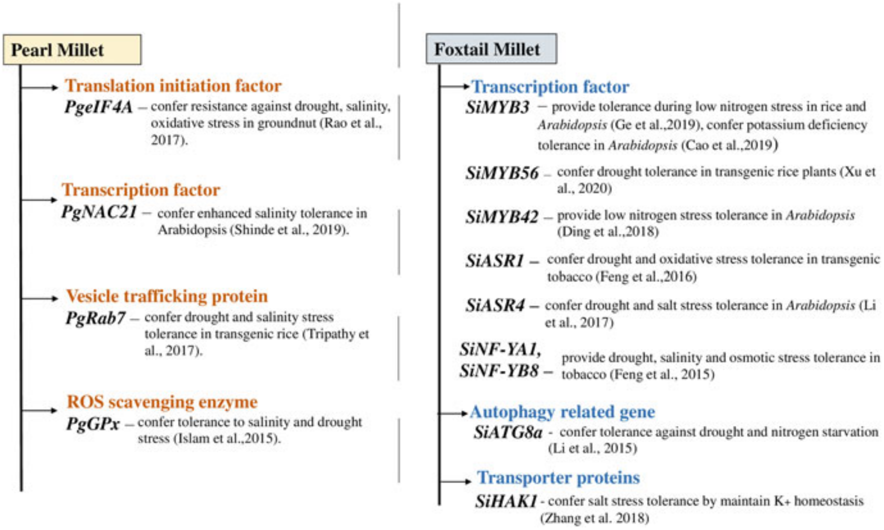

Fig. 22.3 List of candidate genes that are transformed from millet to several other species for enhancing tolerance against abiotic stresses

compiles potential candidate genes of pearl and foxtail millet used to develop transgenic plant with improved abiotic stress tolerance.

22.9 Conclusion

Foxtail and pearl millets are among the natural climate-resilient small grain crops with superior nutritional and therapeutic values. Large numbers of wild and cultivated germplasms stored in various gene banks are like treasure mines to explore the uncovered genetic basis of abiotic stress tolerance, biofuel attributes and advanced nutritional values. This information can further be applied in breeding programme or biotechnology-based improvement of staple cereal crops including wheat, maize, rice and others. Genome-wide analyses of gene families and metabolomic, global transcriptomic and comparative proteomic studies have narrowed down some candidate genes which have proven their potentials in providing abiotic stress tolerance. The availability of different databases would serve the purpose of genomic resources for large-scale genotyping applications. Further, both foxtail and pearl millets are C_4 photosynthetic crops which provide an additional prospect to further characterize the genetics and physiology of C_4 photosynthesis. Altogether, the potential wide range of climatic stress tolerance and large-scale genomic resources in foxtail and pearl millet would definitely expedite various crop improvement programmes in the coming years.

Acknowledgements The work in this area by authors is supported by J.C. Bose National Fellowship Grant of Department of Science and Technology [JCB/2018/000001] and core grant of DBT-NIPGR. RKS acknowledges the DBT Multi-institutional project entitled—'Germplasm Characterization and Trait Discovery in Wheat using Genomics Approaches and its Integration for Improving Climate Resilience, Productivity and Nutritional quality' under mission programme of 'Characterisation of Genetic Resources', [BT/Ag/Network/Wheat/2019-20] for the research grant. JM and SS acknowledge the research fellowship from Council of Scientific and Industrial Research, Govt. of India. The authors are thankful to DBT-eLibrary Consortium (DeLCON) for providing access to e-resources.

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Advancement in Omics Technologies for Enhancing Abiotic Stress Tolerance in Finger Millet

23

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Abstract

Finger millet is an excellent cereal cultivated under rainfed agroclimatic conditions. Special attributes of millets, such as their ability to adapt to harsh climatic constraints, low agro-input necessity and high nutritional content, designate them as prospective food and nutrition security crops. However, when compared to important crops like rice, wheat and maize, the genetic resources available to finger millet are minimal. Therefore, in the bulk of breeding efforts, aiming for climate-resilient agriculture, improving finger millet productivity is still the top priority. Abiotic stresses are the precarious causes of crop failure, growth and productivity, and it has been becoming more vulnerable due to global climate change. Hence, the present era demands development of stress-resistant improved varieties and application of new approach and technology like 'omics' in developing stress tolerance in cereal crops. However, abiotic stresses, like salinity, drought, etc., have imparted physiological, biochemical and metabolic alteration in crops leading to decline in yield and productivity in finger millet. The modern biotechnological approach has tremendous opportunity in understanding and improving crops for stress tolerance and climate resilience. In recent past, omics approaches, viz., genomics, transcriptomics, proteomics and metabolomics, have been considered as important tools in the field of abiotic stress tolerance in finger millets. This chapter provides insight into progress and prospect of genetic manipulation in developing abiotic stress-tolerant finger millet genotypes with the application of omics-based approach.

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_23

Keywords

Abiotic stress · Finger millet · Genomics · Metabolomics · Omics technology · Phenomics · Proteomics · Transcriptomics

23.1 Introduction

Millets are important cereal crops cultivated in semiarid tropical areas of Asia and Africa in rainfed conditions (Numan et al. 2021). Finger millet [*Eleusine coracana* (L.) Gaertn.] is one of the important minor millets, an annual herbaceous, self-pollinated plant, largely cultivated and used by people of Africa and Southeast Asia (Kumar et al. 2016; Gupta et al. 2017; Ceasar et al. 2018). India is considered as secondary centre of diversity for finger millet which also acts as fourth valuable millet crop next to sorghum, pearl millet and foxtail millet, cultivated in more than 25 countries (Upadhyaya et al. 2007; Gupta et al. 2012; Vetriventhan et al. 2016; Hittalmani et al. 2017; Parvathi et al. 2019; Sood et al. 2019). India is considered to be the top producer of finger millet, covering area of about 1.2 million ha with 1.98 million tons of production and 1661 kg/ha of finger millet productivity (Sood et al. 2019). Finger millet is considered as nutritionally potent crop, rich in minerals, proteins, methionine and tryptophan (Gupta et al. 2017). Furthermore, it is a drought-tolerant, climate-smart plant with long shelf life of its grain (Rurinda et al. 2014; Sood et al. 2019). These unique attributes of finger millet with necessity of low agro-input and extraordinary nutritional quality are valued as pivotal plant genetic resources for future food and nutritional security (Panda et al. 2020a, 2021a, b). However, cumulative evidences suggested that climate change has induced the abiotic stress vulnerability like drought, salinity, etc. on growth, yield and productivity of finger millet (Lata 2015; Wambi et al. 2020). Considering current climatic change, it is expected to significantly impact on food production and security in various abiotic stress-affected regions throughout the world (FAO 2005). As per the World Summit on Food Security, the food production needs to enhance by at least 70% by 2050 to cope with climate change and feed the world's growing population. Under this scenario, finger millet is considered to be suitable genetic resource in the modern era for their extraordinary characteristics (Gupta et al. 2017). Stress adaptation is a multilevel process, i.e. controlled at physiological, cellular and molecular levels. Agricultural researchers have long been interested in determining plant stress tolerance mechanism and response (Lata 2015). The degree to which a species is prone to abiotic stress varied among different species. Hence, the present era demands development of stress-resistant improved varieties and application of new approach and technology like 'omics' in developing stress-tolerant cereal crops. Finger millet possesses an excellent carbon-concentrating mechanism through the C_4 route, in addition to having a high nutritional value. One of the explanations for its hardiness subjected to least moisture and harsh climatic constraints could be genes linked in the C_4 pathway. It is worthwhile to compare genes participating in the C_4 carbon absorption network to genes previously identified in other cereal crops

(Wang et al. 2009; Huang et al. 2016; Saha et al. 2016; Hittalmani et al. 2017) and understand the alteration at genomic level. Nowadays, omics technology is assumed as essential tool in improving abiotic stress tolerance in different crop plants. Therefore, omics approaches like genomics, transcriptomics, metabolomics, proteomics and phenomics are useful tools in improving abiotic stresses like drought and salinity in finger millet genotypes (Roychoudhury et al. 2011). Recently, in the year 2017, whole-genome sequence draft of finger millet was released, which will be milestone in the finger millet development for prospective breeding program (Ceasar et al. 2017, 2018; Hittalmani et al. 2017). For that reason, only few investigations regarding genetics and genomics of finger millet have been carried out. Therefore, the present chapter described about involvement of various 'omics' approaches and their advancement in developing abiotic stress-tolerant finger millet genetic resources.

23.2 Finger Millet: An Overview

Finger millet [*Eleusine coracana* (L.) Gaertn.] has 36 chromosomes ($2n = 4x$) and frequently cultivated in Africa and Asia (Kumar et al. 2016; Ceasar et al. 2018). The genus name *Eleusine* is derived from Greek goddess of cereals; popular name finger millet refers to the panicle branching of finger millet (Gupta et al. 2017). The genus *Eleusine* comprises of eight species, and further, these eight species are categorized into four genome groups like AA, BB, CC and DD (Vetriventhan et al. 2016; Odeny et al. 2020). The crop belongs to the Poaceae family and Chloridiodeae subfamily. All other millets belong to the Paniceae tribe, while it is the only millet that belongs to the Chlorideae tribe (Sood et al. 2016). India is regarded as secondary centre and East Africa the primary centre of finger millet diversity and distribution, grown in more than 25 countries (Upadhyaya et al. 2007; Gupta et al. 2012; Hittalmani et al. 2017; Parvathi et al. 2019). Finger millet is regarded as agronomically sustainable, economically important staple food for people in marginal lands and can easily withstand drought and saline conditions, requiring little water (Kumar et al. 2016). Millions of smallholder farmers rely on finger millet as a staple food crop covering around 12% of global millet around the world (Mirza et al. 2015; Kumar et al. 2016; Odeny et al. 2020). It is considered as genetic resources for infertile, dry and marginal lands for agriculture and food security (Odeny et al. 2020). The country that produces major finger millet is India, in Asia continent. Globally, beyond 37,000 wild and cultivated varieties of finger millet germplasm collections have been conserved in various gene banks, and in India, ICRISAT and NBPGR consist of 7519 and more than 10,000 accessions of finger millet, respectively (Odeny et al. 2020). Finger millet grains have abundance in nutritional value such as minerals, proteins, fibre, vitamins and energy which provides economic values for marginal farmers. It also serves as quality diet for children, pregnant and lactating mothers (Gupta et al. 2017; Ceasar et al. 2018).

23.3 Abiotic Stresses as Major Constraints for Finger Millet Production

Abiotic stresses like salinity and drought have significant effect on growth, yield and productivity of finger millet (Saha et al. 2016; Ceasar et al. 2018; Maharajan et al. 2018). The effect of drought stress on finger millet was studied by Parvathi et al. (2013) which revealed that wilting and rolling of leaf occurred due to induction of drought stress causing declining of leaf chlorophyll and solute potential of leaf and also several drought stress-sensitive genes as compared to genotypes grown under control conditions. Panda et al. (2021b) suggested that drought stress caused significant reduction of PSII activity by reducing maximum fluorescence, maximum photochemical efficiency, electron transport rate and photochemical quenching in finger millet seedlings. The authors also reported increasing enzyme activity against PEG-applied drought constraint in finger millet cultivars (Panda et al. 2021b). Similarly, salinity stress is another limitation in productivity of finger millet. Anjaneyulu et al. (2014) reported that salinity stress in finger millet genotypes inhibits growth and physiological activities such as depletion in plant height, leaf enlargement, water availability, length and breadth of grain, diminished grain weight and delayed flowering (Ceasar et al. 2018). Further, Parvathi and Nataraja (2017) concluded from their experiment that plant growth and biomass of root and shoot significantly reduced due to implication of salinity, PEG and oxidative stress in finger millet genotype.

23.4 Finger Millet as Potential Abiotic Stress-Tolerant Crop

Abiotic stressors pose a serious danger to global food security because they reduce crop yield and geographical distribution of agricultural crops, resulting in large economic losses (Gupta et al. 2017). Adaptation to abiotic stress is regulated at physiological, cellular and molecular levels (Roychoudhury et al. 2008). Agricultural researchers have long been interested in determining the mechanisms behind abiotic stress resistance and response in millets (Lata 2015). Finger millet is assumed to be a highly nutritious crop and has the potential to thrive in least water and limited resources; however, it has been ignored for a long time (Gupta et al. 2017). Several researchers such as Bray et al. (2000), Rahman et al. (2014), Odeny et al. (2020) and Shailaja and Thirumeni (2007) reported that finger millet has higher salinity tolerance. The study on salinity tolerance in rice and finger millet by Rahman et al. (2014) reported that finger millet genotypes exhibited elevated degree of salinity resistance as contrasted to rice. Screening of salt tolerance of 80 finger millet accessions (mini-core collection) revealed genotypic variations of finger millet for shoot biomass and yield under salinity (Krishnamurthy et al. 2014). Despite the fact that finger millet is drought-tolerant, its output is severely harmed under extreme drought circumstances. The mechanism behind drought tolerance in finger millet is lagging behind. Bhatt et al. (2011) reported about antioxidant defence system playing important role in scavenging reactive oxygen species and balancing drought resistance in genotypes of finger millet (Odeny et al. 2020). A more targeted study, aimed at abiotic stress tolerance mechanism and associated genes, will make a significant difference towards abiotic stress tolerance in finger millet.

23.5 'Omics' Technology: A Useful Tool in Enhancing Abiotic Stress Tolerance

Omics, also known as high-dimensional biology, comprises cells, tissues and organisms in a way that integrate data from several platforms and aids in its interpretation. This technology works in a non-targeted and non-biased manner, primarily discerning genes (genomics), mRNAs (transcriptomics), proteins (proteomics), metabolites (metabolomics) (Narad and Kirthanashri 2018; Panda et al. 2020b) and plant phenotyping and development (phenomics). Abiotic stress adaptation and regulation is a complex and interdependent process that is necessary for plant viability subjected to suboptimal condition as evidenced by the advancement in omics technology and high-throughput sequencing technologies (Gupta et al. 2017; Banerjee and Roychoudhury 2020). It has been established that stress resistance is mediated by a network of regulatory and signalling molecules that might work antagonistically or synergistically (Gupta et al. 2017). Finger millet, having regulatory proteins and useful genes, can grow in extreme climatic situation, which might be exploited to generate stress tolerance in cereal crops (Gupta et al. 2017). It is necessary to explore and exploit the available broad diversity of finger millet around the world in developing climate-resilient agriculturally important crop. The genome sequence of finger millet must be understood in order to properly comprehend and appreciate abiotic stress resistance capabilities. This chapter describes the recent advancement in omics technologies and approaches such as genomics, phenomics, metabolomics, proteomics and transcriptomics in exploring and exploiting stress tolerance and deciphering improvement of abiotic stress resistance in finger millet in order to enhance its productivity under stressful environment by the application of omics technology (Fig. 23.1). Omics is becoming more mainstream, and several new omics fields are expected to emerge in the near future.

23.5.1 Genomic Approach in Enhancing Abiotic Stress Resistance

Genomics is the interdisciplinary evaluation that focuses on analysing the structure and function of genes and genomes as well as mapping them. Tom Roderick was the first to coin the term 'genomics' in 1986 (Narad and Kirthanashri 2018; Panda et al. 2020b). Genomics includes highly broad areas of functional genomics and structural genomics. The genomes of 6070 eukaryotes, 1,45,357 prokaryotes, 12,924 plasmids, 17,614 viruses and 11,732 organelles have been sequenced and are freely available in the NCBI genome database (https://www.ncbi.nlm.nih.gov/) (Panda et al. 2020b). In comparison to major cereals, finger millet consists of narrow genomic resources as per the National Center for Biotechnology Information

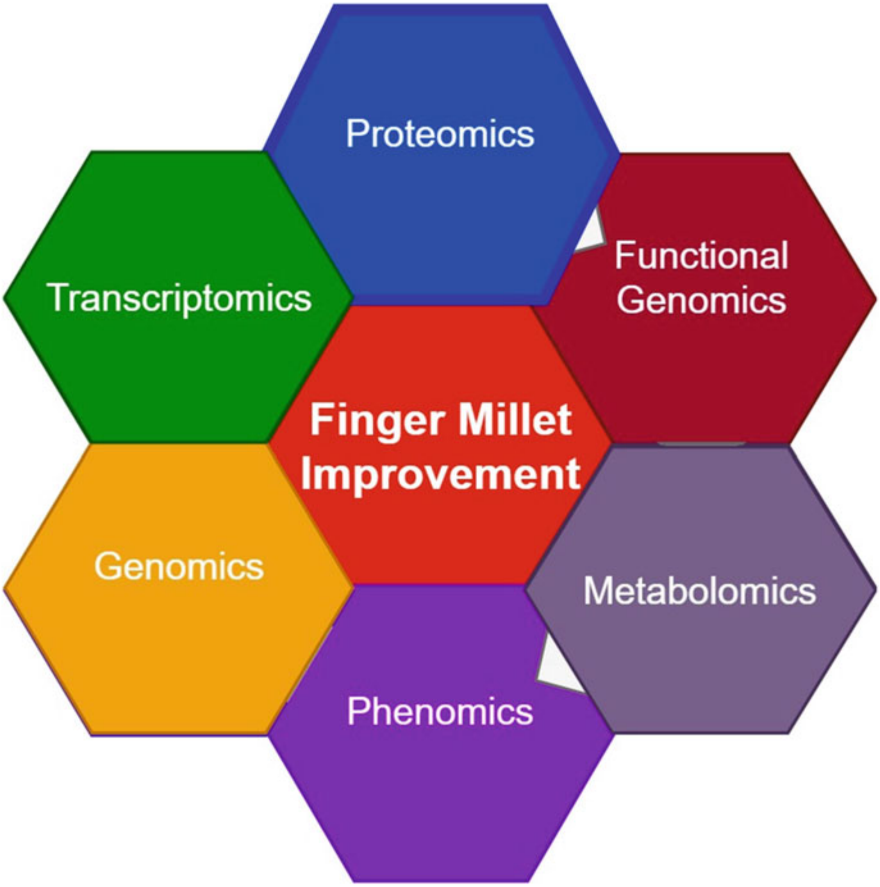

Fig. 23.1 Various omics approaches for improving abiotic stress resistance in finger millet

(NCBI) database (Wambi et al. 2020); this obstructs the further development of the crop (Wambi et al. 2020). In 2018, it was reported that there are only 1934 ESTs linked to salinity, drought and traits related to disease (Wambi et al. 2020) and when comparing with barley, rice and maize, the available genomic resources for finger millet found were to be approximately 434.5, 662.4 and 1046.3 times less (Wambi et al. 2020). Gimode et al. (2016) depicted that 10,327 SSRs and 23,285 non-homeologous first SNPs have been reported in finger millet through Illumina NGS (next-generation sequencing) technologies (Wambi et al. 2020). Recent data on whole-genome sequencing (WGS) of finger millet reported about 146 C4 pathway genes, 1766 R-genes, transcription factor including 56 families, 114,083 SSRs and 2866 drought-responsive genes which were also available in NCBI database (Hittalmani et al. 2017; Wambi et al. 2020). Using RAPD and microsatellite markers, Dida et al. (2008) revealed a higher amount of genetic diversity among different domesticated finger millet varieties (Lata 2015). The first major advance in the field of *Eleusine coracana* genomics was the generation of genetic linkage map of its A and B genomes, using RFLP, AFLP and SSR markers (Dida et al. 2007; Lata 2015). Hence, to identify distinctive features available in finger millet and explore them to boost crop yield, genomic techniques such as structural and functional genomics might be used. Given that, *Eleusine coracana* is a tough, nutritious plant, which claimed that a genomic approach is required for maximum utilization of useful genes for abiotic stress resistance.

23.5.2 Whole-Genome Sequence (WGS) and Genomics-Assisted Breeding of Finger Millets

The creation of genetic and genomic resources is a critical step in enhancing crops for certain features. The genome sequence analysis in major cereals of the world and their timeline was presented in Fig. 23.2. Till date, the genome sequencing of various crops like *Arabidopsis thaliana* (125 mb), *Oryza sativa* (430 mb), *Sorghum bicolor* (818 mb), *Zea mays* (2300 mb), *Setaria italica* (490 mb), *Hordeum vulgare* (5100 mb), *Triticum aestivum* (17,000 mb) and *Eleusine coracana* (1460 mb) has been done (Ceasar et al. 2018). Recently, the whole-genome sequence of 'ML-365' (drought-tolerant) finger millet variety was carried out by the application of Illumina and sequencing technology (Ceasar et al. 2018). According to Hittalmani et al. (2017), 45 and 21 Gb of paired end and mate pair data was gathered. The genomic assembly included a total of 525,759 scaffolds with more than 200 bp size which constituted 2275 bp of mean scaffold length and 23.73 kb of N50 length (Ceasar et al. 2018). The transcriptome of 'ML-365' genotype reported about 53,300 unigenes of well-watered (WW) and 100,046 unigenes of low-moisture stress (LMS) assembles which were about 64% were functionally annotated protein sequences (Ceasar et al. 2018). The unigenes assembled through differential gene expression exhibited WW of 2267 unigenes, LMS-specific 12,893 unigenes and 111,096 genes specific to both well-watered (WW) and low-moisture stress state (Ceasar et al. 2018). The protein-protein homology modelling showed

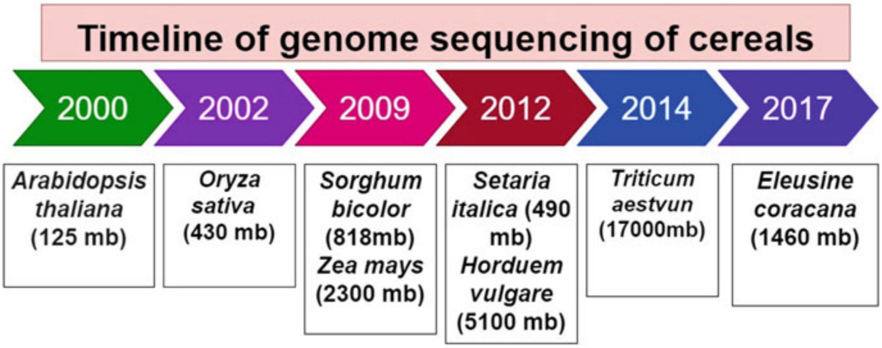

Fig. 23.2 Milestones in genome sequence analysis in major cereals of the world

56 transcriptional factors (TFs) having homology with 11,125 genes. Furthermore, Hittalmani et al. (2017) reported about 2866 of drought-responsive genes associated with 19 Pfam domains of TF families, 1766 R-genes for diseases and 330 genes for transport and accumulation of calcium (Wambi et al. 2020). The authors also suggested that correlation of finger millet WGS with rice and foxtail millet resides under Poaceae family and similar genome size of both subspecies *coracana* and *africana* (Ceasar et al. 2018). Similarly, whole-genome sequence of 'PR-202' genotype was revealed by Hatakeyama et al. (2018). The authors found that 'PR-202' finger millet has genome size of 1.5 Gb and 1189 Mb which is about 78.2% of the genome, and it consists of 2387 scaffolds (Hatakeyama et al. 2018; Ceasar et al. 2018).

23.5.3 Genes Associated with Finger Millets' Abiotic Stress Resistance

Some researchers such as Ramegowda et al. (2012, 2017), Rahman et al. (2014), Babitha et al. (2015a, b), Parvathi and Nataraja (2017), etc. reported about abiotic stress resistance gene such as drought- and salinity-responsive genes in finger millet (Table 23.1). In an experiment on drought resistance in GPU-28 finger millet, Parvathi et al. (2013) reported overexpression of seven drought-responsive genes such as *RISBZ4*, *metallothionein*, *farnesyl pyrophosphate synthase*, *farnesylated*

Table 23.1 Genes associated with finger millet genetic modifications to improve abiotic stress tolerance

Gene name	Stress tolerance	References
Ec-apx1	Gene expression enhanced subjected to drought	Bhatt et al. (2013)
Metallothionein	Prompted under drought stress	Parvathi et al. (2013)
Farnesylated protein ATFP6		
Farnesyl pyrophosphate synthase	Expressed under drought	Parvathi et al. (2013)
RISBZ4		
Protein phosphatase 2A		
Monodehydroascorbate reductase	Overexpression under abiotic stress	Sudan et al. (2015), Ceasar et al. (2018)
EcNAC1	Resistance to abiotic stress	Ramegowda et al. (2012)
EcJAZ	Overexpression of genes prompted stress tolerance in finger millet	Sen et al. (2016), Ceasar et al. (2018)
EcDehydrin7	Overexpression under drought stress	Singh et al. (2015)
EcTAF6	Overexpression under NaCl, PEG and oxidative stress	Parvathi and Nataraja (2017)
EcCIPK31-like	Expressed subjected to drought resistance	Nagarjuna et al. (2016)

protein ATFP6, *protein phosphatase 2A*, etc. in GPU-28 genotype (Ceasar et al. 2018) under drought treatment. Further, the reported genes were found to be linked with drought tolerance in *Eleusine coracana* and need full characterization of such novel genes (Ceasar et al. 2018). Singh et al. (2015) characterized *EcDehydrin7*-drought-responsive gene in finger millet and overexpressed in tobacco plants for drought resistance. Furthermore, Parvathi and Nataraja (2017) examined the effects of NaCl, PEG and oxidative stress (methyl viologen) on GPU-28 finger millet variety and found that normal growth of the finger millet was inhibited and *EcTAF6* (drought-responsive regulatory gene) was significantly expressed (Parvathi and Nataraja 2017; Ceasar et al. 2018) (Table 23.1). *CBL-interacting protein kinase31* (*EcCIPK31*-like) gene in finger millet was discovered and described by Nagarjuna et al. (2016) which is involved in drought resistance (Ceasar et al. 2018). Similarly, a number of salinity-responsive finger millet genes have been discovered. Rahman et al. (2014) identified salt stress-responsive genes by the application of RNA-seq in leaves of finger millet. Ramegowda et al. (2012) reported upregulation of *NAC* gene (*EcNAC1*) against salinity stress. The above-described abiotic stress tolerance genes (Table 23.1) were identified and validated in finger millet. Unfortunately, unlike model plant like *Oryza sativa* and *A. thaliana*, the reported genes are yet to be further described in finger millet genotypes utilizing reverse genetic methods.

23.5.4 Advances in Transcriptomics

The principle behind transcriptomic approach is to examine transcriptome structure and dynamics. In the early 1990s, the term ‘transcriptome’ was coined. All transcripts found in a cell, inclusive of mRNA, miRNA, non-coding RNAs and small RNAs, are included in the transcriptome (Roychoudhury and Banerjee 2015; Rajawat 2018). Transcriptomics is based on mRNA analysis which includes fragmentation, labelling, hybridization and probing once messenger RNA (mRNA) is converted into complimentary DNA (cDNA). Sequencing is next done using next-generation sequencing (NGS), and data is analysed using a bioinformatic technique (Rajawat 2018). Recently, some studies reported that under drought stress constraint, important drought-responsive genes were induced such as *EcDehydrin7*, *EcNAC1*, *metallothionein*, *NAC 67*, *farnesyl pyrophosphate*, *protein phosphatase 2A*, *Ec-apx1*, *RISBZ4* and *EcNAC1* in finger millet (Satyavathi et al. 2019). Similarly, Hittalmani et al. (2017) identified 2866 numbers of genes associated with drought in finger millet through RNA sequencing, assembling and qRT-PCR study, and some identified genes like *ABF*, *AREB*, *MYB*, *MYC*, *GRF* and *NF-Y* encoding different transcription factors (Satyavathi et al. 2019). Rahman et al. (2014) reported that analysis of the transcriptome of a leaf from Trichy 1 finger millet genotype (salinity tolerant) exhibited overexpression of genes from the transporters, cell signalling, compatible solute biosynthesis, transcription factors and osmotic homeostasis families (Satyavathi et al. 2019). The study in GPU-28 finger millet genotype subjected to multiple stress application (drought, salinity, osmotic and methyl viologen stresses) revealed about two transcriptional factors (TFs) belonging to bZIP and basic helix-loop-helix (bHLH) family (Babitha et al. 2015a, b; Ceasar et al. 2018). Nageshbabu et al. (2013) reported that under drought and salinity stress, 12 conserved miRNAs expressed in 8-day-old seedlings of finger millets. Transcription factors associated with finger millet adaptation features, including EcDehydrin7, EcNAC67 and EcbZIP17, were demonstrated to manage with abiotic stimuli in rice and tobacco, including drought, salinity and heat (Rahman et al. 2016; Ramakrishna et al. 2018).

23.5.5 Advances in Proteomics

Proteins are essential molecules which regulate cellular functions. In 1994, the term ‘proteome’ was coined by Marc Wilkins and is defined as the study and exploration of all the proteins present in a single cell (Vaz and Tanavd 2018). Further, in 1997, the word ‘proteomics’ was framed by James (1997) and defined as the large-scale study of proteomes that entails investigating and exploring the structure, function and activity patterns of the proteomes (Vaz and Tanavd 2018). Two-dimensional gel electrophoresis and mass spectrometry are two important techniques used in proteomics (Kumar et al. 2016). 2D electrophoresis is involved in separation of complicated mixture of proteins, whereas mass spectrometry is carried out to identify different proteins (Kussmann et al. 2006; Wang et al. 2006; Kumar et al. 2016). Proteomics is another major functional genomic tool that has shown promise in determining stress-responsive proteins which might be used to improve the abiotic stress tolerance of essential agricultural crops (Gupta et al. 2017). Various ion transporters, signalling cascade and regulatory proteins are expressed under abiotic stress, and understanding the protein functions could be used to improve yield (Gupta et al. 2017; Muthamilarasan et al. 2019). Komatsu and Hossain (2013) emphasized the importance of organ-specific proteome analysis for finding proteins that accumulate in distinct plant organs and intercellular compartments in response to different abiotic stimuli and may thus play a prominent role in plant stress responses. Recently, a study on transcriptome and proteome analysis in finger millet under drought stress by Li et al. (2021) revealed that about 3009 differentially expressed proteins were identified in finger millet (Li et al. 2021).

23.5.6 Advances in Metabolomics

Metabolomics is referred to as high-throughput analysis of metabolites that play an important role in metabolism (Sengupta and Narad 2018). Metabolomics is an interdisciplinary area that relies on data and input from other ‘omics’ studies, including genomics, transcriptomics and to a lesser extent proteomics. This area focuses mainly on metabolic components like enzymes, substrates and products (Sengupta and Narad 2018). The term ‘metabolome’ refers to the entire set of low molecular weight molecules found in a sample that are either substrates or by-products of enzymatic reactions and have a direct impact on the phenotype of a cell, tissue or organism (Lata 2015). Target analysis, metabolite profiling and fingerprinting are some of the conceptual techniques in metabolomics that may be utilized for large-scale applications such as phenotyping, transgenics, gene function identification, significance equivalence testing and stress response monitoring (Lata 2015). Therefore, to have a thorough understanding of both gene function and molecular activities governing complex biological processes, an integrated transcriptome, proteome and metabolome analysis is required (Lata 2015). In rice, most of the metabolomic studies in *A. thaliana* have been done, but such analyses are lagging behind in finger millet. Kim et al. (2013) performed metabolomic profiling of three genotypes of proso millet by the application of GC-TOFMS technique, i.e. gas chromatography-time-of-flight mass spectrometry, showing diversity among phenolic acids and primary metabolites. In another study, polyphenols which include derivatives of benzoic acid, cinnamic acid and quercetin of finger millet were identified by the application of NMR technique, HPLC and electrospray ionization mass spectrometry (ESI-MS) (Banerjee et al. 2012; Kumar et al. 2016). Recent metabolomic experiment on foxtail millet by Pan et al. (2020) reported that phenylpropanoid, flavonoid and lignin synthetic pathway and lysophospholipids are important metabolites which determine salt tolerance of foxtail millet at germination stage (Pan et al. 2020).

23.5.7 Advances in Phenomics

The term phenomics refers to the study of an organism's phenome or physical and biochemical characteristics, as they change in response to environmental stress or genetic mutation. Without phenotypic level of expression, there is no accurate characterization or understanding of genomic, transcriptomic, proteomic and metabolomic profiling (Lata 2015). The way plants respond to environmental variables is determined by their functional features such as morphological, phenological, physiological and nutritional traits (Sood et al. 2019). Variability among the useful characteristics resulted in dominance of one variety over the other. An individual's phenome is a series of reactions against exogenous and endogenous signals over the course of their lives. Nowadays, automation, imaging and software solutions eventually opened the path for a slew of high-throughput phenotyping investigations (Yang et al. 2013; Lata 2015; Sood et al. 2019). Recently, Vadez et al. (2012) reported about phenomic study of pearl millet that revealed that in addition to water use efficiency (WUE) and drought resistance index (DRI), a rigorous phenotyping layout in the form of a dry-down technique for progressive water stress or drought has been established. Further, rooting ability, flowering and tillering have also been used as phenotyping criteria in pearl millet (Vadez et al. 2012; Lata 2015; Sood et al. 2019).

23.6 Genetic Improvement of Finger Millet for Abiotic Stress Tolerance

Genetic improvement in finger millet has been lagging behind as contrasted to other key crops. A study on salinity tolerance was reported by Ceasar et al. (2018) that revealed that *Agrobacterium*-mediated genetic transformation technique was applied to generate a salinity resistance finger millet genotype by the application of *sorghum vacuolar* H^+-*pyrophosphatase (SbVPPase)* gene. Further, under salinity stress, the growth of finger millet was increased due to *SbVPPase* gene overexpression (Ceasar et al. 2018). Similarly, *Agrobacterium*-mediated technique was induced to generate transgenic finger millet genotype resistant to salt by using *AVP1* and *PgNHX1*; further, transgenic finger millet was highly tolerant to salinity (Jayasudha et al. 2014). Mahalakshmi et al. (2006) described that in response to salinity stress, the *PcSrp* gene was over expressed; further, the authors revealed that transgenic finger millet exhibited normal growth and flowering subjected to NaCl stress (250 mM) treatment. Hema et al. (2014) showed that in comparison to wild-type genotypes, transgenic finger millet showed higher growth subjected to abiotic stress (drought and salinity) due to overexpression of *mtlD* gene or *mannitol-1-phosphate dehydrogenase* gene (Ceasar et al. 2018). Hema et al. (2014) also reported that transgenic finger millet showed resistance to osmotic stress.

23.7 Conclusion and Future Perspective

Finger millet is now considered as nutricereal or nutriceutical crop for its higher nutritional value and as drought hardy crop for marginal farmers of Asia and Africa. Till date, only little genomic resources regarding finger millet are available. Recent investigation revealed that finger millet is prone to abiotic stresses like drought, salinity, osmotic stress, etc.; only limited number of studies has been carried out regarding functionality important genes. Recently, whole-genome sequences of two finger millet genotypes 'ML-365' and 'PR-202' were released. Some experiments have suggested induction of some important stress-responsive genes in finger millet under drought and salinity stress. The WGS study might be helpful in designing high-resolution studies in finger millet research. Future research is needed in identification and dissection of key traits involving nutrient enhancement, drought tolerance, salinity resistance, genetic diversity study based on SNP and application of genome editing like CRISPR/Cas9 in the improvement of available genetic resources of finger millet genotypes.

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Buckwheat (*Fagopyrum esculentum*) Response and Tolerance to Abiotic Stress

24

Nidhi Selwal, Mandeep Bedi, Sania Hamid, and Mamta Pujari

Abstract

Common buckwheat (*Fagopyrum esculentum*), a traditional crop belonging to the family Polygonaceae, originated from the northwest of China and is widely cultivated worldwide. In the current scenario of unpredictable changes in the global climate patterns, exploring alternative crops that can withstand hostile environmental conditions and contribute to agricultural sustainability becomes the need of the hour. Common buckwheat seems a good option as it can serve the dual purpose of food security and sustainability at the same time. The major challenges posed by climate change may include frequent drought, flooding, storms and rise in global temperature. The abiotic stress mainly results from water deficit, extremes of temperature, salinity, flooding, waterlogging, etc. A number of responses are generated in plants in response to this stress. Buckwheat is highly sensitive to various stresses, especially at the seedling stage. Salinity stress lowers the germination ratio, weight and height in response to increased concentrations of NaCl. Abiotic stresses like salinity, temperature, cold, drought, flooding and metal toxicity lead to various physiological and biochemical changes in buckwheat.

Keywords

Fagopyrum esculentum · Abiotic stress · Salinity stress · Drought · Heat stress

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_24

24.1 Introduction

Buckwheat plants when cultivated for a long time under high temperature are considered to be under heat shock rather than thermal stress. The optimal temperature required for cell division is normally less than 30 °C. Heat stress is contemplated as one of the detrimental environmental stresses. Different species respond accordingly to temperature conditions, depending on the optimum temperature essential for photosynthesis (Banerjee and Roychoudhury 2019). Formerly, it was delineated that 30 °C is a stress factor for the reproductive expansion of buckwheat. In the later researches, higher temperature was found to be favourable for vegetative growth of the plant. Cell membrane stability is considered a vital indicator to various environmental stresses (Munns and James 2003; Filek et al. 2012). Under extremely unfavourable temperature, plant response to stress involves its ability to rebuild outer and inner distorted membrane. The production of reactive oxygen species (ROS) and abscisic acid (ABA) affects membrane selectivity and causes oxidation of unsaturated fatty acids under strong dehydration (Basu et al. 2010; Das and Roychoudhury 2014). ROS leads to the production of jasmonates (JA) or ethylene which leads to induce premature leaf senescence and plays a major part in signal transmission concerned with plant response to stress. Formerly, it was found that under heat stress, the free ABA content increased in some species and decreased in others. The conjugate ABA-glc concentration enhanced or persisted stably. Enhanced cell membrane permeability not only causes peroxidative damage of cell membranes but also commutes their protein conformation and opening of ion channels (Havaux et al. 1996).

24.2 Photosynthesis Under Stress

Photosystem II (PSII) is intended to be exceedingly sensitive to the effects of environmental factors. As stated from the kinetics of chlorophyll *a*, the damage of proteins building PSII is well-versed by florescence, and changes in fluorescence pattern can be regarded as the response of plants to stress response (Lichtenhaler 1996). In cultivars like Panda and PA15, all variables were higher at 30 °C in relation to the control which showed that it is the required temperature for the proper growth of buckwheat (Hornyák et al. 2020). Energy dissipation gauged was lesser at 30 °C than at 20 °C. The plant stress tolerance may be decreased by greater misspending of surplus energy, and this results in lowering down the photoinhibition damage to PSII (Moradi and Ismail 2007; Banerjee and Roychoudhury 2018a). In retaliation to any kind of stress, the closing of stomata is the most common mechanism (James et al. 2002). At 30 °C, leaves showed lower stomatal conductance than that of control and remarkably reduced transpiration rate. The processes involved in photosynthesis showed tolerance to heat stress in the range of 30–35 °C in different species (Wahid et al. 2007; Jumrani et al. 2017). The photosynthesis disruption under high-temperature stress is caused by stomatal or non-stomatal factors (Athar and Ashraf 2005). Some species demonstrated a higher net photosynthesis efficiency, while others did not show any change at 30 °C compared to control temperature (20 °C) suggesting that stomatal closure did not correlate with net photosynthesis (PN).

Stomatal closure is regarded as a foremost defensive mechanism against water loss as it decreases CO_2 availability in case of C_3 plants, while C_4 plants cope up much efficiently with stress, as phosphoenolpyruvate carboxylase (PEPC), a carboxylation enzyme, shows a negligible K_m for its substrate, HCO_3^- (Hornyák et al. 2020). Thus, according to some of the findings, it is suggested that the temperature of 30 °C is more productive to vegetative growth of common buckwheat than 20 °C. Electrolyte leakage (EL) in plants was significantly higher at 30 °C than that at 20 °C. On the contrary, lower temperature discourages generative development of this species (Płazek et al. 2019) so that 30 °C is more favourable for vegetative growth of common buckwheat than 20 °C. Plants under temperature stress influence the developmental stages of plants and metabolism at cytological, morphological as well as biochemical levels. The plant is sensitive to high-temperature stress, but chilling treatment (1–7 °C) is tolerable; the lowest temperature below 0 °C involving frost condition ceases plant growth and metabolism (Kumar and Srivastava 2015). Optimal temperature is necessary for appropriate plant functioning. If the temperature shifts from optimal threshold level, all the developmental stages and plant metabolism were affected. Generally, higher exposure to any stress inhibits seed germination in angiosperms and gymnosperms (Dhakshanamoorthy et al. 2011).

24.3 Cytological Effects

Cytological results suggested that compared to heat stress, cold stress was more effective to cause chromosomal anomalies in a higher percentage. Heat stress alters changes in the behaviour of chromosomes and cell division. Exposure to heat for long duration may trigger cellular injury and immediate cell death within few minutes which could be attributed to a catastrophic collapse of cellular organization (Schoffl et al. 1999). Under temperature stress, the plant chromosome faces most common anomalies including univalent and multivalent formation, precocious movement, stickiness, laggard and fragmentation at metaphase and anaphase I/II division; these abnormalities lead to the disintegration of the photosynthetic system of which multivalent formation results in primary pairing among two or more homologous chromosomes at zygotene and chiasma formation (Kumar and Srivastava 2015). Another abnormality is formed by improper spindle functioning or complete lacking of homologous chromosome pairing. Laggard formation is mainly due to delayed terminalization, chromosomal stickiness or failure of the chromosomal movement. Laggard formation at anaphase I/II is a result of temperature stress which directly affects spindle fibres due to which chromatin bridge breaks down, resulting in an irregular separation of chromosomes.

24.4 Morphological Symptoms

When the temperature exceeds 40 °C in *Fagopyrum esculentum*, its functioning regarding vegetative growth, seedling and seed germination, seed set, number of flowers and seed ripening is affected destructively (Kumar and Srivastava 2015). When temperature surpasses 35 °C, the growth and developmental stages of the plant are affected, and under such conditions, plant tends to divert resources to cope up with stress and thus restrict photosynthesis (Wahid et al. 2007). An acute damage in vegetative stages causes cessation of stem growth, damaging leaf gaseous exchange property; during the reproductive stage, a brief period of heat stress can cause significant proliferation in floral buds, and the opened flowers abort. Cold stress also holds an effect on the growth and metabolism of the plant, but buckwheat is a winter crop, and 0 °C is habitually the best-foretold base temperature.

24.5 Biochemical Behaviour

In response to environmental stresses, higher plants accumulate proline in large quantities as a mechanism of increasing low molecular weight organic compounds which are signified as compatible osmolytes. The solute accumulation aids them to enhance tolerance which is a significant approach to overcome stress in the plant (Wahid et al. 2007). The heat stress treatment disrupts the proline transport and sugar metabolism, due to which proline content accumulates with the duration of stress (Kumar and Srivastava 2015; Roychoudhury et al. 2015). Water-soluble carbohydrate content decreases in both stresses along with increased duration (Kumar and Srivastava 2015). Although cold stress causes little disturbance in the enzymatic process as compared to heat stress, normal functioning of sugar metabolism occurs. In case of heat stress, pigment alteration shows a direct relationship with growth which is essential for thermo-tolerance in plants. The heat stress causes injury to the photochemical reaction and carbon metabolism. In heat stress, carotenoid content and degradation of chlorophyll also influenced the soluble protein which was more striking with increased duration to heat stress compared to cold stress. This was directly related to the assembly of reactive oxygen species (Guo et al. 2006). Plants affected with cold stress incurred reduced photosynthesis activity because chloroplast and photosynthesis are the major sites of injury (Sanghera et al. 2011).

24.6 Protein and Gene Expression

In plant thermo-tolerance, a major role is played by reactive oxygen-scavenging enzymes, heat-shock proteins (HSPs) and heat stress-responsive transcription factors (HSFs). HSFs induce the communication of HSPs which are regulatory and signal proteins involved in redox homeostasis and metabolism (Banerjee and Roychoudhury 2018b) which in turn regulate the activation of heat-shock transcription factor A1 (HsfA1) that assists in transcriptional networks. The function of

HsfA1 is responsible for coordinating the transcription factor expression including protein called dehydration-responsive element-binding protein 2A (DREB2A) (Janni et al. 2020). The overall protein content augmented and overregulated protein expression shows a positive influence in reproductive and vegetative growth under heat stress (Kopeć et al. 2021).

In response to heat stress, a change is seen by the increase in HSP-70 and HSP-90 proteins and hormones from different plant parts (Banerjee and Roychoudhury 2018b). Protein spots for large RuBisCO subunit were extracted, and some chloroplast-localized proteins functioning in photosynthesis were developed by 2D gel spots (Płazek et al. 2019). The induced changes could be seen based on gel spot pattern of proteins using nano-LS-MS/MS. In floral buds, few proteins were overexposed by heat stress which included 60S ribosomal protein L5-1 and the trihelix transcription factor GTL2, acyl-[acyl-carrier-protein] (ACP) desaturase 6 (localized in chloroplasts) (Kopeć et al. 2021) (Fig. 24.1).

In retaliation to various stresses, unsaturated fatty acids (UFA) function as signalling molecules (Kopeć et al. 2021). During high temperature, plants experience an increase in the UFA content, and change in the UFA content helps in initiation of defensive responses and subsequent release of jasmonic acid (JA) and salicylic acid (SA), specifically during abiotic stresses. ACP desaturase 6 is highly accountable for unsaturated fatty acid synthesis, whereas 60S ribosomal protein L5-1 and the trihelix transcription factor GTL2 are responsible for regulation of gene transcription by binding to DNA sequence. In the former studies, the decrease in contents of JA and its esters while inducing SA content in buds was observed (Płazek et al. 2019).

24.7 Enzyme Activity

In buckwheat, under stress conditions, activity of certain enzymes was upregulated in open flowers. The enzyme 6-phosphogluconate dehydrogenase (6PGDH) plays a crucial role in tolerance response in sustaining the oxidative pentose phosphate pathway (OPPP). This results in withstanding stress conditions by redox balance (Kopeć et al. 2021). The indole-3-glycerol phosphate synthase (IGPS) chloroplastic-like isoform X2 was identified under heat stress by gel spot pattern, and the enzyme produces indole-3-glycerol phosphate (IGP) which contributes to the biosynthesis of glucosinolates, tryptophan, phytoalexin alkaloids and indole-3-acetic acid, playing major role during abiotic stresses (Hornyák et al. 2021). IGP is a compound involved in biosynthetic pathways through tryptophan-dependent and tryptophan-independent routes. Auxins are responsible for high fertility in plants, and under high temperature, the reduction in synthesis of auxin is dwindled due to suppression of auxin biosynthesis genes, YUCCA (Sakata et al. 2010). YUCCA (YUC)-type flavin-containing monooxygenases initiate a cascade of reactions which affect and increase the production of indole-3-acetic acid (Cao et al. 2019). Thus, as heat tolerance mechanism, the accumulation of IGPS enhances in the flowers as a result of decreased auxin content in heat-tolerant species. Many proteins, especially

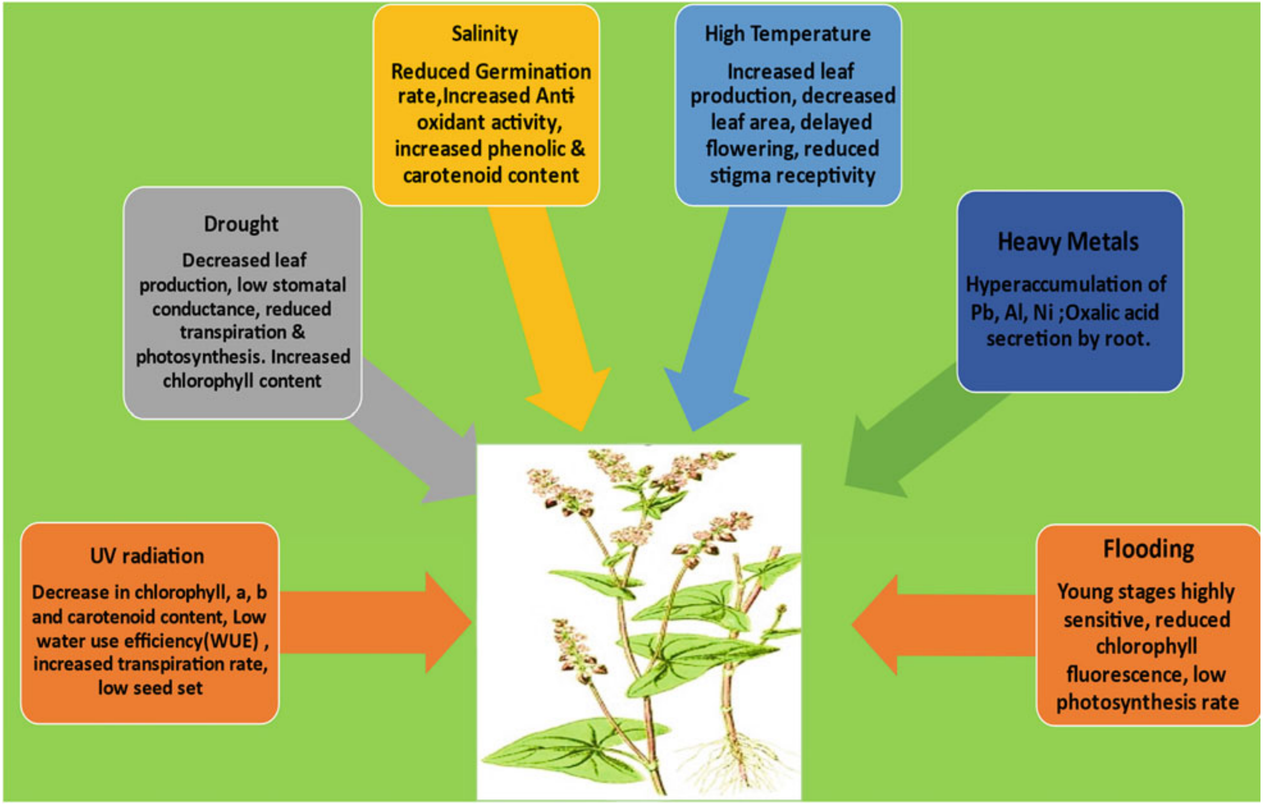


Fig. 24.1 Effect of various types of abiotic stress on *Fagopyrum esculentum*

HSP-70 and HSP-90, were identified from 2D gel spots which were induced by heat changes in different parts of plants (Płazek et al. 2020). During heat stress, proteins were fragmented due to limited repair mechanisms and proteolytic enzyme activity because of generation of high rate of reactive oxygen species (ROS) and proteolytic activity (Kopeć et al. 2021). As senescing tissues do not have efficient antioxidant system and heat stress intensifies the generation of ROS, the control is observed by heat-shock proteins (HSPs). HSP-70 family members work as chaperones to ease multiple functions such as complex assembly, protein folding, translocation and degradation. Thus, the chief role under stress conditions in stabilizing proteins is performed by HSP-70 suggesting definite defence mechanisms (Landi et al. 2019). Furthermore, HSP-100 has a chaperone protein ClpC which works in appropriate chloroplast functioning. Additionally, a protein located at the tonoplast, V-type ATPase, which is a constituent of endomembrane system and membrane-bound catalytic unit, is majorly expressed under heat stress. V-type proton ATPase (V-ATPase) catalytic subunit A is identified to be controlled by environmental stresses (Ratajczak 2000). Alternatively, there is a protein known to be calpain-type cysteine protease DEK1. This is the only calpain protein articulated in heat-affected plants and plays a major role in development of the embryo. A glutamate receptor-like (GLR) channel makes a glutamate receptor protein which works in calcium signalling, and the activity is enhanced under stress conditions. Cytosolic phosphoglycerate kinase (PGK) is an enzyme involved in gluconeogenesis and glycolysis in their plastidial isoforms which is also engaged in Calvin-Benson cycle, glycolytic/gluconeogenic reactions. Chloroplastic fructose-bisphosphate aldolase (FBA) is connected with FBP (fructose-1,6-bisphosphate) and SuBP (sedoheptulose-1,7-bisphosphate) metabolism. All these proteins are involved in carbohydrate metabolism, and their content is overexpressed at the time of stress occurrence, whereas *S*-adenosylmethionine synthetase (SAMS) enzyme was upregulated in leaves only. The enzyme produces L-methionine and *S*-adenosylmethionine (SAM) which are the precursors for the biosynthesis of nicotianamine, polyamines and ethylene and contributes to environmental stress tolerance (Heidari et al. 2020).

24.8 Effect of Temperature on Vegetative Growth

When compared between species, *F. esculentum* and *F. tataricum*, a rise in temperature resulted in enhanced leaf initiation rate and reduced leaf area, but leaf production in both species was differed (Aubert et al. 2020a, b). With increase in temperature, increasing the number of leaves was an adaptation for plants suffering from stress as a tolerance mechanism that allowed to increase transpiration surface which in turn reduced leaf temperature proportionally (Prasad et al. 2008). Previous studies showed a weaker water content in *F. tataricum* compared to *F. esculentum* at 27 °C in the stem and leaves. An increase or decrease in leaf area is not always correlated with the reduction in the leaf area and can vary in different species in response to distinct encountered environmental conditions. Certainly, a change in opening and closing of stomata occurs as a tolerance mechanism in different species. The increase in stomatal opening and for longer duration also influenced the net photosynthesis (PN) rate and leaf gas exchange which led to enhanced photosynthesis rate and upsurge in intercellular CO_2 concentration. Water-use efficiency was higher at 27 °C in relation to increased transpiration rate in *F. tataricum*. These adaptive responses signified the presence and development of tolerance mechanisms concerning reduced photosynthesis rate in high-temperature stress in buckwheat (Aubert et al. 2020a, b). Consequently, the high water-use efficiency ability and CO_2 assimilation rates are an integral response under high-temperature stress in buckwheat species which can be precisely interconnected with tolerance (Hasanuzzaman et al. 2013). During photosynthesis, being one of the utmost heat-sensitive physiological processes (Descamps et al. 2018; Hasanuzzaman et al. 2013), the chlorophyll accumulation between *Fagopyrum* species was contrasting, and an enhancement in temperature affected photosystem II with a decrease of photosystem II efficiency (φPSII), photochemical quenching (qP) and non-photochemical quenching (NPQ), since heat stress disturbs metabolic processes associated with the increase in thylakoid membrane fluidity and electron transport system (Hasanuzzaman et al. 2013).

24.9 Effect of Temperature on Reproductive Growth

Buckwheat species vary in flower production as some species flowered earlier and have extra flowers and spikelet per inflorescences as in *F. esculentum* compared to *F. tataricum* that has delayed flowering and less spikelet per inflorescence. This divulges about the high production rate and increased flower number in *F. esculentum* (Aubert et al. 2020a, b). Certainly, the number of flowers per inflorescence is highly variable in different varieties as ranging from 6 to 33 in *F. tataricum* and 5 to 81 in *F. esculentum* depending on species as inferred from cultivar screening (Cepková et al. 2009). Both the species experience a delay in flowering due to high temperature & the elevation in the first flowering node in *F. esculentum* under high temperature was reported. In buckwheat species, high temperature delayed flowering time, but flower production and number of inflorescences increased comparatively. Also, in *F. tataricum*, the inflorescence rate was doubled at 27 °C than at 21 °C, and the number of inflorescences increased in both the species as a response to high temperature. The increase in flowering time and flower production at high temperature was accompanied with the high number of flowers and spikelet per inflorescences in *F. esculentum*. The approach behind this in *F. esculentum* is the production of large number of flowers so as to counterbalance the lesser flowers at the time of anthesis (Cepková et al. 2009). However, in both *Fagopyrum* species, flower abortion was seen (Aubert et al. 2020a, b), but high temperature affected female organs more than male organs in both species. Temperature had negligible impact on pollen viability but severely decreased stigma receptivity. Buckwheat species are well-known for their inflated pollen viability, but male gametophytes were much less responsive to high temperature in

F. esculentum than female gametophytes (Płazek et al. 2019). *F. esculentum* produces more pollen grains per anther than *F. tataricum* which is pertinent with the requirement of cross-pollination in case of *F. esculentum* and cleistogamy in *F. tataricum* (Wu et al. 2017). Certainly, species diverged by their reproduction approaches as *F. tataricum* is homostylous and self-compatible, whereas *F. esculentum* is heterostylous and self-incompatible (Wu et al. 2017). Although decreased seed set was observed in *F. esculentum*, *F. tataricum* experienced increase in number of aborted seeds by more than 50% under high temperature (Farooq et al. 2016).

24.10 Effect of Temperature on Oxidative Stress and Antioxidant Production

Plants under exceedingly high temperature mainly experience oxidative stress due to the overproduction of reactive oxygen species (ROS). As a measure of oxidative stress, malondialdehyde (MDA) is generated due to membrane lipid peroxidation caused by ROS (Hasanuzzaman et al. 2013; Roychoudhury et al. 2008). An increase in MDA was seen with an increase in temperature in inflorescences and leaves of both the species, but oxidative stress was more pronounced in *F. tataricum* leaves (Aubert et al. 2020a, b). In *F. esculentum*, antioxidant metabolism was enhanced to regulate oxidative stress to defend against the production of ROS. Consequently, the species producing more antioxidants was more tolerant to high temperature (Hasanuzzaman et al. 2013). Both the buckwheat species are known to synthesize a lot of antioxidants under control conditions (Cepková et al. 2009). However, with temperature rise, the antioxidant capacity was enhanced in both species. Antioxidants were lower in the leaves and higher in inflorescences, with more hydrophilic antioxidants being produced than hydrophobic. Diverse antioxidants were produced from both the species, of which one antioxidant family was more pronounced, i.e. the phenolic compounds as hydrophilic antioxidants. In high temperature, concentrations of polyphenols were enhanced in the leaves (Aubert et al. 2020a, b). In previous studies, flavonoids, mainly rutin and quercetin, were described as present in *Fagopyrum* sp. but more in *F. tataricum* (Cepková et al. 2009). Greater concentrations of flavonoids were found in *F. tataricum* inflorescences compared to *F. esculentum* (Aubert et al. 2020a, b). Additionally, antioxidants like glutathione and ascorbate are often involved to counter oxidative stress. They take place in the reaction cycle to bring down one of the ROS, the hydrogen peroxide, which is accumulated in case of oxidative stress. In order to confer defence to the plants, glutathione and/or ascorbate concentrations were enhanced at high temperature. However, it was confirmed that in *F. tataricum*, H_2O_2 detoxification chiefly relied on glutathione, while in *F. esculentum*, ascorbate was mainly involved in H_2O_2 detoxification (Aubert et al. 2020a, b).

24.11 Salinity Stress

Salinity stress refers to the excessive amount of salt in the soil which interferes with the normal crop growth and may eventually result in death. This stress is manifested in many different forms including the osmotic stress, oxidative stress, ionic toxicity, decreased water-use efficiency and inhibition of many metabolic and physiological activities (Roychoudhury et al. 2008). A number of responses are activated in the plants to cope with these stresses. In common buckwheat, a couple of studies have been made to explore the effect of salinity. However, still there is dearth of data with respect to salt tolerance in this plant. The salt tolerance of buckwheat has been reported to be greater than that of many members of family Gramineae and Leguminosae, but lower than certain plants like wheat, barley, cotton and rape (Kobayashi 1954). The highest salinity of soil at which common buckwheat can be sown is 6% (Lu et al. 2018). The saline hydroponic culture of buckwheat has shown that the addition of salt at concentration of 100 mM resulted in significant lowering of plant growth rate, leaf area and net assimilation rate. The rate of photosynthesis and transpiration also showed decline. The decreased rate of transpiration resulted in an enhancement in the levels of efficiency of water use by 1.6 times (Matsuura et al. 2005). The decline in the rate of transpiration resulted from the rise in water potential of leaves with increasing salinity. Further, the excess of Na^+ ions caused damage to the guard cells, resulting in decreased rate of transpiration. The rate of seed germination, fresh weight and growth rate of sprouts were reported to significantly decrease upon treatment with increased concentrations of NaCl (Lim et al. 2012). Salinity not only inhibited the percentage and rate of germination, seedling length and vigour but also led to a decline in the activity of enzyme, superoxide dismutase (Shahverdi et al. 2018). The decline in growth rate was caused by the decrease in turgor resulting from the changes in water potential between the apoplast and the symplast. Salinity stress in buckwheat also led to an increase in the levels of secondary metabolites such as phenolic compounds and carotenoids. The total content of phenolic compounds showed a remarkable increase in a concentration-dependent manner (Lim et al. 2012). This increase was caused by accumulation of rutin, orientin, isoorientin and vitexin. Salinity stress is supposed to stimulate the phenylpropanoid pathway responsible for the formation of phenolic compounds. The enzymes involved in this pathway are stimulated by the phytohormones including jasmonates which in turn are produced in response to the stress. According to Lim et al. (2012), the phenolic compounds produced in response to the salinity stress function as antioxidants. Thus, the increasing NaCl concentration is accompanied by increase in antioxidant activity. Further, the total content of carotenoids also showed an increase due to salinity stress. This is caused due to the stimulation of mevalonic acid pathway aimed at producing abscisic acid from carotenoids. The seeds of common buckwheat contain proteins that have high nutritional value and are gluten-free. The seeds are reported to have properties that lower blood pressure and high cholesterol. Moreover, rutin present in the seeds of buckwheat shows antiplatelet aggregation and antiasthmatic activity. However, the growth and yield of buckwheat were significantly affected by soil salinity.

Therefore, attempts have been made to explore buckwheat varieties with higher salt tolerance in order to fully exploit their economic and medicinal benefits. A transgenic buckwheat plant, overexpressing *AtNHX1* gene, was successfully regenerated using gene from *Arabidopsis thaliana* (Chen et al. 2008). This transformation was mediated through *Agrobacterium tumefaciens*. *AtNHX1* is an antiporter gene which has enabled the buckwheat plant to grow under high salt concentrations without compromising its medicinal value as the content of important nutrients remained unaffected by salt stress. Moreover, the accumulation of rutin, a valuable metabolite, increased under such conditions. Salt-tolerant common buckwheat mutants have been obtained using ethyl methyl sulfone (EMS). These mutants could be screened with 10–20% efficiency using direct precursor 1-aminocyclopropane-1-carboxylic acid (ACC) in combination with ethylene. The advantage of this process lies in its low cost and the stable characters of mutants. Another attempt was made to protect the common buckwheat plant from salinity stress using uniconazole, a triazole derivative (Arya and Singh 2000). Uniconazole, being an anti-transpirant, led to an increase in the relative water content through the closure of the stomata. The decrease in chlorophyll content under salinity stress could also be overcome through the addition of uniconazole. This addition led to a rise in the levels of cytokinins which in turn stimulated the enzyme meant for the synthesis of 5-aminolevulenic acid (ALA), a precursor of chlorophyll. The increased content of cytokinins also led to a rise in the accumulation of carotenoids. The levels of anthocyanin production also showed an increase compared to the saline conditions. In order to have a greater insight into the mechanism of salt tolerance, transcriptomic analysis of common buckwheat on salt response was made. This database contains 43,772 unigenes, out of which 385 differentially expressed genes, which might be relevant to salt stress, were reported (Lu et al. 2018). This information may prove useful in cultivating salt-tolerant varieties in the future.

24.12 *Fagopyrum esculentum*: Response to Drought Stress

Drought stress or water scarcity is among the most commonly encountered environmental stresses that has adverse effects on the growth and yield of crop plants (Ihsan et al. 2016). A number of studies indicated that plants show morphological as well as physiological changes due to drought stress, such as repressed growth rate, enhanced activities of antioxidant enzymes, cell injury, low carbon metabolism, etc. (Wu et al. 2016; Guo et al. 2017). Water shortage can lead to not only mechanical damage to plant cells but also oxidative damage as well as protein denaturation (Al-Abdallat et al. 2014). Drought stress triggers various types of molecular responses in plants such as altered gene expression which might be related to resistance or response to drought (Nakashima et al. 2014). Such abiotic stress can also lead to reduced activities of some chlorophyll-binding proteins like PsbB and PsbQ, which in turn can severely affect the photosynthesis process (Foyer and Noctor 2011). In addition, due to drought stress, there is impact on the mobilization of sugar reserves as the contents of enzymes involved in the carbon metabolism were decreased (Kovács et al. 2007). Many transcription factors (TFs) also play a significant role in drought adaptation and drought resistance in crop plants (Okay et al. 2014; Haake et al. 2002).

24.13 Physiological Effects

Drought stress affects the vegetative growth in *F. esculentum* as it causes less leaf production and reduced leaf weight, in dry as well as fresh form. No significant change was observed in the water content of leaves of the common buckwheat under water stress, but there are reduced rates of stomatal conductance and transpiration. The rate of photosynthesis was also reduced in plants under drought stress with lower intercellular CO_2 concentration. Chlorophyll fluorescence parameters were not affected by drought stress, but there is increase in the chlorophyll content index. *F. esculentum* showed increased water-use efficiency under drought conditions. An increase in malondialdehyde (MDA) content and therefore oxidative stress also occurred in response to the water stress. The activities of catalase and peroxidase antioxidant enzymes also showed increase under drought conditions (Hou et al. 2019).

The reproductive phase of the plant was also affected by water stress. The total number of inflorescences and the number of inflorescences in anthesis were decreased. There was also reduced pollen count per anther, but the number of flowers per inflorescence was not affected much (Aubert et al. 2020a, b). In common buckwheat, drought stress during the vegetative phase was less detrimental as compared to the plants recently engaged in the reproductive phase. So, the plant was less affected when the shoot apical meristem was still initiating leaves as compared to the plant in which SAM was at a stage where it had initiated six to seven inflorescences. Plants subjected to drought at later reproductive phase were also less affected e.g. when the activity was stopped after production of around ten or more inflorescences. The number of cymes and flowers per inflorescence and the number of pollen grains per anther were reduced in response to drought stress at the advent of reproductive phase. The production of seeds was also reduced due to the decrease in cyme number, indicating that drought stress did not affect seed set. Female fertility, seed weight and viability of the pollen grains were not altered by the water deficit. Thus, in common buckwheat, the activity of reproductive meristems, involved in the formation of cymes and flowers as well as the process of male sporogenesis, was affected by drought stress exposure (Cawoy et al. 2006). The seedling stage of common buckwheat is very sensitive to drought stress. Experimental studies have shown that low levels of irradiance modify the effect of water stress during the early developmental stages of buckwheat, mainly the survival and growth-related parameters. Buckwheat seeds are known to accumulate fagopyritol B1 which is an unusual galactosyl cyclitol associated with the acquisition of desiccation tolerance (Delpérée et al. 2003). Therefore, the response of *F. esculentum* to water stress showed characteristics of drought avoidance mechanism rather than drought tolerance as shown by its higher water-use efficiency, low leaf number, reduced stomatal conductance and decreased transpiration rate under drought conditions.

24.14 Gene Expression

Some studies on gene expression profiles of common buckwheat seedlings, growing in normal as well as drought conditions, have been undertaken, and significant difference has been observed between the two. The genome-wide expression patterns have been analysed in common buckwheat showing differentially expressed genes along with their functional characteristics. These studies have suggested that various metabolic pathways such as hormone signal transduction, phenylpropanoid biosynthesis, photosynthesis, carbon metabolism, etc. are highly related to drought adaptation in this plant. There was enhancement of some genes by drought treatment such as brassinosteroid signalling genes (*FeBRI1*, FeBZR*1/2*), genes involved in gibberellic acid signalling (*FeDELLA*, *FeGID2*) and auxin signalling (*FeARF)*. Moreover, it was observed in common buckwheat that drought promotes the expression of gene *c36622_g1* that encodes the DELLA proteins (with aspartate-glutamate-leucine-leucine-alanine or D-E-L-L-A in single-letter amino acid code), which are negative regulators of gibberellin. This indicates that gibberellin signalling plays an important role in drought adaption in buckwheat. Some other genes are also enhanced such as FePAL, FeCYP73A and FeCAD which are involved in phenylpropanoid biosynthesis-related enzyme*s* and cinnamyl alcohol dehydrogenase (CAD), viz., genes *c36561_g1*, *c31631_g1* and *c33174_g1* which are involved in lignin biosynthesis pathway and phenylalanine ammonia lyase (PAL) gene, viz., c34247_g2, linked with the biosynthetic pathway of salicylic acid. The ability of buckwheat plant to tolerate drought stress may also be influenced by certain transcription factors as significant upregulation of the genes encoding ARFs, viz., *c35693_g1*, *c28011_g1*, *c38058_g1* and *c38575_g1*, has been observed. On the other hand, some genes were repressed under drought stress in common buckwheat such as photosynthesis-related genes (*FePSBB*, *FePSBP*, *FePSBM*, *FePSBQ*, *FePSBY*, *FePSBW*) and genes related to carbon metabolism (*FePFKA*, *FeGLYA*, *FeSHMT*, *FeGGAT*, *FeGPMB*). The downregulation of these genes during drought may lead to lower-energy availability, as resources are shifted to stress response in common buckwheat (Wu et al. 2019).

Studies on in situ RNA-RNA analysis have revealed that the localization of buckwheat metallothionein type 3 (*FeMT3*) transcript occurs throughout the whole embryo. It was localized in vascular tissue of roots, xylem, phloem, mesophyll tissue and guard cells of leaves. There were changes in mRNA levels of *FeMT3* as well as reactive oxygen species (ROS)-scavenging abilities of the *FeMT3* protein in response to drought and oxidative stress in yeast. This indicated possible involvement of the gene *FeMT3* in stress defence and ROS-related cellular processes (Samardžić et al. 2010).

24.15 *Fagopyrum esculentum* Moench: Response to Heavy Metal Stress

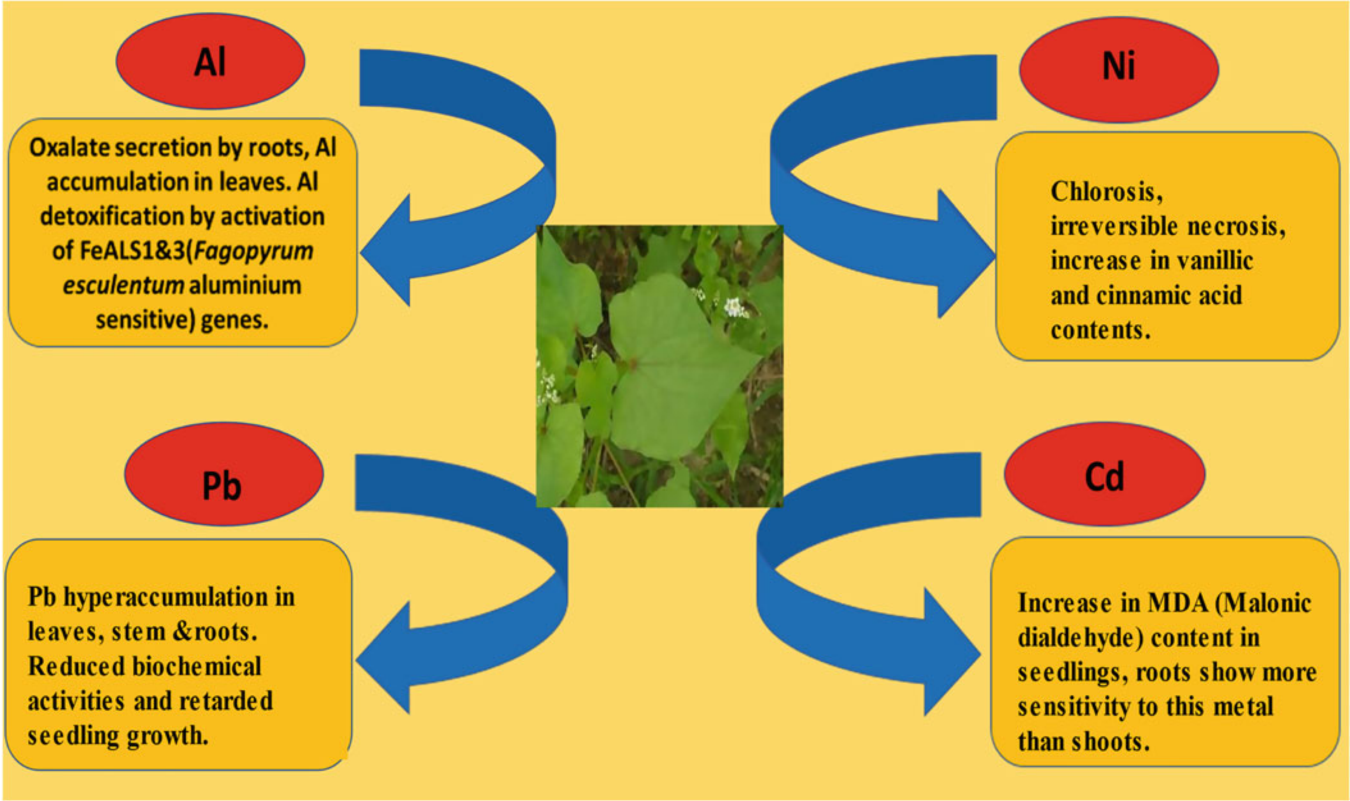
Common buckwheat (*Fagopyrum esculentum* Moench.) is a potential hyperaccumulator of metals like Al, Pb and Ni. The response of *F. esculentum* to heavy metal stress can be used for utilization of this plant in phytoremediation. Metal toxicity also leads to many physiological and biochemical changes in buckwheat, such as non-enzymatic antioxidative systems. The response and tolerance of common buckwheat to various metals are discussed in the next sections.

24.16 Response to (Al) Aluminium

Common buckwheat plant thrives well even on acidic soils, where aluminium (Al) toxicity is usually the main limiting factor for growing crops (Von Uexküll and Mutert 1995). Buckwheat shows secretion of oxalate from the roots in response to Al which is one of the mechanisms for high Al tolerance in this species (Ma et al. 1997) because ionic Al can be chelated by oxalate, thereby detoxifying Al externally in the rhizosphere (Ma 2000). It has been experimentally proved in common buckwheat that when secretion of oxalate was inhibited by phenylglyoxal which is an anion channel inhibitor, the root elongation was inhibited more in the presence of Al (Zheng et al. 1998; Klug and Horst 2010). Thus, oxalate secretion plays a very important role in Al tolerance. No lag phase has been observed between Al treatment and oxalate secretion (Zheng et al. 1998) which suggests that there is no requirement of de novo gene expression for oxalate secretion (Ma et al. 2001) (Fig. 24.2).

F. esculentum plant can also accumulate high Al in the leaves without showing any toxicity symptoms (Ma et al. 1997). Some experiments were performed on a cultivar named Jianxi by Ma et al. (1998). The plant when exposed to high concentrations of Al for 10 days showed accumulation of about 450 mg of Al per kilogram in the leaves. The process of this Al accumulation has also been physiologically characterized (Ma et al. 2001). Buckwheat roots generally take up Al in ionic form, i.e. Al^{3+}, rather than an Al-oxalate complex (Ma and Hiradate 2000). However, some amount of Al-oxalate (1:1) may also be transported by the roots (Klug and Horst 2010). When Al was taken up into the roots, its chelation with oxalate occurs, and an Al-oxalate complex having a ratio of 1:3 was formed in the roots (Ma et al. 1997). Before Al was loaded into the xylem, it underwent ligand change, and Al was present in the form of an Al-citrate complex in the xylem sap (Ma and Hiradate 2000). After Al was transported to the leaves, it was finally sequestrated into the vacuoles in the form of Al–oxalate in a 1:3 ratio (Shen et al. 2002). Once Al accumulated in the leaves, it was no longer mobile. There was more accumulation of Al in the older leaves as compared to the young leaves in this plant (Shen and Ma 2001).

When common buckwheat plants were exposed to Al, there was increase in the content of total phenolics, altered flavonoid as well as anthocyanin content and phenylalanine ammonia lyase (PAL) activity. The most significant effects of Al



Oxalate secretion by roots, Al accumulation in leaves. Al detoxification by activation of FeALS1&3(*Fagopyrum esculentum* aluminium sensitive) genes.

Chlorosis, irreversible necrosis, increase in vanillic and cinnamic acid contents.

Pb hyperaccumulation in leaves, stem &roots. Reduced biochemical activities and retarded seedling growth.

Increase in MDA (Malonic dialdehyde) content in seedlings, roots show more sensitivity to this metal than shoots.

Fig. 24.2 Effect of metal stress on *Fagopyrum esculentum* treatment on the accumulation of phenolic compounds increased in the total phenolic content by around 27.2%, and PAL activity increased by 2.5 times in leaf tissues after 10 days of exposure (Smirnov et al. 2015). The studies on gene expression revealed that in the roots, 4067 annotated genes were upregulated, whereas 2456 were downregulated by Al as compared to the control (without Al exposure). In the leaves, 2663 genes were upregulated, and 2426 genes were downregulated. Out of these, in roots as well as leaves, 602 genes were similarly upregulated, and 401 genes were downregulated (Yokosho et al. 2014). Expression of gene *FeALS3* has also been studied. This gene is an ABC-type membrane transporter which is involved in transporting Al and also internalizing it. It was found that *FeALS3* gene is constitutively expressed in *F. esculentum*, and its expression increased over time. It was upregulated early, in the presence of Al or exogenous ABA before oxalate release was enhanced by the Al supply; then downregulation was observed concurrently with strong Al-induced activation of oxalate exudation (Reyna-Llorens et al. 2015). Thus, in common buckwheat, Al concentration level, above the toxicity threshold, promoted Al detoxification. It was achieved either by activation of *FeALS3* gene directly or by stimulation of higher levels of ABA *(Reyna-Llorens* et al. 2015*)*.

24.17 Response to Pb (Lead) and Cd (Cadmium)

Common buckwheat is a known hyperaccumulator of lead (Pb). When grown in Pb-contaminated soils, it has been found to accumulate a large amount of Pb in its leaves, stem and roots, without significant damage to the plant parts. Pb hyperaccumulators are plants that accumulate more than 1000 mg/kg of Pb in shoots on a dry weight basis. It has also been observed that application of methyl glycine diacetic acid trisodium salt, which is a biodegradable chelator, at concentrations up to 20 mmol/kg, resulted in more than five times higher concentration of Pb in the shoot without any noticeable growth inhibition up to 10 mmol/kg. These results suggested that buckwheat can be used as a potential phytoremediator of soils with Pb contamination (Tamura et al. 2005). Buckwheat seedlings grown in a nutrient medium with Pb showed higher tolerance as compared to those grown in medium having Cd. The effects of short-term exposure to lead (Pb^{2+}) and cadmium (Cd^{2+}) in a nutrient medium in seedlings of two cultivars of common buckwheat (*Fagopyrum esculentum* Moench) have been examined in hydroponic experiments. The elongation of shoots and primary root of buckwheat seedlings was measured, and it was observed that the presence of Pb and Cd in a nutrient medium led to inhibition of the growth of buckwheat seedlings. The study also revealed that more inhibitory effects were seen in the growth of roots than shoots of buckwheat seedlings in the presence of these two ions. The proline and malondialdehyde (MDA) content and the level of photosynthetic pigments were measured, and the result suggested reduction in biochemical activities. Increase in the concentration of both Pb and Cd also caused an elevation in the content of malondialdehyde and proline in common buckwheat seedlings. The accumulation of chlorophylls *a* and *b* and carotenoids in buckwheat cotyledons was stimulated on short-term exposure of seedlings to cadmium and lead ions. Roots were more sensitive to the presence of lead and cadmium in nutrient medium as there was significant correlation between the elongation of primary root and the increase in levels of proline and MDA content (Mitrus and Horbowicz 2020). Functional analysis of the promoter region of common buckwheat metallothionein gene (*FeMT3*) has revealed extremely high inducibility in response to Cu^{2+} and Cd^{2+} treatments. The defence ability of *FeMT3* gene has been confirmed in vivo, and its promoter was found to be highly responsive to heavy metal exposure. Thus, this gene might be used for biotechnological applications (Nikolić et al. 2010).

24.18 Response to Ni (Nickel) and Zn (Zinc)

The response of common buckwheat to Ni has been investigated by many workers. The accumulation of Ni by this plant along with the content of malondialdehyde (MDA), total phenolics and phenolic acids was studied by Sytar et al. (2013). Their study showed that by foliar treatments of Ni at different concentrations, the accumulation of this metal significantly increased with increase in applied concentration. Plants exposed to Ni showed visible symptoms such as chlorosis and tissue damages such as irreversible necrosis. The contents of MDA, total phenolics and chlorogenic, *p*-hydroxybenzoic, hesperetic, *p*-anisic and caffeic acids increased in Ni-treated leaves after 24 h of the treatment. There was also an increase in vanillic and cinnamic acid contents. However, the contents of caffeic acid, *p*-hydroxybenzoic acid, hesperetic acid, *p*-anisic acid and *p*-coumaric acid showed a decrease after 72 h of Ni exposure. Thus, common buckwheat is a possible hyperaccumulator of Ni, because there is high accumulation of Ni in its tissues through foliar treatment. The foliar treatment with different high concentrations of Ni also affected lipid peroxidation process and induced changes in phenolic acids. Also, the analysed phenolic acids showed potential antioxidant role, thus providing tolerance to buckwheat against Ni treatment (Sytar et al. 2013). The effect of zinc oxide nanoparticles (ZnONPs) and microparticles (ZnOMPs) on common buckwheat has been studied & observed impact on plant growth, bioaccumulation and antioxidative enzyme activity in hydroponic culture. Both types of ZnO particles led to significant biomass reduction, but it was more in case of ZnOMPs. Zn bioaccumulation in plants increased with increase in the treatment concentrations. Zn toxicity also caused lower levels of glutathione and reduced catalase activity. The expression of *FeMT3* (*metallothionein-like*) gene was highly stimulated in the early stage of seed development in response to Zn ions, and also expression in leaves was enhanced (Brkljačić et al. 2004).

24.19 Response to Sn (Tin), W (Tungsten), Mo (Molybdenum) and Li (Lithium)

The effect of tin mine waste having high concentrations of Sn, W, Mo and Li has been studied in the common buckwheat plant by Franzaring et al. (2018). It was found that the common buckwheat showed tolerance to tin mine waste at low and medium concentrations. The young plants of *F. esculentum* continued to grow in tin mine waste soil up to seed set as compared to the *F. tataricum* young plants which failed to survive under this stress. Out of the various metals present in tin mine waste, 15% Sn and 11% W could accumulate in the shoot, whereas negligible amounts were translocated to the seeds. Lithium and arsenic also accumulated in the green tissue, but not in the seeds (Franzaring et al. 2018).

24.20 Conclusion

Like any other crop, abiotic stress including drought, heat, cold, metal, salinity, etc. influences the vegetative and reproductive growth of common buckwheat in numerous ways. This plant has however evolved several mechanisms at molecular, biochemical, cellular, genetic and physiological levels to cope with these stresses. Drought stress, which is the most common of all the environmental stresses, causes morphological as well as physiological changes in buckwheat. Drought stress affects both vegetative and reproductive phases of this plant. The gene expression is also altered in response to drought stress. Some genes such as those involved in brassinosteroid, gibberellic acid and auxin signalling are enhanced, and others related to photosynthesis and carbon metabolism get repressed. In response to thermal stress, several changes in photosynthesis and biochemical and morphological activities have been observed in buckwheat. Earlier, 30 °C was considered to be a stress factor for the crop, but later researches have indicated that the different species of buckwheat show tolerance to heat stress in the range 30–35 °C. Rather, at 30 °C, all the variables of growth were found to exhibit better results compared to the control. The response mechanisms of common buckwheat to thermal stress include ability to rebuild distorted membranes, synthesis of abscisic acid and ROS that affect membrane selectivity. Heat-shock proteins, ROS-scavenging enzymes and heat stress-responsive transcription factors also played major role in thermo-tolerance. Cold stress also led to decreased photosynthetic activity because of the injury to chloroplast. Heavy metal stress caused many physiological and biochemical changes in common buckwheat. This plant is a hyperaccumulator of metals like Al, Pb and Ni and thus can be used in phytoremediation of heavy metal-contaminated soil. The tolerance of common buckwheat to salinity stress has been reported to be higher than several graminaceous crops but lower than that of cotton, rape, wheat and barley. It also led to increase in levels of secondary metabolites, i.e. phenolic compounds and carotenoids. Several attempts have been made to protect the plant from salinity stress which include the use of uniconazole and production of salt-tolerant common buckwheat mutant and transgenic buckwheat plant that overexpresses *AtNHX1* gene. As common buckwheat has immense economic and medicinal value, the understanding of the response mechanisms of the plant to various stresses becomes important. This knowledge may provide important insights into the ways and means for addressing and mitigating the negative impact of abiotic stress on common buckwheat.

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Abiotic Stress Response and Adoption of Triticale

25

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A. Roychoudhury et al. (eds.), *Omics Approach to Manage Abiotic Stress in Cereals*,
https://doi.org/10.1007/978-981-19-0140-9_25

Abstract

Abiotic stressors have been demonstrated to have a significant impact on plant development and agricultural production, and yields have been clearly stalled or dropped in economically important crops where only large inputs ensure high yields. The latest manifestations of climate changes are thought to have exacerbated the detrimental impacts of abiotic stressors on plant production. On the other hand, the complexity of plant systems governing essential characteristics and the scarcity of germplasm for stress tolerance have hampered genetic advancements in major crops for greater yields or better features. The study of crop tolerance to abiotic stressors has, on the other hand, provided several results that have been published recently. This chapter discusses recent advances in the study of plant tolerance to abiotic stressors and summarizes the key tolerance pathways and signaling components implicated thus far in triticale.

Keywords

Abiotic stress · Climate change · Triticale · Stress physiology

25.1 Introduction

Abiotic stress is defined as the detrimental effect that nonliving elements have on living organisms in a particular habitat. Poor plant growth and agricultural yields are caused mostly by abiotic stressors such as drought, salt, low or high temperature, and other environmental extremes (Turk and Tawaha 2001; Tawaha and Turk 2002a), which are the most common causes of poor plant growth and crop yield around the world (Turk and Tawaha 2002a, b, c). The majority of plants are more sensitive to salt during the germination period (Tawaha et al. 2003; Turk et al. 2003a, b, c). Drought alone affects 45% of the world's agricultural area, while 19.5% of irrigated agricultural fields are classified as salty, according to the World Resources Institute. Triticale (*Triticosecale* W, 1889, AABBRR, $2n = 6x = 42$) is the first species developed through hybridization between rye (*Secale cereal* L, 1753, RR, $2n = 2x = 14$) and bread wheat (*Triticum aestivum*, AABBDD, $2n = 6x = 42$) (Deng et al. 2020; Mohamed 2020)**.** The name triticale was assigned by merging the scientific names of the two genera, that is, wheat (*Triticum*) and rye (*Secale*) (Mergoum et al. 2009). Triticale was created in the nineteenth century (WYATT 2016), with the aim of combining the high-yielding capacity and good grain quality (high protein and lysine) of wheat and the resistance to biotic stresses (disease and insects) and tolerance to abiotic stresses (drought, salinity, low temperature, etc.) of rye (Mergoum et al. 2019). Triticale contains gluten, minerals and vitamins (Glamoclija et al. 2018). Although it is mainly used in animal feed in the form of grazing, silage, fodder, greenfeed, and hay, a raised interest in using triticale in food production such as bread, cookie, pasta, malt, and yogurt was recorded (Zhu 2018;

Mergoum et al. 2019). Moreover, Eudes (2006) and McKenzie et al. (2014) reported its role in producing ethanol.

According to the Food and Agriculture Organization (FAO), global production of triticale has reached up to 14.06 million tons, with an area of 3.81 million hectares across the world. The main production countries of triticale are Poland (4.5 million tons), Germany (2.2 million tons), and France (1.6 million tons) (FAO 2019). Only 9% of the world's area is suitable for agricultural production, whereas 91% is facing different stresses which usually occur simultaneously. More than 50% of crop production losses occur due to abiotic stresses (Minhas et al. 2017). Triticale is subjected to different stresses that negatively affect the quality and quantity of the crop (Grębosz et al. 2014). Stress is any external factor, whether abiotic (drought, high and low temperatures, waterlogging, salinity, etc.) or biotic (disease and insects), that constricts the rate of photosynthesis and decreases capability of the plants to transform energy to biomass (Grime 1977). Several studies have shown the negative impacts of abiotic stresses (drought, waterlogging, high and low temperatures, and salinity) on crop production. For instance, the severe events during 2001 and 2002, and drought and floods during 2010 and 2011, in Canada, reduced the crop production by 50% (Wheaton et al. 2008). In the USA, drought and high-temperature stress that occurred between 1980 and 2016 caused more than $220 billion loss (Oceanic Atmospheric Administration website 2015). Globally, environmental stressors are a significant constraint on legume and grain production (Al-Rifaee et al. 2004; Tawaha and Turk 2004; Turk et al. 2004; Lee et al. 2005; Supanjani et al. 2005a, b; Abu-Darwish et al. 2009; Al-Tawaha et al. 2006, 2007, 2010, 2018a, b, c, d; Al-Tawaha and Seguin 2006; Al-Tawaha and Odat 2010; Abu Obaid et al. 2018).

25.2 Drought Stress

Drought tolerance refers to the capacity of the plant to survive and generate consistent yields in conditions of water shortage at various phases of crop growth (Ananthi et al. 2017; Al-Juthery et al. 2018; Al-Ghzawi et al. 2019; Amanullah et al. 2019, 2021). Plants respond to drought, like many other abiotic stressors, by generating cellular damages and secondary stresses, such as osmotic and oxidative stress. On the other hand, such kind of stress has been demonstrated in a number of research investigations. Drought stress is the main limiting factor in crop production including triticale mostly in arid and semiarid areas of the world (Arseniuk 2015). Triticale is grown in both rainfed and irrigated environments and exposed to drought stress in both environments which negatively effects on productivity and chemical and physical qualities of triticale, by influencing several processes (biochemical and physiological), for example, photosynthesis, ion uptake, carbohydrates, nutrient metabolism, and growth promoters (Farooq et al. 2008; Silvaa et al. 2020). Drought decreased the leaf-stem ratio, dry matter and dry matter digestibility, and increased crude forage protein, neutral detergent fiber, and ash contents in triticale (Barati and Bijanzadeh 2020). However, the impact of drought stress is not the same as it depends on different factors, (1) the degree and period of drought, and (2) the species and growth phase of crops (Tardieu et al. 2008). If the drought is too severe, it negatively affects the whole plant, irrespective of the growth phase or crop species. In general, triticale performs better than wheat under drought stress conditions (Lonbani and Arzani 2011; Mohammad et al. 2011). Giunta et al. (1993) reported that under different degrees of drought stress, triticale yield showed nonsignificant reduction (8%), while durum wheat was significantly decreased in comparison to the irrigated control. Royo et al. (1996, 2000) Naylor and Su (1998), Zhang et al. (2009), and Grzesiak et al. (2012) reported from their studies that triticale is moderately and maize is highly susceptible to drought. The drought tolerance in triticale in terms of yield was superior to that of durum wheat (Abdalla and Trethowan 1990). Blum (2014) cited that triticale has excellent ability to adapt to drought stress. However, several morphological and physiological traits are correlated to the drought tolerance of crop species which can help the plant to continue growing under water stress, such as chlorophyll content, carotenoid content, proline content, flag leaf area, flag leaf angle, and osmotic adjustment (Serraj and Sinclair 2002; Blum 2005; Jones 2007; Kumar and Sharma 2010). In general, plants can tolerate drought stress in three ways:

1. Escape: Plants can complete their life cycles before the water deficiency occurs. Early flowering and early maturity are strategies that plants follow in order to escape drought stress (Shavrukov et al. 2017).
2. Avoid: By avoiding tissue dehydration by reducing transpiration, which occurs by decreasing leaf area, increasing leaf thickness, and reducing stomatal number (Sicher et al. 2012; Lee and Suh 2013; Goufo et al. 2017).
3. Tolerance: Through plant ability to undergo osmotic adjustments at the cellular level, improve the cell walls, and increase root system (Khan et al. 2015; Blum 2017).

Roohi et al. (2013) reported that triticale was less affected by drought stress than barley and wheat in terms of photosynthetic function and growth development. Akbarian et al. (2011) reported that triticale genotypes are more tolerant to drought stress than wheat cultivars, and proline has a positive role in drought tolerance. Another study by Fayaz and Arzani (2011) to estimate drought tolerance in reproductive growth stages in triticale, and durum and bread wheat genotypes, and to study the correlation between the yield and some morphological traits, including plant height, grain weight spike^{-1}, 1000 grain weight, and harvest index, showed that under both moisture treatments, triticale genotypes performed better than wheat genotypes and the correlation between grain weight spike^{-1} and grain yield was significant and positive. Lonbani and Arzani (2011) indicated that some morphophysiological traits (lower flag leaf angle, lower leaf area, and lower number of stomata) contribute in adaption to drought stress in triticale. Exposing triticale to drought stress at vegetative phase increased cell wall-bound content (CWP) in the drought-tolerant varieties (Hura et al. 2009). Huraa et al. (2012) reported that under soil drought conditions, an increase in the content of cell wall-bound phenolics could affect both water status and triticale production. Hura et al. (2017) pointed to the role of cell wall-bound phenolics (CWP) in the mechanisms of plant adaptation to soil water shortage in triticale. Kumar et al. (2014) reported consistent result with previous studies that under nonirrigated conditions, some morphophysiological traits including (spikes per plant and 1000 grain weight) may respond to higher grain yield. Moreover, Saed-Moucheshi et al. (2021) reported that superoxide dismutase (SOD) improved drought stress tolerance in triticale.

25.3 Waterlogging Stress

Waterlogging is not less dangerous than drought stress as it causes severe damages to plants. About 10% of the world's cultivated areas are affected by waterlogging; it is able to decrease crop production by 15% and 80% depending on the crop species, environmental conditions, development phase, and the waterlogging period (Li et al. 2011; Shabala 2011; Prasanna and Rao 2014). For instance, severe monsoon rains during 2010 and 2014 in Pakistan caused significant loss of at least 11 billion tons of rice, sugarcane, maize, and cotton, resulting in economic losses of over 16 billion dollars (Rehman et al. 2015). In the waterlogged soil, the water fills in the pores of the soil, causing a lack of oxygen, which results in the following effects: (1) lowering of vital nutrients such as N, P, K, Ca, S, Fe, etc., (2) affecting on biological and chemical processes and hormone concentration, (3) terminating aerobic respiration, (4) blocking the Krebs cycle and electron transport system, (5) accumulating ethylene and producing bacterial anaerobic metabolism of root ending up with reduced grain filling and grain weight, and (6), in severe cases, causing death of the plants (Barrett-Lennard 2003; Ashraf 2012; Akhtar and Nazir 2013; Tian et al. 2019). Plants can adapt and react to waterlogging through several morphological, physiological, and biochemical strategies causing differential transcriptomic responses (Fageria et al. 2006; Mommer et al. 2006; Hsu et al. 2011). Changes in structure and role of leaves and root systems are examples of the mechanisms of waterlogging adaptation and response.

In a study which aimed to determine the most effective quantitative trait loci (QTL) associated with waterlogging stress, the results indicated that there is an association between waterlogging stress tolerance and root properties such as aerenchyma and adventitious root formation in the tolerant cultivars of grasses (Mustroph 2018).

Several studies reported that under waterlogging stress conditions, roots spread in the soil profile and that nodals and laterals are shorter and thicker (Yamauchi 1993, Yu et al. 1995, Grzesiak et al. 2002). Grzesiak et al. (2014) reported that drought and waterlogging stresses affected shoot development and morphological root system structure (number and length of roots) of triticale and maize genotypes; moreover, under waterlogging stress condition, the limitation in root propagation was lesser in maize than in triticale. The response of triticale and maize lines to drought or waterlogging is related to the water conditions in the plant, which affect membrane permeability, chlorophyll content, and leaf gas exchange. The degree of effective protection mechanisms against water deficiency, cell membrane status, photosynthesis, and WUE determines the ability to tolerate water stress (drought or waterlogging) in triticale, maize, and wheat genotypes (Grzesiak et al. 2017).

25.4 High-Temperature Stress

High-temperature stress is one of the most severe stresses, threatening crop production and survival all around the world (Nahar et al. 2015a, b). Even short period of heat stress can negatively affect crop productivity (Siebert et al. 2014). For each degree Celsius rise in temperature, global wheat production decreases by 6%. While rising temperature is useful for crop production in some cooler areas of the world, its general influence on global food security remains negative (Challinor et al. 2014). High-temperature stress above the critical threshold causes damages to plant growth (Zhao et al. 2017). High-temperature stress negatively impacts on the morphological, physiological, and metabolic process including (1) root system (physical support, water and nutrient uptake); (2) leaf photosynthesis and leaf senescence rates, water relations, membrane stability, and respiration; (3) flowering; and (4) pollination, seed set, grain filling, seed quality, and seed yield (Porter and Gawith 1999; Balla et al. 2009; Brestic et al. 2014; Nahar et al. 2015a, b; Peer et al. 2020; Jagadish et al. 2020). The injuries caused by heat stress differ among plants, as it is associated with the plant susceptibility to heat stress, degree of heat stress, duration and frequency of exposure, and stage of plant development (Barnabas et al. 2008; Sakata and Higashitani 2008; Hemantaranjan et al. 2018). High-temperature stress is more dangerous for field crops when it occurs during the reproductive phase than the vegetative phase (Ravikiran et al. 2020). Several studies reported that when heat stress occurs earlier or around anthesis stage, it reduces the number of the grains, whereas when it occurs during grain filling, it reduces the weight of grains (Porter and Gawith 1999; Dolferus et al. 2011). Ugarte et al. (2007) reported that exposure of wheat, barley and triticale to heat stress before anthesis stage negatively affects grain weight, which could be because of an indirect impact on the source of assimilates. Ozkan et al. (1999) stated that the occurrence of heat stress in the grain filling period decreased the grain weight in triticale. A large range of physiological and biochemical reactions can help plants adapt (tolerate, escape, or avoid) to heat stress (Jagadish et al. 2020). In general, plants tolerate heat stress by minimizing harm to photosynthetic machinery and increasing biosynthesis of the protective compounds (Bita and Gerats 2013). Triticale is considered to be more tolerant to heat stress compared to wheat (Suresh et al. 2018).

25.5 Low-Temperature Stress

Low-temperature stress is categorized into chilling stress (<20 °C) and freezing stress (<0 °C) depending on the environmental temperature (Mickelbart et al. 2015; Shi et al. 2018). However, low-temperature stress is as harmful as high temperature as it causes various damages and losses to plants (Mboup et al. 2012), which vary according to plant species, for example, cotton, tomato, soybean, and potato are sensitive to chilling stress and do not have the ability to adapt to cold conditions. On the contrary, some crops, for example, oats, are chilling tolerant but sensitive to freezing. Conversely, barley, wheat, and rye adapt well to freezing conditions (Zhang et al. 2011). McIntyre et al. (1988) and Tshewang et al. (2010) reported that wheat, in terms of field survival, was superior to triticale. Nezami et al. (2010) reported that exposure of triticale genotypes to different freezing temperature decreased the dry weight and leaf area by 48% and 42%, respectively; as temperature decreased, the plant height was decreased, along with decrease in the number of leaves, the leaf area, the plant chlorophyll content, the dry weight, and the percentage of electrolyte leakage of all genotypes. Cold tolerance refers to the capability of crops to survive and perform better than other genotypes under low temperature, which can occur through improved germination (Saini and Tandon 1985), increased chlorophyll accumulation (Rasolofo 1986), pollen fertility and seed set (Lia et al. 1998), reduced sensitivity of photosynthesis (Singh 2000), higher superoxide dismutase and catalase activity (Baek and Skinner 2003; Zhang et al. 2016), and increased content of sugars and proline (Ramel et al. 2009; Kocsy et al. 2011), in addition to flavonoid compounds, specially anthocyanins and colorless flavonoids (Gould and Lister 2006; Khlestkina 2013). Kolupaev et al. (2020) stated that under cold stress, the superoxide dismutase and catalase activity improved in the high frost-resistant genotypes of triticale compared to less resistant genotypes; the content of sugar was significantly higher in the resistant varieties than the less resistant ones. Proline content increased in all varieties. Cold hardening significantly affected the content of anthocyanins and flavonoids, in high frost-resistant and facultative genotypes, but not in the less resistant ones. Yazicilar et al. (2020) reported that there is a positive correlation among cold tolerance in triticale genotypes and antioxidant enzyme activities, viz., CAT (catalase), sugar content, and proline.

25.6 Salinity Stress

Salinity in water or in soil is one of the important abiotic stress factors that causes plant growth reduction (Lee et al. 2005; Al-Tawaha and Al-Ghzawi 2013; Abu Obaid et al. 2018; Al-Issa et al. 2020), serious injury to the plants, yield losses in several crops, and plant death in severe conditions (Ashraf and Harris 2004; Hussain et al. 2009; Zhao et al. 2009; Semiz and Suarez 2015; Farooq et al. 2015; Nawaz et al. 2016; Nuriyeva et al. 2016; Bezirğanoğlu 2017). Soil salinity refers to the high level of dissolved salts such as NaCl, Na_2SO_4, and Na_2CO_3 in soil (Munns 2005; Parihar et al. 2015). Salinity stress negatively affects plant growth rate for two reasons: (a) increasing concentration of dissolved salts leads to increases in osmotic pressure, which decreases the plant capability to absorb water and thus decreases the rate of plant growth creating osmotic or water-deficit effect of salinity, and (b) increased salt uptake leads to cell injuries in the transpiring leaves and might lead to decrease in the growth rate. This is referred to as the salt-specific or ion excess effect of salinity (Greenway and Munns 1980). The damages of salinity to plants depend on (a) salt concentration, (b) plant susceptibility, and (c) stage of plant growth (Rozema and Flowers 2008; James et al. 2011). Several studies reported that triticale has the ability to tolerate salinity stress more than rye and wheat (Gorham 1990; Shalaby et al. 1993; Heidari et al. 2016). Richards et al. (1987) stated that salinity reduced the yield of grans and biomasses in triticale, wheat, and barley. Salinity and drought stress decreased the yield and yield components (plant height, number of grains, spikes, weight of 100 grain) in triticale and other cereal and legume crops (Karim et al. 1993). It was reported that by increasing NaCl concentration, the emergence of seedling was delayed, and the root and shoot dry weight was reduced in both wheat and triticale. Kwinta and Cal (2005) reported that growing triticale in a medium containing NaCl decreases plant development, biomass production, and root development. Growing triticale under salinity stress (120 mM NaCl) decreases the dry matter accumulation in shoots and roots and increased the hydrogen peroxide content in shoots and roots (Garifzyanov et al. 2012). Salinity (NaCl) reduced the content of K^+ and enhanced the accumulation of Na^+ in the shoots and roots. Salinity did not affect final germination percentage but significantly affected the germination time mostly because of the increase of Na^+ accumulation in the seeds of triticale genotypes. NaCl negatively affected the seedling characters in triticale (Atakm et al. 2006). Under salinity stress, plant develops several mechanisms which include biochemical and physiological in order to improve salinity tolerance, like (a) increase the activity of antioxidant enzymes, viz., SOD, CAT, glutathione peroxidase (Asada 1999; Gupta et al. 2005), etc.; (b) increase in proline (Hoque et al. 2007; Ahmad et al. 2010), glycine betaine (Khan et al. 2000; Wang and Nii 2000), sugar (Kerepesi and Galiba 2000; Bohnert et al. 1995), and polyol accumulation (Ford 1984; Dopp et al. 1985); (c) increase in endogenous polyamine level (Parida et al. 2004). In study conducted to investigate the impact of NaCl solutions in three levels, i.e. low salinity stress (50 mM), moderate salinity stress (100 mM), and high salinity stress (200 mM), on three triticale varieties, it was confirmed that the three levels of salinity stress caused reduction in seed weight and starch content; the values of reduction were 9–42% under moderate salinity stress and 18–51% under high salinity stress. A rise in amylose synthesis and starch accumulation in triticale was observed under moderate salinity stress and high salinity stress. A reduction in the peak temperature and a rise in the starch were observed under both conditions in the three varieties (He et al. 2013). Salehi and Arzani (2013) reported that salinity stress affects grain quality traits in both triticale and wheat. In a study to evaluate two salt-tolerant and two salt-sensitive triticale lines during salt (200 mM) stress, it was observed that salinity affected more the sensitive genotypes (Kiani-Pouya 2015). Heidari et al. (2016) stated that under salinity conditions, SOD activity was significantly increased in wheat, triticale and rye genotypes. Bezirğanoğlu (2017) noted that salinity increased the accumulation of proline and sugar, along with the rise in antioxidant enzyme activity in triticale genotypes. Demirbas and Balkan (2020) also reported that in response to high NaCl, antioxidant enzyme activity increased in triticale cultivars.

25.7 Conclusions

Throughout this chapter, we have sought to summarize the most recent developments in triticale tolerance to abiotic stressors. Various stress resistance mechanisms employed by triticale are discussed, and some success in improving the tolerance mechanism through upregulation of signaling networks has been highlighted.

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