Shabir Hussain Wani
Manu Pratap Gangola
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Compatible Solutes Engineering for Crop Plants Facing Climate Change

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This book is dedicated to
Prof. P. B. Kavi Kishor
Prof. P. B. Kavi Kishor completed his undergraduate and graduate education at Andhra University and South Gujarat University. After obtaining his Ph.D. in botany from the Maharaja Sayaji Rao University of Baroda, Vadodara, Gujarat, he worked as a postdoctoral fellow and also as a scientist in the Department of Genetics, Osmania University, Hyderabad from 1981 till 1984.
He began his academic career in the Department of Botany, Kakatiya University, for a short stint of 5 years as assistant and associate professor and then moved back to

Osmania University, Hyderabad. After becoming professor of genetics in 1994, he served the Department of Genetics, Osmania University, in several capacities. His teaching and research career spans over 45 years. He worked at the Center for Biotechnology, Ohio State University, Ohio, USA, and produced some seminal papers on proline biosynthetic pathway genes and their validation in plants. He was a visiting fellow at several universities, including the Department of Biochemistry, Emory University, USA; the Division of Biotechnology, Linkoping University, Sweden; and the Leibniz Institute of Plant Genetics and Crop Plant Research, Gatersleben, Germany, where he happened to learn many molecular biological techniques. His interest lies in the broad areas of plant physiology and molecular biology of plants and specifically abiotic stress tolerance in plants. The seminal paper he produced on proline biosynthetic pathway genes and the genes implicated in abiotic stress tolerance in plants were validated. His research work resulted in the publication of as many as 265 papers including book chapters. Notable among them are two papers published in Nature Genetics in 2019 on the genomics of peanut and chickpea in collaboration with Dr. Rajeev Varshney, ICRISAT, Hyderabad. As an author and editor of 7 books, he has been engaged in the services of the academic community. Prof. Kavi Kishor was a member of the Academic Senate, Osmania University, and instrumental in obtaining several grants such as DBT-BUILDER, DST-INSPIRE, and

UGC-CAS. He supervised or co-supervised 49 Ph.D. theses so far. He is an expert committee member of UGC, CSIR, DST, DBT, ICAR, and AICTE.

Prof. Kavi Kishor has been a recipient of many awards for his teaching and scientific contributions in the field of plant biology. He received the Prof. Hiralal Chakravarthy Award in 1990, Rockefeller Foundation Fellowship in 1992, Best Teacher Award from the Government of Andhra Pradesh, Prof. P. Maheswari Gold Medal, Gold Medal from the Association of Biotechnology and Pharmacy, and Life-Time Achievement Gold Medal from the Association of Biotechnology and Pharmacy. Other awards include Dr. Godgil Memorial Lecture and Dr. Diwaker Patel Memorial Lecture.

He is a fellow of the National Academy of Sciences, Allahabad (FNASc), Fellow of National Academy of Agricultural Sciences (FNAAS), Fellow of the Botanical Society, Fellow of the Indian Association of Biotechnology and Pharmacy, Fellow of the A.P. Akademi of Sciences, and Fellow of the Telangana Academy of Sciences. Currently he is working as an emeritus professor at the Vignan's Foundation for Science, Technology and Research, Guntur, Andhra Pradesh.

Foreword

Today, sustainable agriculture and global food security are the prime and formidable challenges. Food production will have to be enhanced, and to feed more than 9 billion, estimated population by 2050 at 1.1–1.3% growth per year, immediate measures are necessary to raise the yield of major crops. One of the major factors limiting crop yield is climate change and ensuing abiotic stresses. This condition may worsen with dwindling agricultural land, water, and biological resources. It is therefore a daunting task for agricultural scientists and plant biologists to understand and develop tolerant/resistant varieties against different abiotic stresses. In this context, there is a greater need to utilize knowledge gained through biotechnological and/or genomic tools to engineer biosynthesis of various abiotic stress responsive biomolecules. Of these, osmolytes with organic chemical nature, termed as compatible solutes, have a vital role to play in structural, physiological, biochemical, and signaling roles during plant growth and development besides adaptation to various abiotic stresses. Research in the past several years has established the significant positive correlation between compatible solutes accumulation and crop yield in cereal, pulse, and oilseed crops. Bioengineering of osmolytes is now explored through coordinated regulation of biosynthetic pathways and genetic machinery to achieve sustainable stress tolerance.

I am pleased to know that this edited volume entitled *Compatible Solutes Engineering for Crop Plants Facing Climate Change* is being published by the authors. The book includes chapters on various compatible solutes, their sensing/signaling, biosynthesis, regulatory components, underlying biochemical mechanisms, crosstalk with other signaling pathways, and recent advances in transgenic development. This is an emerging area of plant biology with considerable scope for engineering of stress-tolerance traits. The editors of this illustrious book have done an excellent job by compiling chapters from contributing authors of international and national repute.

I congratulate the editors for compiling such a book, and I have no doubt that this will become a valuable knowledge resource for researchers, students, and policy makers.

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Contents

Chapter 1
Recent Advances in Plant Adaptation to Climate Change – An Introduction to Compatible Solutes

Muhammad Qudrat Ullah Farooqi, Zahra Zahra, Muhammad Afzal, and Muhammad Imran Ghani

Abstract The phenomenon of climate change has appeared in recent time as an unstoppable and unequivocal event due to the change of environmental condition, rising of sea level, ozone layer fluctuation, and most importantly, it has raised the global apprehension in lowering global food production. Abiotic stress is one of the major factors influencing overall performance of crop plants. The response of crop plant due to environmental alteration could be effective due to the accumulation of compatible solutes or osmoprotetctants, which contain amino acids, polyamines, sugar, and quaternary ammonium compounds. Compatible solutes play important role in mitigating the adverse effect of abiotic stresses in plants. Compatible solutes are low molecular weight compounds stabilizing cellular structure and scavenging reactive oxygen species (ROS) during abiotic stress conditions. The role of compatible solutes is very important in plant growth and development, i.e., glycinebetaine (GB) is helpful in maintaining osmotic potential, protecting proteins from denaturation and restoration of photosystem II complex (PSII) in plants. The biosynthesis of compatible solutes is important in plants especially adapting under stressful environment, in stabilizing the structure of PSII by protecting extrinsic proteins, and the introgression of genes associated with osmoprotactants from one plant to another by genetic engineering.

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Keywords Osmoprotectants · Climate · Compatible solutes · Abiotic stress

1.1 Introduction

A compatible solute is the substance in crop plants that accumulates in cytoplasm during cellular metabolic processes to overcome the imbalance of external osmotic pressure (Lang 2007). Climate change is one of the major factor for reducing crop productivity around the globe due to its impact on crops by enhancing abiotic stress that changes temperature, soil moisture condition, variation in rainfall pattern, nutrient imbalance, and most importantly, it negatively affects the overall plant physiological and metabolic activities during the developmental stages (Khan et al. 2019; Dutta et al. 2018). Overall, the yield losses due to the impact of abiotic stresses on global agricultural reduction approaches 70 percent annually (Acquaah 2009). Therefore, the role of compatible solutes or osmoprotectants should be important to activate plant stress resistances against abiotic conditions.

Plants modify their cellular functions and physiological activities differently due to the exposure of any stressful environment, especially when it comes to abiotic stress (Wang et al. 2018). The production and accumulation of low molecular weight, highly soluble, nontoxic compound generally known as compatible solutes or osmolytes or osmoprotectants are due to the modification of plant cellular function upon abiotic stress exposure (Riaz et al. 2019; Slama et al. 2015). The production and upregulation of these compatible solutes play an important role in minimizing protein denaturation and photosystem II (PSII) activities, reducing the impact of reactive oxygen species (ROS) activation and disruption of cellular structure without interfering the normal activities of plant metabolism (Nahar et al. 2016). The compatible solutes are characterized into three main parts: amino acids (ecotine and proline), ammonium (betaine, polyamines and choline-O-sulphate etc.), and sugar (trehalose, mannitol and sorbitol etc.) compounds (Jain 2013).

In genetic engineering, the manipulation of plant-responsive genes to osmoprotectants is one of the major strategies to resist stress and to improve plant yield. Therefore, transcriptome analysis during the biosynthesis of osmoprotectants made it possible to identify genes that are relevant with compatible solutes (Reguera et al. 2012). In this chapter, we aim to discuss the role of compatible solutes in crop plant to withstand harsh climatic condition, their role in plant adaptability under stressful condition, biosynthesis of compatible solutes under stressful condition. Moreover, it is very important to elaborate the latest development in genetic engineering strategies with enhancing compatible solutes production ability especially under different climatic changes

1.2 An Overview of Compatible Solutes Functions in Plants

The compatible solutes are organic, hydrophilic, small soluble compounds with lower molecular weight. The accumulation of these compatible solutes is very important factor to develop strong mechanism of plant defense under diverse environmental changes that greatly impact overall global crop production (Nahar et al. 2016). Furthermore, these solutes are nontoxic at higher cellular concentration during intercellular metabolic operations activated due to stressful environmental conditions. The osmoprotectants that usually accumulate in plants during unfavorable conditions are generally categorized into three distinct groups based on their chemical composition: amino acid, ammonium, and carbohydrate sugar–related compounds such as fructan, ectoine, sorbitol, polysol, betaine, gamma amino butyric acid (GABA), trehalose, pipecolic acid etc. (Ashraf and Foolad 2007).

The accumulation and concentration of compatible solutes depend on various factors including stress type and its severity to certain plant species for their growth during the exposure of stressful environmental conditions (Kumar 2009). The major reason for accumulating compatible solutes during unfavorable climatic conditions are to regulate osmotic balance by maintaining cell turgor pressure via replacing inorganic ion, alleviate ionic toxicity, osmoregulation, and in protecting cellular components in plants (Slama et al. 2015). Crop plant responses to various stresses due to the accumulation of compatible solutes are briefly presented in a schematic diagram (Fig. 1.1). In brief, compatible solutes play an important role in improving stress resistance by protecting plant cellular membrane, detoxifying reactive

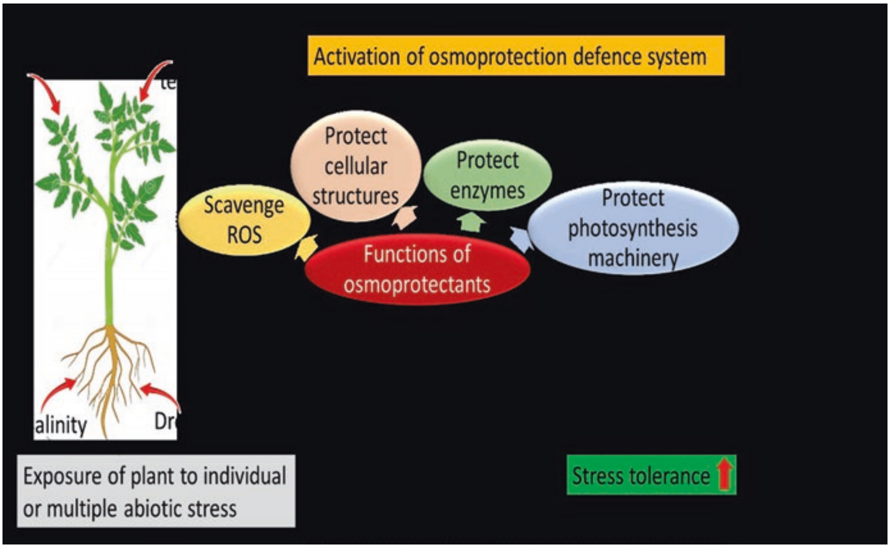

Fig. 1.1 A schematic diagram representing the functions of compatible solutes or osmoprotectants during various environmental changes

oxygen species (ROS), maintaining and stabilizing cellular structure, regulating protein folding to mediate stress signaling, and stabilizing thylakoid membrane to upregulate photosynthesis (Suprasanna et al. 2016; Alam et al. 2014).

Owing to the importance of compatible solutes in plant survival, the studies related to osmoprotectants to play an important role in plant survival as it is considered as central dogma in plant molecular biology and physiology. To understand the diverse role of an individual osmoprotectant, it is therefore important to improve stress tolerance in plants by accumulating compatible solute under stressful environmental conditions (Wani et al. 2018). In this section, we briefly update an overview of compatible solutes' functions and their accumulation under stressful environmental conditions to achieve maximum crop productivity.

1.3 Role of Compatible Solutes in Tolerating Abiotic Stress in Plants

Abiotic stresses are indeed impacted negatively during plant growth and development.

Among abiotic stresses, salinity and drought are the major types that are initiated due to changes in environmental conditions and greatly impacte global crop production in arid and semiarid regions of the world (Chen et al. 2010). Salinity stress is introduced into the environment due to various natural and anthropogenic activities, such as organic waste, industrial discharges, and weathering of parental rocks to release various type of salts (particularly chlorides, calcium, magnesium) in excess to soil (Galvani 2007). While the drought stress can occur across all climatic zones of the world, including area containing high and low rainfall. Drought stress is highly regulated by agronomic, edaphic and climatic factors, and distribution of uneven rainfall during various plant growth stages (Khan et al. 2019).

Crop plants develop a series of developmental mechanisms in response to stresses like drought and salinity at physiological, morphological, and molecular level. In order to develop strong mechanism against salinity and drought stresses, the production and accumulation of compatible solutes, and regulation of nutrient homeostasis is very important at cellular level of plant (Galvani 2007). The role of compatible solutes or osmoprotectants are important to mitigate the risk of ROS, in regulating osmotic adjustment at cellular level, stabilize enzymes and proteins structure, and to prevent plant cell membrane due to the response of abiotic stresses. Furthermore, osmoprotactants could preserve cellular apparatus from damaging risk caused by dehydration by not interfering with normal metabolic activities at cellular level (Suprasanna et al. 2016).

1.4 Physiological Response of Compatible Solutes in Adaptation to Climate Changes

Crop plants are force-exposed to various abiotic stresses due to unfavorable climatic conditions, including extreme temperature, salinity, drought, UV radiation, etc., that could adversely affect plant metabolic activities and yield heavy losses. In this way, compatible solutes are important to play role as safeguard against the impact of stress on plant without disturbing its normal activity related to plant metabolism and its physiology (Song et al. 2008). Evidence shows that compatible solutes are important to combat the ceasing of plant physiological activities, including all key processes like net photosynthesis, biosynthesis of photosynthetic pigments, electron transport rate, protein synthesis, lipid metabolism, signal transduction, and effective quantum yield of photosystem II (PSII) during the development of stress contained by plants (Kalaji et al. 2018). In addition, compatible solutes also increase the expression of some proteins like heat-shock protein (HSP), enzymes, and other macromolecules from denaturation during the process of osmoregulation (Li et al. 2011).

During the exposure of high temperature stress, the PSII complex in photosystem is considered as most susceptible part of photosynthesis to be diminished as the photochemical activity of PSII could reduce drastically during environmental changes (Kalaji et al. 2018). Abiotic stress (heat, drought, and salinity) types could inhibit the synthesis of de novo (D1) protein, reduce relative water and chlorophyll content, and repair of PSII during photoinhibition activities (Pagliano et al. 2013). In this way, compatible solutes could play important roles in physiological activities like osmotic adjustment, signal transduction, stabilizing PSII during the exposure of heat stress (Servillo et al. 2018). Compatible solutes like glycinebetaine (GB) play an important role in stabilizing the structure and function of PSII during the impact of abiotic stresses; furthermore, betaine was proposed as protective substance to secure plant cellular membrane based on previously reported experimental evidences (Murata et al. 2007).

Groups of osmoprotectants are involved to upregulate the physiological activities during various abiotic stresses (Singh et al. 2015). Compatible solutes like polyamines (PAs) are involved in regulating pH of cellular components; binds RNA, DNA, and proteins through electrostatic linkages with a great affinity; and to produce ethylene during unfavorable environmental changes (Hussain et al. 2011). Betaine is another compatible solute that plays an active role in water flow into cells to maintain osmotic equilibrium and regulate signal transduction under various environmental stresses (Wani et al. 2013). Similarly, the group of compatible solutes related to carbohydrate sugar and sugar alcohols is useful to provide carbon and energy to regulate normal functioning of plant metabolism, protect cellular membrane, and to scavenge ROS against various kind of stresses (Koyro et al. 2012). Proline is another compatible solute that belongs to amino acid group that generally accumulates in cytosol and contributes cellular membrane protection, produces various enzymes and proteins, and protects the degradation of plastid and mitochondria during climatic changes in plants (Ashraf and Foolad 2007). Furthermore, these osmoprotectants are essential to produce ion homeostasis and antioxidant defense system due to the severity of oxidative stress caused by salinity and drought stress (Kubiś et al. 2014).

1.5 Enhancing Synthesis of Compatible Solutes Through Genetic Engineering

Low genetic variability is available to compatible solutes breeding in many crop plants to develop their improved cultivars especially under the exposure of varied environmental stress. The technologies that improvise conventional breeding strategies are laborious, time-consuming, and ineffective to develop stress against abiotic (multigenic) and biotic (monogenic) types in plants (Aquino et al. 2011). Furthermore, limited researches have been conducted so far to identify gene of interest related to osmoprotectants to develop through protemic and transcriptomic analyses in plant genome. Therefore, technologies related to genetic engineering like transgenic, genome editing, and transcriptional factors are most abundant to improve stress resistance under varied environmental conditions (Jain 2015). In this way, targeting an individual gene would be more useful to upregulate processes including improve plant water use efficiency, stomatal regulation, and ABA biosynthesis, assimilating $CO_{2,}$ as these could be helpful to increase stress resistance mechanism during unfavorable conditions exposed to plants (Bhatnagar-Mathur et al. 2008).

Transgenic technology has great potential in enhancing stress resistive mechanism in plant that is created due to any unfavorable conditions imposed on plants (Jain 2015). The protocols of genome editing could be employed in plants to accumulate compatible solutes by achieving various processes like insertion and deletion of genes, mutation and alteration of exact sequence in plant genome by tailoring nucleases such as zinc finger nuclease (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat associated with nuclease 9 (CRISPER-Case9) that are promising genome editing tools to accumulate and restore compatible solutes in plants (Jain 2015; Kumar et al. 2004). It has been reported in several studies that osmoprotectants could be accumulated in plant effectively by employing genetic engineering with the modification of chloroplast and plastid genomes in plants (Kumar et al. 2004). Among all these, CRISPER-Cas9 is most effective, inexpensive, simple, and user-friendly genome editing tool in genetic engineering that could be adapted in a variety of plants to fulfil world demand of increasing food productivity in the context of various climatic changes (Khatodia et al. 2016).

Identification and classification of gene is one of the best pathways to develop stress-tolerant species concerted by the combined efforts of cell physiology, plant molecular biology plant genetics, and biotechnology in our research domains. In response to accumulation of compatible solutes in plant cell via genetic engineering tool, two types (directly involve accumulating osmoprotectants, antifreeze protein and chaperons in plants, and another type has to upregulate processes like signal transduction, protein kinases etc.) of genes are involved (Bhatnagar-Mathur et al. 2008). In a broader term, compatible solutes are confined in plant cells with cytosol, chloroplast, and different other cytoplasmic divisions. Due to inability in many plants to upregulate the osmoprotectants production in response to varied stress, the implementation of latest genomic tools is essential to protect plant cellular structure, function, and to improve global food productivity (Wani et al. 2013). Therefore, many studies have been conducted to evaluate the response of increased osmoprotectant production under varied stress through genetic transformation (Cai et al. 2017). However, the phenomenon of incorporation of transgenes into the host genome to gain specific objectives in our conducted research is still considered a great concern in public when it comes to edible crops (Stephens and Barakate 2017).

1.6 Conclusion and Future Prospects of Compatible Solutes in Adapting Climate Changes in Plants

Plants often face a variety of alterations in environmental conditions like temperature, CO_2 level, and water and nutrient availability, which could lead to develop stress, which hampers plant growth at major developmental stages and causes a substantial amount of yield reduction. In this way, compatible solutes or osmoprotectants are one of the influential factors to make a strong defense mechanism in crop plant to reduce the impact of various environmental stresses. A group of osmoprotectatnts belongs to amino acid, sugar, and ammonium group that could enhance stress resistance and to produce defense mechanism especially under the impact of drought and salinity stress. These compatible solutes not only help to upregulate the physiological and metabolic activities in plants but also help in introducing gene(s) of interest to improve stress tolerances in plants. Identifying genetics behind stress tolerance mechanism and gene regulation will aid researchers in translating information for more stress-sensitive crops. Considering the significance of compatible solutes in upregulating plant physiological and biochemical activities, further research dealing with endogenous regulation and omic approaches to accumulate osomoprotectant during stressful conditions would assist to deal with more adverse conditions.

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Chapter 2
Osmosensing and Signalling in Plants: Potential Role in Crop Improvement Under Climate Change

Deepu Pandita and Shabir Hussain Wani

Abstract Osmotic stress and other abiotic stresses have severe impacts on crop productivity. Since climate change is predicted to exacerbate the problem, the development of a new breed of crops, which are tolerant to osmotic stresses, is required. Vicissitudes in contiguous environmental scenarios can be sensed by molecular osmosensors, which sequentially activate cellular signalling proceedings that regulate plant homeostasis for repairs of normal growth and development. However, only limited success has been achieved in agriculture till date primarily because of the lack of a clear understanding of the mechanisms that facilitate osmosensing. In this chapter, we deliberate on the potential mechanisms of osmosensing and signalling in plants, emphasizing the receptor-like kinases, mechanosensitive calcium channels, phospholipase C, aquaporins, and membrane-bound histidine kinases for detection of vicissitudes in extracellular plant osmolarity. Genetic engineering and genome editing of the Osmotic Stress–Regulated (OR) genes and osmosensors including Receptor-Like Kinases (RLKs) and Histidine kinases (HKs) may positively affect stress tolerance by manipulating signalling factors downstream and by elucidating the regulatory mechanism of osmosensors in downstream signalling pathways; designing of the next-generation climate smart crops will be possible.

Keywords Osmosensing · Signalling · Osmotic Stress · Osmosensors · Receptor-like kinases · Aquaporins · Climate change

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2.1 Introduction

Climate change may have antagonistic consequences on the crop production and agricultural sustainability (Porfirio et al. 2018). Global rise in land usage of approximately 100 million hectares with a boosting of global trade is criterion by year 2050 to cater to forthcoming agriculture mandate of 9.8 billion individuals in current cultivable land area (Pastor et al. 2019; Wani et al. 2020). Plants are unremittingly exposed to abiotic stresses which impair plant metabolism, physiology, growth, development, and productivity. The most common adversative abiotic factors faced by terrestrial plants are higher temperature and osmotic stress (Wani and Gosal, 2010). Osmotic stress can be caused by various abiotic factors like drought, salinity, or cold. By year 2050, osmotic stress will be ubiquitous in >50% of arable lands due to severe salinity (Hoffmann, 2002). Osmotic stress in widest sense includes drought and salinity stress–stimulated reduction in water potential, triggering biochemical, morphological, physiological, and molecular fluctuations in a plant cell (Hoffmann, 2002; Wani et al. 2013). Ecological water insufficiency triggers osmotic stress signalling cascades which in turn induce temporary cellular responses to decreased water loss and resilient responses by switching on transcriptional pathways and physiological and developmental processes (Zhu 2002; Cutler et al. 2010; Hsiao 1973; Kim et al. 2010). Initial proceedings of plant adaptation to stresses are signal perception and succeeding signals of transduction. This sequentially activates several physiological and metabolic responses as well as expression of the stress-responsive gene networks (Thomashow 1999; Shinozaki and Yamaguchi-Shinozaki, 2000; Zhu 2002; Zhang et al. 2004; Bray, 2004; Nakashima and Yamaguchi-Shinozaki 2006; Yamaguchi-Shinozaki and Shinozaki 2005, 2006). Turgor loss is in general probable mechanism of perceiving osmotic stress. Nevertheless, cautious extents of cell biochemistry to osmotic stresses designated that osmosensing is not exclusively by turgor fluctuations (Handa et al. 1986), but osmolarity and osmosensors can also be involved. Osmotic stress signalling activates specific kinases comprising 1 to 10 family members (SnRK2.1–2.10) of plant-specific protein kinase, SNF1-related protein kinase (SnRK)2 family in *Arabidopsis thaliana.* SnRK2s members are important kinases in osmotic stress signalling (Fuji and Zhu 2009; Fujii et al. 2009, 2011; Umezawa et al. 2010). Sustainable food sources for the increasing population neeed progress in the crop yield potential in restricted land area and adversative environment (Long and Ort 2010). Genome edited and genetically manipulated crop plants are amongst the swiftest approved in contemporary past with ~100-fold escalation in plant hectares from 1.7 MH (1996) to 189.8 MH (2017) (ISAAA 2017). Genetically modified crop practice escalated production of agriculture by above US$98 billion in a sustainable way and avoided use of about 473 million kg of pesticides (Gilbert 2013).

The osmolarity alteration leads to stretching of plasma membrane and that activates osmosensors. Osmosensors are mechanosensing channel subtype: for instance DEG/ENaC, K2P, TRP, MscS-like, and Piezoinnon in eukaryotes (Kung 2005; Arnado'ttir and Chalfie 2010; Coste et al. 2012; Wu et al. 2010). Plants lack DEG/

ENa Cor TRP (Hedrich et al. 2012; Ward et al. 2009). *Arabidopsis thaliana* has 10 MscS-like and one Piezo homolog (Monshausen and Gilroy 2009), but their osmosensor nature remains to be elucidated. *Arabidopsis* MCA1 and MCA2 (MID1-complementing activity 1 and 2) proteins homologous to *Saccharomyces cerevisae* stretch-activated Ca2+ channel MID1 mediate hypo-osmolarity-induced Ca2+ escalations and mechanical responses (Nakano et al. 2011). *A. thaliana* histidine kinase AtHK1 functions like *Saccharomyces cerevisae* osmosensor histidine kinase SLNI (Wohlbach et al. 2008). To manage osmotic stress, it is imperative to comprehend the mechanisms of perception, signalling, and responses to drought, salinity, cold-osmostresses which interrupt cellular homeostasis for stress acclimation and tolerance for improving crop production. This chapter highlights the characterization of osmosensors, and molecular and physiological osmosensing mechanisms in plants for future utilization in genetically modified osmotic stress–tolerant crops required to cope with climate change.

2.2 Lexicon and Conception of Plant Osmosensing

The term “osmosensing” denotes steadfast perception of osmolarity of exterior or interior cell environment. The mechanical stimuli triggered due to modifications in plasma membrane tension, cell wall mutilation, or interruption of plasma membrane–cell wall networks are applicable aspects in osmosensing, besides changed extracellular osmolarity intrinsically (Fig. 2.1). Osmosensing includes direct perception of osmotic imbalance across plasma membrane besides perception of indirect influences of osmotic imbalance on plasma membrane, cell wall, or plasma membrane–cell wall system. The elevated water permeability and solute discrimination of plasma membranes safeguards fluctuations in the extracellular osmolarity. The forces plasma membrane and cosolvents trigger widespread variations in the chemistry and physical structure of cell. So, in principle, osmosensors detect fluctuations in extracellular water activity (direct osmosensing) or detect and elicit responses personalized to cater to the secondary consequences of osmotic shifts (indirect osmosensing). Thus, it can be predicted that osmosensor arrays may help in detection and regulation of temporal cascade of cellular changes and osmoregulatory responses (Garcia-Romeu et al. 1996; Strange 1994). Accordingly, “osmotic history” of every cell may decide its response to novel osmotic stimuli (Garcia-Romeu et al. 1996; Strange 1994). The downstream influences of water constraint on plants include compatible solutes accumulation, induction of protective proteins, production of ABA, and enhanced extracellular sensitivity to abscisic acid (Christmann et al. 2013; Verslues and Juenger 2011; Wani et al. 2013).

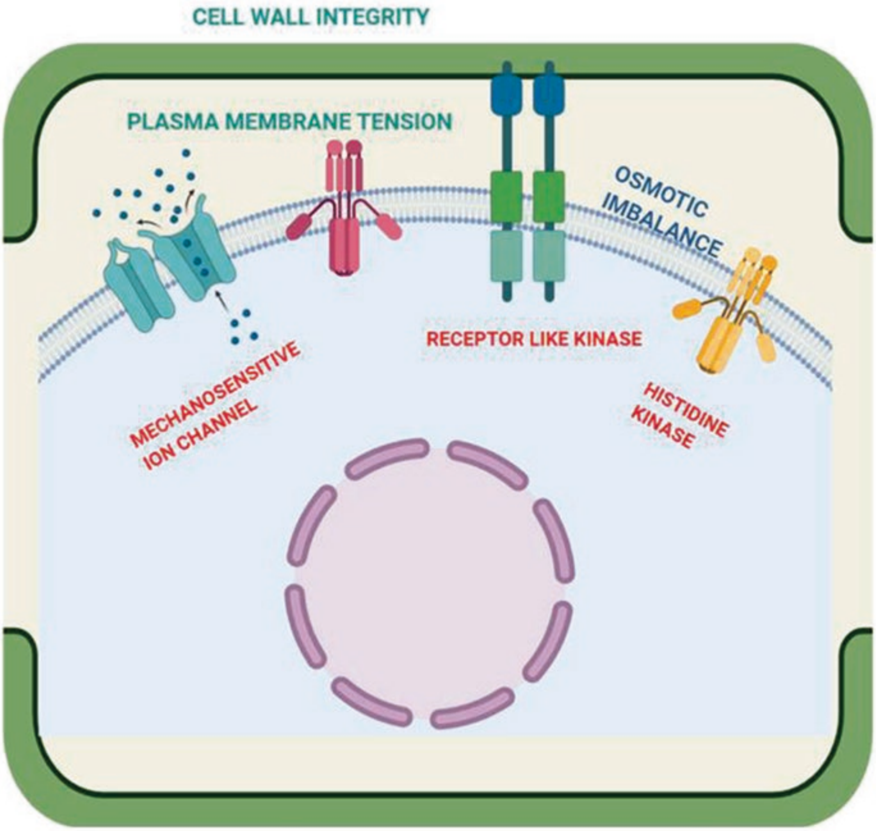

Fig. 2.1 Molecular models for mechanism of osmosensing in plants

2.3 Probe of Plant Osmosensors

In *Saccharomyces cerevisiae*, hyperosmolarity is perceived via a two-component system which comprises YPD1 phosphorelay intermediate known as SLN1 His kinase and response regulator known as SSK1. These activate HOG1 MAPK pathway. AtHK1 of *Arabidopsis thaliana* is homolog of yeast SLN1: it suppresses salinity-sensitive phenotype of yeast double mutants' ln1D sho1D, deficient of *Saccharomyces cerevisiae* osmosensors (Urao et al. 1999). Straight-forward substantiation of AtHK1 as plant osmosensor is till now missing. AtHK1 interacts with phosphorelay AtHP1 in *Saccharomyces cerevisiae* two-hybrid system but AtHP1 interaction with RRs (response regulators) was undetected (Urao et al. 2000). Cytokinin receptor CRE1 kinase complements yeast sln1D mutant during cytokinin existence (Inoue et al. 2001). Reiser et al. (2003) reported that in *Saccharomy cescerevisiae* SLN1 His kinase and CRE1 kinase senses osmotic stress through turgor sensing. For sensor function, integrity of SLN1 periplasmic region is vital. Osmotic stress induces alteration in SLN1 conformation because of stress generated by the alterations in cell wall–plasma membrane interactions. May be an analogous turgor-sensing mechanism controls plant hyperosmotic signalling. Instead, connection of membrane-located receptor-like kinases (RLK) to osmosensing came into light after augmented osmotic stress tolerance due to NtC7 overexpression in *Nicotiana tabacum* (Tamura et al. 2003). RLK protein forms dimers with conformation sensitive to structure modifications in plasma membrane to sense hyperosmolarity. There is no kinase catalytic domain present in NtC7 and transduces osmotic signal to cytoplasm through interaction with protein partners by C-terminal tail regions. Therefore, numerous osmosensors play roles in different plant-induced stress signal perceptions, even if definitive confirmation is limited. Abiotic stress responses because of heat, cold, drought, and salinity have diverse stress signalling pathways and thus may be distinct osmosensors as well (Kumar et al. 2018, Wani et al. 2020, Wani and Kumar 2020). In various plant genera, manifold “signalling-circuitries” which crosstalk and over-abundance of “osmotic-stress-response proteins” have been recognized. In plants, the three indispensable constituents of osmotic stress response machinery are perception, signalling, and response.

Eight diverse categories of genes which respond to stress were designated (Bhatnagar-Mathur et al. 2008). Out of which stress sensor and receptor protein comprised class involved in the osmotic stress perception is subjected to less investigation and characterization because of which engineering of osmotic stress-tolerant crop plants is challenging. Sensor proteins are imminent master regulators to trigger and coordinate stress response. Multiple signalling pathways operative during plant osmotic stress indicate that there is probably over one mechanism for perceiving osmotic stress. Substantiation from bacteria, mammalian cells and lower eukaryotes also emphasize that manifold osmosensing approaches operate at cell level (Häussinger and Sies 2007). Research studies disclose that plant osmosensing includes detection of consequences of osmotic stress on cellular components, for instance, mechanical stress subsequent to plasmolysis, cell membrane depolarization, and mutilation of cell membrane and cell wall. In this chapter, analysis of only representative proteins with a role in osmosensing and probable mechanisms of action will be discussed.

2.3.1 Two-Component System or Membrane-Localized Kinases

Osmosensing in the bacteria and *Saccharomyces cerevisiae* is facilitated via histidine kinases of TCS (two-component system) phosphorelay (Hall and Silhavy 1981; Maeda et al. 1994; Forst et al. 1989). Machinery designated as prototypical two-component system is present primarily in prokaryotes which includes mainly first identified sensor member Histidine kinases (HKs) and proteins known as response regulators (RRs). This two-component system is comprehensively understood (Hoch 2000; Hwang et al. 2002; Stock et al. 2000; Nongpiur et al. 2012). Histidine kinases (HKs) mediate bacterial surrounding warning signs to downstream RRs (response regulators) (Ha et al. 2012; Mizuno 2005). In osmosensing, cellular membrane or endoplasmic reticulum–localized kinases known as histidine kinases (HKs) originally characterized as first member of two-component system sense and perceive osmotic stress changes and plant hormones during signalling mechanism and subsequently auto-phosphorylation occurs at conserved histidine residues, which downstream transfers to conserved aspartate residue of response regulator.

According to Tran et al. (2007) and Wohlbach et al. (2008), non-CK receptor kinase AtHK1/AHK1 recognized as osmosensor acts as positive controller of osmotic stress signal transduction. An instance of two component systems is Envz-Ompr which takes part in *E. coli* osmosensing (Forst et al. 1989). The alternative category of TCS signalling is labelled as multistep Histidine to Aspartate phosphorelay, histidine phosphotransfer proteins (HPs) and response regulators (RRs) (Mochida et al. 2010; Schaller et al. 2008; Lee et al. 2011; Ha et al. 2012). Hybrid histidine kinase (HHK) auto-phosphorylates conserved histidine residue and relocates phosphoryl group to conserved aspartate residue in receiver domain of same histidine kinase which is then transferred to conserved histidine residue of histidine phosphotransfer protein (HPT), from there phosphoryl group is relocated to conserved aspartate group of cognate response regulator. This type of Histidine to Aspartate phosphorelay occurs in prokaryotes as well as few eukaryotes, alongside *Saccharomyces cerevisiae* and higher plants. *Saccharomyces cerevisiae* SLN1-YPD1-SSK1 two-component system is one mechanism of osmosensing (Posas et al. 1996). In plants, His-Asp phosphorelay regulates several processes along with hormone perception and responses to environmental stimuli and abiotic stresses, for instance, cold, salinity, and drought (Mochida et al. 2010; Jeon et al. 2010; Lee et al. 2011; Tran et al. 2007, 2010; Schaller et al. 2008; Ha et al. 2012; Wohlbach et al. 2008; Pham et al. 2012). Changed phosphorelay component expression due to abiotic stress occurred in numerous plants, for example soybean, *Arabidopsis thaliana*, and *Oryza sativa* (Osakabe et al. 2002; Tran et al. 2007; Jeon et al. 2010; Karan et al. 2009; Lee et al. 2011). Functional loss of ARR5, ARR6, or ARR7 increased tolerance towards cold stress and ARR1 and ARR12 increased tolerance towards salinity (Jeon et al. 2010; Mason et al. 2010). *Arabidopsis* two-component system gene identification logically led to functional characterization of histidine kinases, primarily to determine whether TCS performs an analogous function in osmoregulation as in counterparts of bacteria and *Saccharomyces cerevisiae*. *Arabidopsis* has 8 HK members. Five CK receptor AtHKs (ETR1, ERS1, AHK2, AHK3 and AHK4) perceive ethylene and CKs hormones (Schaller et al. 2008). AHK2, AHK3, and AHK4 take part in phosphorelay (Tsai et al. 2012). The non-hormonal receptor HKs are AHK1, AHK5, and CKI1. *Arabidopsis thaliana* histidine kinase ATHK1 is cell membrane bound with N-terminal half having 2 hydrophobic regions (Urao et al. 1999; Tran et al. 2007). *Arabidopsis* HK AtHK1/AHK1is a unique non-hormonal receptor kinase which functionally complements histidine kinase (SLN1) in *Saccharomyces cerevisiae* osmosensing-deficient mutant SLN1-ts by functioning as a putative osmosensor and exhibits positive regulation of osmotic stress signal transduction and tolerance in *Arabidopsis thaliana* as a unique osmosensor (Tran et al. 2007; Urao et al. 1999; Wohlbach et al. 2008). The putative his or asp phosphorylation sites on substitution with different amino acid residues made ATHK1 incapable of complementing SLN1 mutant (Urao et al. 1999).

AHK5 is cytoplasm and cell membrane bound at putative N-myristoylation sites (Desikan et al. 2008) and regulates stomata closing under H_2O_2-generated ROS (Desikan et al. 2008). The guard cells of ahk5 knock-down mutants were less sensitive to ROS-induced stomata closing (Desikan et al. 2008). AHK5 also is negative controller of salinity in *A. thaliana* seed germination and young seedlings and plant growth (Pham et al. 2012). Other HKs are plasma membrane bound (Inoue et al. 2001; Mähönen et al. 2000; Kim et al. 2006). AtHK1 transcription was controlled by peripheral osmolarity (Urao et al. 1999). Overexpression 35S:ATHK1 in *Arabidopsis* transgenic plants resulted in augmented expression of nine-cis-epoxycarotenoid dioxygenase-3 (NCED3) and more levels of ABA and enhanced osmotic and water stress tolerance, whereas athk 1 null mutants showed two-fold decrease in expression of NCED3 and decreased sensitivity to ABA levels and higher sensitivity to osmotic stress (Tran et al. 2007; Wohlbach et al. 2008). These conclusions suggest that AtHK1 osmosensor works upstream of NCED3 induction and ABA biosynthesis during onset of osmotic stress and further that osmosensing mechanism mediated by histidine kinases is conserved in bacteria, *Saccharomyces cerevisiae*, and higher plants and as a positive osmotic stress regulator regulates downstream gene expression in ABA-dependent and ABA-independent mode (Tran et al. 2007; Wohlbach et al. 2008). In epidermal guard cells of *Arabidopsis thaliana*, AHK1 has additional function downstream of H_2O_2 in ABA-dependent calcium channel regulation for regulating opening of leaf stomata (Lü et al. 2013). AtHK1 primarily regulates stomata density and avoidance of the dehydration (Kumar et al. 2013). AtHK1 regulates NCED3 and P5CS1 transcriptionally, whereas AHK1 mutants lack changed ABA and proline amounts (Kumar et al. 2013). AtHK1-mediated perception of osmotic stress and osmosensing in *Arabidopsis thaliana* is no single mechanism. ABA levels were not under AHK1 regulation, but ABA biosynthesis and regulation of stomata take place due to reduced turgor pressure of leaves (Sussmilch et al. 2017). Therefore, AtHK1 as an osmosensor is in conflict. Probably, ABA biosynthesis and accumulation is under regulation of multiple proteins (few with redundant functions) and AtHK1's function loss is recompensed by supplementary proteins. AHK1-AHP2 interaction sequentially shows interaction with many type-A and type-B response regulators. Thus, this multistep-phosphorelay (MSP) intermediated by AtHK1 functions in *A. thaliana* osmosensing (Urao et al. 2000; Dortay et al. 2006). AtHK1 supplements a HK osmosensor SLN1 deletion in *Saccharomyces cerevisiae* by playing role of osmosensor by activation of downstream cascades (Urao et al. 1999). *Populus tricocarpa* two-component system circuitry mediated by HK osmosensor PtHK1 (orthologue of AtHK1) has been characterized and decoded which show connexions to SLN-YPD1-SSK1/SKN7 osmosensor-deficient mutants of *Saccharomyces cerevisiae* (Bertheau et al. 2013; Héricourt et al. 2013). Diagrammatic representation of osmosensing mechanism intermediated by TCS in *Saccharomyces cerevisiae* and AtHK1 in *Arabidopsis thaliana* is given in Fig. 2.2.

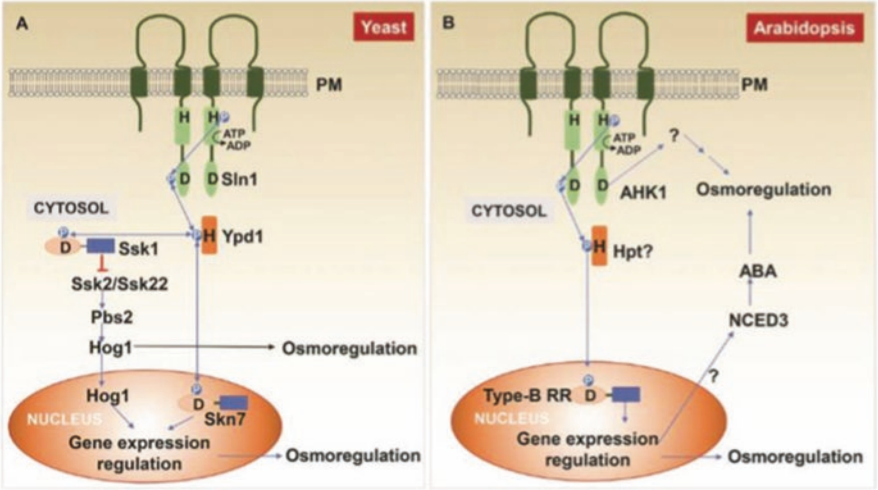

Fig. 2.2 Diagrammatic representation of two-component system osmosensing mechanism intermediated by TCS in *Saccharomyces cerevisiae* and AtHK1 in *Arabidopsis thaliana* (Source: Nongpiur et al. 2020)

2.3.2 *Mechanosensitive (MS) Channels*

MS ion channels present in all kingdoms of life are composed of multimeric proteins that remain implanted in plasma membrane and mediate ion flux transversely through it during lateral membrane tension (Ranade et al. 2015; Martinac 2012; Basu and Haswell 2017). Mid1 complementing activity (MCA) overexpression causes enhanced Ca2+ uptake under hypo-osmotic shock in A. *thaliana*, *Nicotiana*, and *Oryza sativa* (Nakagawa et al. 2007; Kurusu et al. 2012a; Kurusu et al. 2012b). *Arabidopsis thaliana* showed significant detectable events of transient boost of cytosolic Ca2+ [Ca2+ (cyt)] after approximately 5 seconds of osmotic stress due to drought and salt treatment (Yuan et al. 2014; Knight et al. 1997). These characteristic transient increased alterations in cytosolic Ca2+ responsive to a drought or salt stimulus Ca2 + (cyt) are designated as cytosolic Ca2+ [Ca2+ (cyt)] signatures (Whalley and Knight 2013). Diverse cytosolic Ca2+ [Ca2+ (cyt)] signatures lead to induction of diverse plant gene sets (Whalley and Knight 2013). Proteins which contribute to cytosolic Ca2+ [Ca2+ (cyt)] signatures might act as osmosensors. OSCA1(REDUCED HYPEROSMOLARITY-INDUCED [CA2+]i INCREASE 1), a hyper-osmolarity gated calcium-permeable channel, is one such plasma membrane protein identified in *Arabidopsis thaliana* and causes hyper-osmolarity-induced transient upsurges in cytosolic Ca2+ [Ca2+ (cyt)] (Yuan et al. 2014). OSCA1 is an integral protein with 9–10 transmembrane α-helices from a gene family which has fifteen members in *A. thaliana* and OSCA homologs show presence in different plants. *Arabidopsis thaliana* osca1 mutant showed normal cytosolic Ca2+ [Ca2+ (cyt)] in non-stress situations while after induction with a sorbitol stimulus and osmotic stress, cytosolic Ca2+ [Ca2+ (cyt)] levels lowered compared to wild plants and exhibited reduced root growth and stomatal response and enhanced water loss and wilting of plants (Yuan et al. 2014). Thus, there is a strong possibility of OSCA1 as plant osmosensor machinery for different osmotic stress stimuli like the role of calcium channels as animal osmosensors and future avenues of prospective molecular targets for designing stress-resistant crops (Pedersen et al. 2011). MCA1 allows integrity of plant cell wall signalling (Engelsdorf et al. 2018; Denness et al. 2011; Wormit et al. 2012). AtCSC1 (Arabidopsis Calcium permeable Stress-gated cation Channel 1) is one more integral plasma membrane calcium channel (Hou et al. 2014). AtCSC1 on expression in Chinese hamster ovary (cells) displayed amplified cytosolic Ca2+ [Ca2+ (cyt)] and reached peak in some seconds and decreased significantly after one minute of hyperosmotic stress (Hou et al. 2014). AtCSC1channel on expression in *Xenopus* oocytes exhibited permeability to K+, Ca2+, and Na + and belongs to unknown osmosensitive calcium-permeable cation channel family. AtCSC1C-terminal domain has 6 transmembrane helices labelled as Domain of Unknown Function 221(DUF221). *Arabidopsis* has 15 AtCSC1 homologs. OSCA1 also belongs to this osmosensor protein family (Pedersen and Nilius 2007; Hou et al. 2014). MscS-like (MSL) family members play role in osmotic homeostasis in chloroplasts (Haswell and Meyerowitz 2006; Veley et al. 2012) and pollen (Hamilton et al. 2015). In plant root epidermal cells immediately after hyperosmotic stress imposition, Cl-, Na + ,and K+ ions influx through mechanosensitive (MSL) channels which reinstate the lost cell turgor (Shabala and Lew 2002). *Arabidopsis* sign posted no contribution of MSL for restoring cell turgor during osmotic stress. *A. thaliana* msl4–1; msl5–2; msl6–1; msl91; msl10–1 quintuple mutants showed growth like wild-type plants under osmotic stress, salinity, drought or rehydration, and mechanical stress (Haswell et al. 2008). The voltage-gated K+ channels regulate intake under osmotic stress (Shabala and Lew 2002). Very recently, MscS-like10 (MSL10) has been reported to function as a phospho-regulated membrane-based sensor connecting perception of cell swelling to downstream signalling cascades and programmed cell death in *Arabidopsis thaliana* in response to hypo-osmotic treatment (Basu and Haswell 2020).

In prokaryotes, mechanical stress and amplified cell membrane tension due to hyperosmotic stress signals mechanosensitive channels to open up (Rohacs 2014). In eukaryotes, mechanosensing mechanism involves interactions of scaffolding network, actin cytoskeleton, extracellular matrix, plasma membrane and cell wall and proteins for instance integrins, TRPs (non-selective cation channels permeable to Ca2+), receptor kinases, cell wall–associated proteins, and G-protein-coupled receptors (Christensen and Corey 2007; Lü et al. 2007; Knepper et al. 2015; Rohacs 2014; Danziger and Zeidel 2015). TRP family is not identified in plants yet (Nilius and Owsianik 2011). CSCs are analogous to Transient Receptor Potential (TRP) channels of animals with role in (osmo) mechanosensing. CSCs have traits reminiscent of TRPs, so can be part of osmo-mechanosensing plant machinery (Hou et al. 2014). OSCA1 and OSCA3 are mechanosensitive channels, but their structure and pore-opening mechanism bears resemblance with TMEM16 channels by forming

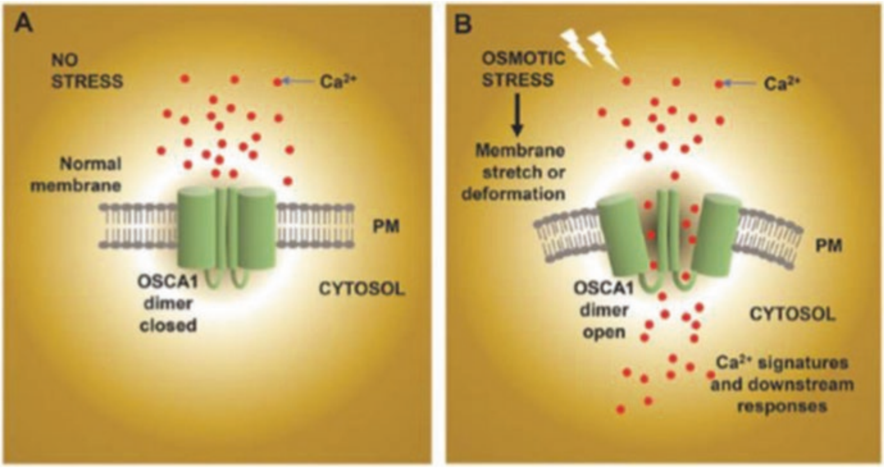

Fig. 2.3 Diagrammatic representation of mechanosensitive calcium OSCA1 channel osmosensing (Source: Nongpiur et al. 2020)

dimers, whereas the TRPs form tetramers (Zhang et al. 2018). The diagrammatic representation of OSCA1 osmo-mechanosensing is depicted in Fig. 2.3.

2.3.3 *Phospholipase C*

In rice within seconds under salinity, phosphoinositide-specific OsPLC1 (phospholipase C) stimulates osmotic stress–induced Ca2+ (cyt) in stomata. The osplc1 mutant rice exhibited sensitivity to salt with proliferated conductance of stomata and transpiration rates than wild type plants. Thus, OsPLC1 was suggested having a role in Ca2+ (cyt) generation under water deficiency and ABA. OsPLC1 responds to manifold signals, and salt stress triggers OsPLC1 translocation from cytoplasm to cell membrane (Li et al. 2017). The plant roots subjected to salt exposure caused lowered water potential resulting in reduced water supply to shoot tissues and further changes in the properties of cell membrane and cell wall which may potentially trigger recruitment and activity of OsPLC1 (Maathuis 2014). Plant PI-PLCs and OsPLC1 belong to PLC family and could be regulated by calcium-permeable channel(s) under osmotic stress. Rice osplc1 mutant under salt stress show transient proliferation in cytosolic Ca2+, suggesting that osmo (mechano) sensitive calcium channel for Ca2+ influx into cytosol was functioning, but maintenance of Ca2 + (cyt) signature has prerequisite of OsPLC1 which is an integral osmosensing component (Li et al. 2017). The enzymatic activity on PLC produces DAG and IP3 secondary messengers. In animals, IP3 binds to calcium channel receptors of ER and releases Ca2+ into cytosol (Hamada et al. 2017). IP3 receptors are unidentified in higher plants (Krinke et al. 2006). PLC undergoes regulation and activates cytosolic Ca2+

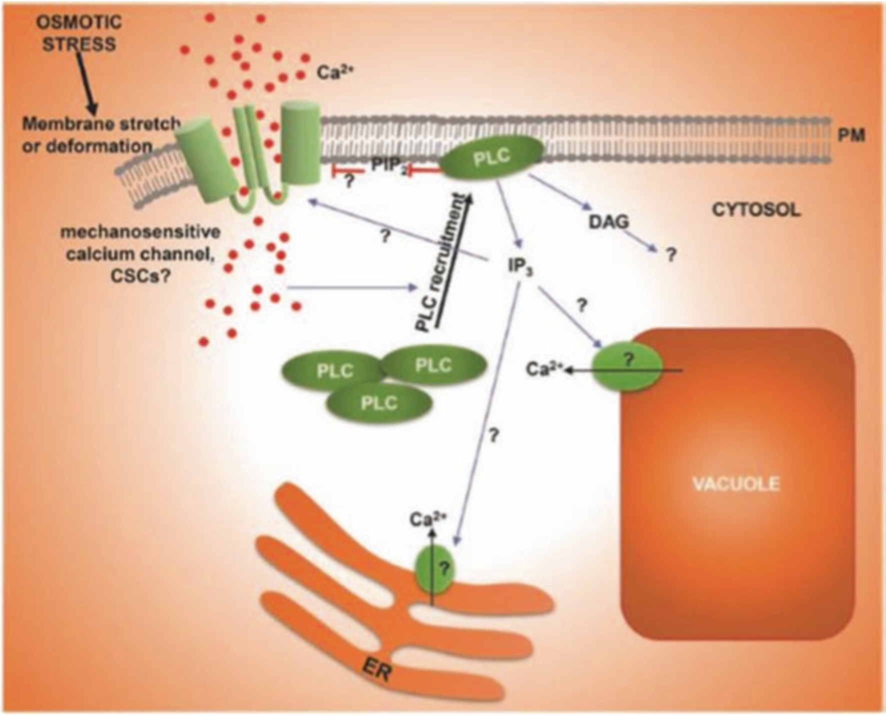

Fig. 2.4 Model of PLC osmosensing (Source: Nongpiur et al. 2020)

influx through mechanosensitive channels and drifts PLC to altered plasma membrane (Rupwate and Rajasekharan 2012). IP3-mediated cytosolic Ca2+ release from intracellular stores in the cytosol regulates pollen tube growth and maintenance of stomata pore (Franklin-Tong et al. 1996; Staxen et al. 1999). *Arabidopsis* PLCs increase cytosolic Ca2+ under salinity and temperature stress, whereas its plc3 mutant showed hypersensitivity to heat stress and a drop in heat-induced increase in Ca2+ and hyposensitivity to salinity (Gao et al. 2014; Xia et al. 2017). The PLC4-over-expressed plants were hypersensitive (Xia et al. 2017). AtPLC4 regulates salt-induced increase of Ca2+ levels (Xia et al. 2017). Postulate of probable PLC osmosensing is provided in Fig. 2.4.

2.3.4 Observation of the Cell Wall and Receptor-like Kinases (RLKs)

Osmotic fluctuations regulate stretching of cell wall for the growth of cells (Brett and Waldron 1996). Plant cell wall remains submerged in a watery matrix responsible for its various characteristics and osmotic stress causes matrix water loss which in turn leads to substantial fluctuations in properties and polymerization of the cell wall (Huang et al. 2018; Moore et al. 2008).When fully plasmolysed plant cells with disrupted hechtian strands get deplasmolysed via rehydration with isotonic solution during hypertonic stress treatment, influx of Ca2+ into the cytosol does not take place. Conversely, under hypotonic stress to deplasmolysed cells, hypotonicity-induced Ca2+ influx was observed (Hayashi et al. 2006; Hayashi and Takagi 2003). The cellulase pre-treatment of the plant cells leads to comprehensive inhibition of Ca2+ influx under only hypertonic treatment (Hayashi et al. 2006). Cell wall integrity or adhesion of plasma membrane with cell wall (PM-CW) plays a fundamental function in mechanosensitive Ca2+ influx into cytosol in hyperosmotic stress. Cell membrane remains linked at hechtian strands to the rigid cell wall anchor. Hyperosmotic stress causes plasmolysis which in turn leads to plasma membrane stretching due to hechtian strand attachments. This leads to opening of the mechanosensitive Ca2+ channels. The deplasmolysed cells lose hechtian strand attachment and a decrease in cell volume because of succeeding hyperosmotic stress that doesn't unavoidably include stretching of the plasma membrane as cell plasmolysis is of convex type. Ca2+ transients were present in deplasmolysed cells after hypotonic stress. This hypertonicity induces plasma membrane stretching by increasing the cell volume which may open mechanosensitive Ca2+ channels. Hyperosmotic stress–induced plasmolysis would also cause internal pressure on cell wall at plasma membrane–cell wall attachment sites leading to subsequent mechanical bending, destruction, and extra changes and modifications to plant cell wall which may be identified by cell wall–monitoring proteins (Feng et al. 2016). Membrane-localized cell wall monitoring receptor-like kinases (RLKs) are fascinating contestants for the perception, osmosensing, and mechanosensing of the osmotic stress (Haswell and Verslues 2015; Monshausen and Haswell 2013; Feng et al. 2016). The abiotic stress perception and environmental stimuli in cell wall takes place by a family of integral cell membrane proteins known as Receptor-like kinases (RLKs) which perceive extracellular ligands and environmental fluctuations in extracellular region and communicate the signal downstream into intra- and intercellular signalling pathway by second messengers or ROS (reactive oxygen species) meddling with ABA signalling or through phosphorylation of the transcription factors and intracellular serine/threonine kinase domains or supplementary uncharacterized signal proteins (Lindner et al. 2012; Osakabe et al. 2013; Wolf et al. 2012; Doblin et al. 2014). Receptor-like kinases are part of sensing external signals and abiotic stress signalling pathways in plant species like *A. thaliana*, *Oryza sativa*, *Medicago truncatula*, and *Glycine soja* (Yang et al. 2004; Bai et al. 2009; Osakabe et al. 2005, 2010; Lee et al. 2011; Ouyang et al. 2010; Tanaka et al. 2012; Yang et al. 2010; de Lorenzo et al. 2009). RLKs genes are expressed under abiotic stress and increase signal for stress response (Lindner et al. 2012). RLKs have extensin-like or cysteine-rich LRR extracellular domains. *Arabidopsis* RLK family comprises >600 members (Shiu and Bleecker 2001a, b, 2003; Gish and Clark 2011). The major RLK family is Leucine-Rich Repeat RLKs (LRR-RLKs) with >200 members and subdivided in A. *thaliana* into 15 sub-families (LRR I–LRR XV) (Shiu and Bleecker 2001a, b, 2003). Rice genome constitutes 1100 RLKs members and is categorized on basis of extracellular structures (Shiu and Bleecker 2001a, b, 2003; Morillo and Tax 2006; Gish and Clark 2011). Some 267 abiotic stress-responsive RLK genes are present in rice, out of which *Oryza sativa* stress-induced protein kinase gene 1 (OsSIK1) is triggered by drought, salt, and oxidative stress (Ouyang et al. 2010). Rice transgenic which overexpress OsSIK1 is salt and drought tolerant and OsSIK1 knock-out mutant is sensitive. RLKs like PROLINE RICH EXTENSIN LIKE RLK4 (PERK4), CYSTEINE-RICH RLK (CRK36), RPK1, and GUARD CELL HYDROGEN PEROXIDE-RESISTANT 1(GHR1) regulate water stress signalling in *A. thaliana* (Osakabe et al. 2005; Y. Osakabe et al. 2010; Bai et al. 2009; Lee et al. 2011; Tanaka et al. 2012; Hua et al. 2012). RECEPTOR-LIKE PROTEIN KINASE1 (RPK1) which is LRR-RLK from *A. thaliana* on expression constitutively up-regulates many stress-induced genes and enhances stress tolerance (Osakabe et al. 2010). RPK1 also determines regeneration frequency of shoots from *A. thaliana* calli (Motte et al. 2014). Here question arises whether RPK1 is primary receptor of abiotic stress or related to abscisic acid signalling. RPK1 *Arabidopsis* transgenic possess enhanced drought and oxidative stress tolerance. RPK1, mainly identified in *Brassica* species and *Arabidopsis thaliana* RPK1 homologous protein RPK2/TOAD2, was recognized in various plants (Sawa and Tabata 2011). *Arabidopsis* CRK36 knock-out mutant has enhanced sensitivity to abscisic acid and osmotic stress in post-germination growth phase and up-regulates ABA-responsive LEA genes, ABA-INSENSITIVE 4 (ABI4), oleosin, and ABI5 (Tanaka et al. 2012).

Another cell wall integrity receptor group is CrRLK1 family initially characterized from *Catharanthus roseus*. The well-characterized group members in *Arabidopsis thaliana* include FERONIA (FER), THESEUS (THE1), and HERKULES (HERK1, HERK2) (Huck et al. 2003; Hematy et al. 2007; Guo et al. 2009). FERONIA (FER) which is a cell membrane spanning receptor-like kinase functions as osmosensor of salt stress in epidermal cells of *Arabidopsis thaliana* roots (Feng et al. 2018). FERONIA is amongst seventeen members of CrRLK1L gene subfamily of *A. thaliana* which comprises an extracellular pectin-binding Malectin-like domain, which senses mutilation triggered to pectin-associated cell wall and regulates numerous processes of development (Li et al. 2016; Feng et al. 2018). THESEUS 1, HERKULES 1 and HERKULES 2of CrRLK1L subfamily monitors cell wall shortcomings (Hématy et al. 2007). In epidermal cells of *Arabidopsis thaliana* roots, extracellular salt leads to cell wall relaxing by interference of pectin crosslinking which is perceived by FERONIA (senses cell wall-associated changes, involved in recovery process), which stimulates cell-specific Ca2 + (cyt) transients and maintains cell wall integrity during salt stress–induced wall damage (Feng et al. 2018). FERONIA is also activated by the binding of rapid alkalinisation factor 1 (RALF1)-peptide ligand (Haruta et al. 2014). FERONIA malectin-like domain may not show interaction with RALF1 and possibly each of CrRLK1L kinases (the family of RLKs which contain the malectin-like domain) as well as FER may possess manifold chemically varied ligands, for instance, pectin (Wolf and Hofte 2014). FERONIA regulates numerous processes like growth and development, responses to pathogens, pollen tube growth, and rupture in fertilization in which modifications of cell wall are integral components (Duan et al. 2014;

Li et al. 2016). FERONIA responses are not stimuli specific but show activation under record protagonists of cell wall modifications and interaction with Rop-guanine nucleotide exchange factors (RopGEFs) which causes activation of RAC/ROPs, which sequentially interacts with numerous proteins, together with NADPH oxidases for ROS production (Berken et al. 2005; Wong et al. 2007; Nibau et al. 2006; Duan et al. 2010). Fer-mutants show decreased hypocotyl cell elongation (Deslauriers and Larsen 2010). CrRLK1L genes and proteins and members of RLKs subfamilies, Wall-Associated Kinases (WAKs), Proline-rich Extensin-like Receptor-Kinases (PERKs), and lectin-like RLK subfamilies play roles in osmotic stress signalling (Gish and Clark 2011). CrRLK regulates integrity of the plant cell wall. WAKs bind cell wall pectin and stimulate pectin-mediated signalling through the kinase activity of their cytosolic domains but WAKs proteins do not have role as sensors (Kohorn and Kohorn 2012). *A. thaliana* translates above 610 RLK members, out of which 78 up-regulate within 1 hour in response to drought (Kilian et al. 2007; Shiu and Bleecker 2001a, b; Marshall et al. 2012). Arabidopsis RLKs mutant lines (13) showed tolerance to both salt and mannitol and thus regulate tolerance of the osmotic stress (ten Hove et al. 2011). RLKs play significant role in response to initial stages of drought and salt stress in plants involving ABA-dependent and ABA-independent signalling pathways or downstream ABA signalling (Feng et al. 2014; de Lorenzo et al. 2009; Deng et al. 2009; Ouyang et al. 2010; Osakabe et al. 2010; Dievart et al. 2016; Zhang and Chen 2017; Sun et al. 2013; Li et al. 2014; Lim et al. 2015; Vaid et al. 2015; Kang et al. 2017). Direct connection between RLKs and perception of osmotic stress involves cell wall–binding RLKs which function independent of mechanosensitive Ca2+ channels and detect cell wall alterations and successively phosphorylation of downstream target proteins occurs. RLKs repair cell wall in growth recovery phase of cell elongation and show sensitivity to turgor changes with roles in cell elongation (Cosgrove 2016), regulate growth and development on plants, and integrate environmental and hormone signalling (Shiu and Bleecker 2001b; Dievart and Clark 2004). Hyperosmotic stress reduces turgor in root cells (Shabala and Lew 2002) which activates turgor-sensitive RLKs and downstream signalling ultimately upkeep cell wall integrity and stress adaptation (Fig. 2.5).

2.3.5 Aquaporins

Aquaporins (AQPs) are ubiquitous in eukaryotic cells, transmembrane channels, act as passive transporters of water (water channels) and additional trivial neutral solutes for instance CO_2, H_2O_2, and nutrients and fast-track transportation of water above native lipid bilayer (Agre et al. 2002). Plants have huge aquaporin sets (38 for *Arabidopsis thaliana*) subdivided into 4 subfamilies based on amino acid sequences similarities. Angiosperms possess about 35 diverse aquaporin genes, and their proteins are subdivided into 4 varied subfamilies. Aquaporins are members of Major Intrinsic Proteins (MIPs) superfamily. Two of aquaporin subfamilies Plasma

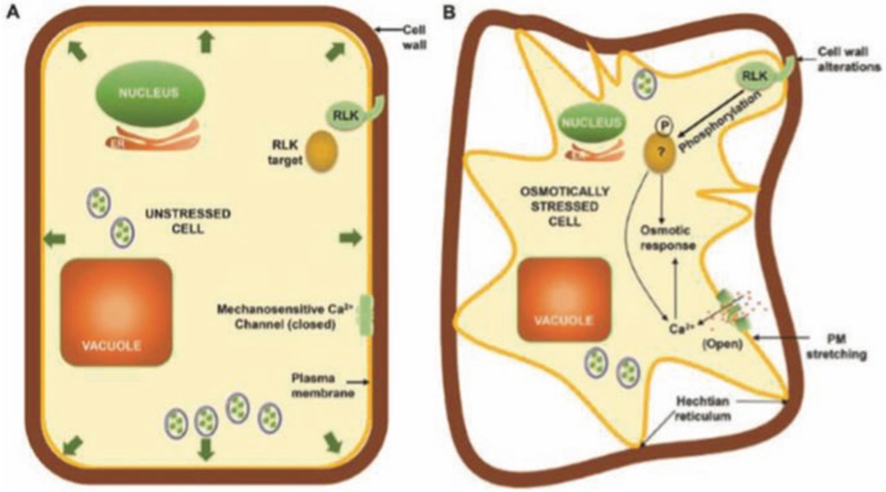

Fig. 2.5 Diagrammatic representation of cell wall (CW) and associated proteins in osmosensing (Source: Nongpiur et al. 2020)

membrane Intrinsic Proteins (PIP) and Tonoplast Intrinsic Proteins (TIP) relate to subcellular localization of plasma membrane and tonoplast. NIP aquaporins were originally reported from nodulated roots of soybean localized in the peribacteroid membrane (Niemietz and Tyerman 2000). Small and basic intrinsic proteins (SIPs) subfamily lack characterization and integrate into ER membrane (Ishikawa et al. 2005). This aquaporin classification reports exceptions (Barkla et al. 1999). PIP has been found located in the organellar membranes and TIPs are also reported from plasma membrane. From 1990s, functional characterization of the aquaporin-facilitated membrane transport reports substrates for instance glycerol, NH3/NH4þ, urea, carbon dioxide, malate, silicon, methylammonium, formamide, glycine and hydrogen peroxide besides water in plants (Kaldenhoff and Fischer 2006; Bienert et al. 2006). Hill et al. (2004) proposed that aquaporin molecules possess osmosensory function and directly sense transmembrane transferals in water potential and signal to other molecules. AQPs may serve as TMOs and osmosensors. Limited scientific data shows that aquaporin activity depends on plant transmembrane hydrostatic and osmotic pressures (MacRobbie 2006; Postaire et al. 2010; ShacharHill et al. 2013). Aquaporins might act as true membrane osmosensors and directly perceive difference in water potential spanning a membrane as opposed to osmotic stress–induced detection of plasma membrane and cell wall alterations (Hill and Shachar-Hill 2015). Direct evidence on prospective function of aquaporins in osmosensing is currently limited. However, viewpoints connecting aquaporins with osmosensing are hypothetical and depend on broad-spectrum inhibition of water channels via mercury and/or molecular modelling versus direct genetic substantiation (Maurel et al. 2015). Byrt et al. (2017) reported that aquaporins transport Na + and K+ charged solutes. *Arabidopsis thaliana* aquaporin AtPIP2; 1 regulates monovalent cation Na + influx and K+ efflux in *Xenopus* oocytes and *Saccharomyces cerevisae* under NaCl treatment. But whether it plays any role in initiation of osmotic stress responses is not recognized yet. Na + import shows inhibition due to additional aquaporins, pH, Ca2 +, and Cd2+. Na + transport inhibition by Ca2+ suggests that AtPIP2; 1 activity may be inhibited at initial phases of osmotic stress response (Byrt et al. 2017).

2.4 Molecular Mechanism of Osmosensing: An Overview

How plants at first perceive osmotic challenges is still a question mark. The 3 broad molecular models for osmosensing mechanisms in plants (Fig. 2.1) are summarized below:

2.4.1 Osmotic Imbalances Across Cell Membrane

A protein integrated in cell membrane could directly perceive osmolarity fluctuations exterior to cell due to osmotically synchronized variations in conformation of the extracellular domains (Fig. 2.1) (Parsegian et al. 1995). Though this model lacks experimental validation in any eukaryote, innumerable hyperosmotic signalling-based osmosensors via *S. cerevisiae* high osmolarity glycerol response (HOg)1 pathway as well as HK Synthetic Lethal of n-end rule 1 (SLn1) were initially anticipated to act via this mode; however, currently, any other mechanism enumerated below might alternatively take in (Saito and Posas 2012). Histidine kinase SLn1 plant homologs take part in regulation of water stresses (Tran et al. 2007; Wohlbach et al. 2008). But, it is still debated whether histidine kinases act as main osmosensing system in plants (Kumar et al. 2013). Reduced Hyper Osmolarity-Induced [Ca2+]i Increase (OSCA)1 calcium channel are activated by hyperosmolarity and genetically obligatory for stress-linked phenotypes (Yuan et al. 2014; Hou et al. 2014).

2.4.2 Increased Cell Membrane Tension

Hypo-osmotic shock leads to stretching of cell membrane. During plasmolysis, protoplast detaches from cell wall but remains attached by Hechtian strands of cell membrane. Protoplast attenuation and inflammation enhance plasma membrane tension. Mechanosensitive (MS) channels sense membrane tension and facilitate osmolyte flux across cell membrane (Fig. 2.1). Manifold mechanosensitive ion channel families are reported in plant genomes along with several MS ion channel activities in cell membranes (Monshausen and Haswell 2013). MS putative ion channel family known as MId1 Complementing Activity (MCA) proteins promote Ca2+ influx against hypo-osmotic shock and mechanical stimulus (Kurusu et al. 2013). *Arabidopsis thaliana* MCA1 plays role in obliteration of cell wall and effective growth of the plant roots (Hamann 2012; Kurusu et al. 2013).

2.4.3 Changed Integrity of the Cell Wall

Interface of cell wall–plasma membrane may sense stimuli created by fluctuating water status by Focal adhesion–like complexes (Canut et al. 1998). Molecular modelling has discovered an integrin-like structure in *Arabidopsis thaliana* for osmoregulation (Knepper et al. 2011). Osmosensing may resemble cell wall monitoring in *Saccharomyces* (Hamann 2012; Monshausen and Haswell 2013). The osmosensor can adhere to cell wall protein or mechanical alteration may discharge molecules to be perceived by osmosensor (Fig. 2.1). *Arabidopsis* genome encodes above 600 receptor-like kinases for stress signalling (Marshall et al. 2012). THESEUS and FERONIA observe position of the cell wall through carbohydrate or glycoprotein binding (Cheung and Wu 2011). Wall-Associated Kinase family helps in cell wall integrity and binds to cell wall pectin to regulate cell expansion by some signal transduction pathway (Kohorn and Kohorn 2012).

2.5 Osmotic Stress Perception, Sensing, and Signalling in Plants

Signalling components were characterized by widespread genetic monitors for changed sensitivities to osmotic stress. Signal perception at plant cell membrane produces second messengers that trigger cascades of downstream signalling events. Calcium ions and ROS are vital monitoring secondary messengers which induce under stress (Takahashi et al. 2011). Calcium is one such comprehensively studied second messenger with a role in osmotic signalling (Chinnusamy et al. 2004). Osmotic stress triggers calcium fluxes and calcium signatures unique to specific stress and cell category. The calcium signal upshots from extracellular calcium influx and/or calcium discharge from intracellular storages. Variations in calcium concentration are perceived by sensor relays (CBL proteins) and sensor responders (CDPKs). CDPK's role in osmotic signalling of salt, cold, or drought was confirmed (Saijo et al. 2000; Chehab et al. 2004). Hyperosmolarity and other osmotic stress stimuli trigger acceleration in cytosolic-free calcium concentration [Ca21]I (Knight et al. 1997; Dodd et al. 2010). Osmosensing Ca21 channels function as osmosensors in bacteria and animals (Kung 2005; Arnado'ttir and Chalfie 2010). In the same way, hyperosmolarity-induced [Ca21]I escalations are supposed to be important in plant osmosensing (Zhu 2002; Monshausen and Gilroy 2009). But molecular character of analogous Ca21 channels is indistinct (Dodd et al. 2010; Hedrich 2012; Ward et al. 2009). In *Mesembryanthemum crystallinum* CSP1 gene was isolated (Patharkar and Cushman 2000) which encodes pseudo-response regulator of nucleus. McCPK1 phosphorylates CSP1 in a calcium-dependent manner and shifts from subcellular localization of cell membrane to nucleus under salinity and water stresses (Patharkar and Cushman 2000; Chehab et al. 2004). McCPK1 regulates CSP1 function by phosphorylation under hyperosmotic stress. Plant cells sense and progress information through receptors present on cell surface. Receptor-like Ser/Thr kinases (RLKs) and receptor Hiskinases (HKs) function as receptors in osmotic stress (Urao et al. 2001; Shiu and Bleecker 2001a, b). *Arabidopsis thaliana* has AtHK1 osmosensor homologous to SLN1of *Saccharomyces cerevisiae* which perceives osmolarity deviations and activates two-component system and which in turn triggers Mitogen-Activated Protein Kinase (MAPK) pathways. AtHK1 expression rises under salinity or temperature reduction to 4 °C (Urao et al. 1999). AtHK1 didn't interact with any of 5 *Arabidopsis thaliana* RRs (Urao et al. 2001). *Saccharomyces cerevisiae* SHO1 plays role of the osmosensor at higher osmolarity. SHO1 has 4 transmembrane domains and SH3 domain exposes towards cytoplasm without enzyme activity. SHO1 starts a signalling pathway like eukaryotic cells (Reiser et al. 2000; Raitt et al. 2000). Membrane cytokinin receptor HK CRE1 in *Arabidopsis thaliana* alternates HK SLN1 in sln1D *Saccharomyces cerevisiae* activation with its ligand zeatin (Inoue et al. 2001). SLN1 and CRE1 are analogous in domain structure and homologous in cytoplasmic kinase and sensor domains. CRE1 perceives fluctuations in turgor pressure. CRE1 facilitates somatic connection of cell membrane to cell wall (Reiser et al. 2003). To function as osmosensor permutation of CRE1, periplasmatic and transmembrane domains and integrity of total periplasmatic domain is needed. Thus, plants possess osmosensing system with structure and function analogous to yeast analogue. OsMCA1 regulates cell membrane Ca2+ influx and reactive oxygen species generation prompted by hypo-osmotic stress in *Oryza sativa* (Kurusu et al. 2012a). Downstream signalling pathways of osmotic stress have been more studied than initial water deficit perception. Stress-responsive solute accretion mechanisms are common in bacterial and plant cells. The signalling initiates switching on of expression array in stress signalling genes of osmolytes and chaperones (Vierling and Kimpel 1992; Wang et al. 2004; Lee et al. 2008, Dutta et al. 2019). Initiation of defensive proteins and stress responsive expression of the genes are unique to plants (Yoshida et al. 2014). Alterations in cytosolic calcium, regulation of transcription, epigenetic and posttranslational protein modification of plants against hyperosmolarity, deficiency of water, and abscisic acid are studied (verslues and Juenger 2011; Christmann et al. 2013). The mechanism of ABA-mediated perception at molecular levels is characterized (Cutler et al. 2010). Genes or proteins expressed under water restraint are active in signalling. Several members of plant-specific kinases SnrK2 family play role in ABA signalling. Some cannot be activated by ABA but via osmotic stress (Boudsocq et al. 2007), the mechanism of which is unknown. ABA and defensive solute accumulation and stimulation of defensive proteins are not surely connected to signalling events. Supplementary investigation is required for establishing accurate pathway and identification of components. Under hypo-osmotic stress, suspension culture cells stimulate quick oxidative burst dependent on phosphorylation cascade linking Mitogen-Activated Protein Kinase and/or Calcium-dependent Protein Kinase activation (Cazale et al. 1999; Romeis et al. 2001). Finally, anion efflux is induced for guard cell osmoregulation, but molecular mechanisms have not been deduced yet. Hyperosmotic stress induces upsurge in activator of vacuolar calcium channels, inositol 1, 4, 5-trisphosphate (IP3) which gets gridlocked by phospholipase C inhibitors (Takahashi et al. 2001). IP3 activates calcium release from intracellular stores under hyperosmotic stress. Phosphatidic acid (PA) imperative in osmotic transduction pathways amass under cold and water deficiency (Frank et al. 2000; Munnik and Meijer 2001; Ruelland et al. 2002). PA forms after diacylglycerol phosphorylation directly by phospholipase D or indirectly by phospholipase C under osmotic stresses (Ruelland et al. 2002). PA activates alfalfa MAPK (SAMK) and targets kinases, phosphatases, and 14–3-3 transduction proteins (Lee et al. 2001; Testerink et al. 2004). Osmotic stress triggers oxidative damage against which antioxidant enzymes and osmolytes act as reactive oxygen species (ROS) scavengers (Kumar et al. 2019). ROS act as second messenger and are produced at intracellular sites, which activates diamine oxidase and cell membrane NADPH oxidase by hyperosmolarity and drought, respectively (Lin and Kao 2002; Jiang and Zhang 2002). H2O2 induced Catalase gene CAT1 and activates osmotic stress-responsive AtMPK6 in *A. thaliana* (Guan et al. 2000; Yuasa et al. 2001). To conclude synchronized action of gene networks leads to stress tolerance (Fig. 2.6).

2.6 Potential Role in Crop Improvement Under Climate Change

Climate has revolutionized with an outstanding speed in preceding centuries. Capricious weather proceedings such as drought, gradually increasing global mean temperature (2 to 5 °C by 2100), and seasonal floods are recurrent (Christensen and Corey 2007; Kadam et al. 2014; Quesada-Montano et al. 2018; Herring et al. 2018; Mukherjee et al. 2018). Antagonistic consequences of climate change factors, for instance enhanced CO_2 and ambient temperature, have previously been reported on agriculture, biological diversity, and distribution of plant species and biological processes, for example, time of flowering, pollination through pollen grains, and dormancy of the plant seeds (Dale et al. 2001; Doney et al. 2012; Craufurd and Wheeler 2009; Jagadish et al. 2016). Agricultural ecosystem compared to natural ecosystems is more susceptible to climate change as majority of the agricultural crops are enhanced for larger biomass and yield quantity which expectedly materializes by generation of abiotic stress-tolerant characteristics (Mayrose et al. 2011; Zivy et al. 2015). Quite a few miscellaneous classes of Osmotic Stress-Regulated (OR) genes are named in Fig. 2.7**.**

Stress-inducible gene products can be categorized into the following:

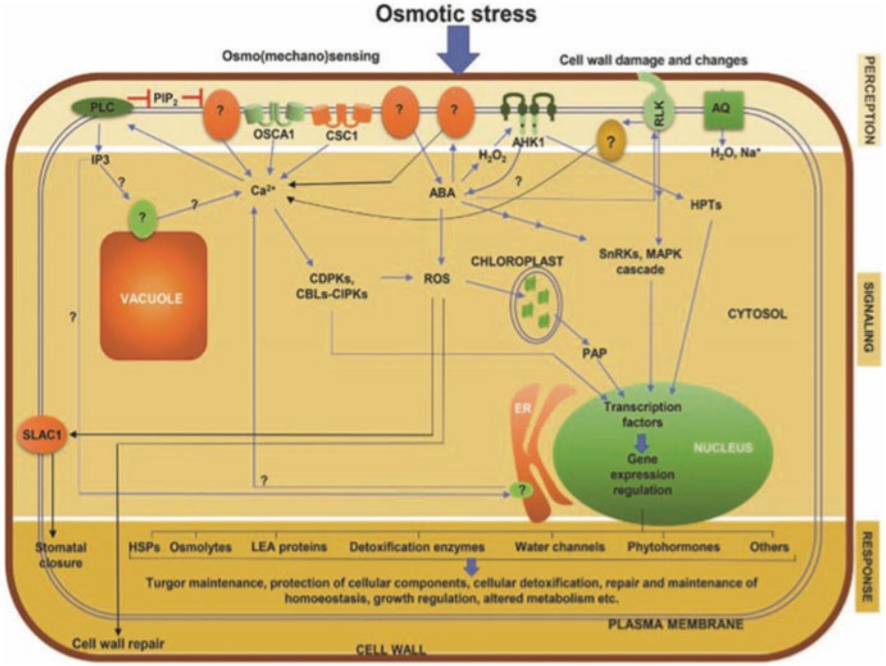

Fig. 2.6 Diagrammatic representation of 3 major stages: Perception, signalling, and response of osmotic stress in plants (Source: Nongpiur et al. 2020)

(i) **Functional metabolic proteins which defend cells from stress.**

Enzymes necessary for osmoprotectant biosynthesis and fatty acid metabolism, detoxification, late embryogenesis, chaperones, antifreeze mechanisms, proteinase inhibitors, sugar and proline transporters, ferritin, water channel and lipid-transfer proteins are included here.

(ii) **Regulatory proteins control gene expression and signal transduction under stress.**

Transcription factors, protein kinases, and enzymes are involved in phosphoinositide metabolism and ABA biosynthesis (Nakashima and Yamaguchi-Shinozaki 2006; Yamaguchi-Shinozaki and Shinozaki 2006). Genes encoding regulatory proteins are triggered promptly and transitorily against drought, salt, and cold stress and genes which encode functional proteins amass steadily and progressively within 10 h of stress (Kreps et al. 2002; Fowler and Thomashow 2002; Seki et al. 2002; Vogel et al. 2005, Joshi et al. 2016, Banerjee et al. 2017, Ganie et al. 2020). Genetic modification approaches to produce osmotic stress-tolerant transgenics have focused on gene expression of superoxide dismutase (SOD), glutathione reductase, glutathione S transferase, glyoxylases and genes encoding late embryogenesis protein-LEA, HVA1, LE25, dehydrin and DREB1A, CBF1, Alfin1

Fig. 2.7 Osmotic stress–regulated (OR) genes in Plants

transcription factors, cell membranes and ion homeostasis proteins and genes involved in biosynthesis of osmolytes like glycine betaine, mannitol, and proline. Therefore, there is requisite for identification, characterization, and utilization of the master regulators or osmosensors of osmotic stress for genetic and metabolic engineering of crop plants with tolerance to osmotic stress. OsCDPK7 overexpression in *O. sativa* confers drought, cold, and salt stress tolerance (Saijo et al. 2000). AtCPK10 and AtCPK30 of *A. thaliana* activate HVA1 promoter active under ABA and abiotic stress (Sheen 1996). Transgenic *Arabidopsis* releases SOS2 auto-inhibition via interacting with SOS3 which translocate kinase to cell membranes (Guo et al. 2004). SOS2/SOS3 complex activates SOS1 Na1/H1 antiporter and SOS2 regulates CAX1 Ca21/H1 and AtNHX1 Na1/H1 vacuolar antiporter (Qiu et al. 2004; Cheng et al. 2004). In *A. thaliana* RNAi cbl1 mutant, expression of AtCBL2 and AtCBL3 remains unaffected, while co-suppression of AtCBL1 and AtCBL9 resulted in phenotype (Pandey et al. 2004). AtCBL1 overexpression triggered drought and salt stress genes leading to increased drought and salt stress tolerance, whereas mutants showed conflicting phenotype (Cheong et al. 2003; Albrecht et al. 2003). *Arabidopsis* transgenic expressing ZmHK9, a maize histidine kinase gene, showed drought tolerance by controlling stomata opening (Wang et al.

2012).OsGATA8 overexpression in Saltolp QTL in *Arabidopsis thaliana* and *Oryza sativa* provides salt and drought tolerance and enhanced yield (Nutan et al. 2020). CRISPR-Cas9-based editing of histidine kinase osmosensor hos1 gene of *Leptosphaeria maculans*, which causes blackleg disease of *Brassica napus*, led to lethal impact on pathogenicity and saved plants from infection (Idnurm et al. 2017). With CRISPR-Cas9, new AtHK3 null mutants were identified (Hodgens et al. 2017). Proteins of plant sensory histidine kinases have positive roles for improving crops (Chakradhar et al. 2019). Rice genome has 51 gene members for Two Component System among which 14 genes translate putative HKs, 5 Hpts and 32 RRs (Pareek et al. 2006). The positive association of AtHKs with growth indicates that Histidine Kinases may be prospective targets for crop improvement. Few examples of Receptor-like kinases and HKs with use in crop improvement prospects are enlisted in Table 2.1**.**

2.7 Conclusion

The concept of how plants perceive osmotic stress is not yet fully understood. Plants utilize multiple proteins and mechanisms for detection of osmotic stresses. Ca2+ signatures play significant roles in osmosensing and osmotic signalling. Plant osmotic stress perception shows similarity to other eukaryotes. Yeast SLN1-YPD1-SSK1 two-component system resembles *Arabidopsis* AtHK1-mediated TCS. Mechanosensing calcium channels occur in both plants and animals (Choe and Strange 2007; Christenson and Corey 2007; Gresset et al. 2010; Harden et al. 2011; Thakur et al. 2016; Ozu et al. 2018). Plant cell wall affords superfluous surface compartment for osmosensing and signalling by binding with receptor-like kinases which counter to its fluctuations in composition, organization, and mutilation due to osmotic stress. Principal to signalling pathways are Ca2+, ROS, and ABA secondary messengers which show regulation of osmotic stress-responsive genes (Fig. 2.6). Osmosensors regulate transcription factors which amplify signals for regulation of gene expression under osmotic stress. Consequently, putative osmosensors like OSCA1, histidine kinases, CSCs, and RLKs with prospective to function as master regulators of osmotic stress are model and beneficial targets of manipulation through advanced genome editing techniques like CRISPR-Cas for designing of the next-generation osmotic stress resistant crop plants.

Table 2.1 Prospects of receptor-like kinases and HKs for climate smart crops

Gene		Plant	Function	References
Receptor-like kinases Osmosensor	Salt-induced receptor-like kinase (SRLK)	*Medicago truncatula*	RNAi prevents inhibition of root growth under salt stress.	de Lorenzo et al. 2009
	Receptor-like kinase 7 (RLK7)	*Arabidopsis thaliana*	Regulates speed of the seed germination and osmotic stress tolerance.	Pitorre et al. 2010
	O. sativa stress induced protein kinase gene 1 (OsSIK1)	*Oryza sativa*	OXa plants are tolerant to salinity and drought stress with enhanced SOD levels and reduced ROS accumulation.	Ouyang et al. 2010
	Receptor-like protein kinase 1 (RPK1)	*Arabidopsis thaliana*	OXs plants show increased sensitivity to abscisic acid and drought tolerance by enhanced SOD levels and reduced ROS accumulation.	Osakabe et al. 2005, 2010
	Cysteine-rich receptor-like kinase 36 (CRK36)	*Arabidopsis thaliana*	CRK36 complexes with ARCK1 and exhibits negative regulation of abscisic acid and osmotic stress.	Tanaka et al. 2012
	O. sativa receptor-like cytosolic kinase 253 (OsRLCK253)	*Oryza sativa*	OsRLCK253 mutants show enhanced drought and salinity tolerance.	Giri et al. 2011
	G. soja calmodulin-binding receptor-like kinase (GsCBRLK)	*Glycine soja*	OX plants are tolerant to high salinity and ABA.	Yang et al. 2010
Histidine kinase Osmosensor	Specific ETR1 ethylene receptor	*Solanum lycopersicum*	Mutants result in extended shelf life of 4–5 days.	Mubarok et al. 2015
	ahk2/3 and ahk3/4 double mutants	*Arabidopsis thaliana*	ahk2/3 and ahk3/4 mutants were cold tolerant and ABA sensitive during germination.	Jeon et al. 2010
	ahk2/ahk3 mutants	*Arabidopsis thaliana*	Reduced vegetative growth and chlorophyll levels in leaves.	Riefler et al. 2006
	AHK2 and AHK3 gain-of-function mutants	*Arabidopsis thaliana*	Increased yield in mutants than wild-type plants.	Bartrina et al. 2017
	AHK4 loss-of-function mutants	*Arabidopsis thaliana*	Cytokinin-induced resistance in calli greening and inhibition in shoot and root development.	Ueguchi et al. 2001

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Chapter 3
Amino Acids Other Than Proline and Their Participation in Abiotic Stress Tolerance

Showkat Ahmad Ganie

Abstract Ensuring food supply to the increasing global population has remained a daunting task for agriculturalists and plant scientists, and abiotic stress only exacerbates this problem. Abiotic stress adversely affects many aspects of plant biology that predominantly govern growth and yield. Continued research efforts have revealed that plant response to abiotic stress is very complex and involves precise changes in several physiological, metabolic, and molecular facets. One of the basic and ubiquitous responses includes the synthesis and accumulation of compatible solutes. Amino acids are primary metabolites that, besides other functions, act as compatible solutes and play decisive roles in the cell life under various abiotic stresses. Although, proline has been vastly studied for its role as a compatible solute in mitigating abiotic stress effects in plants, other amino acids (both proteinogenic and nonproteinogenic) have received considerably less attention in this context. This chapter provides the current state of knowledge of the role of amino acids, excluding proline, in abiotic stress tolerance of crops. It provides a comprehensive overview of how these crucial metabolites improve the abiotic stress tolerance of crop plants when overproduced (using transgenic approaches) or supplied exogenously. Further, the potential of amino acid–based biostimulants in improving the biochemical, physiological, and molecular responses regulating plant adaptation and tolerance to various abiotic stresses is described. Based on the information in this chapter, it is deduced that proline is unquestionably not the only amino acid accumulated by plants in response to different abiotic stresses, and that genomic resources and related pathways associated with the other amino acids must be characterized to further our understanding of crop abiotic stress tolerance. This will facilitate the genetic engineering efforts for the improvement of abiotic stress tolerance and productivity of crop plants for sustainable agriculture.

Keywords Abiotic stress tolerance · Amino acids · Biostimulants · Crop improvement · Endogenous accumulation · Exogenous application · Proline

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3.1 Introduction

Mechanisms of plant resistance to abiotic stresses have been actively explored over the past few decades. Abiotic stresses are the major factors that negatively affect the agricultural crop yield, imperiling the agricultural capacity to sustain the rapidly growing human population (Ganie et al. 2020). Abiotic stresses jeopardize all the major biological processes in plants, such as photosynthesis, transpiration, metabolism of proteins, lipids and other biomolecules, cell division, etc. (Cramer et al. 2011; Ganie and Ahammed 2021; Qi and Zhang 2020; Waqas et al. 2019). For surviving and growing under these unfavorable environmental conditions, plants have evolved a variety of protective mechanisms (Ganie et al. 2019; Ganie and Reddy 2021; Ganie et al. 2021; Waqas et al. 2019). Metabolic acclimation via the synthesis and amassing of the low-molecular-weight compatible solutes is often regarded as a basic and ubiquitous mechanism for tolerance to abiotic stresses, especially the osmotic imbalance–inducing stresses (Chen et al. 2007; Sharma et al. 2019; Slama et al. 2015). For increasing the ability of cells to retain water, these hydrophilic and osmotically active solutes can be accumulated to considerably high levels without jeopardizing the normal cytosolic or organelle metabolism (Shabala and Shabala 2011). These solutes include sugars (e.g., glucose, fructose, sucrose, trehalose), polyols (e.g., mannitol, sorbitol, glycerol), betaines (e.g., glycine-betaine), and amino acids (e.g., proline, glutamate, glycine, alanine, asparagine) (Sharma et al. 2019). The degree of accumulation of these organic solutes depends profoundly on the stress severity and may occur in magnitude of several to hundred folds (Shabala and Shabala 2011). Accordingly, it establishes a relationship between the degree of compatible solute accumulation and osmotic stress tolerance (Sakamoto and Murata 2002). Consequently, traditional crop breeding programs adopt the capacity of compatible solute accumulation as one of the selection criteria for enhancing the crop osmotic stress tolerance (Ashraf 2004; Zhang et al. 1999).

Amino acids are primary metabolites that play decisive roles in the cell life under abiotic stresses. Amino acids influence wide-ranging physiochemical characteristics of plant cells, tissues, and organs (Batista-Silva et al. 2019; Hildebrandt 2018; Rai 2002). Besides their conventional role in the cellular osmoregulation, amino acids, like other compatible solutes, are also suggested to protect enzymes and membranes from denaturation, stabilize photosystem II complex and other macromolecules, and scavenge ROS to protect vital cellular structures (Ali et al. 2019; Carillo et al. 2011a, b). Amino acids play various other crucial roles in plants including optimizing nutrient uptake and metabolism, vitamin biosynthesis, and growth promotion (Khan et al. 2019; Noroozlo et al. 2019). They also regulate stomatal-movement, which helps in maintaining the cellular water balance (Kamran et al. 2009). These crucial metabolites are amphoteric in nature, which makes them ideal buffers protecting plant cells from abiotic stress–induced effects by maintaining favorable cellular pH (Davies 1982; Khan et al. 2020). They also serve to store carbon and nitrogen to be used by plants for the synthesis of specific enzymes and secondary metabolites, which are useful when the stress is relieved (Rhodes and Hanson 1993). Amino acids have a close metabolic connection with TCA cycle. It is suggested that the reduction in electron supply from TCA cycle under oxidative stress is compensated by catabolism of amino acids (Obata et al. 2011). Further, amino acids are also involved in providing rigidity to plant cells for withstanding the mechanical constraints imposed by abiotic stresses (Ali et al. 2019). Moreover, due to their biostimulant nature, amino acids have beneficial effects on plant growth and yield under abiotic stresses (Van Oosten et al. 2017).

Although, attention has been paid greatly to the role of proline in abiotic stress tolerance of plants, remarkable stress-induced changes in the levels of other amino acids also take place (Das et al. 2017; De Leonardis et al. 2015; Kusaka et al. 2005; Ogura et al. 2001; Ricoult et al. 2006). Therefore, proline is certainly not the only amino acid that is accumulated at high levels in response to abiotic stresses. Under water-limited conditions, the accumulation of other amino acids is more important than that of proline in some plant species, and in some other species, these amino acids accumulate to the similar levels as that of proline (Naidu 1987). Together, this suggests that other amino acids play as important role as that of proline in alleviating the stress-induced effects, and that in terms of nitrogen requirements of plants, such accumulations merit equal research attention and interest.

This chapter focusses on the role of different amino acids, excluding proline, as compatible solutes in alleviating the abiotic stress–induced effects in crop plants. Since, *Arabidopsis* is a model species and since the molecular experiments standardized with this plant species have direct relevance for cereal crops, the chapter will also review the different studies about the role of amino acids in abiotic stress tolerance of this model plant. The accumulation of amino acids is one of the vital responses of plants to abiotic stresses, and in stressed-plant tissues, the amino acids may accumulate up to the millimolar range (Planchet et al., 2011). Abiotic stresses can have substantial effects on the composition of crops, because amino acids accumulate to high extents in different plant tissues in response to these stresses (Halford et al. 2015). Extensive literature survey revealed that different groups of amino acids, such as aspartate-derived, branched chain, aromatic, sulfur-containing, and assimilation pathway amino acids, accumulate under different abiotic stress conditions, especially drought, high salinity, and extreme temperatures. Many scientific endeavors have also identified specific amino acid biosynthesis genes associated with abiotic stress tolerance phenotypes and have used them for synthesis and accumulation of corresponding amino acids for improving the crop abiotic stress tolerance through genetic engineering approaches. Knowing that endogenous and genetic engineering–mediated accumulation of amino acids improve the plant abiotic stress tolerance, several studies have used exogenous amino acids (either individually or as biostimulants) through different modes and in different formulations for improving the abiotic stress tolerance in crop plants.

3.2 Drought and Salinity Tolerance

3.2.1 Endogenous Accumulation

The processes of regulation of free amino acid levels and the maintenance of their concentration within plant cells are accomplished by many different metabolic and developmental factors. However, their endogenous accumulation in plants occurs in response to various abiotic stresses, including drought and high salinity. The accumulation of various amino acids is progressively catching more attention as a crucial physiological mechanism in response to different abiotic stresses in many crop plants. With the increasing need for developing abiotic stress–tolerant crop cultivars, it is being suggested to select the cultivars based on morphological and physio-biochemical parameters. Ability to accumulate amino acids could be used as a potential parameter to select cultivars for abiotic stress breeding programs. The studies concerning the roles of amino acids, including their accumulation, under drought and salt stress conditions, have been reported for different types of amino acids. Role of glutamate, aspartic acid, methionine, and lysine in the plant abiotic stress tolerance has recently been reviewed (Ali et al. 2019), and thus will not be included in this chapter.

Due to its high nitrogen-to-carbon ratio and nonreactive nature, asparagine is an ideal compound for transporting and storing nitrogen in plants (Lea et al. 2007), which is a crucial mechanism for responding to abiotic stresses (Rhodes and Hanson 1993). Asparagine has been found to accumulate to a considerable extent in plants under drought and high salinity conditions. Under salt stress, the shoot metabolic phenotypes of *Lotus* species revealed that asparagine accumulated to a higher extent in tolerant genotypes than sensitive ones (Sanchez et al. 2011a). In drought-stressed pearl millet, the accumulation of asparagine has been found to increase to 15-fold (Kusaka et al. 2005), whereas in salt-stressed *Pandanus odorifer*, it increased almost to 29-fold (Deo et al. 2019). It has also been found to accumulate in *Hordeum* species under salt stress (Garthwaite et al. 2005). In *Lupinus albus*, the drought has been found to cause accumulation of asparagine in the stem and the levels of asparagine were higher than proline even after relieving the stress (Pinheiro et al. 2004). Together, this suggests that plant stem plays a crucial role in plant strategy to cope with drought and that asparagine is an essential metabolite synthesized in response to water deficit. Also, asparagine, being the predominant amino acid in potato tubers (Halford et al. 2012), accumulates further in tubers under severe drought (Muttucumaru et al. 2015). Metabolic profiling of drought-stressed roots and shoots of traditional aus-type rice plants has shown that aspartate family amino acids, especially asparagine, accumulated to significant levels (Casartelli et al. 2018). The inoculation of bacterial endophyte *Bacillus subtilis* B26 has been reported to alleviate the stress of drought in *Phleum pratense*, which was accompanied by, among other factors, the accumulation of higher levels of asparagine, glutamate, and glutamine (Gagné-Bourque et al. 2016). Salt stress also causes a dramatic increase in the aspartate family amino acids, including asparagine, isoleucine, and threonine in maize shoots (Guo et al. 2017). Free asparagine concentration has also been found to accumulate predominantly under water shortage conditions in *Vicia faba* and *Glycin max* (Fukutoku and Yamada 1981; Venekamp and Koot 1988). Further, in some plants, asparagine accumulates to a higher extent than other amino acids. For instance, in young leaves of wheat, asparagine concentration increases more than proline under salt stress (Colmer et al. 1995). Similarly, in the nonphotosynthetic sink tissues of *Coleus blumei*, asparagine levels have been found to be significantly more than arginine under salt stress (Gilbert et al. 1998). Taken together, these results indicate that asparagine plays a vital role in drought and salt tolerance, perhaps by serving as a reserve for nitrogen and carbon to support the core nitrogen metabolism.

Branched-chain amino acids (BCAAs) (valine, leucine, and isoleucine) are essential in agriculture and their role in plant abiotic stress tolerance has gained a profound research interest. They have been the subject of extensive research particularly because they serve as precursors for various plant metabolites (Lea and Ireland 1999). Under drought, BCAAs have been found to accumulate considerably in *Arabidopsis* leaves, flowers, and siliques, and also in the tomato leaves (Joshi et al. 2010). Further, the study found that drought-induced increase in BCAA concentration was significantly higher than that of proline. The higher accumulation of BCAAs than proline under drought stress was also reported by Shen et al. (1989) who found that BCAAs contributed highly to the drought-induced total free amino acid concentration, more than the increase in proline. Similarly, the greater increase in the foliar BCAA levels over proline levels has also been reported (Nambara et al. 1998). The high accumulation of BCAAs under dehydration stress has been found to be regulated by ABA (Urano et al. 2009). The regulation of BCAA accumulation under drought has been reported to occur due to the increased protein degradation through ABA-dependent manner, and this accumulation was found to be higher than that of proline (Huang and Jander 2017). In concert, these results implicate that ABA plays a crucial role in regulating the accumulation of BCAAs under drought and that proline is not the only amino acid that is substantially accumulated in response to drought. The highly increased levels of BCAAs have also been found in the leaves of soybean and wheat under drought (Bowne et al. 2012; Das et al. 2017). The accumulation of valine and isoleucine has also been found to be evoked by drought and high salinity in maize and wheat, respectively (Elshintinawy and Elshourbagy 2001; Obata et al. 2015). Further, isoleucine levels of plants under osmotic stress have been reported to be maintained by methionine gamma-lyase and threonine deaminase, because both threonine and methionine act as substrates for the synthesis of isoleucine (Joshi et al. 2010).

Aromatic amino acids (phenylalanine, tyrosine, and tryptophan) serve as precursors for several crucial metabolites important for plant growth, development, and stress responses (Tzin and Galili 2010). Growing body of evidence suggests that endogenous accumulation of aromatic amino acids plays a vital role in abiotic stress tolerance of plants. Levels of aromatic amino acids are reported to increase in the leaf tissues of wheat in response to drought; however, tryptophan accumulation in drought-tolerant cultivar was more dramatic than that of phenylalanine and tyrosine

(Bowne et al. 2012). In maize, the accumulation of tryptophan and phenylalanine is reportedly induced under drought conditions (Witt et al. 2012). Further, the high tryptophan-accumulating rice plants have been found to exhibit improved growth under stress conditions (Dubouzet et al. 2007). The induction of high levels of tryptophan under drought has been reported to be controlled by ABA in the primary leaves of barley (Ogura et al. 2001). Besides, drought stress also metabolically activates the wheat leaves in the form of high accumulation of tryptophan and several other metabolites, which enhances the water uptake and growth for post-anthesis drought tolerance (Kang et al. 2019). Since, tryptophan is a target for oxidative modifications, it may protect the proteins from ROS-induced damage by serving as a buffer for improved plant growth (Bowne et al. 2012). Metabolomic analysis of *Malus halliana* under salt-alkali stress has revealed that among other proteins and metabolites, accumulation of phenylalanine and tryptophan contributed to the adaptation of *M. halliana* to salt-alkali stress by maintaining osmotic balance and scavenging ROS (Jia et al. 2019). Endogenous levels of aromatic amino acids have also been found to increase following salt stress treatment, and all the three aromatic amino acids were coinduced (Kim et al. 2007). This coinduction may be explained from the fact that tryptophan acts as a positive feedback activator for the synthesis of the other two amino acids (Bentley 1990). Drought also affects leaf metabolites, including phenylalanine, in potato cultivars, and it is suggested that accumulation of phenylalanine may contribute to strategy to conserve leaf water in potato under drought (Barnaby et al. 2019). Although, amino acids accumulated in response to environmental stresses are L-isomers, in the wilted tomato leaves, drought reportedly induces the accumulation of D-isomers only for tryptophan in the malonylated form (Rekoslavskaya et al. 1992), which may be regarded as a specific adjustment in the leaf metabolome to survive drought. Besides, drought has also been reported to evoke the accumulation of aromatic amino acids in pigeon pea and sesame under high salinity and drought conditions, respectively (Biswas et al. 2018; You et al. 2019). Salt-induced accumulation of tyrosine has been shown to be strongly increased by high light in durum wheat, whereas under the same conditions, the glycine-betaine synthesis was inhibited (Carillo et al. 2011a, b), indicating that under high light conditions, amino acid accumulation may be more important than that of glycine-betaine for tolerating high salinity in wheat. Tyrosine contents also increase in the maize roots and leaves in response to salt stress (Azevedo Neto et al. 2009).

Drought and high salinity cause a profound reduction in the photosynthetic rate with a concomitant increase in the rate of photorespiration (Acosta-Motos et al. 2017; Kangasjärvi et al. 2012; Wingler et al. 1999). Since, under drought, the electron consumption by photosynthetic CO_2 assimilation is reduced (Haupt-Herting and Fock 2002), the photorespiration can exert protective effects on photosynthesis by providing a sink for photosynthetic electron transport, resulting in the dissipation of excess photochemical energy (Di Martino et al. 2003; Wingler et al. 1999). Therefore, increased rate of photorespiration could in turn lead to the reduced stress-induced effects on plants. Glycine and serine are actively involved in photorespiration. Besides their role as osmolytes, their accumulation under stress conditions evidences for the increased photorespiration. These two amino acids have been found to significantly increase in barley and spinach under drought and salt stress, respectively (Di Martino et al. 2003; Wingler et al. 1999), indicating high rates of photorespiration as vital responses to these stresses. Since glycine and serine are the precursors for the synthesis of osmolyte glycine-betaine (Waditee-Sirisattha et al. 2015), photorespiration has been suggested to involve in osmotic adjustment during high salt stress in spinach (Di Martino et al. 2003). Glycine acts as a crucial metabolite in the nucleic acid biosynthesis, which is the ultimate source of energy for the synthesis of various biomolecules in a cell (Stasolla et al. 2003). The accumulation of glycine has been suggested to enhance cellular osmotic balance, and the purine biosynthesis under drought in soybean (Das et al. 2017), indicating the importance of nucleic acid synthesis in the drought tolerance. Metabolite analysis of shoots of salt-acclimated *Lotus* indicated that among other soluble metabolites, glycine and serine accumulated to considerable extent to account for the osmotic adjustment (Sanchez et al. 2011b). Besides, the levels of these two amino acids have also been reported to increase in maize and barley under salinity and drought (Azevedo Neto et al. 2009; Wingler et al. 1999). Despite the drought- and salinity-induced accumulation of glycine and serine, some studies report the decline in their levels under these stresses: e.g., glycine and serine contents decreased in canola and barley under salt stress and drought, respectively (Ashraf and McNeilly 2004; Templer et al. 2017).

Besides glycine and serine, alanine is also involved in the process of photorespiration, because it donates the amino group to the photorespiratory metabolism (Ta and Joy 1986). Under severe salt stress, alanine accumulates to a great extent in spinach leaves, which is suggested to be due to the increased glycolysis for furnishing carbon skeletons to the photorespiration for minimizing the severe salt stress–induced damage to photosynthetic apparatus (Di Martino et al. 2003). In other study, alanine accumulation was found to decrease in drought-stressed barley, which was suggested to be due to the high rate of photorespiration (Wingler et al. 1999). Together, these studies indicate that glycine, serine, and alanine may play a crucial role in alleviating the effects of drought and salt stress not only by involving in osmoregulation but also by involving in photorespiration to protect the photosynthetic apparatus from photoinhibition. Metabolomics analysis for investigating salt tolerance in maize has indicated that increased levels of alanine and other metabolites represented the most significant metabolic perturbations in the salt-stressed roots (Gavaghan et al. 2011). The study suggests that high levels of alanine may sustain the process of glycolysis under salt stress for the energy production, because alanine acts as a storage form of pyruvate (Kim et al. 2007). Alanine has also been found to be highly accumulated in drought-tolerant chickpea as one of the major metabolic products of nitrogen fixation (Thavarajah and Ball 2006), indicating alanine may be crucial for sustaining the process of nitrogen fixation under drought. Foliar application of 5-aminolevulinic acid has been reported to effectively mitigate the salinity-induced physiological damage in creeping bentgrass, which was found to be associated with the accumulation of alanine and other soluble metabolites (Yang et al. 2014). In a metabolic analysis of contrasting lentil genotypes, alanine was identified as a marker of salt stress (Muscolo et al. 2015). Besides, the accumulation of alanine has also been found to increase in maize, barley, sweet potato, and rapeseed under drought or salt stress (Azevedo Neto et al. 2009; Good et al. 1994; Wang et al. 1999; Wu et al. 2013).

Besides the proteinogenic amino acids, some nonproteinogenic (or nonprotein) amino acids (NPAAa) also play important roles in the resistance of crop plants to abiotic stresses. The role of some nonprotein amino acids (such as canavanine, mimosine, L-DOPA, β-DOPA, 5-hydroxynorvaline, m-tyrosine, etc.) in the abiotic stress tolerance of plants has recently been comprehensively reviewed (Rodrigues-Corrêa and Fett-Neto 2019). Here, the role of other NPAAs such as ornithine, citrulline, pipecolic acid, etc., in the plant tolerance to abiotic stresses will be discussed. NPAAs are a large group of heterogenous nitrogen-containing specialized metabolites ubiquitously present in plant lineage and most prevalent in family Fabaceae (Rodrigues-Corrêa and Fett-Neto 2019). However, citrulline is commonly found in family cucurbitaceae. Several studies report that citrulline serves as a crucial metabolite to subside the abiotic stress–induced effects on plants. Particularly, citrulline accumulates to a considerable extent in the foliage of watermelon and other allied species under drought and high salinity. For example, high levels of citrulline accumulate in the drought-tolerant watermelon leaves, and it has been suggested to alleviate drought-induced oxidative stress damage in green tissues by serving as an efficient and potent hydroxyl radical scavenger (Akashi et al. 2001; Yokota et al. 2002). The accumulated citrulline has also been shown to serve as an osmolyte to maintain the cellular water status in the leaves of drought tolerant watermelon (Kawasaki et al. 2000). Similarly, strikingly higher concentrations of citrulline have also been found to be accumulated by drought- and salinity-tolerant melons than sensitive melons under combined drought and salt stress (Kusvuran et al. 2013). Seedlings of melon have also been found to accumulate increased levels of citrulline under salt stress conditions with salt-tolerant melon accumulating higher concentration (Dasgan et al. 2009). The study also found that as compared to citrulline, relatively low levels of proline were accumulated, and it was suggested that higher values of citrulline-to-proline ratios are associated with salt tolerance of melon. Besides the drought- and salinity-induced citrulline accumulation in cucubitaceae, several studies also suggest its accumulation in non-cucurbitaceae plants under drought or high salinity (Joshi and Fernie 2017; Khan et al. 2019). Taken together, the results from these studies indicate that citrulline plays crucial role in osmotic adjustment and abating oxidative stress, and as such, it can be used an efficient biochemical indicator in screening the germplasm for drought and salt stress.

Ornithine is another NPAA which is synthesized from glutamate in plants (Slocum 2005). It is a metabolic intermediate of polyamine and amino acid biosynthetic pathways contributing to the production of osmolytes such as proline, arginine, and polyamines for osmoregulation under stress conditions (Minocha et al. 2014). Accumulation of endogenous ornithine levels has been shown to increase abiotic stress tolerance in crop plants. In drought-stressed rice plants, ornithine levels have been found to increase, which led to the increase in proline accumulation (Hsu et al. 2003). In a metabolic analysis of contrasting lentil genotypes under drought, high accumulation of ornithine was observed, and ornithine was identified as a marker of drought (Muscolo et al. 2015). Overproducing ornithine (a nontoxic metabolite) has been suggested as an alternative strategy to enhance abiotic stress tolerance in plants (Kalamaki et al. 2009a). This can be explained from that fact that proline accumulation under nonstress conditions proves to be toxic (Maggio et al. 2002); therefore, overproduced ornithine (precursor of proline) can be converted to proline for osmoregulation under osmotic stress conditions (Kalamaki et al. 2009a). Tolerance of apple cultivars to water deficit has been suggested to be governed by pronounced accumulation of amino acids including ornithine (Sircelj et al. 1999). Similarly, pipecolic acid represents another NPAA that has been found to accumulate in plants under abiotic stresses. Pipecolic acid acts as an osmoprotectant in bacteria (Gouesbet et al. 1992), and it has been shown to accumulate via the catabolism of lysine in rapeseed leaves under osmotic stress (Moulin et al. 2006). The significance of its synthesis and accumulation via osmoregulated catabolism of lysine was explained on the basis of noncompatible nature of lysine (Rishi et al. 1998), which is therefore catabolized and channeled into the synthesis of compatible pipecolic acid under osmotic stress (Moulin et al. 2006). This NPAA has also been found to accumulate under the conditions of high salinity in plants (Mansour 2000).

3.2.2 Amino Acid Biosynthetic Genes and Their Use in Engineering Plant Drought and Salt Tolerance

For explicating the potential molecular mechanisms underlying crop growth and development during abiotic stresses, researchers have widely employed various transcriptomic methods to identify the stress-responsive differentially regulated genes. Likewise, several amino acid biosynthetic genes have been identified to be differentially regulated in different crop species under drought and high salinity conditions by using transcriptomic and other gene expression methods (Table 3.1). Knowing that levels of amino acids significantly increase under various abiotic stress conditions in plants, numerous studies have functionally analyzed the amino acid biosynthesis genes in different plant species by manipulating their expression through genetic engineering approaches for augmenting the endogenous amino acids levels to produce drought- and salinity-tolerant plants. These genetic engineering attempts have been driven by the fact that biosynthetic pathways of compatible solutes are easy to manipulate, because the accumulation of compatible solutes is usually controlled by a single gene (Serraj and Sinclair 2002). On that account, these attempts also open up exciting avenues for the identification of compatible solute synthesis-linked molecular markers and QTLs in plants (Shabala and Shabala 2011). Further, the manipulation of biosynthetic pathways of compatible solutes is relatively easy, because the stress-mitigating effects of these solutes are not species-specific (Kumar et al. 2018). Therefore, compatible solute-coding genes from one

Table 3.1 Differentially regulated abiotic stress-responsive amino acid biosynthetic genes identified from the different crop species through transcriptomics and other expression studies

Gene	Amino acid biosynthesis	Species	Abiotic stress	Regulation	Approach	References
BCAT2	Valine, leucine, isoleucine	*Sesamum indicum*	Drought, salt stress	Upregulated	RNA-seq	You et al. (2019) and Zhang et al. (2019)
Cystathionine beta-lyase	Methionine	*Zea mays* var. B73	Drought	Upregulated	GS FLX Pyro sequencing	Batlang et al. (2014)
Cystathionine beta-lyase	Methionine	*Solanum pennellii*	Drought	Upregulated	Microarray	Gong et al. (2010)
Cystathionine-γ *-synthase*	Methionine	*Corchorus olitorius* var. TC	Salt stress	Upregulated	RNA-seq	Yang et al. (2017)
Carbamoyl-phosphate synthetase	Citrulline	*Citrullus colocynthis*	Water-deficit	Upregulated	RNA-seq	Wang et al. (2014)
Diaminopimelate decarboxylase	Lysine	*Camellia sinensis*	Drought	Upregulated	SSH	Gupta et al. (2012)
Dihydrodipicolinate synthase 2	Lysine	*Setaria italica* cv. Prasad	Salt stress	Upregulated	cDNA macro array	Sreenivasulu et al. (2004)
GS	Glutamine	*Solanum tuberosum* cv. Désirée	Drought, salt stress	Varied expression	Dot-blot	Teixeira and Pereira (2007)
GS1	Glutamine	*Helianthus annuus* cv. SH222	Salt and osmotic stress	Upregulated	Dot-blot	Santos et al. (2004)
HAS1	Asparagine	*Helianthus annuus* cv. HA-89	Salt and osmotic stress	Upregulated	Northern-blot	Herrera-Rodríguez et al. (2007)
IGPS, TS1	Tryptophan	(*Vitis vinifera*) x (*V. labrusca*)	Drought	Upregulated	RNA-seq	Haider et al. (2016)
IGS	Tryptophan	*Saccharum* sp. (Co 740 x Co 775)	Drought	Upregulated	qRT-PCR	Simon and Hemaprabha (2012)
Isopropylmalate dehydrogenase	Leucine	*Setaria italica* cv. Prasad	Dehydration stress	Upregulated	SSH	Lata et al. (2010)

Isopropylmalate synthase	Leucine	*Oryza sativa* cv. LC8866	Water-deficit	Upregulated	cDNA-AFLP	Rodríguez et al. (2006)
Isopropylmalate synthase	Leucine	*Camellia sinensis* cv. TV-23	Drought	Upregulated	SSH	Das et al. (2012)
BjGS1, BjGDH1, BjASN2	Glutamine, glutamate, asparagine	*Brassica juncea* cv. Varuna	Salt, osmotic, cold and heat stresses	Downregulated	qRT-PCR	Goel and Singh (2015)
Ketol-acid reductoisomerase	Valine, isoleucine	*Oryza sativa* cv. IRAT109	Drought	Upregulated	qRT-PCR	Shu et al. (2011)
GS-1	Glutamine	*Lycopersicon esculentum* cv. Moneymaker	Water stress	Upregulated	Northern-blot	Bauer et al. (1997)
Methionine synthase	Methionine	*Solanum lycopersicum* cv. LA2711 and ZS-5	Salt stress	Downregulated	Microarray, SSH	Ouyang et al. (2007)
Methionine synthase	Methionine	*Oryza sativa* cvs. IR64 and Pokkali	Salt stress	Downregulated	SSH	Kumari et al. (2009)
Methionine-γ-lyase	Isoleucine	*Hordeum vulgare* cv. Haruna Nijo	Drought	Upregulated	qRT-PCR	Sugimoto et al. (2017)
VvSERAT2;1	Cysteine	*Vitis vinifera* cv. Touriga Nacional	Drought	Upregulated	qRT-PCR	Tavares et al. (2015)
TaASN1	Asparagine	*Triticum aestivum* cv. Keyi26	Salt and osmotic stress	Upregulated	Differential display RT-PCR	Wang et al. (2005)
Threonine synthase 1, aspartate amino-transferase	Threonine, aspartate	*Sesamum indicum* cv. WZM3063	Salt stress	Upregulated	RNA-seq	Zhang et al. (2019)
ZmGln1;3, ZmAS3	Glutamine, asparagine	*Zea mays* var. ZD958 and NH101	Drought	Upregulated	qRT-PCR	Li et al. (2016)
Aspartate amino-transferase	Aspartate	*Oryza sativa* var Dongan	Salt stress	Upregulated	Microarray	Lee et al. (2016)

(continued)

Table 3.1 (continued)

Gene	Amino acid biosynthesis	Species	Abiotic stress	Regulation	Approach	References
Chorismate synthase	Phenylalanine, tyrosine, tryptophan	*Solanum lycopersicum* cv. 1260	Drought, salt stress	Upregulated	RT-qPCR	Filiz et al. (2019)
Chorismate mutase	Phenylalanine, tyrosine	*Musa acuminata* cv. Berangan	Salt stress	Upregulated	RNA-seq	Lee et al. (2015)
MLT92	Alanine	*Vigna radiata* cv. Nampyoung	Cold stress	Upregulated	SSH	Chung et al. (2009)
GmASP1	Aspartate	*Glycine max* cv. Sinpaldal 2	Cold stress, NaCl	Upregulated	SSH	Cho et al. (2007)
Cystathionine -γ-synthase	Methionine	*Cicer arietinum* var. Azad	Cold stress	Upregulated	cDNA-AFLP	Dinari et al. (2013)
VvLCD, VvDCD	Alanine	*Vitis vinifera* var. F-242	Chilling stress	Upregulated	qRT-PCR	Fu et al. (2013)
Methionine-γ-lyase	Isoleucine	*Citrullus lanatus* var. Zhongke 6	Heat shock	Upregulated	RNA-seq	Zhu et al. (2020)
DAHPS	Phenylalanine, tyrosine, tryptophan	*Nicotiana tabacum* cv. Bel W3	Ozone stress	Upregulated	Northern-blot	Janzik et al. (2005)
AlaAT1	Alanine	*Triticum aestivum* cv. Jasna	Hypoxia	Upregulated	qRT-PCR	Kendziorek et al. (2012)
alt	Alanine	*Zea mays* cv. W22 and Crusader	Hypoxia	Upregulated	Northern-blot	Muench et al. (1998)
m-AlaAT	Alanine	*Medicago truncatula* cv. Paraggio	Hypoxia	Upregulated	qRT-PCR	Ricoult et al. (2006)
Ntgdh-NAD, GLN1.5, AS	Glutamate, glutamine, asparagine	*Nicotiana tabacum* cv. Gatersleben	Boron deficiency	Upregulated	qRT-PCR	Beato et al. (2014)
Branched-chain amino acid aminotransferase	Valine, leucine, isoleucine	*Solanum tuberosum* var. Kufri Jyoti	Nitrogen stress	Upregulated	RNA-seq	Tiwari et al. (2020)

CsGS	Glutamine	*Camellia sinensis*	Cadmium and salt stress	Upregulated	RT-PCR	Rana et al. (2008)
Diaminopimelate epimerase	Lysine	*Saccharum* spp. var. BO 91	Waterlogging stress	Upregulated	SSH	Khan et al. (2014)
ScASN1	Asparagine	*Secale cereale* cv. Askari	Sulfur deficiency	Upregulated	qRT-PCR	Postles et al. (2016)
SAS1, SAS2, SAS3	Asparagine	*Glycine max* cv. IAC-17	Nitrogen stress	Downregulated	Northern-blot	Antunes et al. (2008)

SSH Suppression subtractive hybridization, *AFLP* Amplified fragment length polymorphism, *qRT-PCR* quantitative real time-polymerase chain reaction, *RNA-seq* RNA-sequencing plant species (or non-plant species) can be more confidently used for engineering abiotic stress tolerance in other different plant species.

Glutamate is an important proteinogenic amino acid which plays a crucial role in regulating many plant metabolic activities during abiotic stress conditions, because it is a precursor of some other important amino acids such as proline, lysine, arginine, ornithine, and GABA (Slocum 2005; Bown et al. 2006). Besides, glutamate also plays important roles in photosynthesis, stomatal movements, carbon-nitrogen metabolism, antioxidation, stress signaling, etc. (Ali et al. 2019). Glutamate dehydrogenase (GDH) has been reported to be a stress-responsive enzyme, which plays important role in detoxifying the high intracellular ammonia levels and producing glutamate in response to abiotic stress (Zhou et al. 2015). Overexpression of GDH genes from bacteria (*gdhA*) and fungi (*MgGDH*) has been found to confer tolerance to drought and dehydration stress in transgenic maize and rice plants, respectively, by increasing the germination, biomass, and the capacity to prevent the accumulation of toxic ammonium (Lightfoot et al. 2007; Zhou et al. 2015). Overexpression of *Nicotiana plumbaginifolia* GDH gene (*NpGHD*) has been demonstrated to enhance the salt tolerance in transgenic tobacco plants by inducing changes in their leaf metabolome and increasing their biomass production under salt stress conditions (Tercé-Laforgue et al. 2015). Another enzyme involved in glutamate synthesis is ornithine-δ-aminotransferase (δ-OAT). It is actually reported to be involved in the process of proline synthesis from ornithine, during which it also produces glutamate (Adams and Frank 1980). Although, it is reported that *δ-OAT* is involved in proline synthesis, a separate study has shown that this gene is not essential for proline biosynthesis but for arginine catabolism to produce glutamate, and that it does not contribute to stress-induced proline accumulation (Funck et al. 2008). Overexpression of an Arabidopsis *δ-OAT* gene has been shown to confer tolerance to drought and salt stress in rice and *Nicotiana plumbaginifolia* plants by improving their proline-content, germination, growth, and yield under stress conditions (Roosens et al. 2002; Wu et al. 2003; Wu et al. 2005). Similarly, the rice plants overexpressing *OsOAT* have been shown to exhibit improved tolerance to drought and osmotic stress due to the enhanced proline content and ROS-scavenging capacity (You et al. 2012).

Glutamine is another important amino acid which plays a crucial role in plant metabolic adaptation to abiotic stresses. The gene coding for glutamine biosynthetic enzyme, glutamine synthetase (GS), has been functionally characterized for its role in drought and salt tolerance in various plant species. This enzyme is very crucial for nitrogen metabolism assimilating the inorganic ammonium into glutamine (Cai et al. 2009). In rice, overexpression of chloroplastic *OsGS2* has been demonstrated to result in enhanced salt tolerance due to the enhancement of photosystem II activity and increased photorespiration capacity (Hoshida et al. 2000). The study suggested that increased photorespiration could shield the electron transport systems of rice from over-reduction under salt stress. A separate study has shown that transgenic rice plants concurrently overexpressing cytoplasmic *OsGS1;1* and chloroplastic *OsGS2* showed increased salt and drought tolerance by exhibiting highly enhanced photosynthetic and agronomic performances in response to salinity and drought (James et al. 2018). However, these results obtained for rice drought and salt tolerance contrast with those of Cai et al. (2009) who found that overexpressed *OsGS1;2* conferred high sensitivity to salt, drought, and cold stresses in rice. Decreased expression of *OsGln1-5* (encoding cytoplasmic OsGS1) through RNA antisense strategy has been found to result in the pronounced salt sensitivity in transgenic tobacco plants due to the wilting and bleaching of foliage (Brugière et al. 1999). Similarly, *gln2-2* mutant lotus plants are subject to more intense cellular stress under drought than wild-type plants as indicated by higher oxidative membrane damage, diminished proline levels, and compromised recovery after drought (Díaz et al. 2010. In addition to these studies, overexpression of another GS gene from *Arabidopsis AtGS1.5* is reported to improve the germination and decrease toxic ammonium ions in transgenic plants under salt stress (Liu et al. 2017), resulting in their enhanced salt tolerance. Together, these results reveal a deep involvement of glutamine in the mitigation of drought- and salinity-induced harmful effects in plants.

Genetic engineering of BCAA production for enhancing plant drought and salt tolerance has also been accomplished by manipulating the expression of genes coding for BCAA biosynthetic enzymes. The branched-chain-amino-acid aminotransferases (BCATs) are crucial enzymes involved in BCAA synthesis. The *bcat3* mutant plants have been found to be more sensitive to water deprivation than wild-type plants (Scarpeci et al. 2017). Further, the dehydration-inducible expression of *BCAT2* in *Arabidopsis* has been found to correlate with the accumulation of BCAAs under dehydration stress (Urano et al. 2009). These results indicate the importance of BCAA accumulation in the plant tolerance to water-deficit stress. However, contrary to these results, it is reported that osmotic stress-inducible *BCAT2* is involved in the degradation of BCAAs, and its overexpression was shown to prevent BCAA accumulation under osmotic stress (Huang and Jander 2017). Nonetheless, a separate study found that *bcat2-1* knock-out mutant plants did not exhibit an increase in BCAAs under osmotic stress (Angelovici et al. 2013). So, there is some controversy in the role of *BCAT2* in accumulating the BCAAs under osmotic stress and needs to be resolved. Dihydroxyacid dehydratase (DHAD) is another vital enzyme in the BCAA biosynthetic pathway. Knock-down of *DHAD* has been found to cause reduced accumulation of BCAAs in the roots, resulting in the shorter root phenotype. The knock-down mutants were also found to exhibit hypersensitivity to salt stress, indicating the osmotolerance ability of BCAAs under salt stress (Zhang et al. 2015).

Arginine is another important amino acid in higher plants which plays an important role in nitrogen transport and storage due to its high N:C ratio. In the arginine biosynthesis from glutamate, ornithine is formed as an intermediate (Kalamaki et al. 2009b). Both arginine and ornithine play crucial metabolic roles in plant growth, development, and abiotic stress responses. Through genetic engineering, arginine biosynthetic genes have been used for improving arginine and ornithine production titers for enhanced drought and salt tolerance of plants. *N*-acetylglutamate synthase (NAGS) catalyzes the first step of arginine biosynthesis pathway. The overexpression of *SlNAGS1* from tomato has been reported to significantly increase ornithine accumulation and tolerance to drought and high salinity in transgenic *Arabidopsis* plants (Kalamaki et al. 2009b). The high stress tolerance of these transgenic plants was found to be due to the increase in seed germination, chlorophyll content, and ability to re-establish the growth after the stress was relieved. The second step of arginine biosynthesis pathway is catalyzed by *N*-acetylglutamate kinase (NAGK). It has been demonstrated that transgenic tobacco plants overexpressing a maize NAGK gene (*ZmNAGK*) exhibit improved tolerance to drought as indicated by the enhanced capacity of antioxidant defense, alleviation of the oxidative damage, and increased arginine and NO levels (Liu et al. 2019). Together, these results indicate that plant tolerance to drought and high salinity can be substantially improved by the genetic modification of the arginine biosynthetic pathway. Arginase represents an important enzyme in the arginine metabolism, which breaks down the arginine. The importance of arginine accumulation in drought and salt tolerance can be recognized from the finding of Shi et al. (2013), who showed that knock-out *Arabidopsis* mutants for arginase genes *(AtARGAH1* and *AtARGAH2)* exhibit high tolerance to drought and salt stress, whereas the overexpression lines of these genes were sensitive to these stresses. The stress tolerance of the knock-out lines was found to be due to the significantly decreased rates of electrolyte leakage and water loss as well as due to the high survival rates. This study indicates that arginine accumulation is critical to the stress tolerance, and the accumulated arginine in the knock-out lines is then channeled into synthesis of polyamines and nitric oxide, which modulate ROS accumulation, resulting in enhanced abiotic stress tolerance.

The plant tolerance to drought and high salinity has also been modulated through manipulating the expression of genes involved in glycine and serine biosynthesis. As mentioned in the earlier parts of this chapter, the importance of glycine and serine in the plant stress responses is primarily because they are very closely involved in the process of photorespiration. The photorespiratory enzyme serine:glyoxylate aminotransferases catalyzes the synthesis of glycine in peroxisomes. The overexpression of gene coding for this enzyme (*AGT1*) has been shown to confer salt tolerance to transgenic duckweed as indicated by strengthening of antioxidant system, increased membrane integrity, and higher maximum quantum yield of photosystem II (Yang et al. 2013). Another photorespiratory enzyme serine hydroxymethyltransferase (SHMT) converts glycine to serine in mitochondria. Mutations in *SHMT1* have been reported to result in the plants exhibiting hypersensitivity to salt and drought, owing to the excessive ROS production, inability to recover from the stress, and insensitivity to the stomatal closure (Moreno et al. 2005). In agreement with these results, the heterologous expression of *OsSHMT3* from rice has been found to confer salt tolerance to *Arabidopsis* and *E.coli* (Mishra et al. 2019). In concert, these results point toward the fact that photorespiration is recognized as an important pathway for alleviating the harmful effects of drought and salt stress in plants. Since this pathway acts as an electron sink for mitigating the stress-induced photooxidative damage and attenuating the ROS production (Di Martino et al. 2003), the accumulation of important intermediates serine and glycine in photorespiration can further complement the energy dissipation mechanisms to minimize ROS production (Leegood et al. 1995). Cysteine is another important amino acid which is derived from serine and plays a central role in plant metabolism due to its ability to donate sulfur for the synthesis of a wide variety of essential biomolecules (Romero et al. 2014). The accumulation of cytosolic cysteine is reported to profoundly affect the plant responses to abiotic stresses and the increased, but nontoxic, cysteine levels induce stress tolerance (Romero et al. 2014). The first step of cysteine biosynthesis from serine is catalyzed by serine O-acetyltransferase (SAT). Overexpression of a SAT gene, *BvSAT*, from sugar beet has been shown to confer tolerance to osmotic stress in osmosensitive yeast (Mulet et al. 2004). The second step in cysteine biosynthesis is catalyzed by O-acethylserine (thiol) lyase (OASTL). The overexpression of *Atcys-3A*, coding for OASTL, has been reported to enhance salt tolerance in transgenic yeast (Romero et al. 2001). Together, these studies imply that cysteine biosynthesis is a limiting factor for resistance against osmotic and salt stress, and increased endogenous availability of cysteine is responsible for imparting stress tolerance.

Besides, the genetic manipulation of aromatic amino acid biosynthetic genes has been delineated to influence the drought and salt tolerance of plants. Chorismate mutase (CM) catalyzes the first step of shikimate pathway for the synthesis of phenylalanine and tyrosine. The application of *Trichoderma parareesei* fungus is reported to enhance the productivity of rapeseed under drought and salinity conditions, and it was shown through gene silencing that *CM* of fungus enables it to promote the stress tolerance in rapeseed plants by increasing the expression of ABA- and ethylene-responsive genes (Poveda 2020). A dominant point mutation (*adt2-1D*) in the regulatory domain of phenylalanine biosynthetic enzyme arogenate dehydratase 2 (ADT2) has been demonstrated to cause feedback insensitive phenylalanine biosynthesis leading to pleiotropic physiological effects, including enhanced salt tolerance due to improved root growth (Huang et al. 2010). These studies indicate the importance of aromatic amino acid accumulation for acclimation of plants to the conditions of severe drought and salt stress.

3.2.3 Exogenous Application

Despite the results obtained for drought and salt tolerance of transgenic plants by genetically modifying the expression of amino acid biosynthetic genes, it is, however, believed that concentrations of compatible solutes due to the genetic manipulations are not sufficient for the full osmotic adjustment in transgenic plants (Chen et al. 2007). Besides, producing transgenic crop plants with high levels of compatible solutes by itself may prove counterproductive in the long run due to high crop yield penalties. Moreover, there have been several arguments against the endogenous compatible solutes in maintaining the osmotic adjustment under stress conditions (reviewed excellently by Shabala and Shabala 2011). According to these arguments, the levels of compatible solutes accumulated in plants under hyperosmotic stress are usually not osmotically significant; *de novo* endogenous production of these organic solutes is energetically highly expensive, which can result in substantial yield penalties; and the process of *de novo* compatible solute synthesis is very much slow, taking hours to days, whereas the fluctuations in the surrounding environment can be very rapid. Further, some crop species are unable to accumulate specific compatible solutes normally synthesized by their tolerant counterparts (Mellacheruvu et al. 2019). All these factors favor the exogenous application of compatible solutes as an alternative strategy for improving the stress tolerance of plants. Exogenously applied organic and inorganic compounds play a crucial role in mitigating the abiotic stress–induced effects in plants by modulating a complex array of physio-biochemical and molecular processes (Ahmad et al. 2019). As compatible solutes, amino acids are no exception to this approach. Exogenous applications of different groups of amino acids (as seed priming, foliar spray, soil drench, or medium supplementation) have been used for the alleviation of drought- and salt-induced effects in various crop species, which is detailed in Table 3.2. Exogenous amino acids provide protection against drought and high salinity in different crop species by imparting positive and beneficial effects on different morphological, physio-biochemical, and molecular aspects such as chlorophyll-content, antioxidant defense system, membrane integrity, polyamine and proline levels, seed vigor, germination, K/Na ratio, water status, biomass production, overall growth, nutrition composition and quality, stomatal-movement and density, transpiration, photosynthesis, expression of stress-responsive genes, and yield (Table 3.2). So, it is evident that L-amino acids have the tremendous potential in modulating the abiotic stress tolerance of crops, which can encourage their establishment in soils with stress issues. These results also suggest potential avenues for the follow-up research, which may produce useful information about stress responses during different plant developmental stages. Further, it also points toward the prospects of commercial applications of amino acids in promoting growth and stress tolerance of crops.

3.3 Temperature Stress Tolerance

3.3.1 Endogenous Accumulation

During heat and cold stresses, significant alterations in the accumulation of different types of amino acid take place in crop species, suggesting the close relationship between stress adaptation and the amino acid levels. Metabolite profiling of temperature-stressed plants has provided beneficial information regarding changes in the levels of amino acids related to the heat and cold stress adaptation. This information suggests that amino acid metabolism contributes to the mechanisms by which plants adapt to the extreme temperatures. Heat shock of cowpea cells has been found to lead to the accumulation of BCAAs and several other amino acids (Mayer et al. 1990). Heat stress at the early seed development stage of wheat has been reported to elevate the levels of several amino acids including valine, isoleucine, and aromatic amino acids, which had the strong effects on the nutritional and

Table 3.2 Effects of various exogenously applied amino acids on the performance of different crop species submitted to abiotic stress conditions

Amino acid	Applied to	Mode of application	Tolerance to	Beneficial effects in the tested plants	References
Arginine	*Triticum aestivum* L. cv. Prodip	Foliar spray	Drought	Reduces oxidative stress and methyl-glyoxal toxicity, increases proline content, and restores relative water content (RWC)	Hasanuzzaman et al. (2018)
Arginine	*Helianthus annuus* cv. Sakha 53	Seed soaking	Salt stress	Enhances antioxidant defense, K^+/Na^+ ratio, phosphorus and fatty acid contents, growth and yield parameters	Ramadan et al. (2019)
Arginine	*Eruca sativa* Mill.	Seed soaking; Added to nutrient media	Salt stress	Enhances percentage of seed germination, dry weight as well as chlorophyll and ascorbic acid contents in seedlings	Yagi and Abdulkareem (2006)
Arginine	*Lycopersicon esculentum* Mill cv. Alicante	Foliar spray	Drought	Alleviates oxidative damage by increasing the activity of antioxidative enzymes and content of antioxidants	Nasibi et al. (2011)
Arginine	*Phaseolus vulgaris*	Seed soaking	Salt stress	Improves germination and seedling growth by increasing polyamine content, total soluble sugar content, and K^+/Na^+ ratio	Zeid (2009)
Arginine	*Vigna radiata* var. Kawmy-1	Foliar spray	Salt stress	Significantly decreases Na and Cl concentration, increases uptake of N, P, and K, as well as K^+/Na^+ ratio; enhances growth, yield, nutrient composition, and nutritional value	Qados (2010)
Arginine	*Lycopersicon esculentum*	Foliar spray	Drought	Significantly increases chlorophyll, carbohydrate, and proline contents, improves plant growth and yield	Hamid and Idan (2019)
Arginine	*Helianthus annuus* cv. Giza 102	Seed soaking	Drought	Significantly reduces H_2O_2 and MDA contents; increases total biomass, shoot-root length, leaf area, succulent value	Hassan and Mohamed (2019)

(continued)

Table 3.2 (continued)

Amino acid	Applied to	Mode of application	Tolerance to	Beneficial effects in the tested plants	References
Arginine	*Vigna radiata*	Seed soaking	Salt stress	Significantly increases concentration of N, P, and K, plant height, dry weight, nutritional value, and yield	Hozayn et al. (2013)
Arginine	*Brassica napus*	Seed soaking	Salt stress	Improves seedling growth and alleviates oxidative damage	Nasibi et al. (2014)
Arginine	*Lupinus termis*	Foliar spray	Salt stress	Improves different growth parameters by enhancing antioxidative enzyme activity and photosynthetic pigment content; induces stress-responsive gene expression	Akladious and Hanafy (2018)
Arginine, cysteine	*Triticum aestivum*	Seed soaking	Salt stress	Improve growth parameters; increase reduced-glutathione amount in roots; decrease malondialdehyde and hydrogen peroxide content	Nasibi et al. (2015)
D-asparagine	*Oryza sativa* cv. Taichung Native 1	Added to the media	Salt stress	Reduces shoot growth inhibition	Lin and Kao (1995)
Asparagine	*Zea mays* cv. DK 847	Foliar spray	Salt stress	Reduces shoot growth inhibition; increases K^+, Ca^{2+}, P, and chlorophyll contents; reduces Na^+ content	Kaya et al. (2013)
Aspartic acid	*Lycopersicon esculentum* cv. Castle rook	Foliar spray	Salt stress	Increases plant growth and contents of anthocyanin, α-tocopherol, ascorbic acid, and total proteins; increases endogenous amino acid levels for improved stem anatomy	Akladious and Abbas (2013)
β-Aminobutyric Acid (BABA)	*Oryza sativa* var. Neeraja, Vaisakh and Vyttila 6	Seed soaking	Drought and Salt stress	Increases seedling growth, photosynthetic pigment content, photosystem activities, mitochondrial activities, proline content, and antioxidation	Jisha and Puthur (2016)

BABA	*Vigna radiata* var. Pusa Ratna, Pusa 9531, Pusa Vishal	Seed soaking	Drought and salt stress	Increases seedling growth, photosynthetic pigment content, photosystem activities, mitochondrial activities, proline content, and antioxidation	Jisha and Puthur (2015)
BABA	*Brassica napus* cv. BARI Sharisha-13	Added to the nutrition solution	Salt stress	Improves antioxidant defense system; increases growth, photosynthetic pigments, proline content, and K^+/Na^+ ratio	Mahmud et al. (2020)
BABA	*Brassica napus* cv. Madonna	Soil drench	Drought	Increases contents of ascorbate, anthocyanin, flavonoid, and calcium; enhances antioxidant activity	Rajaei and Mohamadi (2013)
BABA	*Solanum tuberosum* cv. Desirée	Soil drench	Drought	Reduces drought-induced effects on metabolite composition; improves yield by decreasing the loss of leaf RWC	Sos-Hegedus et al. (2014)
BABA	*Triticum aestivum* cv. Gansu 96 and Longchun 8275	Soil drench	Drought	Enhances drought-induced ABA accumulation for better water-use efficiency and rapid stomata closure; reduces oxidative damage	Du et al. (2012)
BABA	*Nicotiana tabaccum* cv. Yunyan 97	Foliar spray	Low-potassium stress	Increases primary root length, chlorophyll content, proline accumulation, peroxidase activity, and K^+ uptake	Jiang et al. (2012)
Cysteine	*Glycine max* cv. Giza 111	Foliar spray	Salt stress	Improves growth and yield through increasing the contents of photosynthetic pigments, proline, proteins, N, P, and K; enhances the antioxidative activity	Sadak et al. (2020)
Zinc-chelated lysine	*Raphanus sativus* cv. mono-early	Seed soaking	Drought	Enhances germination attributes and plant biomass production; improves leaf photosynthetic pigments, final root yield, nutritional quality, and mineral status; reduces oxidative damage	Noman et al. (2018)

(continued)

Table 3.2 (continued)

Amino acid	Applied to	Mode of application	Tolerance to	Beneficial effects in the tested plants	References
Glutamate, Aspartate	*Fagopyrum esculentum* var. Chuanqiao No.1 & 2	Foliar spraying	Saline-alkali stress	Significantly increase the seed mineral-content and crop yield	Song et al. (2019)
Leucine, Phenylalanine	*Lactuca sativa*	In nutrient solution	Salt stress	Significant increased the cell and seedling length	Mills (2014)
Glutamine, Glutamine, Lysine, Serine, Arginine etc.	*Hordeum vulgare* cv. Franklin	Added to the hydroponic solution	Salt stress	Mitigating NaCl-induced K^+ efflux; therefore, maintaining optimal K^+/Na^+ ratio	Cuin and Shabala (2007)
Ornithine	*Anacardium occidentale* var. CP 06	Foliar spraying	Salt stress	Induces proline synthesis and accumulation both *in situ* and *in vivo*	Da Rocha et al. (2012)
D-Ornithine	*Nicotiana tabaccum* L. cv. Burley 21	Added to suspension culture	Salt stress	Improves growth; enhances antioxidation enzyme activity, and contents of proline and polyamines	Ghahremani et al. (2013)
Ornithine	*Beta vulgaris* var. saccharifera	Foliar spray	Drought	Reduces the effects of drought on root length, root, and shoot weight; increases photosynthetic pigments, protein profile, antioxidation, total soluble sugars, and total amino acids	Hussein et al. (2019)
Ornithine	*Nicotiana tabaccum* L. cv. Burley 21	Added to suspension culture	Salt stress	Increases antioxidation enzyme activity; stimulates the production of proline, putrescine, and spermidine	Ghahremani et al. (2014)
Phenylalanine+calcium nitrate	*Zea mays* cv. 2B512	Seed soaking	High humidity and temperature	Increase seed vigor and gemination percentage	Gouveia et al. (2017)

Phenylalanine	*Zea mays, Vicia faba*	Spraying	Salt stress	Restricts Na^+ uptake, enhances K^+ and Ca^{2+} uptake, increases saccharides and protein content	El-Samad et al. (2010)
Tryptophan	*Chenopodium quinoa* Willd.	Foliar spray	Water deficit	Markedly improves different growth parameters; increases photosynthetic pigments, amino acids, antioxidant enzyme activity, yield, components, and nutritional value of the yielded seeds	Bakry et al. (2016)
Tryptophan	*Allium cepa* cv. Behary Red	Foliar spray	Salt stress	Increases plant growth, biomass, bulb dry weight; mitigates the oxidative damage	Hussein et al. (2014)
Tryptophan+salicylic acid	*Zea mays* cv. Islamabad Gold	Foliar spray	Drought	Increase leaf membrane stability index, RWC, chlorophyll and potassium content	Rao et al. (2012)
Tryptophan	*Triticum, aestivum* cv. MH-97, Inqlab-91	Seed soaking	Salt stress	Improves germination percentage and growth, reduces Na^+ uptake; increases Ca^{2+} partitioning, accumulation of free salicylic acid and grain yield	Iqbal and Ashraf (2007)
Tryptophan+*Rhizobium phaseoli*	*Vigna radiata*	Added to the slurry	Salt stress	Enhance nutrient-uptake and rhizosphere auxin production, which increases the plant height, number of nodules, plant biomass, yield and quality of grains	Zahir et al. (2010)
Poly-γ-glutamic acid	*Brassica napus*	Added to the hydroponic solution	Drought	Enhances fresh weight, RWC, and antioxidant enzyme activity; increases contents of chlorophyll, proline, and ABA; upregulates ABA-responsive drought-induced genes	Xu et al. (2020b)
Arginine	*Triticum aestivum* cv. Giza 168	Foliar spray	Heat stress	Increases the endogenous contents of polyamines, total amino acids, and essential amino acids; decreases ethylene and NH_4^+ contents	Hassanein et al. (2013)

(continued)

Table 3.2 (continued)

Amino acid	Applied to	Mode of application	Tolerance to	Beneficial effects in the tested plants	References
Phenylalanine	*Lycopersicon esculentum* cv. Izmir	Fruits immersed in phenylalanine solution	Chilling stress	Profoundly enhances antioxidative defense system and maintains membrane integrity by ensuring the supply of reducing power NADPH in fruits	Aghdam et al. (2019)
Arginine	*Triticum aestivum* cv. Giza	Foliar spray	Heat stress	Increases antioxidative enzyme activity, DNA/RNA content, and synthesis of new proteins; reduces oxidative damage	Khalil et al. (2009)
Arginine	*Triticum aestivum* cv. DBW 14 and K 307	Foliar spray	Heat stress	Reduces chlorophyll degradation in flag leaf, and relative permeability; improves different yield parameters for increased yield	Roy Chowdhury et al. (2019)
Arginine	*Pistacia vera* cv. Ohadi	Added to the growth media	Cold stress	Decreases lipid peroxidation, protein oxidation, hydrogen peroxide levels, and proline content; increases antioxidant enzyme activity	Barand et al. (2015)
Arginine	*Pistacia vera*	Seed soaking	Freezing stress	Improves germination, growth, and soluble sugar content; reduces concentration of phenols and oxidation of proteins	Nasibi et al. (2013)
Glutamine	*Brassica napus* cv. Hornet H and Sunday	Foliar spray	Freezing stress	Increases the production of free proline and ethylene	Gavelienė et al. (2014)
Poly-γ-glutamic acid	*Brassica napus* var. Suyou No. 1	Added to the hydroponic solution	Cold stress	Increases fresh weight, proline and chlorophyll content, activates antioxidant enzyme system, and regulates Ca^{2+} levels and expression of calcium-dependent protein kinase genes	Lei et al. (2015)

Aspartic acid	*Oryza sativa* cv. Kainat	Foliar spray	Cadmium stress	Increases plant height, biomass, chlorophyll content, photosynthesis, gas exchange, and antioxidative capacity; reduces oxidative damage, cadmium concentration and translocation	Rizwan et al. (2017)
Glutamate, phenylalanine, cysteine, glycine	*Glycine max* cv. NS 7901 RR	Foliar and seed spray	Oxidative stress	Increase the antioxidation potential by increasing the activity of phenylpropanoid pathway enzymes and other antioxidative enzymes	Teixeira et al. (2017)
Histidine	*Solanum lycopersicum* cv. Cal-J N3 and Petoearly CH	Added to the hydroponic media	Nickel stress	Reduces oxidative markers and nickel accumulation in roots and shoots; enhances antioxidative enzyme activity, Ca^{2+}, ascorbate, and total protein contents	Mozafari et al. (2014)
Zinc-lysine chelate	*Oryza sativa* cv. Kainat	Foliar spray	Chromium stress	Increases the plant height, biomass, photosynthesis, and zinc uptake; reduces the chromium concentration and oxidative damage	Hussain et al. (2018)
Tyrosine	*Zea mays* cv. Sadaf and S-2002	Seed soaking	pH stress	Improves antioxidative potential, seedling vigor, and growth by modulating the phenylpropanoid pathway	Mahmood et al. (2017)
Tryptophan	*Oryza sativa*	Roots dipped in Tryptophan solution	Cadmium stress	Improves different growth and yield parameters; reduces cadmium translocation to rice grains	Farooq et al. (2015)

antioxidant properties of mature grains (De Leonardis et al. 2015). Increased levels of BCAAs and aromatic amino acids have also been observed in heat- and cold-shocked *Arabidopsis* plants, and this accumulation of amino acids was suggested to support the elevated production of secondary metabolites for mitigating the harmful effects of thermal stress (Kaplan et al. 2004). Valine and leucine have been found to markedly increase in their abundance during cold temperatures in the tobacco, and were suggested to act as compatible osmolytes and as alternative donors of electrons to mitochondrial electron transport chain for sustaining the cellular energy production during cold acclimation (Xu et al. 2020a). Metabolic responses of cold-stressed *Arabidopsis* plants have revealed that among other metabolites, BCAAs and aromatic amino acids significantly increased in abundance and this increase was found to be correlated with the corresponding cold-regulated gene expression (Kaplan et al. 2007). Besides, valine and isoleucine have also been found to be upregulated in the grapevine leaves during cold acclimation, which was suggested to contribute to the osmotic adjustment in the cold-acclimated leaves (Chai et al. 2019). However, leucine and isoleucine levels have also been found to significantly reduce under heat stress in wucai and soybean leaves, respectively (Das et al. 2017; Yuan et al. 2017), which may indicate a direct effect of heat stress on the enzymes for BCAA biosynthesis in these crop species. Metabolomic analysis of tobacco during cold acclimation has revealed that abundance of aromatic amino acids tyrosine and phenylalanine substantially increases, which was suggested to promote the secondary metabolism in tobacco for alleviating the cold-induced damage (Xu et al. 2020a). In a separate metabolic analysis, the glucosinolate biosynthesis pathway has been shown to play a crucial role in metabolic adaptation of sweet potato roots to low temperature. Further, the study reported that levels of BCAAs, tyrosine, and tryptophan were significantly upregulated in the glucosinolate biosynthesis, suggesting that these amino acids play a leading role in the metabolic adaptation of sweet potato roots to low temperature (Yang et al. 2020). Similarly, thermopriming has been shown to restructure the metabolic networks to impart tolerance to heat stress in *Arabidopsis* (Serrano et al. 2019). The study revealed that better performance of the thermoprimed plants than nonprimed plants was attributed to the increased production of BCAAs, aromatic amino acids, and other essential metabolites, which served as compatible osmolytes, antioxidants, and growth promoters to help plants withstand heat stress. The results from this study suggested that the increased accumulation of these amino acids constituted a part of metabolic reprogramming to help maintain the metabolic homeostasis in response to heat stress. Despite the increased accumulation of aromatic amino acids in response to temperature stress in various plants, their levels have also been reported to diminish considerably in heat-stressed cowpea cells (Mayer et al. 1990) and soybean leaves (Das et al. 2017), indicating that biosynthesis of aromatic amino acids in these plants may be more prone to thermal stress.

In addition, crop plants have also been found to increase the abundance of several other types of proteinic and nonproteinic amino acids when they face heat or cold stresses, such as glycine, serine, alanine, methionine, cysteine, aspartate, glutamate, ornithine, pipecolic acid, etc. (Purdy et al. 2013; Kaplan et al. 2004, 2007;

Thind and Malik 1987; Tao et al. 2018; Yuan et al. 2017; Singh et al. 2016; Simon-Sarkadi and Galiba 1996; Huang et al. 2019; Zuther et al. 2019; Müller 2017).

In concert, results from all these studies indicate that extreme temperatures stimulate a defense response at metabolic level in plants by regulating the abundance of amino acids (other than proline) that act as osmoprotectants, antioxidants, secondary metabolite precursors, and growth stimulants. Further, they also suggest that metabolomic analyses are very beneficial for providing comprehensive insights into the amino acids involved in various stress-responsive processes, which could expedite plant breeding for improved crop yields under abiotic stresses.

3.3.2 Amino Acid Biosynthetic Genes and Their Use in Engineering Plant Heat and Cold Tolerance

As compared to drought and salt stress, studies on identification of heat and cold stress-responsive amino acid biosynthetic genes are very limited. In particular, the studies concerning the functional characterization of such genes for their role in engineering temperature stress tolerance in plants are absolutely unavailable. Nonetheless, some amino acid biosynthesis genes have been reported to be temperature stress-responsive. Using global transcriptome analyses and other gene expression studies, several amino acid biosynthesis genes have been identified as potential genomic resources for heat and cold stress tolerance of different crop species (Table 3.1). For example, genes for biosynthesis of aspartate and aspartate-derived amino acids (such as lysine, methionine, and isoleucine) have been found to be differentially induced in response to extreme temperatures. L-asparaginase-encoding gene (*GmASP1*) is reported to be rapidly induced under low-temperature in soybean (Cho et al. 2007), indicating that *GmASP1* may facilitate the early and rapid response of soybean plants to cold stress. Since L-asparaginse catalyzes the production of aspartate and ammonia from L-asparagine, the study suggests that ammonia may serve to fulfill the nitrogen demands of cold-stressed plants and the aspartate production may serve to maintain the cellular osmolarity (Cho et al. 2007). A gene for methionine biosynthetic enzyme cystathionine gamma-synthase (*CgS*) has been found to be significantly expressed in the cold-tolerant chickpea leaves exposed to the cold stress (Dinari et al. 2013). Similarly, the expression of a gene for lysine biosynthetic enzyme, diaminopimelate epimerase, has been found to be upregulated under heat stress (Lin et al. 2016). Another gene *DHPS1* coding for a different lysine biosynthetic enzyme, dihydrodipicolinate synthase, has been found to be colocalized with a QTL associated with frost damage in pea (Dumont et al. 2009), indicating that lysine biosynthesis is closely linked with the physiological adaptation of plants to temperature stress. The expression of a gene for methionine-gamma-lyase, catalyzing isoleucine biosynthesis, is reportedly upregulated under heat shock in the unfertilized ovules of watermelon (Zhu et al. 2020), indicating that increased isoleucine content may contribute to the promotion of ovule growth under heat stress. Additionally, the expression of genes involved in the synthesis of alanine, cysteine, and glutamate has been demonstrated to be highly upregulated in crops such as mungbean, soybean, and rapeseed under extreme temperatures (Chung et al. 2009; Heis et al. 2011; Goel and Singh 2015; Yi et al. 2013). Altogether, these results indicate that alterations in the amino acid levels due to the differential expression of their biosynthetic genes contribute to a profound restructuring in the plant metabolome in response to extreme temperatures, which is a crucial factor in the stress tolerance. Further, functional characterization of these genes can provide potential insights into the connection between specific amino acid biosynthesis pathways and other metabolic pathways activated under temperature stress, which would in turn be useful for the genetic improvement of heat- and cold-tolerant crop cultivars.

Although no plant amino acid biosynthetic genes have been functionally characterized for temperature stress tolerance, a bacterial gene, *panD*, for L-aspartate-α-decarboxylase (catalyzing the decarboxylation of L-aspartate into non-proteinic β-alanine and CO_2) has been analyzed and it has been found to enhance thermotolerance in transgenic tobacco (Fouad and Rathinasabapathi 2006). The better performance of transgenic plants over the wild-type was attributed to the enhanced germination and growth, higher fresh and dry weight, and significant stress recovery. As β-alanine is a precursor for vitamin B5 and therefore essential for normal plant growth and development (Parthasarathy et al. 2019), the enhanced growth of transgenic lines under heat stress might be due to the enhanced levels of β-alanine.

3.3.3 Exogenous Application

Global need for the novel temperature stress-resilient crops and for the compounds mitigating the harmful effects of heat and cold stress is intensely increasing for better crop yield. Like other natural products and synthetic chemicals, application of amino acids has also been used to enhance the thermotolerance of crop plants through modulation of stress-responsive molecular and physiological mechanisms. Exogenous applications of different amino acids have been found to be beneficial for alleviating the temperature stress–induced effects in different crop species (Table 3.2). However, the progress in this approach for crop thermotolerance has been much lesser than that for drought and salt tolerance. The application of arginine improves thermotolerance in heat-stressed wheat plants by exerting beneficial effects on various physio-biochemical parameters such as increasing the endogenous polyamine and amino acid contents, promoting the synthesis of new proteins and nucleic acids, decreasing the extent of oxidative damage and chlorophyll degradation, and increasing the grain yield (Hassanein et al. 2013; Khalil et al. 2009; Roy Chowdhury et al. 2019). Similarly, arginine pretreatment of pistachio plants submitted to cold stress has been reported to increase their tolerance to low temperature by boosting the antioxidant defense system, and by enhancing germination, growth, and soluble sugar content (Borand et al. 2015; Nasibi et al. 2013a). Further, exogenous glutamine has been found to enhance cold tolerance in rapeseed and rice by inducing the production of proline and ethylene, and by upregulating the expression of cold-responsive transcription factor DREB1A, respectively (Wu et al. 2010; Kan et al. 2015), indicating that glutamine may act as a signaling molecule to interact with other signaling pathways for regulating the cold stress-responsive gene expression in plants. Likewise, poly(γ-glutamic acid) also protects the rape seedlings from the cold-stress-induced damage by increasing the fresh weight, proline and chlorophyll levels, activating the antioxidant enzyme system, and by regulating Ca^{2+} levels and calcium-dependent protein kinases (Lei et al. 2015), indicating that Ca^{2+}-dependent signaling pathway possibly mediates the effect of poly(γ-glutamic acid) on the cold tolerance. Besides, the exogenous phenylalanine application has been found to be a promising procedure in promoting chilling injury tolerance in tomato (Aghdam et al. 2019), and this was attributed to the ability of phenylalanine to activate the ROS scavenging system ensuing in the higher membrane integrity in tomato submitted to cold storage.

From these studies, it is evident that application of amino acids has huge prospects for promoting the thermotolerance of crops, and as such, the effect of other amino acids should be tested on the performance of crop plants submitted to heat or cold stresses.

3.4 Tolerance to Other Abiotic Stresses

3.4.1 Endogenous Accumulation

Accumulation of specific amino acids is a crucial metabolic adaptation, which has been implicated also in increased plant tolerance to other adverse environmental stresses such as hypoxia, submergence, oxidative stress, metal stress, light stress, etc. However, the research interests have remained underappreciated for these types of stresses probably because of the poor financing by the stakeholders. Alanine (both L-alanine and β-alanine) plays a crucial role in modulating plant physiology and metabolism to enhance plant tolerance to various abiotic stresses, especially hypoxia and flooding. During hypoxia, pyruvate derived from active glycolysis is normally metabolized into the ethanol, which is a dead-end product, resulting in the net loss of carbon (Albrecht et al. 2004). To counteract this problem, plants accumulate alanine under hypoxia by competitively synthesizing it from the glycolysis-derived pyruvate, which safeguards carbon in nitrogen store and saves energy for the sustenance under hypoxia (Diab and Limami 2016). The saved carbon can readily be utilized and fed into TCA cycle upon reoxygenation. Alanine has been shown to accumulate in crop species such as *Medicago truncatula*, *Glycine max*, and *Lotus japonica* subjected to hypoxia (António et al. 2016; Ricoult et al. 2005, 2006; Rocha et al. 2010). In common sunflower, the roots flooded with water were found to exude more β-alanine into the water (Drakeford et al. 1985). Later, hypoxia was also found to induce alanine accumulation predominantly in the roots of model plant *Arabidopsis* (Miyashita and Good 2008). Conjointly, these results suggest that alanine accumulation in roots may protect plants from the harmful effects of hypoxia or flooding. Further, the osmoprotectant β-alanine-betaine (synthesized from β-alanine) is proposed to provide more efficient osmoprotection to plants than glycine-betaine under the hypoxic conditions because of the requirement of oxygen for glycine-betaine synthesis, and for this reason, many plants accumulate this compound during hypoxia (Parthasarathy et al. 2019). Besides, alanine has also been shown to accumulate in plants submitted to heavy metal stress, such as cadmium stress (Sun et al. 2010).

As discussed in the earlier sections, asparagine accumulates under abiotic stress conditions to maintain the nitrogen needs of plants and also the osmotic adjustment. Likewise, the accumulation of asparagine under other abiotic stresses is also evidenced. Asparagine plays an essential role in mitigating the metal toxicity by binding to certain toxic metals such as cadmium, sulfur, and lead (Navrotskyi 2016). For example, asparagine has been found to highly accumulate in tomato roots subjected to cadmium stress (Chaffei et al. 2004). Differential accumulation of asparagine together with glutamate, glutamine, and aspartate has been reported in two Brassicaceae species under cadmium stress (Zemanová et al. 2013), suggesting that plants require assimilated nitrogen remobilization to cope with the metal stress. Also, the increased accumulation of cysteine is reported to support the survival of *Arabidopsis* plants submitted to acute cadmium stress, because cysteine serves as a precursor for the synthesis of glutathione and phytochelatin – compounds important for metal sequestration (Dominguez-Solis et al. 2004). Asparagine concentration in crop plants can also significantly increase under the conditions of sulfur deficiency. In wheat plants grown in the sulfur-deficient regions, asparagine levels were found to be significantly high, which was correlated with the yield (Navrotskyi 2016; Curtis et al. 2018). The accumulation of histidine is also considered as an active response to heavy metal stress. The nickel-hyperaccumulation trait is specifically associated with the free histidine production (Krämer et al. 1996). Under nickel stress, *Brassica juncea* has been reported to increase the histidine levels to chelate the nickel for increasing tolerance to applied nickel stress (Kerkeb and Krämer 2003). These findings indicate an important association between amino acid accumulation and alleviation of metal stress, particularly, because amino acid–metal complexes (especially amino acid-nickel complexes) are significantly more stable (Homer et al. 1997).

Additionally, in CO_2 -stressed plants, the accumulation of NPAAs ornithine and citrulline has been shown to increase, which was suggested to facilitate the nitrogen storage during photorespiration (Blume et al. 2019) and may perhaps promote photosynthesis under favorable conditions.

3.4.2 Amino Acid Biosynthetic Genes and Their Use in Engineering Plant Tolerance to Other Abiotic Stresses

Alterations in different amino acid levels in plants exposed to heavy metals are controlled by the differential regulation of genes coding for their biosynthetic enzymes. Different amino acid biosynthetic genes responsive to plant metal tolerance have been identified by employing transcriptomics and other gene expression analyses (Table 3.1). In this section, only those studies which have functionally analyzed some of these genes by manipulating their expression for heavy metal tolerance will be appraised. Most of the studies on these genes have focused the attention particularly on cadmium stress. Cadmium is highly toxic even at low levels due to its high mobility in the soil (Shahid et al. 2016). Cadmium toxicity causes concerning effects on different morphological and physio-biochemical aspects of plant growth and development ensuing in decreased crop yield (Ranieri et al. 2005). Different amino acid biosynthetic genes have been functionally characterized and used to engineer cadmium tolerance in plants. Cysteine plays a pivotal role in endogenous heavy metal detoxification, because several plant molecules (such as metallothioneins and phytochelatins) that are crucial for heavy metal homeostasis and detoxification are cysteine rich, and these molecules also have very high affinity for cadmium and sequester it in the vacuoles (Kuramata et al. 2009). Overexpression of a rice cysteine biosynthetic gene *RCS1* is reported to impart cadmium tolerance in transgenic tobacco plants by increasing the production of phytochelatins, which provided cadmium detoxification by buffering high cytosolic cadmium concentrations in shoots (Harada et al. 2001). Consequently, the transgenic plants showed improved growth over wild-type when exposed to high levels of cadmium. Tobacco plants overexpressing another cysteine biosynthetic gene from soybean (*GmOASTL4*) exhibited significant increase in cysteine levels, antioxidation capacity, root length, fresh weight, and chlorophyll content, resulting in the high cadmium tolerance (Ning et al. 2010). Similarly, the *Arabidopsis* overexpression and mutant lines for cysteine biosynthetic genes *Atcys3-A* and *OAS-A1* have been reported to be highly tolerant and sensitive to cadmium stress, respectively (Domínguez-Solís et al. 2001; López-Martín et al. 2008). Besides cysteine, tryptophan and glutamine biosynthetic genes are also reported to contribute to plant tolerance to cadmium stress. Overexpression of a tryptophan biosynthesis gene *tryptophan synthase beta 1* (*AtTSB1*) has been demonstrated to increase accumulation of tryptophan, decrease accumulation of cadmium, and impart tolerance to cadmium stress in transgenic *Arabidopsis* and tomato (Sanjaya et al. 2008). The *AtTSB1*-transgenic plants were found to have higher chlorophyll content and lower oxidative damage when exposed to cadmium. Further, the same study suggested that high tryptophan concentration in transgenic plants restricted the cadmium transport by suppressing the expression of metal transporter genes *ZIP4* and *ZIP9*. Likewise, rice plants expressing glutamine synthetase gene (*OsGS*) show significantly high antioxidation defense when submitted to cadmium stress (Lee et al. 2013). Apart from cadmium stress tolerance, manipulation of amino acid biosynthetic gene expression also alleviates the harmful effects induced by other heavy metals in transgenic plants, such as *CSase*, *AtCpNifS* and *StHisG* conferring tolerance to nickel and selenium toxicity (Kawashima et al. 2004; Van Hoewyk et al. 2005; Wycisk et al. 2004).

Conjointly, these compiled studies highlight the significance of amino acids as crucial metabolites in plant responses and acclimation to heavy metal toxicity, convincingly revealing a mechanistic link between amino acids and heavy metal tolerance. They also point toward the functional diversification of amino acids, mainly addressed as metabolites in the context of drought, salinity, and temperature stresses, whereby their biosynthetic pathways can interact with other metabolic pathways to mount a response specific to each particular stress.

Oxidative stress is a very common situation for field-grown plants. Amino acid biosynthetic genes are also involved in regulating the plant responses to this stress. Transgenic tobacco plants overexpressing cysteine biosynthetic genes from wheat (*cys1*) and *E. coli* (*cysE*) exhibit increased resistance to oxidative stress imposed by exogenous methyl viologen and $H_2O_{2,}$ respectively, as indicated by high chlorophyll content and reduced membrane damage (Blaszczyk et al. 1999; Youssefian et al. 2001). However, tobacco plants overexpressing methionine biosynthetic gene (*AtCGS*) have been found to exhibit sensitivity to oxidative stress (Hacham et al. 2017). Altogether, these studies indicate that alterations in the sulfur metabolite levels can have profound effect on the oxidative stress tolerance of plants.

Due to their structural diversity, the role of amino acids is not only limited to the abovementioned abiotic stresses but is also involved in several other processes. This functional diversification has sparked great interest in targeting the amino acid metabolism for plant tolerance to several other abiotic stresses. To cut the long story short, through functional studies, different types of amino acid biosynthetic genes have been substantiated to be involved in some other important but poorly studied abiotic stresses in plants, such as desiccation (Cohen et al. 2014), nitrogen stress (Fuentes et al. 2001; Lam et al. 2003), ammonium toxicity (Guan et al. 2016), sulfur deficiency (Postles et al. 2016), herbicide stress (Pasquer et al. 2006), environmental pollution (Noji et al. 2001), and elevated CO_2 (Gao et al. 2019).

3.4.3 Exogenous Application

Like their role in the amelioration of other abiotic stresses, the application of exogenous amino acids through different modes has also been found to improve the heavy metal stress tolerance in plants (Table 3.2). For example, the foliar applications of aspartic acid and alfalfa leaf extract (containing different metabolites and amino acid profiles) have been shown to induce cadmium tolerance in rice and wheat plants, respectively, as indicated by reduced oxidative damage to photosynthetic organs, and increased growth, free proline, total soluble sugars and yield (Rizwan et al. 2017; Perveen et al. 2016). Similarly, the pretreatment of tomato and henbane plants, respectively, with histidine and arginine alleviates the nickel stress–induced oxidative stress by inhibition of nickel translocation and improving the nickel chelating mechanism (Nasibi et al. 2013b; Mozafari et al. 2014). Foliar application of zinc-lysine chelate ameliorates the stress induced by another heavy metal chromium in rice plants by increasing their photosynthesis, biomass, and zinc content while reducing the chromium concentration (Hussain et al. 2018). Further, exogenous tyrosine improves the antioxidative potential and growth of maize seedlings subjected to different pH regimes by modulating the phenylpropanoid pathway (Mahmood et al. 2017). The high antioxidation potential, also due to the increased activity of phenylpropanoid pathway enzymes and other antioxidant enzymes, of soybean plants is induced by the foliar and seed application of glutamate, cysteine, phenylalanine, and glycine (Texeira et al. 2017). Together, these results bear witness to the versatility of amino acids and to the dynamics of amino acid metabolism, which is modulated stress-specifically. Undoubtedly, plants reap huge number of benefits from these soluble organic compounds whether they are under stress or relaxed.

3.5 Amino Acid–Based Biostimulants and Abiotic Stress Tolerance

Increasing the crop resilience to wide range of environmental conditions is not an issue to be resolved in the future, it actually is very concerning for the crop production right now. For fixing this issue, biostimulants can contribute to a remarkable extent. Biostimulants, also known as bioeffectors, represent an array of natural or synthetic environment-friendly substances that stimulate the plant growth and development as well as maximize the yield when applied in the right way (Popko et al. 2018). Besides, their application also helps increase the resistance of plants to different abiotic stresses. This relatively new group of products, differing from traditional fertilizers, comes in various formulations and may contain different organic compounds and other chemicals that vary depending on the manufacturer (de Vasconcelos and Chaves 2019). However, I am going to give a brief account of only the ones whose preparations are based on the amino acids. As we saw in the earlier sections that applications of individual amino acids have an array of beneficial effects that mitigate the stress on plants, therefore, the combination of different amino acids should ideally have additive beneficial effects on plant growth, yield, and quality, especially under abiotic stress conditions. During favorable environmental conditions, plants use carbon, nitrogen, oxygen, and hydrogen from their surrounding environments to biosynthesize the endogenous amino acids, which are required for the normal plant growth and development. However, when the plants are submitted to unfavorable conditions, limiting the absorption or uptake of these elements, the amino acid biosynthesis, and therefore the fitness and productivity of plants is seriously affected. This is where the application of amino acid biostimulants could prove handy, which are being incorporated into the production systems for optimizing the crop metabolic efficiency and yield. Moreover, as mentioned earlier, the *de novo* biosynthesis of amino acids is energetically expensive to plants; therefore, biostimulants serve as ready-for-uptake sources of amino acids that stimulate growth of plants during critical times. Some of the amino acid–based biostimulants that are available in market and have been used and recommended for improving the abiotic stress tolerance of crops include Amino Plus Foliar Fertilizer for drought (Campomanes and Porter 2016), Terra-Sorb® Foliar for temperature stress (Botta 2013), Alethea for salinity and oxidative stress (Wargent et al. 2013), AminoKelp^{TM} for drought, frost and heat stress (GrowGreen, Australia), Perfectose for various abiotic stresses (sofbey, SA, Switzerland), Aminototal for salt stress (Sadak et al. 2015), Aton AZ for drought and UV-B radiation (Januškaitienė and Kacienė 2017), Botamisol for salt stress (Jannesari et al. 2016), Bridgeway for drought, heat and nutrient stress (Interagro, UK), Delfan Plus for drought, heat and cold stress (Tradecopr, Australia), Fitomare® for drought (Atlántica Agrícola SA, Spain), Megafol® for drought (Petrozza et al. 2014), Ruter AA and Razormin for cold stress (Gavelienė et al. 2018), and CycoFlow for high temperature (Francesca et al. 2020). These biostimulants have been found to enhance the performance of crop plants under various abiotic stresses by exerting highly beneficial effects on various morphological and physio-biochemical features such as antioxidation; chlorophyll, mineral, carotenoid, proline, protein, carbohydrate, and nucleic acid contents; photosynthesis; leaf water potential, growth, vigor, biomass production, stress-induced gene expression, yield, and fruit nutritional quality.

3.6 Concluding Remarks

Amino acids are amazing biomolecules which have reduced the need for synthetic agrochemicals such as fertilizers and pesticides, and have high potential to take part in plant responses mounted against various abiotic stresses. Being compatible solutes, they not only contribute to the maintenance of cellular osmoregulation, but also protect vital biomolecules and cellular structures from the abiotic stress-induced oxidative damage. They also serve as potential signaling molecules and also as precursors of numerous secondary and energy-associated metabolites, which are usually crucial components of metabolic networks restructured during abiotic stresses. For these reasons, profile of plant amino acids is modified and their accumulation is elevated as a crucial mechanism for abiotic stress tolerance. Moreover, amino acids are differentially accumulated in different species and in response to various stress conditions, which is governed by the differential regulation of their biosynthetic genes. Although, numerous abiotic stress-responsive amino acid biosynthetic genes have been identified, the majority of them have remained functionally uncharacterized. More particularly, studying the genetic and epigenetic regulation of these genes will undoubtedly provide some deep insights into the interaction of amino acid biosynthetic pathways with other metabolic pathways and also with various important traits under critical stress conditions. It can also divulge the major pivotal factors regulating the amino acid accumulation under abiotic stresses. On that account, amino acid biosynthetic genes could indisputably prove as potentially beneficial genomic resources for engineering the tolerance to various abiotic stresses in crop species. Additionally, a close examination of the effects of amino acid–based biostimulants on plant life processes indicates that amino acids are in the premier league of pivotal metabolites that enhance plant performance under environmental perturbations. Therefore, application of amino acid biostimulants may offer a potentially novel approach that can help optimize the metabolic efficiency of crops for making agriculture more resilient and sustainable. However, the mechanism of action of these amino acid formulations is relatively indecisive, which precludes harnessing their potential in boosting the agricultural production. Moreover, in order to produce the highest impact on abiotic stress tolerance of crops, it is very essential to define the specificities of biostimulants to crop species, because a particular biostimulant can have significantly dissimilar effects on different species (de Vasconcelos and Chaves 2019). Apart from all this, there is a severe deficiency of studies for divulging the role of amino acids in the plant tolerance to temperature and other stresses as compared to drought and salt stress, which thus necessitates considerably more work to be done in these fields. Last but not the least, all other amino acids deserve the same research interest shown to proline for managing the abiotic stress tolerance of plants, given the reason that proline is certainly not the only amino acid accumulated in plants during abiotic stress conditions.

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Chapter 4
Engineering Glycine Betaine Biosynthesis in Alleviating Abiotic Stress Effects in Plants

Bidisha Bhowal, Prakash Chandra, and Saurabh C. Saxena

Abstract The severe impact of climate change on crop production is already evident; there are strong chances of further changes occurring. Therefore, there is an urgent need to address agricultural adaptation more coherently. Adapting agriculture to the increasing scale of climate risks will ensure food security and sustainability. An increase in intensity and frequency of temperature extremes such as heat and cold along with other abiotic stresses such as salinity and drought are all manifestations of a global environmental change. Owing to their sessile nature, plants have developed several strategies to adapt to the adverse effects of abiotic stresses, synthesis, and build-up of osmoprotectants being one of them. Osmoprotectants are soluble organic compounds compatible with the cellular metabolism and hence are also known as compatible solutes. Among the various compatible solutes, Glycine betaine (GB) is most effective in imparting abiotic stress tolerance to plants. There is enough evidence to suggest the protective role of GB owing to its versatility and ability to accumulate under unfavourable conditions. GB, however, does not accumulate in many crop plants such as rice, tobacco, etc. Tailoring the GB biosynthetic pathway has thus been one of most promising strategies in reducing susceptibility of such crops to abiotic stresses. A number of crop plants have been engineered since then with an objective to increase the accumulation of GB to acquire improved stress tolerance. In this chapter, we shall discuss about the role of GB in ameliorating stress tolerance in plants, major breakthroughs in engineering its pathway, magnitude and mechanism of protective effects, challenges faced, strategies to overcome limitations and future prospects.

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Abbreviations

ABA	Abscisic acid
AREB/ABF	ABA responsive element binding
CAT	Catalase
DMSP	Dimethylsulfoniopropionate
DREB	Dehydration Responsive Element Binding
FAO	Food and Agricultural Organisation
FBPase	Fructose 1,6-bisphosphatase
GB	Glycine betaine
H_2O_2	Hydrogen peroxide
HK	Histidine Kinases
MAPK	Mitogen Activated Protein kinase
MDA	Malondialdehyde
NAC	NAM/ATAF/CUC
NaCl	Sodium chloride
NADP	Nicotinamide adenine dinucleotide phosphate
NT	Non-Treated
PSII	Photosystem II
ROS	Reactive Oxygen Species
Rubisco	Ribulose-1,5-biphosphate Carboxylase/Oxygenase
SOD	Superoxide dismutase
T-DNA	Transfer DNA
ZF-HD	Zinc finger Homeodomain

4.1 Introduction

Climate change has severely impacted agricultural productivity across the globe. According to the latest report of the IPCC (Intergovernmental Panel on Climate Change), average global temperature has risen 1.5 °C in the recent past, leading to an overall environmental change across the world. Physical and biogeochemical changes either due to natural phenomena or driven by human activities such as consumption of non-renewable sources such as coal and petroleum, deforestation, agricultural intensification, urbanization, and waste production have primarily contributed to the global environmental change.

The terrestrial ecosystem has been majorly affected by climate change which includes increases in frequency and intensity of temperature variation. This has led to desertification, land degradation and food insecurity. In other words, climate change is making a challenging situation worse and insidiously undermining food security. It is projected that climate change would adversely impactavailability, access, utilization of food security, its stability, and their interactions. Therefore, climate change and food security is a global concern, whereas sustainability in food production is the need of the hour.

Agricultural productivity is primarily governed by climate change. Given the significance of agriculture in human well-being, there is an ever-growing concern on possible effects of climate change on agriculture. Crop production one of the major components of agricultural system is anticipated to be influenced by climate change. Therefore, there is urgent need to address agricultural adaptation more coherently. The question is how it can be done. A solution that frequently appears in the latest IPCC's Special Report on Climate Change and Land is the 'genetic improvement of crops' that can make crops more resilient to tolerate the increased intensity and frequency of abiotic stresses. Technological solutions like 'new cultivars from breeding or biotechnology' can help increase food productivity and will be an integral and crucial part of climate change adaptation. Genetic engineering of important commercial crops, i.e. introduction of a gene of interest to target crops, can be an important tool to develop climate change ready crops.

Plants have developed a wide range of coping mechanisms in response to adverse environmental conditions such as heat, cold, salinity, and drought. One of the many responses is the synthesis and build-up of organic compounds known as osmolytes or osmoprotectants (Johnson et al. 1968; Yancey et al. 1982; Serraj and Sinclair 2002). These are low-molecular-weight, highly soluble compounds also known as compatible solutes. They are neutral at physiological pH and are non-toxic at increased concentrations. Osmoprotectants have been reported to be ectopically expressed in several crop plants. The most commonly found osmoprotectants in plants are proline, glycine betaine (GB), and mannitol. Researchers worldwide have been greatly interested in improving plant tolerance by expressing biosynthetic enzymes involved in the synthesis of osmolytes, thereby leading to its accumulation. Accumulation of osmoprotectants is believed to impart tolerance to plants, thereby increasing their survivability. Amongst the commonly available osmolytes, GB provides enhanced tolerance to plants against environmental stress (Sakamoto and Murata 2000, 2001, 2002; Saxena et al. 2013) either when applied exogenously or when its biosynthetic pathway is engineered. There is enough evidence suggesting the diverse functions of osmoprotectants in stress tolerance. However, several dimensions related to osmolyte-moderated abiotic stress responses and stress tolerance mechanisms require more research. In this chapter, we review and update on the importance of engineering glycine betaine biosynthesis in alleviating abiotic stress effects in plants.

4.2 Osmoprotectants

Osmoadaptation in plants involves metabolic acclimation via accumulation of low-molecular-weight organic compounds called compatible solutes or osmoprotectants. These compounds build up in the cytoplasm to maintain external osmotic pressure. Osmoprotectants are accumulated either de novo or due to transport from the medium. Cell viability and proliferation due to osmolyte accumulation is possible because electrolyte concentration, cell volume, and turgor pressure are maintained in the cell (Saxena et al. 2013). Since most organisms are not halotolerant, the accumulation of osmoprotectants is widely prevalent. This strategy mainly helps various life forms to modulate their intracellular electrolytic balance according to their surroundings. Apart from osmotic adjustment, these solutes are effective in scavenging harmful reactive oxygen species (ROS). They have also been reported to stabilize complex protein structures and help in metabolic detoxification (Serraj and Sinclair 2002).

Osmolytes, based on their chemical nature, can be grouped into free amino acids (proline), quaternary amines (glycine Betaine and polyamines), sugar alcohols, and sugars (trehalose and mannitol) (Yancey 2005; Roychoudhury et al. 2015).

The membrane integrity of cells and quaternary structure of proteins are stabilized by GB and trehalose. Proline works as an important storage sink for carbon and nitrogen, and inhibits ROS production and accumulation (Kaur and Asthir 2015). Mannitol is an effective free radical scavenger. A vital defence mechanism of plants to cope with the morphological, physiological, metabolic, and molecular changes associated with environmental stress factors that limit plant growth, and thus, agricultural productivity is the production and accumulation of compatible solutes.

4.2.1 Mechanism of Osmoprotectant Action

Stress perception and signalling cascades are activated when plants are subjected to stress conditions. These cascades induce specific stress-responsive genes to help plants adapt to changing environmental conditions (Vinocur and Altman 2005). Osmotic stress inflicted by drought or salinity is perceived by primary sensors like HKs. The stimuli are then transferred to specific transcription factors (MYC/MYB, AREB/ABF, NAC/ZF-HD, DREB, etc.) that moderate transcription of osmolyte biosynthetic genes (Zhou et al. 2016). The MAPK pathway drives and regulates the entire pathway (Dutta et al. 2019).

Osmoprotectant or compatible solutes are generally compartmentalized at various sub-cellular levels. This results in reduction of water potential and thus, enhancement of osmoregulation leading to osmoadaptation. Osmolytes reduce protein misfolding owing to their chaperone-like activity, thereby stabilizing complex protein structures. Compatible solutes lower lipid peroxidation levels owing to their ability to scavenge ROS (Roychoudhury and Chakroborty 2013).

4.2.2 Osmoprotectant Accumulation in Response to Adverse Environmental Conditions

4.2.2.1 Proline

Proline is one of widely distributed osmolytes in plants. It is highly water soluble, helps in osmotic adjustment in the cells, and has a stable conformation (Yancey 2005). Proline biosynthesis in plants occurs via glutamate or ornithine pathway. Ornithine pathway is followed when nitrogen in the cells is available at higher amounts, whereas glutamate pathway is followed in cells which are either nitrogen deficient or osmotically imbalanced (Delauney and Verma 1993). Its levels in plants are strictly regulated by degradation or metabolism of proline. In this mechanism, proline dehydrogenase oxidizes proline to L-pyrolline-5-carboxylate (P5C) and finally P5C to L-glutamate by the action of P5C dehydrogenase (Boggess and Koeppe 1978; Elthon and Stewart 1981). The Proline degradation pathway leads to elevated levels of proline under stress conditions (Delauney and Verma 1993; Peng et al. 1996).

It has diverse functional roles, such as increased cellular proline content helps in stabilizing protein structure. ROS scavenging is an important function of proline. It also acts as a vital storage sink for organic elements and energy during stress recovery (Tyagi and Sairam 2004). It also aids in stabilizing the NADP$^+$/NADPH ratio in the cell (Hare and Cress 1997).

Proline accumulation is found to be important for plant development and survivability under various environmental stresses (Hong et al. 2000; Mattioli et al. 2008; Szekely et al. 2008). Rice, wheat, and carrot cell line over-expressing moth bean P5CS exhibited reduced susceptibility to salinity (Zhu et al. 1998; Sawahel and Hassan 2002; Han and Hwang 2003). Numerous studies showed up-regulation of P5CS in rice and *Arabidopsis* subjected to osmotic stress, salinity, and dehydration (Yoshiba et al. 1995: Igarashi et al. 1997). *Arabidopsis* plants carrying antisense proline dehydrogenase encoded by *AtProDH* showed enhanced tolerance to freezing due to accumulation of proline in the cells (Nanjo et al. 1999).

4.2.2.2 GB and Polyamines

Glycine betaine (GB) is a widely distributed quaternary ammonium compound known for its diverse function in plant cells. It maintains stability of the quaternary structure of enzyme, complex proteins, and membrane integrity under stress conditions (Sakamoto and Murata 2000). The role of GB in ameliorating stress effects in plants is discussed in detail in the following section of the chapter.

All eukaryotic cells consist of small organic compounds known as polyamines. Among the major polyamines, putrescine, spermidine, and spermine are found in plants. These polyamines have crucial role to play in overall cell growth and programmed cell death (Yamaguchi et al. 2007; Alcázar et al. 2010a). Free or conjugated forms of polyamines are most widespread. Their biological activity is attributed to its polycationic nature at physiological pH (Gill and Tuteja 2010).

The accumulation of these polyamines is well studied under adverse environmental conditions (Evans and Malmberg 1989; Alcazer et al. 2006, 2010b). Polyamines are components of the cellular antioxidant system besides being involved in osmotic adjustment of cells. They are also ROS scavengers. Cadaverine prevents DNA oxidative degradation *in vitro* via putrescine and other polyamines. Spermine or spermidine was found to scavenge singlet oxygen at higher concentration (Das and Misra 2004). Transgenic plants expressing polyamine biosynthetic enzymes had better stress tolerance capacity (Gill and Tuteja 2010; Ruben et al. 2010).

4.2.2.3 Sugar and Sugar Alcohols

Mannitol

Mannitol is a six-carbon sugar alcohol, extensively found in nature. Transgenics engineered for mannitol-1-phosphate dehydrogenase in *Nicotiana tobacum* and *Arabidopsis thaliana* resulted in increased salt tolerance. Mannitol biosynthesis targeted in chloroplasts resulted in reduced susceptibility to oxidative stress induced by methyl viologen and improved photosynthetic efficiency (Shen et al. 1997). Transgenic sorghum over-expressing this gene grew well in comparison to control under salinity stress (Maheswari et al. 2010). Tailoring mannitol biosynthesis in potato led to salt tolerance under controlled conditions as well as in hydroponic culture (Rahnama et al. 2011). Eggplants expressing the same gene were found to be less susceptible to both abiotic and biotic stress (Prabhavati et al. 2002).

Trehalose

Trehalose is a non-reducing disaccharide. Its unique bonding properties make it resistant to acid hydrolysis (Richards et al. 2003). The uniqueness in the bonding properties can be attributed to glycosidic bonding between the reducing ends of the molecule. It is also known as a membrane or molecule stabilizer (Crowe 2007). Trehalose biosynthesis is achieved by an OtsA-OtsB pathway in most of the higher plants (Grennan 2007). Metabolism and engineering trehalose biosynthetic pathway for reduced stress susceptibility has been of great interest to researchers. When yeast or bacterial TPS and TPP genes were over-expressed in tobacco and potato plants (Holmström et al. 1996; Romero et al. 1997; Goddijn and Van Dun 1999; Goddijn et al. 1997; Paul et al. 2001), stunted growth of transgenics and abnormal metabolism was observed. Thereafter, transgenic rice was developed using a fusion construct of *OtsA* and *OtsB*CDS with stress-inducible (ABA) and tissue-specific (rice rbcs) promoter. These transgenics were found to be phenotypically normal and fertile and accumulated significant amount of trehalose. Accumulation led to increased tolerance to stresses like salt, drought, and low temperature. Photosynthetic capacity in transgenics was greatly enhanced (Garg et al. 2002). Transgenic *Arabidopsis* over-expressing *AtTps1* under the constitutive promoter resulted in reduced susceptibility to dehydration stress without compromising on plant growth parameters (Avonce et al. 2004). The tolerance extent of these transgenics negatively correlated with the amount of accumulated trehalose, indicating diverse roles of trehalose (Iordachescu and Imai 2008).

4.3 Glycine Betaine

Betaines are zwitterionic compounds consisting of a positively charged group such as a quaternary ammonium in place of a hydrogen atom. These are amino acid derivatives wherein the nitrogen atom is fully methylated. GB, stachydrine, βeta-Ala betaine, choline-*O*-sulphate, and DMSP are the well-known plant betaines (Rhodes and Hanson 1993). DMSP and choline-*O*-sulfate cannot be termed as betaines, since there is a tertiary sulfonium instead of quarternary ammonium in case of DMSP and sulphate ester group instead of carboxyl group in case of choline-*O*-sulfate. However, they share similar chemical and physiological properties owing to close structural analogy to betaines. Betaines start accumulating when subjected to salinity, drought, and cold, because the biosynthetic enzymes of these solutes are maximally expressed under stress. They have been found to be largely confined in the cell cytoplasm (Matoh et al. 1987). Several investigations into the physiology, genetics, biophysics, and biochemistry of plants strongly suggest the importance of GB, amongst the other betaines in plants under various environmental factors, such as high salinity and low temperature (Sakamoto and Murata 2002).

Glycine betaine (N,N,N-trimethylamine) is a dipolar, quaternary ammonium compound, electrically neutral at physiological pH. It is found to accumulate majorly in the chloroplasts and plastids. GB is of utmost importance as it is involved in stabilization of quaternary structures of enzymes and complex proteins, maintenance of membrane integrity at non-physiological conditions. The protective properties of GB were first understood in a study where it was incorporated in bacterial growth media (Le Rudulier et al. 1984). In 0.6 M NaCl media, the bacterial growth was slow until supplied with GB which they later took up from media and accumulated it to intracellular levels of greater than 1 M. Diverse plant species synthesize GB, along with halo-tolerant cyanobacteria (Jones and Storey, 1981). However, commercially important crop plants like rice, potato, and tomato are known as non-accumulators because of their inability to synthesize GB.

GB is one of the most comprehensively studied compatible solutes owing to its versatility. Studies show that both the exogenous application and engineering of

GB-biosynthetic pathway into non-accumulators such as rice, soybean and potatoes increase the tolerance of such plants to various types of abiotic stress (Chen and Murata 2011). GB has also been reported to stabilize photosystemII-protein complex under high salt concentration (Murata et al. 1992, Papageorgiou and Murata 1995) Extent of GB accumulation in response to abiotic stress is generally correlated with the extent of stress tolerance (Rhodes and Hansen 1993).

4.3.1 Biosynthesis of GB

The biosynthetic pathway for GB in plants differs from that of bacteria and animals. GB biosynthetic pathway in plants comprises of two steps. Choline is first converted into betaine aldehyde (Cromwell and Renni 1953) catalyzed by a Rieske type iron-sulphur [2Fe-2S] containing enzyme CMO. Conversion to GB is catalyzed by BADH, a NAD$^+$-dependent dehydrogenase having functional similarity to aminoaldehyde dehydrogenase (Woods et al. 1996, Fitzgerald et al. 2009). The first step is the rate-limiting step of GB biosynthetic pathway in higher plants. The biosynthetic pathway is elucidated in Fig. 4.1.

CMO and BADH are found to localize in the chloroplasts. Compartmentalized cellular localization of GB has not yet been widely studied. In spinach plants, approximately half of the total amount of GB in leaves is found to be localized in the chloroplasts (Robinson and Jones 1986). These observations were made close to three decades ago; therefore, new and relevant methods should be employed for studying the compartmentalization of GB in plant cells.

4.3.1.1 Comparative Analysis of the GB Biosynthetic Pathway

In most of the biological systems, choline is utilized for biosynthesis of GB. However, in cyanobacteria such as *Actinopolyspora halophila* and *Ectothiorhodospira halochloris*, glycine is used as a precursor molecule for GB biosynthesis (Nyyssölä et al. 2000). These are extremely halophilic microorganisms. In fact, accumulation of GB in *A. halophila* is one-third of total cell dry weight and therefore is highly efficient GB-accumulating bacteria. (Nyyssöläet al. 2000). In this pathway, glycine is methylated thrice sequentially to yield GB. The intermediate molecules formed are sarcosine and dimethyglycine (Fig. 4.2). The first step is catalyzed by glycinesarcosine

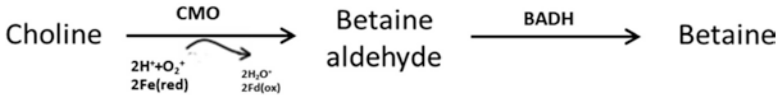

Fig. 4.1 A two-step oxidation reaction for biosynthesis of GB in plants: Oxidation of choline to betaine aldehyde catalyzed by choline mono-oxygenase which is then converted to yield GB, catalyzed by BADH

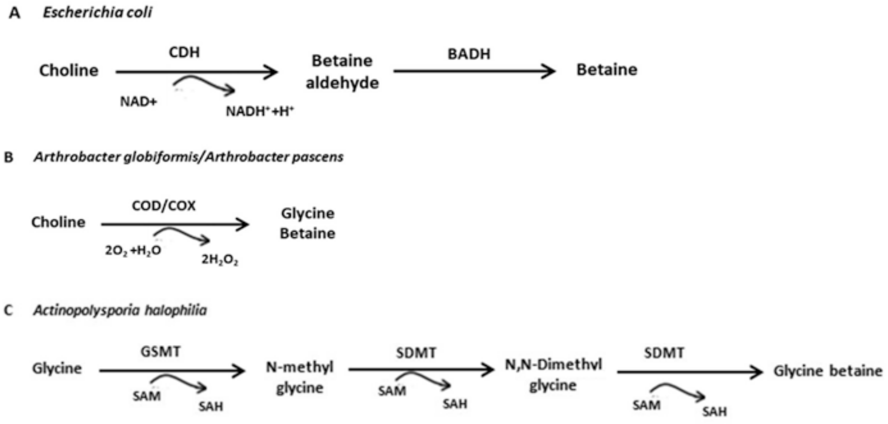

Fig. 4.2 (**a**) GB biosynthetic pathway in *E.coli*steered by CDH and BADH enzymes. (**b**) One-step GB biosynthetic pathway in *Arthrobacter globiformis* and *Arthrobacter pascens* driven by COD/COX (**c**) Three-step methylation pathway in *Actinopolyspora halophilia* and *Ectothiorhodo spirahalochloris*

methyltransferase (GSMT), the second by both GSMT and sarcosine dimethylglycine methyltransferase (SDMT), and third by SDMT alone. Transformation of these genes in *Arabidopsis* yielded more GB as compared to transgenics expressing CMO (Waditee et al. 2005).

4.3.2 Glycine Betaine: Targets for Metabolic Engineering Toward Enhancing Stress Tolerance

GB has long been a target for imparting stress tolerance in plants owing to its ability to accumulate under unfavourable conditions (Le Rudulier et al. 1984). The rationale behind introducing the GB pathway into non-accumulators was based both on comparative physiology (Yancey 1994) and on mutation studies in maize (Saneoka et al. 1995; Yang et al. 1996). Further, physiological-genetic evidence of salt tolerance conferred by GB was provided by work on wide-crossing on *Lophophytum elongatum* and wheat (Colmer et al. 1995). Since GB does not get catabolized in plants (Rhodes and Hanson 1993; Nuccio et al. 1998), it has been rationalized that tailoring the GB biosynthesis will result in its accumulation.

Several groups from thereon have made steady and significant progress in this direction. Many expressed bacterial GB biosynthetic enzymes (Lilius et al. 1996; Hayashi et al. 1997; Alia et al. 1998; Sakamoto et al. 1998) or spinach CMO (Nuccio et al. 1998) in tobacco and other non-accumulators.

Engineering the GB biosynthetic pathway into non-accumulators or exogenously applying it reduces the susceptibility of such plants to various forms of abiotic stress.

4.3.2.1 Exogenous Application of GB

Plant susceptibility to cold, drought, and salinity can be significantly reduced by exogenously applying GB to plants. It can also improve water use efficiency and can increase subsequent growth and yield of plants. GB can be taken up both by leaves and roots of plants.

A study on tomato shows that upon foliar administration, GB was promptly translocated in higher amounts to younger tissues besides other organs. Most of the localization of GB took place in the cytosol followed by chloroplast. Upon exposure to cold stress, catalase activity and expression of *CAT1* gene was significantly enhanced in plants supplied with GB (Park et al. 2006). Exogenous application of 50 mM GB resulted in decrease in proline, MDA, and Na^+ content in soybean plants, thereby improving growth and survivability under salt stress (Malekzadeh, 2015). A similar study in rice showed 5 and 10 mM GB to effectively ameliorate damages caused by high salinity, resulting in improved plant morphological traits and total chlorophyll in both varieties tested, namely, MR220 and MR253 (Yao et al. 2016).

Another study showed that exogenous foliar application of 100 mM GB at the booting stage improved growth, yield attributes, and net photosynthetic rate in two indica rice varieties, namely, RD43 and SPR1 under drought conditions (Tisarum et al. 2019). Similarly, exogenous application of GB improved water use efficiency in *Triticum aestivum* L. due to enhanced photosynthetic efficiency and anti-oxidative capacity (Ahmed et al. 2019). Table 4.1 summarizes the data.

Table 4.1 Exogenous application of GB to enhance stress tolerance in plants

Plant species	Tolerance developed against	References
Oats *(Avena sativa)*	Drought, salt stress	Makela et al. (1996)
Arabidopsis thaliana	Salt, freezing, oxidative, chilling	Hayashi et al. (1998), Xing and Rajashekar (2001), Cuin and Shabala (2007) and Einset et al. (2007)
Mustard *(Brassica rapa)*	Drought, salt	
Fragaria x ananassa	Freezing	Rajashekhar et al. (1999)
Barley *(Hordeum vulgare)*	Drought, salt	
Tomato *(Lycopersicon esculentum)*	Chilling, drought, salt	Park et al. (2006)
Alfalfa *(Medicago sativa)*	Freezing	Zhao et al. (1992)
Rice *(Oryza sativa)*	Salt	Harinasult et al. (1996)
French bean *(Phaseolus vulgaris)*	Drought	Xing and Rajeshekar (1999)
Wheat *(Triticum aestivum)*	Freezing, drought	Allard et al. (1998) and Ahmed et al. (2019)
Maize *(Zea mays)*	Chilling	Alia et al. (1999)

4.3.2.2 Spatial and Temporal Distribution of GB in Plants Under Abiotic Stress

Exogenous application of GB to the older damaged tissues results in its quick re-translocation to younger meristematic tissues, as they require protective functions of GB the most. This leads to a spatial discrepancy. In addition to this, temporal delay of GB biosynthesis could be caused possibly because GB cannot be catabolized. GB synthesis and accumulation occurs during prolonged stress exposure, particularly in younger tissues, and at low N nutrition, thereby suggesting a pivotal role of GB in protecting young and growing tissues. Indeed, osmotic adjustment, and ionic and oxidative stress defence by GB is more in young leaves than in older leaves (Annunziata et al. 2017).

4.3.2.3 GB Biosynthetic Genes Tailored for Improved Plant Stress Tolerance

Numerous transgenics have been raised by cloning GB biosynthetic genes with an overall aim to enhance plant tolerance. These include genes for *CMO* and BADH from higher plants (Rathinasabapathi et al. 1997); *CDH* and *BADH* from *Escherichia coli* (Landfald and Strom 1986); COD (i.e. *coda* from *Arthrobacter globiformis* (Patcharaporn Deshnium et al. 1995) and *COX* from *Arthrobacter pascens* (Rozwadowski et al. 1991)); and GSMT and SDMT from both *Actinopolyspora halophila* and *Ectothiorhodospira halochloris* (Nyssola et al. 2000). Expression of these genes individually in plants accumulates GB to various extents and thus exhibits tolerance to several types of stress. Table 4.2 summarizes the data.

4.3.3 Transgenic Plants Engineered to Synthesize GB for Enhanced Tolerance to Stress

4.3.3.1 Rice *(Oryza sativa)*

Cereals crops like barley, wheat, and maize do not accumulate significant amount of GB naturally which could be due to the production of truncated transcripts for GB synthesizing enzyme BADH**.** Even though, rice has two BADH- and one CMO-encoding genes, it is the only cereal that does not accumulate GB naturally under stress. This is because BADH transcripts are processed in an unusual manner in rice resulting in removal of translational initiation codon, loss of functional domains, and premature stop codons (Niu et al. 2007).

The ability to synthesize GB in rice was established by introducing the *coda* gene for choline oxidase from the soil bacterium *Arthrobacter globiformis*. This was the first report of the successful establishment of transgenic plants of

Table 4.2 Transgenic plants tailored to express GB biosynthetic genes

Plant species	Gene	Sub-cellular location	Tolerance against	References
Arabidopsis thaliana	codA	Chloroplasts	Chilling, salt, heat, strong light, freezing	Alia et al. (1998), Hayashi et al. (1997) and Sakamoto and Murata (2000)
	Cox	Cytosol	Salt, drought, freezing	Huang et al. (2000)
	CMO + BADH	Unknown	Salt	Hibino et al. (2002)
	ApGSMT+ApDMT	Cytosol	Salt, chilling	Waditee et al. (2005)
Brassica napus	Cox	Cytosol	Drought, salt	Huang et al. (2000)
Lycopersicon esculentum	codA	Choroplasts, Cytosol+chloroplasts	Chilling Oxidative, salt	Park et al. (2004) Park et al. (2007)
Nicotiana tobacum	Cox betA BADH CodA	Cytosol -do- Chloroplasts Cytosol	Salt Salt, chilling Heat Salt, chilling	Huang et al. (2000) Holmstrom et al. (2000) Yang et al. (2005, 2007) Sakamoto et al. (1998)
Oryza sativa	codA betA CMO BADH	Cytosol, chloroplasts Chloroplast Mitochondria Chloroplasts Peroxisomes	Salt, chilling Salt Salt, drought Salt Salt, cold, and heat	Sakamoto et al. (1998) Mohanty et al. (2002) Takabe et al. (1998) Shirasawa et al. (2006)
Solanum Tuberosum	codA BADH	Chloroplasts - Chloroplasts	Oxidative, salt and drought Salt and drought Salt, oxidative and cold	Ahmad et al. (2008) Zhang et al. (2010) Fan et al. (2012)
Zea mays	betA BADH	Cytosol	Chilling, drought Salt	Quan et al. (2004) Wu et al. (2007)
Gossypium hirsutum	betA	Cytosol	Drought	LV et al. (2007)

(continued)

Table 4.2 (continued)

Plant species	Gene	Sub-cellular location	Tolerance against	References
Diospyras kaki	codA	Chloroplasts	Salt	Gao et al. (2000)
Triticum aestivum	betA BADH	- - Cytosol	Salt Cold Salt	He et al. (2010); Zhang et al. (2010)

agricultural importance with the acquired ability to synthesize GB. Levels of GB were as high as 1 and 5μmol per gram of fresh weight of leaves in two types of transgenic plants, one in which choline oxidase was targeted to the chloroplasts (ChlCOD plants) and the other in which the protein was targeted to cytosol (CytCOD plants). The transgenic plants picked up growth at normal rate more rapidly as compared to wild-type plants after elimination of salt stress. When the photosynthetic activities of the two transgenics were compared under high salinity, choline oxidase targeted to the chloroplasts provided a higher degree of protection than that targeted to the cytosol. The tolerance of the photosynthetic machinery of ChlCOD rice plants to low-temperature and photo-inhibition was enhanced to a greater extent than that of CytCOD plants, suggesting that sub-cellular compartmentalization of the biosynthesis of GB was a critical element in the efficient enhancement of tolerance to stress in the engineered plants (Sakamoto et al. 1998).

In a similar study, transgenic rice with chloroplast-targeted choline oxidase maintained higher activity of photosystem II and showed better physiological performance such as detoxification of ROS under water stress. Transgenics fared better in terms of survival rate and agronomic performance under prolonged water stress. Microarray-based transcriptomic analysis of the transgenic rice unravelled altered expression of many genes, most of which are known to be involved in stress response (Kathuria et al. 2009).Apart from *coda* gene, a gene encoding choline monooxygenase from spinach was introduced in rice by *Agrobacterium*-mediated transformation. Accumulation of GB was higher in transgenics carrying a single copy of the transgene and expressing spinach CMO. These plants also exhibited enhanced tolerance to salt stress and temperature stress in the seedling stage (Shirasawa et al. 2006). In another study, transgenic rice was developed by introducing cDNA for BADH of barley, localized in peroxisomes. Herein, transgenics efficiently converted high levels of exogenously applied betaine aldehyde to GB. The elevated level of GB in transgenic plants conferred salt, cold, and heat stress tolerance (Kishitani et al. 1994).

4.3.3.2 *Arabidopsis thaliana*

Several genes that catalyze the biosynthesis of GB have been cloned in *Arabidopsis*, since it is a natural non-accumulator of GB. Amongst the different GB biosynthetic genes, *coda* gene encoding choline oxidase from *A. globiformis* has been widely used for GB production in transgenic *Arabidopsis*. Transgenic *Arabidopsis* engineered to express *codA* gene from *A.globiformis* showed enhanced tolerance to cold and salt stress. The concentration of betaine in the chloroplasts of the transformed plants was estimated to be 50 mM. The accumulation of GB rendered *Arabidopsis* tolerant to salt stress. Transformed plants exhibited better growth characteristics when exposed to increased concentration of NaCl. In fact, when treated with 400 mM NaCl for 4 days, PSII system in transformed plants remained relatively high as compared to wild-type plants, suggesting that GB synthesized in vivo conferred strong protective effect against salt stress. When exposed to low temperature, leaves of wild-type plants showed symptoms of chlorosis, whereas transformed plants did not (Hayashi et al. 1997). In another study, *Arabidopsis* plants transformed with the same gene showed significantly enhanced tolerance towards light stress caused by acceleration of the recovery of the PSII complex from the photoinactivated state. Transformed plants synthesized GB but changes in relative levels of fatty acids or membrane lipids were not observed. However, there was increase in low levels of hydrogen peroxide by 50–100% in stressed and non-stressed plants. The study concluded that in vivo production of GB protected the PS II complex in transformed plants from damage due to light stress. Further studies with the same transgenic lines showed improved tolerance to heat stress, both at the germination and young seedling stage. The extent of enhancement of the tolerance to high temperature was correlated with levels of choline oxidase expressed and of GB accumulated in the transformed plants. Homologues of heat shock protein 70 at high temperature were less induced in the transformed plants as compared to wild-type plants, suggesting that the transformation conferred heat tolerance to transgenics (Alia et al. 1999).

Arabidopsis contains two genes, *ALDH108* and *ALDH109*, that code for betaine dehydrogenase, known to catalyze the last step of the GB biosynthetic pathway. Upon studying the functional properties of *Arabidopsis* BADH genes, it was found that *ALDH108* and *ALDH109* are targeted to leucoplasts and peroxisomes, respectively. Both the genes were weakly induced by cold, salt, ABA, dehydration, and methyl viologen. T-DNA insertion mutation showed ALDH108seedlings to be sensitive to dehydration and salt stress as compared to wild-type plants. It was hypothesized that either of the genes may serve as detoxifying enzymes controlling the level of aminoaldehydes which are produced under stress conditions (Missihoun 2015).

In another study, betaine-biosynthesizing enzymes, *Mpgsmt* and *Mpsdmt*, encoding glycine sarcosine *N*-methyltransferase (GSMT) and sarcosine dimethylglycine *N*-methyltransferase were introduced into *Arabidopsis*. Increased accumulation of betaine and slower water loss was observed in the homozygous lines and their cross as compared to the wild-type plants. Transgenics were able to cope with dehydration and salinity stress better than the wild-type plants. The homozygous lines and their cross also maintained higher relative water content and photosystem II activity under salt stress. This study showed that introduction of stress-activated enzymes can confer tolerance in stressful environments (Lai et al. 2014). In a recent study, novel member of the ALDH10 gene family, SpBADH isolated from *Sesuvium* *portulacastrum*, was introduced into *Arabidopsis* plants. Over-expression of the gene in Arabidopsis resulted in higher BADH activity and GB content and increased tolerance to drought/osmotic stresses, specifically strong tolerance to drought stress. Transgenic lines exhibited lower MDA, and H_2O_2 contents but higher proline, POD, SOD, and CAT contents than the wild type under stressed condition. ROS-related genes including *AtSOD*, *AtPOD*, *AtCAT*, *AtAPX*, and *Atpsb* were expressed under drought and osmotic stresses. Thus, *SpBADH* increased plant tolerance to drought or osmotic stresses by reducing H_2O_2, increasing proline, and activating antioxidative enzymes to improve ROS scavenging (Yang et al. 2005).

4.3.3.3 Tobacco (*Nicotiana tabacum)*

Transgenic tobacco expressing bacterial *betA* gene encoding choline dehydrogenase (CDH) showed both salt and choline resistant phenotypes. Although choline is ubiquitous in higher plants, tobacco growth was severely retarded at external concentrations greater than 10 mM. The toxic influence of 30 mM choline reduced the yield of wild-type tobacco to less than 10% as compared to non-treated plants. However, an improvement of three-four fold change was observed in transgenic lines. Enhanced resistance to choline could be correlated to higher CDH activity and, thus, greater tolerance towards choline: 80% increase in salt tolerance, measured in terms of dried weights, was observed between the transgenic and wild-type plants at a salt concentration of 300 mM (Lilius et al. 1996).

Transformation of tobacco with the *E.coli* genes, *betA*- and *betB*-encoding betaine aldehyde dehydrogenase (BADH), for GB biosynthesis resulted in transgenics accumulating GB and exhibiting enhanced tolerance to salinity and cold stress. 1HNMR analysis revealed that plants producing both CDH and BADH accumulated higher levels of GB than plants producing CDH alone. Experiments conducted with leaf discs from GB-accumulating plants indicated enhanced recovery from photo-inhibition caused by high light, salinity as well as cold stress. Introduction of GB production in tobacco is associated with stress tolerance owing to partial improved protection of the photosynthetic apparatus (Holmstrom et al. 2000).

In another study, tobacco plants transformed with BvCMO gene from beet encoding for choline monooxygenase (BvCMO) via plastid genetic engineering were able to accumulate GB in leaves, roots, and seeds, and showed improved tolerance to toxic level of choline, salt, and drought stress as compared to wild-type plants. Transplastomic plants demonstrated higher net photosynthetic rate and apparent quantum yield of photosynthesis in the presence of 150 mM NaCl. No significant change was observed in the maximal efficiency of PSII photochemistry in wild-type and transplastomic plants. However, the actual efficiency of PSII (UPSII) dropped more in wild-type plants than transgenics. The results demonstrated the feasibility of plastid transformation in improving salt and drought tolerance in plants (Zhang et al. 2010). In a similar study, *OsBADH1* gene expression in transgenic tobacco was studied in response to salt stress. Real Time-PCR results and western blot analysis indicated that the expression of *OsBADH1* gene in transgenic tobacco lead to accumulation of GB. Transgenic lines exhibited normal seed germination, morphology, and growth rate under salt stress conditions, thereby suggesting that *OsBADH1* gene could be an excellent candidate for producing plants with osmotic stress tolerance (Hasthanasombut, 2009). Transgenic tobacco expressing *CMO* gene from rice encoding for choline mono-oxygenase resulted in elevated levels of GB and enhanced tolerance to salt stress. Immuno-blotting results demonstrated that a functional OsCMO protein was present in transgenic tobacco but rarely accumulated in wild-type rice plants, suggesting that it is the lack of a functional OsCMO protein that probably results in non-accumulation of GB in the tested rice plant (Luo et al. 2012).

4.3.3.4 Potato (*Solanum tuberosum)*

Transgenic potato cultivar bearing *codA* gene was raised under moderate-to-severe drought stress to study the effects of the codA transgene for choline oxidase on the drought resistance and recovery. The plants were pre-treated with 20% PEG followed by four-day-water stress combined with two-day-recovery treatment. Pretreatment resulted in accumulation of glycine betaine (GB): higher leaf water potential. During stress-recovery-treatment, stronger antioxidant ability, efficient photosynthetic system, higher chlorophyll content, lower malondialdehyde content, and better recovery from water deficit stress was shown by transgenics as compared to control plants, suggesting that the exogenous *codA* gene provided potato a stronger drought resistance and recovery ability (Cheng et al. 2013).

Transgenic potato expressing *BADH* gene from spinach under the control of *rd29A* from *Arabidopsis* exhibited significant tolerance to salt and drought stress. Northern blot analysis revealed the induction of the BADH gene by drought and NaCl stress. The BADH activity was found to be higher in the transgenic potato plants. The transgenic plants showed better growth characteristics such as increased plant height, fresh weight under stressed conditions as compared to wild-type plants, indicating that the ability to tolerate stress increased with increase in their BADH activity (Zhang et al. 2010). To evaluate the performance of transgenic potato plants expressing betaine-synthesizing genes against salt and cold stresses, transgenic potato plants were exposed to salt and cold stresses. Upon salt stress, transgenic plants maintained better growth, tuber yield as compared to control plants. Transgenic plants showed improved membrane integrity and less chlorophyll bleaching against cold stress. In addition, control plants accumulated significantly less biomass, and yielded fewer tubers as compared to transgenic plants after cold stress treatment (Ahmad et al. 2014).

In another study, it was reported that the *codA* gene from *Arthrobacter globiformis*, which encoded choline oxidase to catalyze the conversion of choline to GB, was successfully introduced into potato (*Solanum tuberosum*) plastid genome by plastid genetic engineering. Southern blot analysis confirmed that the mRNA levels of *codA* were much higher in leaves than in tubers. Both leaves and tubers of *codA*-transplastomic potato plants accumulated similar levels of GB. The GB content was moderately increased in transplastomic plants, and compartmentation of GB in plastids conferred considerably higher tolerance to drought stress compared to wild-type (WT) plants. Higher levels of relative water content and chlorophyll content under drought stress were detected in the leaves of transgenic plants compared to WT plants. Significantly higher photosynthetic performance as well as anti-oxidant enzyme activities during drought stress was observed in the transplastomics, suggesting that chloroplast engineering of GB biosynthesis was an effective method to increase drought tolerance (Cheng et al. 2013). In another study, a chloroplastic BADH gene from *Spinacia oleracea* (*SoBADH*) was transformed into the sweet potato cultivar. The over-expression of *SoBADH transgene* improved tolerance to salt, oxidative stress, and low temperature. Protection against cell damage through the maintenance of cell membrane integrity, stronger photosynthetic activity, reduced reactive oxygen species (ROS) production, and induction or activation of ROS scavenging by the increased activity of free radical-scavenging enzymes was conferred by the increased BADH activity and GB accumulation (Fan et al. 2012).

4.3.3.5 Wheat *(Triticum aestivum)*

Transgenic wheat expressing *betA* gene encoding choline dehydrogenase from *E. coli* accumulated higher levels of GB and chlorophyll. Transgenic lines had lower Na^+/K^+ ratios, solute potential, and less cellular membrane damage. At a concentration of 200 mM NaCl, these lines also retained moderately high proportions of photosynthetic rates and better growth compared to wild-type plants. When grown in a high salt field, transgenic lines had better germination rates, more tillers, and higher grain yields, suggesting that the transgenic plants were more tolerant to salt stress and have potential for breeding salt-tolerant wheat. Transgenic lines upon exposure to drought stress exhibited greater root length, improved growth, and less injury as compared to wild type. It was concluded that amount of injury to wheat plants was negatively correlated to levels of GB accumulated in the plants (He et al. 2010).

Wild-type winter wheat (*Triticum aestivum*) and 3 transgenic lines expressing *BADH* gene from *Artiplexhortensis* L. were used to study the protection of wheat plasma membrane under cold stress. Under cold stress (0–2 °C), the transgenic lines with higher GB content maintained better membrane integrity and higher H^+/ATPase activity, ROS production, and membrane lipid peroxidation levels were lower, while anti-oxidative enzyme activities were found to be higher than the wild-type plants (Zhang et al. 2010).

In another study using the same transgenic lines and wild-type plants, seedlings exposed to conditions of drought (30%, PEG-6000), heat (40 °C), and their combination showed ultra-structural damage to the chloroplast and thylakoid lamellae and withered phenotype by both drought and heat stress, and the damage being worsened by the combination of drought and heat. Greater damage was found in wild type than transgenic line. Over-accumulation of GB in the transgenic line could protect lipids in the thylakoid membrane from damage and stabilize the index of unsaturated fatty acids under stress. GB over-accumulation decreased photoinhibition of PSII under stress (Wang et al. 2010).

In another study, *HvBADH1* gene from *Hordeum vulgare* encoding a cytosolic BADH was transformed into wheat plants. Physiological indicators that reflected abiotic stress tolerance were measured in individual transgenic plant lines to detect the efficacy of the *HvBADH1* transgene in the transformants. These indicators included intracellular K^+ and Na^+ contents or K^+/Na^+ ratio, relative conductivity, and MDA, and glycine betaine (GB) concentrations in cells. All the tested transgenic lines showed significant increase in the concentration of K^+ in their cytosol than the wild-type seedlings. Similarly, greater accumulation of GB, higher calli relative growth rates, and moderately high seedling survival rates were found in transgenic lines under 150 mM NaCl stressed conditions. The study demonstrated that the overall salt tolerance could be increased by over-expressing the *HvBADH1* transgene in genetically transformed wheat (Wang et al. 2010).

4.3.3.6 Maize (*Zea mays*)

BADH-encoding gene from *Atriplex micrantha* was introduced into maize inbred lines under the control of maize ubiquitin promoter. Transgenic maize plants expressed higher amount of betaine aldehyde dehydrogenase and exhibited better growth characteristics under salt stress than the wild-type plants (Di et al. 2015). Similarly, transgenic inbred maize lines DH4866 over-expressing choline dehydrogenase showed improved tolerance to drought than wild-type plants, both at the germination and young seedling stage (Quan et al. 2004).

In another study, *BADH* gene from *Suaeda liaotungensis* kitag was transformed into maize by pollen tube pathway, using the principle of minimum linear length of the transformation element. Transgenic lines were subjected to salinity stress for 15 days. Most of the transgenic seedlings survived and grew well, whereas most wild-type seedlings wilted and showed loss of chlorophyll. The transgenic progeny had higher GB and chlorophyll content than wild type after salt stress (Wu et al. 2007).

4.3.3.7 Tomato (*Lycopersicon esculentum)*

Transgenic tomato plants transformed with bacterial *codA* gene for choline oxidase resulted in the accumulation of GB and enhanced chilling tolerance during their entire life cycle, from seed germination to reproductive stage. Targeting of choline oxidase in the chloroplast, however, elicited only a very low level of GB accumulation in transgenic leaves (Park et al. 2004). Therefore, in the next study, transgenic tomato plants were generated with choline oxidase targeted to cytosol (Cyt-CodA), chloroplast (Chl-CodA), or both chloroplast and cytosol (Chl-Cyt-CodA) simultaneously. The Cyt-CodA and ChlCyt-CodA accumulated 5–6 folds higher GB over that measured from the chloroplast-targeted lines. Chloroplastic GB was found to be more effective in providing tolerance against chilling, salinity, and oxidative stress (Park et al. 2007).

In another study, genetically engineered tomato hairy root lines were established with the ability to synthesize GB. This was done by the introduction of BADH-1 gene from sorghum plants. H-NMR analysis was carried out to detect the accumulation of GB. The accumulation of GB in the transgenic roots led to the maintenance of the osmotic potential of the cells under salt stress (Moghaieb et al. 2000). A recent study has shown that the accumulation of GB in vivo in codA transgenic tomato plants is related to maintaining Na^+/K^+ homeostasis in salt stress by the regulation of Na^+ and K^+ channels and transporters. GB also enhances the activities of anti-oxidant enzyme which scavenges ROS with resultant alleviation of the effect of salt stress on the PSII machinery (Wei et al. 2017).

4.3.4 Mechanisms of Protection Against the Damaging Effects of Stress

4.3.4.1 Protective Effect of GB in Reproductive Organs of Plants Under Abiotic Stress

Reproductive stage is the most sensitive and crucial stage in the life cycle of a plant, especially when subjected to abiotic stress. Plant yield is severely compromised under harsh environmental conditions due to limited growth of reproductive organs. Protection of plants against abiotic stress at the reproductive stage is therefore most essential for improved growth and high yield. Various studies indicate the possible role of GB in the protection of reproductive organs.

Analysis of transgenic *Arabidopsis* engineered to synthesize choline oxidase revealed differential levels of expression of GB in various organs despite constitutive expression of *codA* gene. In mature plants, five-fold higher expression of GB was measured in flowers, siliques, and inflorescence apices as compared to leaves, which could have contributed to the increased number of flowers, siliques, and seeds when the plants were grown under stress conditions:100 mM NaCl treatment to wild-type plants for 3 days resulted in the abortion of flower buds and decreased numbers of seeds per silique. However, in transgenic plants, GB accumulation led to reduced effects of salt stress. Moreover, transgenic plants produced 21% more siliques and 45% more seeds than WT plants after treatment with 100 Mm NaCl for 3 days. Thus, the accumulation of GB in the reproductive organs effectively protected the formation of flowers and seeds against salt stress (Park et al. 2003).

Transgenic maize transformed with *beta* gene, upon exposure to drought stress, accumulated large amounts of GB in the leaves as compared to leaves of wild-type plants (Quan et al. 2004). There was reduction in number of pollen grains and delayed formation of ears in both types of plants on prolonged exposure to drought stress. However, reproductive development, as measured by the number of pollen grains and the silking time of transgenics, was much less affected by drought than wild-type plants. The weight of grains per plant in transgenic maize was 10–23% higher than that of wild-type plants due to an increase in both the number and weight of grains.

Tomato plants are most susceptible to chilling stress at the reproductive stage. Transgenics harbouring the *codA* gene from *A. Globiformis* exhibited a significant increase in flower retention and fruit set after exposure to chilling stress. GB-accumulating transgenic tomato produced an average of 10–30% more fruits on exposure to chilling stress as compared to wild-type plants. Thus, from all the studies done so far, it can be concluded that reproductive organs accumulate significantly higher amounts of GB among the various organs of the transgenic tomato and *Arabidopsis*. Tolerance to stress in these plants can be attributed to the protective effects of GB.

4.3.4.2 Protection of the Photosynthetic Machinery and Detoxification of ROS During Abiotic Stress

In lines with a scheme proposed by Asada, regarding the interrelation among the electron transport, fixation of CO_2 in the Calvin cycle, and the inhibition of the synthesis of D1 protein, a key protein of the PSII component, interruption of the photosynthetic fixation of CO_2 accelerates the production of H_2O_2, which, in turn, inhibits protein synthesis and, thus, the repair of PSII.

At high temperatures, activation of Rubisco is hampered due to the association of stromal Rubisco activase to the thylakoid membranes (Yang et al. 2005). GB apparently supports the activation of Rubisco by preventing the sequestration of Rubisco activase from stromal fractions and, in this way, it enhances the tolerance of CO_2 assimilation to high-temperature stress.GB can render enhanced thermotolerance by suppressing ROS accumulation and, also, via the enhanced repair of PSII, inactivated by light stress.

A number of transgenic plants that have been engineered to accumulate GB in vivo exhibit enhanced PSII activity under salt stress. These include transgenic *Arabidopsis* lines that express a bacterial *coda* gene (Hayashi et al. 1998), the *CMO* gene from spinach (Hibino et al. 2002) and the *ApGSMT* and *ApSDMT* genes from *A. Halophytica* (Waditee et al. 2005); rice plants that express the bacterial *coda* gene (Sakamoto et al. 1998); and tobacco plants that express the bacterial *beta* gene from *Escherichia coli* (Holmstrom et al. 2000) or the *BADH* gene from spinach (Yang et al. 2007). In a study performed by Yang et al. (2007) to examine the effects of salt stress on the growth of seedlings and on photosynthetic activity in WT and transgenic tobacco plants, it was found that presence of the *BADH* transgene resulted in better growth of seedlings under salt stress. However, salinity stress–induced suppression of CO_2 accumulation was less significant in transgenic than in WT plants. Salt stress also decreased the maximal rate of electron transport in PSII and increased the extent of non-photochemical quenching, with less significant changes in transgenic than in WT plants. Salt stress inhibited the activity of Rubisco, fructose 1,6-biphosphatase (FBPase), fructose 1,6-biphosphate aldolase (FBP aldolase), and phosphoribulokinase (PRKase) of chloroplasts, and the extent of inhibition was also less significant in transgenic than in WT plants. Salt stress did not, however, affect the activities of phosphoglycerate kinase, triose phosphate isomerase, ribulose-5-phosphate isomerase, transketolase, and sedoheptulose-1,7-biphosphatase in either WT or transgenic plants. These results suggest that the GB-enhanced tolerance of the assimilation of CO_2 to salt stress might be one of the physiological bases for the increased tolerance of the growth of transgenic plants to salt stress. Yang et al. (2007) proposed that the accumulation of GB in transgenic plants could result in stable conformation of Rubisco, FBPase, FBP aldolase, and PRKase and this in turn might maintain these enzymes in a functionally active state under salt stress, thereby acting as a molecular chaperone.

ROS is effectively generated by methyl viologen (MV). Park et al. (2004, 2007) demonstrated that GB-accumulating transgenic tomato plants were more tolerant to MV-induced oxidative stress than WT plants. In the presence of MV in moderate light (100 mmol photons m^{-2} s^{-1}), PSII activity fell significantly in both WT and transgenic plants, although the inactivation of PSII was much slower in transgenic than in WT plants. A positive correlation was found to exist between the level of GB in the chloroplasts and the tolerance limit to oxidative stress–enhanced photo-inhibition.

4.3.5 GB-Induced Expression of Specific Genes

Mechanisms for GB-mediated abiotic stress tolerance include stabilization of native structure of proteins and enzymes, osmoregulation, membrane integrity, and protection of photosynthetic machinery against the synergistic effects of light stress and other abiotic stress. The stress tolerance observed in transgenic plants cannot be completely explained by these mechanisms alone, given the low levels of accumulation of GB in transgenic plants. Therefore, it is reasonable to hypothesize that the effects of GB might be manifested through the induction of expression of specific stress-responsive genes.

Transcriptomic analysis of transgenic rice encoding *codA* gene from *A. globiformis* showed altered expression of several transcripts under non-stressed conditions: 50 genes known to be involved in one or more biotic and abiotic stress were up-regulated, together with other genes involved in cellular processes like transcription, signalling, membrane transport, metabolism, and growth. Similarly, DNA microarray analysis of *Arabidopsis* plants treated with 100 mM GB showed up-regulation of genes encoding for transcription factors, membrane trafficking components, ROS scavenging enzymes, and NADP-dependent ferric reductase located in the plasma membrane. Expression of four genes was significantly enhanced in *Arabidopsis* roots during treatment with GB for 24 h as revealed by Northern blot analysis. The study concluded that RabA4C is required for the effect of GB on chilling tolerance and that treatment with GB enhances stress tolerance, at least to some extent via gene activation (Einset et al. 2007). A model was also proposed to explain how GB-up-regulated genes in roots of *Arabidopsis* might prevent the accumulation of ROS in cell walls, thereby conferring tolerance to abiotic stress (Eisen et al. 2007).

In order to compare gene expression in flower buds of wild-type and *codA*-transgenic tomato, tomato cDNA microarray was used. It was found that the expression of 30 genes was enhanced and that of 29 genes was repressed by the transgene. Exogenous application of GB in tomato leaves resulted in up-regulation of genes involved in electron transport chain (Park et al. 2006). Proteomic approach was applied to investigate the effect of exogenously applied on the salt stress–induced inhibition of growth of seedlings of a salt-sensitive and salt-tolerant variety. It was found that GB could alleviate the growth inhibition via changes in levels of six proteins and two proteins in salt-tolerant and salt-sensitive variety, respectively. Major regulatory networks analysed by computational bioinformatics revealed the role of PSII, Rubisco, and SOD. In wheat plants, exogenous application of 250 mM GB increased the tolerance limits to freezing stress by activation of genes encoding low temperature–inducible proteins. WCOR410, accumulated in the presence of GB and its final level, also depended upon the concentration of GB. Enhanced tolerance to freezing was observed due to induction of the expression of the low temperature–responsive genes by GB. Thus, exogenous application or in vivo synthesis of GB in transgenic plants can activate specific genes. The functions of these genes contribute, at least in part, to a reduction in the stress-induced accumulation of ROS.

4.4 Limitations to the Engineering of the GB Biosynthetic Pathway

Till date, plants and/or bacteria have been the major source of genes and cDNAs used for cloning enzymes involved in the GB biosynthetic pathway. The most commonly used strategy for the molecular genetic manipulation of the biosynthetic pathway in cyanobacteria and plant non-accumulators has been the introduction of the relevant gene under the transcriptional control of a strong promoter that ensures high level of expression of the gene in the transgenic plants. To ensure appropriate localization, the genes have often been modified so that the encoded polypeptide is transported post-translationally to the chloroplasts of the engineered plants (Sakamoto and Murata, 2000). Low levels of GB in the chloroplasts of transgenic plants can be attributed to the availability of choline itself (Huang et al. 2000, Nuccio et al. 1998) and the transport of choline across the chloroplast envelope (McNeil et al. 2000). In a study on transgenic tobacco over-expressing choline mono-oxygenase, Nuccio et al. (1998) reasoned that the absence of CMO is the primary constraint of GB production in non-accumulators such as tobacco, but the endogenous supply of choline is equally and potentially problematic. A similar observation was made even in *Arabidopsis* and *B.Napus* (Huang et al. 2000). In transgenic tobacco, the first of the three successive methylations of ethanolamine was found to be the major constraint. The activity of phosphoethanolamine

N-methytransferase, the enzyme catalyzing this step, was found to be 30–100 folds lower in tobacco compared to spinach, a natural betaine producer. These reactions that produce and consume choline in non-accumulators appear to constitute a rigid metabolic network (Stephanopoulus and Vallinio 1991) evolved to supply lowered novo flux of choline moieties for synthesis of phosphatidyl choline and to salvage choline. It is therefore appropriate to reason that such a network resists increase in the total flux of choline for synthesis of GB. As a result, there has been no instance wherein a transgenic plant attained GB level approaching those in natural accumulators during stress (Nuccio et al. 1998). In rice, however, choline supply was found not to be the limiting factor, because free choline levels were unaltered in CodA+ transgenics (Sakamoto and Murata 2000).

4.5 Methods to Overcome Limitations

One of the methods employed to overcome the limited accumulation of GB was choline supplementation. In the study carried out by Huang et al. (2000), various concentrations of choline were tested to find a suitable level that did not adversely affect the seedling growth and, thereafter, exogenously applied to the plants. The concentration varied for the three species, namely, *Brassica*, *Arabidopsis*, and tobacco. The *Arabidopsis* lines when supplemented with 10 mM choline showed the largest increase: 30–37 folds over the un-supplemented levels. The other two species also showed significant increase. *B. napus* lines accumulated progressively higher content of betaine with increasing supply of choline. Thus, it was concluded that choline supplementation increases *in planta* accumulation of betaine.

Metabolic engineering of the choline-betaine network in a systemic approach could be another way to overcome the limitations of the engineered GB biosynthetic pathway. This will enable generation of transgenic systems that support the synthesis of significant level of betaine and thus more information can be drawn from the stress tolerance measurements.

4.6 Conclusion

The primary objective of exogenous application and metabolic engineering of the GB biosynthetic pathway has been to increase the synthesis and/or accumulation of GB to alleviate abiotic stress effects in plants tissues. This has led to improvement in growth and survival of plants, ROS scavenging, osmoregulation of the cytosolic compartments, membrane stabilization, buffering of redox potential, and induction of stress-responsive genes that help in alleviating stress. Various genes have been employed to generate transgenic plants that accumulate GB and are tolerant to abiotic stress. The physiological and morphological aspects of stress tolerance have been investigated most extensively in codA transgenic plants at all stages of the life cycle of plants. Tolerance to stress might have been mediated by the high levels of GB in the plant tissues. Protection of the photosynthetic machinery, reductions in levels of ROS under stress, induction of specific genes whose products are involved in stress tolerance, and regulation of the activities of ion-channel proteins either directly or via protection of the plasma membrane are some of the possible mechanisms of GB-induced tolerance (Chen and Morata 2011).

The past decade has seen a number of transgenics being developed employing several other genes for accumulation of GB in plants like *BADH*, *CMO* individually and in combination, *GSMT, DMT*, *cox*, *betA*, isolated from microbes or plants, all of which have contributed to stress tolerance at varying levels. However, the efficacy of GB metabolism transformation for plant crops has not been fully demonstrated possibly because even if the GB concentration in transformed plants is significantly raised, it is still lower than that of a natural high accumulator species. Therefore, it is important to generate transgenics that have the capacity to express increased levels of the transgene without taxing the metabolic flux. Also, field trials of such transgenics must be carried out to verify their potential utility in crop-improvement programs.

4.7 Future Prospects

The generation of transgenic lies accumulating GB in model species such as *Arabidopsis* and tobacco has allowed these stress defence mechanisms to be extended to various crop plants such as rice, potato, and sugar beet with varying degrees of success. Therefore, it is important to carefully identify regulatory factors that can drive the expression of these key genes following any abiotic stress. Also, further research is necessary in order to genetically manipulate tissue-specific and stress-inducible osmo-protection without exhausting the metabolic flux.

For practical application in crop plants, availability of the appropriate substrate in a specific sub-cellular compartment should be improved further so as to facilitate generation of plants that produce elevated levels of GB, consequently enhancing tolerance to stresses. Different strategies that are individually effective against stress can be combined to make further progress in this regard. Targets for this approach could be *Cold Regulated* (*COR*) and *Late Embryogenesis Abundant (LEA)*, and genes for regulatory proteins (Ottosen et al. 1998). Such combinations can significantly impact the attempts to increase the stress tolerance of agriculturally important crops.

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Chapter 5

Improvement of Abiotic Stress Tolerance by Modulating Polyamine Pathway in Crop Plants

Anshu Alok, Akshay Nag, Jitesh Kumar, Phanikanth Jogam, Kashmir Singh, and Sudhir P. Singh

Abstract Various abiotic stresses severely affect crop plant germination, growth, and productivity worldwide. Plants exhibit numerous mechanisms to battle against hostile abiotic stress. Out of several mechanisms, here we focused on the polyamine metabolic pathway. Polyamines exist in the plants with free or covalently/noncovalently conjugated forms and mainly three types, i.e., putrescine, spermidine, and spermine. These are involved in various cellular processes such as DNA replication, transcription, cell membrane stability, cell division, regulation of enzyme activity, and in abiotic stress, etc. Polyamines biosynthesis pathways in plants use two critical precursor substrates, i.e., L-arginine and methionine. In the present chapter, we will focus on genes and enzymes involved in polyamines biosynthetic and catabolism. Multiple functional roles of polyamines at the cellular level and during the developmental stage, during high-temperature stress, during cold and chilling stress, during water and drought stress are discussed in detail. Apart from these, we also focused on genetic engineering in polyamines pathways to develop abiotic stress-tolerant crops. Genetic manipulation using plant genetic engineering tools by targeting *ADC*, *ODC*, *SPDS*, *SAM*, and *SAMDC* in different plants improved abiotic stress tolerance. Apart from these genes, transcription factors regulating these pathways need to be explored to achieve better abiotic stress-tolerant plants.

Keywords Abiotic · Polyamines · Cold · Drought · Salt stress · Polyamines biosynthesis · Polyamines catabolism · Genetic engineering

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5.1 Introduction

Abiotic stress, such as heat, salinity, cold, drought, and freezing, etc., severely affects plant germination, growth, and productivity worldwide. To battle against the hostile abiotic stress, the plant has evolved several responses and molecular mechanisms. The molecular responses and mechanisms may vary among plant species. Of the multiple existing mechanisms, production and accumulation of low-molecular-weight, organic metabolites/compatible solutes are universal in plant species. These small molecules are extremely soluble, nontoxic, and do not interfere with other metabolic pathways of the cells (Slama et al. 2015). The compatible solutes are also well known as osmoprotectants due to their role as osmolytes (which protect cells during extreme oxidative and osmotic stress). There are various types of compatible solutes present into plants such as amino acids and their derivatives, betaines and related compounds, polyols and nonreducing sugars, polyamines, tertiary sulphonium compounds, and quaternary ammonium compounds, etc. (Bohnert and Jensen 1996; Slama et al. 2015; Dutta et al. 2018).

Polyamines are polycationic aliphatic amines consisting of two or more amino groups with a nitrogenous base (Vuosku et al. 2012; Chen et al. 2018). These low-molecular-weight polyamines are present in all plant species and animals (Hussain et al. 2011; Liu et al. 2015). The polyamines are not only present in eukaryotic cells, but it is also found in prokaryotic cells (Michael 2018). In plants, mostly putrescine, spermidine, and spermine are common polyamines and ubiquitously found in free or bound form (Gill and Tuteja 2010). Soluble polyamines are attached to phenolic compounds, whereas insoluble polyamines are attached to nucleic acids or proteins. The different types of polyamines present in plants play a significant role during growth and development. At the cellular level, they are involved in various biological processes, i.e., transcription, translation, cell division, expansion, cell differentiation, and cellular apoptosis (Masson et al. 2017). Furthermore, they are actively involved during leaf senescence, floral development, and fruit ripening (Guo et al. 2018; Sobieszczuk-Nowicka 2017).

During various abiotic stresses such as salt, cold, drought, and heat, etc., the plant increases the biosynthesis and accumulation of polyamines to overcome these stresses (Chen et al. 2018). Various studies have suggested that the exogenously applied polyamines drastically induce tolerance in the chilling-, salt-, and drought-stressed plants. Effect of polyamines treatments to various plants such as zoyia grass, bentgrass, cucumber, and mung bean, etc. has been explored against multiple abiotic stresses (Zhang et al. 2009; Li et al. 2015; Nahar et al. 2016; Li et al. 2016). Increasing the endogenous polyamines within plants is highly in demand by means of genetic engineering.

Therefore, plant genetic engineering of the polyamines pathway is another approach to improve plants to cope against various abiotic stress. The potential genes such as (*Arginine decarboxylase*) *ADC*, (*SAM decarboxylase*) *ODC*, (*Spermidinesynthase*) *SPDS*, (*S-adenosylmethionine synthase*) *SAM*, and (*SAM decarboxylase*) *SAMDC* encoding the enzymes involved in polyamine biosynthetic pathways have been utilized for generating abiotic stress-tolerant plants. Transcriptome analysis of plants during different stresses also explored the potential genes involved in different polyamine pathways.

5.2 Different Form and Types of Polyamines

Polyamines exist in the free form in most plants. Whereas in other living organisms, it exists either as in free or covalently/noncovalently conjugated forms. There are mainly three types of polyamines, i.e., putrescine, spermidine, and spermine, and they are almost present in all plants. Apart from these, other polyamines such as agmatine and cadaverine are also found in lower amounts during the different developmental stages of plants such as tillering, heading, and anthesis (Hura et al. 2015). The covalently conjugated polyamines are further subdivided into perchloric acid-soluble covalently conjugated and perchloric acid-insoluble covalently conjugated polyamines (Chen et al. 2018).

Putrescine, spermidine, and spermine can exist as free, soluble conjugated and insoluble bound forms in higher plants. Soluble conjugated polyamines are covalently attached to different phenolic compounds, whereas insoluble bound polyamines are covalently attached to nucleic acids or proteins (Gill and Tuteja 2010). The phenolic compounds such as hydroxycinnamic acid, coumaric acid, caffeic acid, and ferulic acid are well known to be bound with the polyamines. This complex compound is known as phenolamides, a class of secondary metabolites (Bassard et al. 2010). Few uncommon polyamines, such as homospermidine, cadaverine, 1,3-diaminopropane, and canavalmine are also present in plants (Kuehn et al. 1990). Other uncommon polyamines norspermidine and norspermine are detected in the callus of heat-tolerant rice cultivar N22 (Roy and Ghosh 1996). Free polyamines are positive in nature and, therefore, electrostatically bind with negatively charged molecules (e.g., nucleic acids, acidic proteins, and membrane phospholipids, etc.) and form noncovalently conjugated-polyamines (Igarashi and Kashiwagi 2015). These types of noncovalently conjugated-polyamines are involved in DNA replication, transcription, cell membrane stability, cell division and regulation of enzyme activity, etc.

5.3 Polyamines Biosynthetic Pathways in Plants

The biosynthetic pathways of polyamines have been explored extensively at molecular levels in various plants. The biosynthesis from its initial precursor, conjugation, transport, and catabolism plays an important role in polyamines homeostasis. There are two precursor key substrates, i.e., L-arginine and methionine, which are found to be present in plants for polyamines biosynthesis (Fig. 5.1).

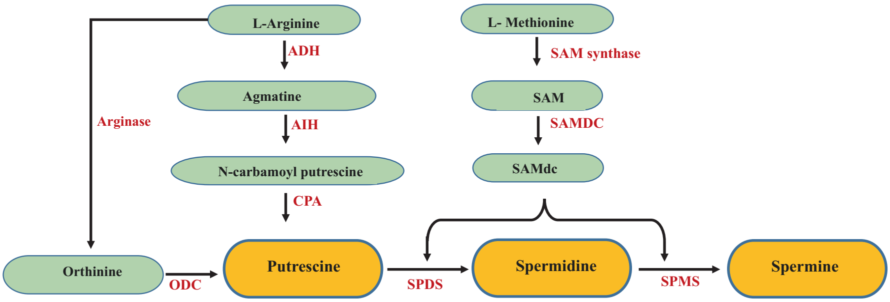

Fig. 5.1 Biosynthetic pathways of polyamines. *ADC* arginine decarboxylase, *AIH* agmatine iminohydrolase, *CPA* N-carbamoylputrescine amidohydrolase, *ODC* ornithine decarboxylase, *SPDS* spermidine synthase, *SPMS* spermine synthase, *SAM* S-adenosylmethionine, *SAMDC* S-adenosylmethionine decarboxylase, and *SAMdc* decarboxylated Sadenosylmethionine

L-arginine is the starting substrate for two pathways via ornithine decarboxylase (ODC) pathway and arginine decarboxylase (ADC) pathway. The expression pattern of the genes invovled in these pathways may vary into different tissues and abiotic stress. In the ODC pathway, ornithine is synthesized from L-arginine by arginase enzyme and further metabolized to putrescine and proline via ODC enzyme and ornithine-d-aminotransferase, respectively (Shi and Chan 2014; Majumdar et al. 2016). Initially, it was known that only arginine decarboxylase pathway exists in *Arabidopsis thaliana*, as nucleotide sequence of *ornithine decarboxylase* gene was not known due to unavailability of complete genome information (Hanfrey et al. 2001). Soon after the availability of genome information, *arginine decarboxylase* 2 (*ADC2*) gene was explored to be involved in polyamine biosynthesis in response to mechanical injury and methyl jasmonate treatment using microarray and RNA gel-blot analysis (Perez-Amador et al. 2002). Further, the enzyme activity of ornithine decarboxylase was performed from the leaves of *A. thaliana* (Tassoni et al. 2003).

Methionine is another precursor for the biosynthesis of polyamines. Adenosylmethionine is synthesized from methionine using S-adenosylmethionine (SAM) synthase, which is further converted into decarboxylated S-adenosylmethionine (dcSAM) with the help of SAM decarboxylase (SAMDC). S-adenosylmethionine synthase is the only enzyme that synthesizes SAM from L-methionine and ATP, and is also called methionine adenosyltransferase (MAT) (Binet et al. 2011). SAM synthase in *A. thaliana* is encoded by four genes, i.e., *MAT1* to *MAT4*. Out of these four *MAT1, 2, 4* are ubiquitously expressed in all different tissues, while *MAT3* is expressed mostly in pollen (Chen et al. 2016). The *SAMDC* gene family encodes SAMDC enzyme. The *A. thaliana* genome consists of four members of *SAMDC* gene family, i.e., *SAMDC1*, *2, 3,* and *4*. The *SAMDC4* gene is also known as *BUD2* in *A. thaliana* (Ge et al. 2006).

The enzyme spermidine synthase (SPDS) is responsible for the conversion of putrescine to spermidine using aminopropyl donated from dcSAM. Further, the spermidine is converted to spermine by spermine synthase (SPMS). *A. thaliana* genome consists *of SPDS1* and *SPDS2* genes, which encode the enzyme spermidine synthase. In *A. thaliana*, it was shown that SPDS enzyme is vital for the survival of plants (Imai et al. 2004). In *Arabidopsis* single copy of *AtSPMS* is present, whereas three copies are present in rice namely, *OsSPMS1*, *OsSPMS2*, and *OsSPMS3*. The *AtSPMS* gene was induced with abscisic acid and salt stress treatments in *Arabidopsis*, whereas in rice, *OsSPMS1* plays a vital role in seed size, yield, and germination (Alet et al. 2012; Tao et al. 2018).

5.4 Polyamines Catabolism

The cellular pool of polyamines is dependent on the regulation of its biosynthesis, transport, and catabolism. Different genes and pathway of polyamines catabolism in plants species including the model plant *Arabidopsis*, crop plants such as *Oryza sativa*, and fruit plants such as sweet orange, etc. have been explored (Liu et al. 2014; Kamada-Nobusada et al. 2008; Wang and Liu 2015). However, the genes and enzymes involved in these pathways and their putative functions are poorly understood. However, there are mainly two types of amine enzymes responsible for polyamines, i.e., copper-containing amine oxidases and FAD-dependent polyamine oxidases. Copper-containing amine oxidases mainly catalyze putrescine and cadaverine to produce 4-aminobutanal, H_2O_2, and ammonia (Fig. 5.2), whereas FAD-dependent polyamine oxidases primarily oxidize spermidine and spermine. The enzyme flavin adenine dinucleotide (FAD)-dependent polyamine oxidases are also known as flavin-containing polyamine oxidases encoded by *the PAO* gene. Both

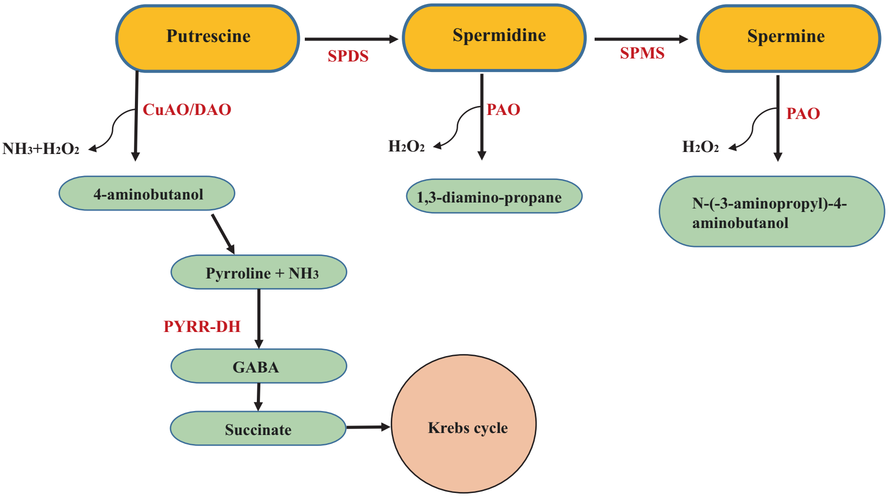

Fig. 5.2 Catabolism of polyamines. *SPDS* spermidine synthase, *SPMS* spermine synthase, *CuAO* Copper-containing amine oxidase, *PAO* polyamine oxidase, *DAO* D-amino acid oxidase, and *GABA* γ-Aminobutyric acid

Cu- and flavin-containing amine oxidases (CuAOs and PAOs) are highly present in the cell wall of *Fabaceae* and *Poaceae* plant families (Tavladoraki et al. 2016). In plants, the enzyme PAOs are categorized into two groups according to the mechanism of the reaction. The terminal catabolism produces 1,3-diaminopropane, H_2O_2, and their respective aldehydes. At the same time, the back-conversion reaction produces spermidine from tetraamines, spermine, and thermospermine and/or putrescine from spermidine, along with 3-aminopropanal and H_2O_2 (Liu et al. 2014). Terminal catabolism of spermine by PAOs yields 1,3-diaminopropane, H_2O_2, and N-(3-aminopropyl)-4-aminobutanal. Similarly, terminal catabolism of spermidine by PAOs yields 1,3-diaminopropane, H_2O_2, and 4-aminobutanal (Wang et al. 2019).

The diamine oxidase (DAO) catalyzes the putrescine and form 4-aminobutanal, H_2O_2, and NH_3 (ammonia). Further, 4-aminobutanal undergoes cyclization to form pyrroline. The enzyme pyrroline dehydrogenase (PYRR-DH) converts pyrroline into γ-aminobutyric acid (GABA). Later, GABA is converted into succinate, which enters into the Krebs cycle (Chen et al. 2018) (Fig. 5.2).

In *Arabidopsis* genome, five genes *AtPAO1, 2, 3, 4,* and *5* are present. All five AtPAOs enzymes encoded by these genes catalyze the back-conversion of polyamines (Fincato et al. 2010). AtPAO1 and AtPAO4 are able to convert spermine to spermidine, whereas AtPAO2 and AtPAO3 convert spermidine from spermine and further produce putrescine (Moschou et al. 2008). *Oryza sativa* consists of seven members of the *OsPAO* gene family. Out of these seven, OsPAO1, OsPAO3, OsPAO4, and OsPAO5 are involved in back-conversion reactions of polyamines, whereas others catalyzed the terminal catabolism of polyamines (Ono et al. 2012). The PAO isolated from maize shoots was characterized for terminal catabolism of polyamines (Federico et al. 1990). The *Brachypodium distachyon* BdPAO2 is able to catalyze the spermine or thermospermine to spermidine, and spermidine to putrescine. Whereas BdPAO3 preferred spermine as substrate and converts tetraamines to spermidine (Takahashi et al. 2017).

5.5 The Functional Role of Polyamines at the Cellular Level and During the Developmental Stage

Polyamines are associated with various cellular progressions such as replication of DNA, transcription of mRNA, translation of protein, cell proliferation, and regulation of various enzyme activities. Few polyamines act as growth regulators in plants, and exogenous application of polyamines enhanced flower bud differentiation. In Chrysanthemum, apical buds enhanced content of polyamines; this was reported for the initiation, maintenance, and differentiation of flower bud (Xu et al. 2014). Polyamines are polycation in nature; therefore, they interact with negatively charged DNA, RNA, proteins, and phospholipids. Polyamine interacts with RNA to form polyamine–RNA complexes in the presence of physiological K and Mg ions. Further, in this way, structural changes in RNA (m-, r-, and t-) occur. Polyamines contribute to an important role in zygote polarity, apical axis, differentiation for cell layer, meristem formation, and somatic embryogenesis (Vondráková et al. 2015). The content of polyamines and polyamines synthetase is higher in the meristem region while it is lowest in senescent tissues. Exogenous application of spermidine and spermine increased the polyamines content and delay the senescence in cut flowers and enhance shelf-life. Various mechanisms and effects of polyamines were examined at the low-temperature storage of flowers (Han 2016). Polyamines also play essential roles in seed germination, dormancy break of tubers, stimulation, and fruit set and ripening.

5.6 Role of Polyamines Within Plants During Abiotic Stress

Polyamines are playing an important role during abiotic stresses such as chilling, heat, water logging drought, salt, UV radiation, and heavy metal toxicity. Exogenous application of polyamines and its effect in abiotic stress tolerance is tabulated in Table 5.1. Polyamines interact with various molecules such as abscisic acid, and nitric oxide during response against abiotic stress. Reactive oxygen species (ROS) and hydroxyl radicals are produced from polyamines catabolism during oxidative stress abiotic stress. Polyamines are positively charged, hence interact electrostatically with ion channels at physiological pH, which is involved during water stress. Here, in this section, we have focused on the molecular mechanism of polyamines underlying various abiotic stresses.

Table 5.1 Exogenous application of polyamines and its effect in abiotic stress tolerance

Plant name	Effect observed	References
Vicia faba (L.)	Salt stress tolerance	Mahdi (2016)
Capsicum annuum	Extended the shelf life	Patel et al. (2019)
Mangifera indica	Extended storage period	Chakraborty et al. (2017)
Triticum aestivum L.	Cadmium stress tolerance	Rady and Hemida (2015)
Citrus sinensis L.	Improved fruit quality and yield	Saleem et al. (2006)
Nymphoides peltatum	Copper stress tolerance	Wang et al. 2007
Pistacia vera L.	Salt stress tolerance	Kamiab et al. (2014)
Punica granatum L.	Extended shelf life	Mirdehghan et al. (2007)
Gossypium hirsutum L.	Increased seed set	Bibi et al. (2010)
Phaseolus vulgaris L.	Drought stress tolerance	Abass and Mohamed (2011)
Oryza sativa L.	Drought stress tolerance	Farooq et al. (2009)
Malus hupehensis Rehd.	Cadmium stress tolerance	Zhao and Yang (2008)
Cucumis sativus L.	Chilling stress tolerance	Zhang et al. (2009)
Solanum lycopersicum	Chilling stress tolerance	Aghdam et al. (2019)
Prunus armeniaca L.	Ovule development	Alburquerque et al. (2006)

5.6.1 Functional Role During High-Temperature Stress

The polyamines play a direct protective role as well as control essential cellular processes as signaling molecules. Various studies have suggested that the abiotic stress tolerance induced by the polyamines is mostly related to their signaling role. Nevertheless, the exact functional role of polyamines in heat stress has not yet been characterized (Janda et al. 2019; Jahan et al. 2019). Recently, the wheat plant was subjected to moderately elevated temperature (30 °C) to investigate the potential protective mechanism. They found that the content of putrescine, spermine, and spermidine either decreased or did not change during the heat. However, the content of 1,3-diaminopropane and expression of *TaPAO* gene was increased (Janda et al. 2019). Recently, in tomato, the connection between melatonin and polyamines metabolism via the nitric acid pathway was studied for the plant protection at high temperature. The heat stress to tomato increased the endogenous levels of polyamines (putrescine, spermidine, and spermine) (Jahan et al. 2019). In rice, the exogenous application of spermidine enhances heat tolerance by regulating endogenous starch and polyamine metabolism (Fu et al. 2019). These findings suggest that polyamine metabolisms and its subsequent signaling processes might be involved in heat tolerance.

5.6.2 Functional Role During Cold and Chilling Stress

Under cold conditions, polyamines interact with phospholipid of the cell membrane to avoid cytolysis and increase cold resistance (Chen et al. 2018). Whereas in case of cold stress, the phase of the cell membrane changes from liquid crystalline to gel. Due to this, the cell membrane loses fluidity and becomes more rigid. Apart from these during chilling stress, the overproduction of ROS causes lipid peroxidation and solute leakage. Chilling reduces the activity of the enzyme result in slow metabolism of plants (Zhao et al. 2017). Sweet orange callus increased the content of spermine, as well as the expression of *ADC* and *SAMDC* genes, which were also enhanced during cold and salt stress (Wang and Liu 2009). External application of polyamines to stevia decreased H_2O_2 and malondialdehyde content. However, polyamine supplementation increased the saturated long-chain fatty acids, and therefore, a significant tolerance was observed in stevia at cold stress (Peynevandi et al. 2018). Melatonin treatment to cucumber conferred chilling stress tolerance by increasing the content of putrescine and spermidine (Zhao et al. 2017). Putrescine application to *Anthurium andraeanum* under chilling stress (6 °C in winter) increased antioxidant activity, N_2 metabolism, chlorophyll, and proline content whereas decreased malondialdehyde content. Further, exogenous application of 1.0 mmol/L putrescine showed less chilling damage (Sun et al. 2018).

5.6.3 Functional Role During Water and Drought Stress

Polyamines regulate potassium ion channels and pores size in the cell membrane of guard cells. In this manner, polyamines control the opening and closing of pores and regulate water loss from the plant's cells. The various findings showed that the foliar spray of polyamines starts multiple cellular processes such as biosynthesis of amino acids, osmotic-related molecules, soluble sugars, proline, etc. These all may compensate for the biomass loss of plants during drought conditions (Peng et al. 2016; Chen et al. 2018).

In rice, the role of polyamines during drought and water stress was studied. During this stress, the activities of enzymes involved in polyamines pathways, i.e., spermidine synthase, arginine decarboxylase, and S-adenosyl-L-methionine decarboxylase were increased. Apart from these, the content of putrescine, spermidine, and spermine also increased (Yang et al. 2007). Nitric oxide is a key signaling molecule induced by polyamines and plays a significant role in plants during drought conditions. Exogenous application of spermidine improved drought tolerance in white clover by increasing the relative water content and decreasing electrolyte leakage and malondialdehyde (Peng et al. 2016).

Ectopic expression of *ADC* and *SAMDC* genes increased polyamine content in rice and *Arabidopsis* to enhance drought tolerance (Capell et al. 2004; Wi et al. 2014). In agreement with this, when *SAMDC* gene downregulated, the content of spermidine and spermine decreased, and further, the rice plants showed less drought tolerance (Chen et al. 2014).

5.7 Genetic Engineering of Polyamines Pathways for Abiotic Stress Tolerance

The genes and their families, such as *ADC*, *ODC*, *SPDS*, *SAM*, and *SAMDC*, etc., encode the enzymes involved in polyamine biosynthesis and are targeted for engineering in plants. The transcriptome and promoter studies also revealed various transcription factors regulating the polyamine biosynthetic genes. In the recent few years, various transgenic plants including *Arabidopsis*, tobacco, rice, and eggplant, etc., have been generated for abiotic stress-tolerant (Perez-Amador et al. 2002; Tassoni et al. 2003; Liu et al. 2014a; Ono et al. 2012; Raman and Rajam 2007; Zhuo et al. 2018). Genetic engineering in polyamines biosynthetic pathway and its effect in different crop plants are tabulated in Table 5.2. The ectopic overexpression of *Cucurbita ficifolia SPDS* gene under the regulation of CaMV35S promoter was done in *Arabidopsis*. The transgenic *Arabidopsis* plants increased (two-fold) the content of spermidine and conferred resistance against salt, drought, and low temperature (Kasukabe et al. 2004). Transgenic eggplant carrying *ADC* gene under the control of CaMV35S showed an enhanced ADC enzyme activity as well as increased

Table 5.2 Genetic engineering in polyamines biosynthetic pathway and its effect in different crop plants

Targeted genes	Targeted plants	Effect observed	References
Arginine decarboxylase	*Medicago truncatula*	Higher seed yield	Duque et al. (2016)
Arginine decarboxylase	*Lotus tenuis*	Salt stress tolerance	Espasandin et al. (2018)
Arginine decarboxylase	*Oryza sativa L*	Salt stress tolerance	Roy and Wu (2001)
Spermidine synthase	*Pyrus communis L.*	Salt and heavy metal stress tolerance	Wen et al. (2008)
Spermidine synthase	*Arabidopsis thaliana L*	Salinity and drought stress tolerance	Kasukabe et al. (2004)
Spermidine synthase	*Solanum lycopersicum*	Increasing fruit shelf life	Nambeesan et al. 2010
Spermidine synthase	*Solanum melongena*	Resistance to *R. solanacearum*	Qiu et al. (2019)
S-adenosyl methionine decarboxylase	*Nicotiana tabacum*	Salinity stress tolerance	Waie and Rajam (2003)
S-adenosyl methionine decarboxylase	*Lycopersicon esculentum*	High temperature stress tolerance	Cheng et al. (2009)
S-adenosyl-L-methionine synthetase	*Arabidopsis thaliana L*	Salt and oxidative stress tolerance	Ma et al. (2017)

content of polyamines. Transgenics showed tolerance against several abiotic stresses and fungal wilt diseases (Raman and Rajam 2007).

Polyamine pathway engineering was done even in woody fruit trees such as citrus, pears, and apple. Overexpression of the apple *SPDS* gene into transgenic pear showed enhanced heavy metal tolerance probably due to enhanced antioxidant activity and spermidine as a metal chelator (Wen et al. 2010).

In silico analysis of rice *SAMDC* promoter showed the occurrence of ABRE, LTRE, MYBR, and W-box, which are related to abscisic acid signaling, drought, and cold. These facts suggest that the genes involved in the polyamine biosynthesis pathway might be controlled by a specific transcription factor or its family. Apart from the above-mentioned pathway genes, few transcription factors were also targeted for plant engineering for generating abiotic stress-tolerant. Ethylene responsive factor (ERF) is a subfamily transcription factor, which plays a significant role in abiotic and biotic stress tolerance in plants. The ERF1 of *Medicago falcata* was overexpressed in tobacco that conferred enhanced tolerance to freezing and chilling stress via stimulated polyamine and proline accumulation (Zhuo et al. 2018). MYB transcription factors play an important role during the development of plants as well as abiotic stress condition. The *MYB21* gene of *Pyrus betulaefolia* was overexpressed in tobacco, which showed increased tolerance to dehydration and drought stresses (Li et al. 2017).

5.8 Conclusion and Future Perspectives

Current findings in the area of plant abiotic stress biology showed that polyamines are involved in various abiotic stress conditions. However, polyamine and its subsequent signaling transduction pathway are poorly understood. Apart from this, the correlation between polyamine and other hormones is still mostly unknown. Genetic engineering of the polyamine pathway-related genes is an effective method for generating abiotic stress-tolerant plants. Further, transcriptome and metabolome analysis of abiotic stress-tolerant plant species is needed to investigate the interaction between polyamines and other stress-responsive molecules.

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Chapter 6
Engineering Fructan Biosynthesis Against Abiotic Stress

Gourav Choudhir and Neeraj K. Vasistha

Abstract Many plant species contain a human health beneficial component called fructans. Fructans are fructose-based polymers sugar and synthesized from sucrose by an enzyme called as fructosyltransferases (FTs). Enhancement in the level of fructan molecules in engineering plants is one of the most critical areas of research. Several studies have been conducted to correlate the fructans content with various abiotic stresses like heat drought, chilling, etc. It has been confirmed that fructans may work as cryoprotectants and can stabilize the plasma membranes during the dehydration after the incorporation of polysaccharide into the lipid headgroup region of the membrane. This mechanism maintains the water level and protects the plant tissues from leakage during abiotic stresses. The level of fructans in certain plant species cannot be easily improved using conventional methods of breeding due to the low genetic diversity of this trait in the germplasm of certain species. However, fructans levels in plants can be enhanced using the biotechnological tools for the biosynthesis of fructans against the abiotic stresses. The abiotic stress tolerance is a complex mechanism of plants, and engineering fructans biosynthesis may protect the plant from stresses incorporation with some other genetic factors. Due to the importance of high fructans content in plants for potential physiological benefits during the stresses, this trait should be taken into mainstream breeding programs at a large-scale for developing abiotic stress tolerance and nutritionally improved crop varieties.

Keywords Fructan · Biosynthesis · Fructosyltransferase genes · QTLs · Transgenic · Abiotic stress

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6.1 Introduction

Plants are vital for the survival of the human race as they create an aerobic environment, which is compulsory for our existence, along with the beginning essential source of food and medicine. Plants grow in an open environment where they directly interact with the climate condition, which affects the growth and development of the plants in both a positive and negative manner. The favorable climate leads to higher metabolic activity, which promotes exponential growth of the plants while adverse conditions such as distinct types of biotic and abiotic stress drought, heat, heavy metal toxicity, and oxidative stress, etc. lead to hindered plant growth. Excessive stress due to external stimuli leads to stunted growth, lower yield, permanent damages to plant tissues, and, ultimately necrosis (Mittler 2006; Zhu 2016). The stimuli for stress can be classified as either biotic factors or abiotic factors. The term biotic factors refer to any stress stimuli, which arise due to animals (i.e., grazing) or other plants (i.e., secrete allelochemicals). In contrast, abiotic factors refer to environmental stress due to a lack of essential elements needed for plant survival. Due to human intervention, most of the crops are protected against biotic stress; however, abiotic stress is still a challenge, which leads to significant economic losses for the growers all over the world. Plant productivity is affected mainly by abiotic stresses (Haynes, 1996).

Survival, biomass production, accumulation, and grain yield are severely affected by most crops. Drought and salinity are two significant abiotic stresses, which adversely affect at least 20% of the world arable land and approximately 40% of irrigated land to various degrees. About one-third of irrigated land is believed to be affected by salinity. As a consequence of stress conditions, physiological and biochemical activities in plants vary, because cellular aqueous and ionic equilibriums are disrupted. The United Nations Environment Program state that approximately 20% of agricultural land and 50% of cropland in the world is salt-stressed and salinized areas are increasing at a rate of 10% annually (Usman et al. 2020). Abiotic stresses are responsible for the degradation of various structural and functional components that takes participate in different processes, which leads to the growth and development of plants such as photosynthesis assemblies, respiratory assemblies, and structural protein (Feller et al. 2008; Wang and Blumwald, 2014; Zhang et al. 2015; Hildebrandt, 2018). Maintaining proteins in their functional conformations and preventing the aggregation of nonnative proteins are necessary for cell survival under stress.

Fructans with a short chain length are known as fructooligosaccharides. The sucrose unit (i.e., a glucose–fructose disaccharide) of fructose residues is used to build up fructans. The type of fructans is determined by the linkage position of fructose residues. The fructans are categories into five groups i.e. (i) 1-linked: in inulin, the fructosyl residues are linked by β-2,1-linkages (ii) 6-linked: in levan and phlein, the fructosyl residues are linked by β-2,6-linkages (iii) graminin type (Chibbar et al. 2016) contains both β-2,1-linkages and β-2,6-linkages (van Den Ende et al. 2013) (iv) neo-inulin type (also called "inulin neoseries"; Chibbar et al. 2016), predominant β-2,1-linkages and (v) neo-levan type (also called “levan neoseries” Chibbar et al. 2016), predominant β-2,6-linkages. It is present in over 12% of the angiosperms including both monocots and dicots. Its abundantly present in agave, artichokes, asparagus, leeks, garlic, and onions (including spring onions) and also in barley and wheat. Recent advances have identified fructans as potent prebiotic. Deit rich in fructans tends to reduce the risk of diseases by modulating the gut microbiome composition, and regulating the host epithelial response and immune-pathological conditions. These changes are brought about due the fact that breakdown products of fructans tend to increase the count of beneficial bacteria and their associated immunomodulatory metabolic activity (Galgano et al. 2012). Fructans play vital role in survival of plants during stress conditions by acting as free radical scavengers and protectants (Van den Ende and Valluru 2009) and perform significantly better as compared to other disaccharides.

6.2 What Is Abiotic Stress?

Abiotic stress refers to all nonliving factors that affect the plant’s growth and development in a negative manner, such as reduction in growth, depletion of energy production, and reduction in the length of plants. The performance of a plant is negatively affected as a repercussion of various abiotic stresses in the surrounding environment. Plants get a wide range of environmental insults during a typical life cycle and have evolved mechanisms by which they increase their tolerance to these through physical adaptations, interactive molecular and cellular changes that initiate after the onset of stress (Mittler, 2006). An array of abiotic factors affects the plants grown directly in a complicated manner, depending on the plant part being affected by abiotic stress. Abiotic stress occurs due to depletion/ excess of the resources, such as water deficiency, which leads to drought stress. In contrast, the excess of the water leads to waterlogging and, therefore, lack of oxygen. Different types of abiotic stress are described below.

6.2.1 Drought Stress

Drought stress is the leading form of abiotic stress, which affects 14% of the agricultural land in the world (Hao et al. 2018; Hossein Azadi et al. 2018). The drought stress has a multifactorial impact on the plants via the modulation at morphological, physiological, biochemical, and molecular levels (D’Alessandro and Havaux, 2019; Mendiburu 2019).The intensity of the drought stress depends on several factors such as low precipitation, salinity, while low temperature and robust influence the intensity of the drought stress (Salehi-Lisar and Bakhshayeshan-Agdam 2016). Drought stress starts when the available water in the soil is reduced to critical levels plus atmospheric conditions contribute to continuous loss of water (Parkash and Singh, 2020). Drought stress tolerance is found in all plants, but its extent varies from species to species. The reason behind drought stress is the water deficit, generally due to high temperature and solar radiation (Bartosiewicz and Jadczyszyn, 2021). Water deficit and salt stress are global issues to assure the survival of field crops and sustainable food production. The effect of oxidative stress in willow leaves was exaggerated by drought, leading to increase in the amount of flavonoids and phenolic acids. Drought stress is responsible for changes in the ratio of chlorophyll "a" and "b" as well as carotenoids (Mihaljević et al. 2021). A reduction in chlorophyll content was reported in cotton under the influence of drought stress, and it was also reported in *Phaseolus vulgaris*, *Cicer arietinum* (Cornic and Massacci, 1996; Mafakheri et al. 2010). Drought conditions reduced the content of saponins in *Chenopodium quinoa* from the dry weight (DW) in plants growing under low water deficit conditions compared to plant in high water content (Gómez-Caravaca et al. 2012). Anthocyanins are found to accumulate under drought stress and at cold temperatures (Cirillo et al. 2021). Plant tissues containing anthocyanins are generally slightly resistant to drought. For example, a purple cultivar of chili resists water stress far better than a green cultivar. Flavonoids have protective functions under the influence of drought stress (Ramkrishna and Ravishankar 2011). The drought stress promotes negative morphological changes, such as decreased rate of root proliferation, stem shortening, and leaf deformities. At a physiologically level, it also interferes with water utilization efficiency as well as impedes plant-water relations. The compromised water-utilization efficiency affects the plant from the cellular level to the organism level. The drought stress causes closure of stomatal guard cells with the intension of reducing water loss, which in turn negatively affects the CO_2 assimilation (Dias and Brüggemann, 2010; Rahbarian et al. 2011). Additionally, drought stress leads to membrane damage that causes loss of functionality of membrane-bound enzymes, which are involved in the ATP formation and CO_2 fixation (Sahsah et al. 1998; Li et al. 2016). Drought stress also promotes the photorespiratory pathway, which leads to the generation of reactive oxygen species (ROS), Qi et al. 2018) ROS are highly reactive and exhibit their harmful effects by modulation of gene expression and regulation by the interaction with the biological macromolecules. (Farooq et al. 2009).

6.2.2 Heat Stress

The excessive increase in temperature in short periods causes heat stress. The heat stress affects growth and development negatively. It permanently damages plant tissues if there is an increase of 10–15 °C in temperature from the optimum level. Heat stress affects the primary and secondary metabolism of the plants adversely (Macedo, 2012; Mittler et al. 2012). It also inspires the perturbation of morphological features of the plants such as leaf etiolation and wilting.

Additionally, high temperature also affects the anatomy and physiology of heat stress–bearing plants; for example, the opening of the stomata is reduced. Heat stress leads to the overproduction of the reactive oxygen species, which damage photosynthesis assembly and biological macromolecules (Bukhov and Mohanty, 1999). The intensity of heat stress reduces the efficiency of photosynthesis and increases the rate of photorespiration, which affects the binding of RuBisCo protein (Demirevska-Kepova et al. 2005; Sharkey, 2005). The reduction of photosynthetic efficiency by the heat stress is due to the damage to the photosynthetic apparatus and also the degradation of the carotenoids (Georgieva, 1999; Sinsawat et al. 2004). The heat stress has a maximum impact on the reproduction of plants, since reproductive organs are most sensitive to heat stress (Hasanuzzaman et al. 2013). The modulation of gene expression is a vital process for the response of abiotic stress.

6.2.3 Chilling Stress

The lowering of the temperature below a limit is defined as chilling stress. Exposure of the temperature below the 0 °C, which is freezing temperature for water, is termed as chilling stress. Although chilling stress impacts plants through different processes such as morphological, anatomical, physiological, and molecular levels, it is more hazardous for the physiological process of the plants (Nie et al. 1992; Bertrand and Schoefs, 1999). Chilling stress is characterized by the loss of viscosity of the membrane by the elevation in the concentration of the unsaturated fatty acids (Quinn, 1988). The chilling stress confers leakage of solutes from plasmalemma and causes swelling in the membrane of various cell organelles such as mitochondria, plastids membrane as well as chloroplastids thylakoids (Abe, 1990). Due to the depletion of the membrane fluidity, the permeability of water is lost (Murata, 1990). The chilling stress upholds the level of the oxidative stress generation that leads to activity modulation of the enzymes that participate in photosynthesis and respiration (Einset et al. 2007). The rate of photosynthesis is demoted, whereas the rate of the respiration promoted by the low temperature (Martin et al. 1981). The overall water level of plants decreases rapidly; however, the level of the bound water is higher compared to the reasonable condition. The chilling stress harms the yield of the crop by the depletion of grain fling activity of the enzymes.

6.2.4 Salt Stress

Elevation of the salt molecules in the soil above-average concentration causes salt stress, which leads to a negative impact on the growth and development of the plants (Jouyban, 2012). Salt stress has an impact on the structural and functional unit of the plant at the cellular level. The salt stress affects the plants in multiple ways such as membrane disorganization, ion toxicity, nutritional deficient condition, alteration of metabolic processes, oxidative stress, genotoxicity, compromisation of cell division, and expansion long with water stress (Zhu, 2001; Azevedo Neto et al. 2004). The salt stress affects the root development as well as also has negative impacts on the other plant parts such as leaf and stem. The elevation of a salt level decreases water potential that plays a leading role in salt accumulation in the plant. High salt stress disturbs homeostasis in water potential (osmotic homeostasis) and ion distribution (ionic homeostasis). This disruption of homeostasis initiates at both the cellular and whole plant levels. Significant changes in ion and water homeostasis induce molecular damage, growth arrest, and even death (Serrano et al. 1999). To achieve salt tolerance, three interconnected conditions of plant activities are essential. First, the damage must be prevented or mitigated. Second, homeostatic conditions must be restored in a new, stressful environment. Third, growth must resume, admitting at a reduced rate (Zhu, 2001).

6.2.5 Heavy Metal Toxicity

The heavy metal toxicity is a condition where the concentration of heavy metal ions in the soil is beyond the minimum tolerance level of plants. Heavy metals such as Zn, Cu, Mo, Mn, Co, and Ni play vital roles in the development and biological processes such as photosynthesis, respiration, etc. Their ionic species also act as a cofactor of the various enzymes, which is essential for the survival of the plants. The soil, which is excessively polluted with heavy metals, promotes the production of ROS in the plant, which grows in them (Keunen et al. 2011). Mani and Sankaranarayanan (2018) explained that heavy metal toxicity triggers the perturbation of the rice plants from the cellular level to the morphological levels. Among heavy metals, Cd, Hg, Pb, and As are dominant in their contribution toward heavy metal–inspired toxicity. These four heavy metal affects the morphological, biochemical, and molecular level of the plant in a different manner.

6.2.5.1 Cadmium (Cd)

Cd is considered as highly toxic heavy metal compared to others due to its high solubility in the water. Even a minute amount of the Cd causes harm to the activity of enzymes that are related to the metabolism of nutrients (i.e., carbohydrates and phosphorus) and Calvin cycle (CO_2) fixation (Dias et al. 2013). The elevation in the level of Cd concentration inspires the structural transition of chloroplast structure and antioxidant machinery. Cd toxicity affects plant growth and development negatively (Ding et al. 2014).

6.2.5.2 Mercury (Hg)

Hg^{2+} is a heavy metal that plays significant roles in heavy metal toxicity. At lower concentrations, it does not show any effects on the growth and development in the plant. However, at higher concentration, it adversely affects the plant morphology as well as the physiological processes of plants (Patra and Sharma, 2000). Hg^{2+} ions mostly affect the water transport that, in turn, negative impact on the stomatal closing and opening. Hg^{2+} stimulates ROS generation that leads to the elevation enzyme activity of the various system such as SOD, POD, and APX (Patra and Sharma, 2000).

6.2.5.3 Lead (Pb)

Pb-inspired toxicity affects various biological processes of the plants such as transpiration, chlorophyll biosynthesis, seed germination, and cell division of the plants. It also perturbs the membrane permeability and alters the activity of enzymes, that play vital roles in the.

metabolism.

6.2.5.4 Arsenic (As)

Arsenic is a toxic metal that is present in the two forms As (V) and As (III) as interferes with the various biological process of plants by modulating the activity of the enzyme such as pyruvate dehydrogenase and 2-oxo-glutarate dehydrogenase. The toxicological events of As are initiated by the generation of oxidative stress (Sneller et al. 1999; Mascher et al. 2002).

6.2.6 *Oxidative Stress*

Oxidative stress conditions refer to a lack of proportion among the systemic alignment of reactive oxygen species. Plant under oxidative stress fails to detoxify the biological system that is needed to reverse the injury caused by damage of reactive oxygen species (Inzé and Van Montagu, 1995). Most of the time, oxidative stress is generated by the cross-talk between biotic and abiotic stress. The oxidative stress symptom might overlap with another abiotic stress. Additionally, oxidative stress plays a vital role in the modulation of the enzyme activity as well as the regulation of the gene (Demidchik, 2015). ROS inspires the interaction with the biopolymer that leads to the destruction of DNA, RNA, and protein. Demidchik (2015) reviewed oxidative stress and revealed that oxidative stress leads to the lipids peroxidation that affects membrane stability, which stimulates the loss of structural integrity of the cell organelles. ROS has an immense oxidative capacity, which can oxidize any macromolecules at the building unit level (i.e. nucleotides and amino acid), which leads to the functional perturbation at the cellular level. Oxidative stress reduces the rate of photosynthesis by the modulation of structural and functional components of photosynthesis-associated assemblies and biological processes (Sedigheh et al. 2011).

6.2.7 Signal Transduction Pathways

Abiotic and biotic stresses produce the most rapid modifications in higher plants, including changes in the electrical potential difference across the plasma membrane. Signal transduction networks for cold, drought, and salt stress could be further classified as three major signaling types: (A) osmotic/oxidative stress signaling that uses MAPK modules, includes the generation of ROS scavenging enzymes and antioxidant compounds and osmolytes; (B) Ca^{2+}-dependent signaling that initiates the activation of late embryogenesis abundant (LEA)-type genes (such as the DRE/CRT class of genes) and involves the formation of stress-responsive proteins mostly of anonymous functions; and (C) Ca^{2+}-dependent salt overly sensitive (SOS) signaling that controls ion homeostasis. It includes the SOS pathway, which is specific to ionic stress (Rodriguez et al. 2005; Kour and Gupta 2005). The sensitivity of the electrical membrane potential to other stimuli suggests that the electrogenic exchange of ions across the plasma membrane could be responsible for the transduction of signals perceived at the plasma membrane. Therefore, a hyperpolarization-activated influx of Ca^{2+} into the host cell could give a pathway for the increase of cytosolic free Ca^{2+} concentrations that mediate the induction of many biochemical pathways that are part of the plant defense response. Biochemical responses are also associated with the plant defense mechanisms. These responses may be inhibited by the depletion of extracellular Ca^{2+} or stimulated in the presence of ionophores (Joseph et al. 1985). This phenomenon permits the entry of Ca^{2+} into the cells. It has also been shown that the fluctuation in cytosolic Ca^{2+} in the cells is required for an effective defense response (Joseph et al. 1985).

It was suggested that drought, salt, and cold stresses bring out transient Ca^{2+} influx into the cell cytoplasm derived from either influx from the apoplastic space or release from internal stores. Channels responsible for the Ca^{2+} influx represent a type of sensor for the stress signals. Ligand-sensitive Ca^{2+} channels manage internal Ca^{2+} release. These ligands are secondary messengers. An important characteristic of the role of Ca^{2+} as a signal is the presence of repetitive Ca^{2+} transients. These transients may be formed both by first-round second messengers and by signaling molecules such as abscisic acid (ABA) that may themselves be produced as a result of cascades of early Ca^{2+} signals. Receptor-like kinases (RLKs) are found in both animals and plants. Structurally, they consist of an extracellular domain that may be responsible for ligand binding or protein-protein interactions, a transmembrane domain, and an intracellular kinase domain. The two-component sensor-response regulator systems, including histidine kinases that were initially found in prokaryotes for the perception of various environmental signals, also exist in eukaryotes, involving plants. When the extracellular sensor domain perceives a signal, the cytoplasmic histidine residue is autophosphorylated, and the phosphoryl moiety is then transferred to an aspartate receiver in a response regulator, which may constitute part of the sensor protein or a separate protein. The sensors may bind with a downstream mitogen-activated protein kinase (MAPK) cascade or directly phosphorylate specific targets to initiate cellular responses. Upon receiving a signal from membrane receptors, cells often use multiple phosphoprotein cascades for the transductions well as amplification of the information. Protein phosphorylation and dephosphorylation may be the most common intracellular signaling modes. They control a wide range of cellular processes such as enzyme activation, assembly of macromolecules, protein localization, and degradation. Secondary signals [i.e., hormones and second messengers: inositol phosphates and reactive oxygen species (ROS)] could start another cascade of signaling events, which may differ from the primary signaling in time and space.

6.3 Mechanism Evolved by the Plants to Combat Abiotic Stress

The plant stress response is a dynamic process that depends upon stress intensity and stress duration. Several stages of plant stress response can be distinguished: an initial alarm phase when stress creates a shock to a nonacclimated plant and the level of plant stress tolerance reduces, an acclimation phase, which remains several days and which further leads to an establishment of new homeostasis in plant metabolism under the influence of stress (the level of plant stress tolerance increases during acclimation phase), a maintenance phase when a newly established homeostasis is regulated under stress conditions (the level of plant stress tolerance remains stable upon maintenance phase), and an exhaustion phase if a stress treatment remains too long. A plant is unable to maintain stress-induced homeostasis (the level of plant stress tolerance declines during the exhaustion phase). After the completion of plant stress treatment, a recovery phase might be seen when a re-establishment of cellular homeostasis under nonstressed conditions occurs (Kosová et al. 2011).

Plants have a well-developed defense system that combats with the mild level of stress by the modulation of the structural and biochemical processes at the cellular level as well as morphological features. At the morphological level, stressed plants inspire the modification of the outer parts of the anatomy that interact directly with the environment such as roots, stems, and leaves. The perturbation of the biochemical process of plants leads to the internal protection of the plants from the abiotic stress. Several biochemical factors play a vital role in the protection from the abiotic stress, such as accumulation of fatty acid, ROS, reactive carbonyl species, osmolytes, secondary metabolites, upregulation of the stress protein, and the stress hormone (He et al. 2018).

6.4 Molecular Mechanisms of Plants During the Abiotic Stress

Abiotic stress also triggers the perturbation of the gene response that may be upregulated/downregulated, which leads to the protection against the abiotic stress. Some examples of genes that participate prominently in the response of abiotic stress via the overexpression are fatty acid–condensing 3-ketoacyl-CoA synthase CER6, alcohol-forming fatty acyl-CoA reductases (FARs), transporters, e.g., nonspecific lipid transfer proteins (nsLTPs), unsaturated fatty acids-3 fatty acid desaturases (FAD3, FAD7, FAD8), lipid biosynthetic glycerol-3-phosphate acyltransferases (GPATs) enzyme that acts as a cofactor of acyl-carrier proteins (ACPs), H_2O_2 reducing ascorbate peroxidases (APXs), GSH conjugating glutathione S-transferases (GSTs), HSP70, HSP16.4, glycine betaine (GB), raffinose biosynthetic galactinol synthases (GOLSs) (Wang et al. 2004; Kim et al. 2005; Timperio et al. 2008; Upchurch 2008; Lee et al. 2009; Hu et al. 2010; George and Parida 2010; Zhou et al. 2014; Singer et al. 2016; Lee et al. 2020). An example of the negative impact of stress on plant genes would be the suppression of genes catabolic proline dehydrogenases (ProDHs) (Hurkman 1992).

Additionally, sugar plays a vital role in the protection against abiotic stress. During the abiotic stress, sugar acts as an osmoprotectant that stabilizes the membrane (Hincha et al. 2000; Hincha, 2002). Sugar plays a dual role in plant growth and development via the upregulation of growth-related genes as well as the downregulation of stress-related genes (Rosa et al. 2009).

6.5 Sugar and Its Role in Growth and Development as Well as Abiotic Stress

Sugar is a nutrient source for plants that are essential for growth and development, which is synthesized by photosynthesis. Mainly two types of sugar molecules are present in the plants, namely, disaccharides and oligosaccharides. The primary function of the sugars is to maintain the structure and functional component of the plant at the cellular level. Sugar assists in the formation of a large and complex polymer that provides morphological strength to the plants (Horacio and Martinez-Noel, 2013). It also helps in the various physiological processes, such as seed germination, promotion of growth, flowering, and delay of senescence (Sami et al. 2016). Sugar acts as the substrates of cellular respiration as well as an osmoprotectant during both cases, either plant's healthy lifespan or stress condition. Sucrose is the leading form of sugar, which is made of two monomeric units, i.e., glucose and fructose. Glucose assists in the initial organ development and expansion of the cell after the division by the maintaining osmosis. At the same time, sucrose plays a leading role in the prime condition of plants (Horacio and Martinez-Noel 2013).

The sugar plays a vital role in the transition stages of the development from the vegetative part to the floral part (Eveland and Jackson, 2012).

Gene involved in the regulation of the sugar biosynthesis also participates in environment-associated stress mitigation (Gill et al. 2001). The compromised sugar level affects the plants in an adverse way: the germination of seed does not take place as well as the growth of seedlings is affected adversely (Gill et al. 2001). The amino acids and sugar maintain the metabolic homeostasis under increasing abiotic stress (Richter et al. 2015). However, sugar level also depletes in the case of heavy metals pollutants, high light irradiance (PAR, UVBR), ozone, and nutrient shortage, (Dubey and Singh 1999). The enhancement of sugar level depends on the genotypes of the plants and diversity of the stress (Castonguay et al. 1995; Morsy et al. 2007). Sucrose upregulates the expression of a gene, which leads to the biosynthesis of various functional components such as starch biosynthetic gene and fructan biosynthesis gene (Wang et al. 2001). The upregulation of stress-responsive gene by the sugar sensing leads to combat in the abiotic stress. The distinct forms of sugar assist in the mitigation of abiotic stress, for example, directly as an osmoprotectant or promoter of other factors such as gene expression and upregulation of enzymes activity.

6.5.1 Sugar Response to Abiotic Stress

Sugar assists in combat against abiotic stress by modulation at various levels, such as physiological, biochemical, and molecular (Gangola and Ramadoss 2018). Different types of abiotic stress deplete the rate of photosynthesis by using several mechanisms. A decrease in the rate of photosynthesis leads to the depletion of sugar generation, and as a result, plants have to compromise with their growth and development. Sugar maintains homeostasis of the plants during the stress condition. The elevation of the soluble sugar level stimulates the tolerance to various types of abiotic stress, such as drought, heavy metal toxicity, salt, heat, and oxidative stress (Dubey and Singh 1999). Sugar acts as signaling molecules, which regulates genes positively as well as adversely and leads to the fitness of plant in the abiotic stress condition (Sami et al. 2016). Sugar improves the tolerance of plants from several types of abiotic stress. The sugar molecules act as an antioxidant indirectly via the modulation of another antioxidant system (Gangola and Ramadoss 2018). Disaccharides commonly found in the plants (sucrose, trehalose, maltose, and lactose) have shown antioxidant potential under in vitro condition (Wehmeier and Mooradian 1994). Sugar acts as a scavenger of free radical in the plant system, which leads to protection from the various types of abiotic stress. Sugar also functions as Osmoprotectants, which protect plants from the different abiotic stresses via the regulation of turgor pressures in the internal environment of the plant. Hydrogen bond formation capability of the sugar leads to the protection from the abiotic stress via the stabilization of native macromolecules structures as well as membrane integrity (Pukacka et al. 2009). At the same time, they are simultaneously assisting in growth and development in order to combat abiotic stress by promoting the upregulation of the ABA hormone-related gene expression (Dekkers et al. 2008). Dekkers et al. (2008) reported that the gene related to ABA and glucose signaling functionally overlaps.

6.5.2 Sugar-Associated Gene Regulation in the Abiotic Stress

Sugar plays a vital role in abiotic stress through the enhancement of plant fitness via several mechanisms. Sugar upregulates the expression of the various phytohormones, which is a leading mechanism for combat with abiotic stress. Rolland et al. (2006) revealed that the sugar controls various genes at distinct levels, such as regulation, transcription, translation, and posttranslation modification. The glucose-regulated gene upholds the regulation of ABA expression coupled with osmotic stress that leads to the fitness in the abiotic stress (Dekkers et al. 2008).

6.5.3 Fructan and Its Biosynthesis Mechanism and Metabolism

A fructan is the main form of sugar, which plays an essential role in the abiotic stress defense. The fructan has been classified into four classes based on the linkage of monomeric unit (Table 6.1). The formation of structurally diverse fructans has been taking place by the activity of the elongation of specific enzymes.

6.5.3.1 Biosynthesis of Fructan

The sucrose is a leading substrate for fructan biosynthesis via the activity of the different enzymes along with the other molecules. The diversity of the structural features of fructan takes place by the activity of 4 enzymes that catalyzes the formation of diverse structural fructans, such as 1-SST (sucrose-sucrose fructosyltransferase), 6-SFT (sucrose: fructan 6- fructosyltransferase), 1-FFT (fructan fructosyltransferase), and 6G-FFT (6G- fructan 6- fructosyltransferase). Various fructosyltransferase

Table 6.1 Heterologous expression experiments using fructosyltransferase genes in crop plants

Type	Representative species	Linkage (β)	Initial trisaccharide
Inulin	Chicory, Jerusalem artichoke	2-1	1-kestose
Levan	Dactylis glomerata	2-6	6-kestose
Branched	Wheat, barley	2-1 and 2-6	1- and 6-kestose
Inulin neoseries	Onion, asparagus, Lolium	2-1	6G-kestotriose (neokestose)

enzymes work simultaneously on the functional groups of sucrose to convert it to a fructan molecule.

Biosynthesis of the various fructan molecules has been taking place by the activity of the 1-SST enzyme, which catalyzed sucrose conversion into 1-Kestotriose by releasing one glucose molecules. 1-Kestotriose, along with sucrose molecules, can convert the different forms of fructan intermediates via the activity of the various enzymes. The activity of 1-FFT enzymes converts β (2-1) linkage containing inulin by the shifts of a single terminal fructose molecule from an oligosaccharide to the same carbon position on another molecule, thus producing the fructan. In contrast, the activity of 6-SFT enzymes inspires the formation of 6-Kestotriose by using sucrose as a substrate. Secondary 6-Kestotriose convert into the Levan β (2-6), which are new types of fructans.

Alternatively, 1-Kestotriose is a molecule that acts as substrates of the multiple fructans along with the activity of enzymes. The activity of 6G-FFT enzymes leads to the formation of 6 G -Kestotriose (Neokestose) by using 1-Kestotriose and sucrose. The 6G-Kestotriose acts as the substrates of the two enzymes, which convert it into the two products such as Levan neo-series β (2-6) as well as inulin neo-series β (2-1). The conversion of Levan neo-series is catalyzed by the activity of 6-SFT by using 6 G-Kestotriose as a substrate meanwhile the conversion of inulin neo-series β (2-1) takes place by the activity of the 1-FFT enzymes.

Additionally, the combination of 1-Kestotriose and sucrose molecules gets converted to bifrucose by the activity of 6-SFT enzymes. MBifrucose acts as substrates of the diverse structural forms of fructans such as Mixed-type of Levan β (2-1) and β (2-6) as well as levan β (2-6). The activity of the two enzymes 6-SFT and 1-FFT forms Mixed-type of Levan that have both linkage β (2-1) and β (2-6) by using bifrucose as a substrate. However, the combined activity of 1-FFT and 6-SFT, along with the FEH (Fructan Exo hydrolase) enzyme, leads to the formation of Levan β (2-6).

The enzymes involved in fructan biosynthesis function by englongating the sucrose molcelues that later converts into distinct forms of fructan while FEH hydrolase the β (2-1) linkage and β (2-6) linkage (Chalmers et al. 2005). The regulation of the activity of these enzymes may lead to the accumulation of distinct classes of fructan.

6.5.3.2 Role of Fructan in Different Forms of Abiotic Stress

The upregulation of fructan metabolism takes place in response to cold temperatures. The utilization of the glucose molecules leads to the fructan biosynthesis. Fructan also plays a vital role in the stabilization in the biological membrane. Van den Ende and Valluru (2009) reported that fructans stabilized the membrane of the tonoplasts by the clarification of the free radical. An elevation of the fructan level also stimulates the reservoir of ascorbate and glutathione, which assists in the protection from the abiotic stress (Bolouri-Moghaddam et al. 2010). Fructans act as potent antioxidant that scavenges ROS more efficiently when compared to other disaccharides. Fructans, fructan exohydrolases, and freezing mechanism Various cell organelles could get some significant damage due to abiotic stresses, especially, the plasma membrane; this can be resolved by plasma membrane–protecting compounds, such as various sugars. However, this does not signify that all other cellular membranes (e.g., the tonoplast) do not need to be stabilized under stress conditions. Liposomes are spherical vesicles composed of bilayer membranes that are often utilized to study membrane stabilization in vitro. Both mono- and disaccharides are useful in membrane stabilization, whereas polysaccharide (hydroxyethyl starch, glucan, and dextran) does not show similar protection capability. Nevertheless, the behavior of fructans is different, and it is capable of reducing the leakage of the soluble contents from liposomes, probably by decreasing the 'transition temperature' (TM) at which a phospholipid membrane changes from a packed, ordered, solid-like state into a more random, fluid-like state. A study with inulins isolated from chicory (Cichorium intybus) roots and Dahlia (Dahlia variabilis) tubers revealed that inulins stabilize egg phosphatidylcholine (PtdCho) during freeze-drying by maintaining a direct hydrogen-bonding interaction between the phosphate groups of egg PtdCho and the inulin hydroxyls.

Fructans behave more like low molecular mass sugars, reducing leakage from liposomes after freeze-drying. This may be due to the greater mobility of fructan chains, with a $-O-CH_2-$ between each constituent monosaccharide, as compared to glucan chains. The breakdown of fructans is carried out by fructan exohydrolases (FEHs). Apoplastic FEHs are proposed to depolymerize apoplastic fructans and thus generate a mixture of sugars (hexoses, sucrose, and fructan oligosaccharides) that could be optimal for efficient membrane stabilization in vivo. Measurements of fructan protection of membranes in vitro showed that a low degree of polymerization (DP) in fructans from oat (Avena sativa) and rye (Secale cereale) was more protective than higher DP fractions. Mixtures of fructans, as they occur in living cells, might have protective properties that differ significantly from those of the purified fractions (Valluru et al. 2008).

6.6 Fructan Bioengineering

The biosynthesis of fructan molecules is initiated by using sucrose as primary substrates by the catalyzation reaction of several enzymes. The activity of different enzymes on the same substrates transforms it into a different form of fructan end products. The positive and negative regulation of a gene related to the fructan biosynthesis pathway leads to the expression of the enzyme, which plays leading roles in the management of the abiotic stress. A study conducted by Vijn et al. (1997) focussing on expression of engineered 6G-FFT sourced from onion plant identified several folds higher accumulation of neokestose and fructans on inulin neo-series. It was also revealed that the protein was homologous with the 6-SFT and plant invertase which is expressed in tobacco along with trisaccharide 1-kestose and sucrose. Knipp and Honermeier (2006) studies revealed that the transformation of

SST/FFT enhances the proline accumulation in the potato plants and also strengthens the level of soluble sugar. Tobacco plants do not have the capacity to the synthesis of the fructans; however, the expression of the barley 6-SFT gene leads to the biosynthesis of trisaccharides kestose as well as phlein type (beta-2,6 linkages) which is an unbranched fructan (Sprenger et al. 1997).

Additionally, the transformation of barley 6-SFT leads to the biosynthesis of branched graminan fructan that is tetrasaccharides fructans (Sprenger et al. 1997). The functional characterization Fructan: fructan 6G-fructosyltransferase (6G-FFT)/ fructan: fructan 1-fructosyltransferase (1-FFT) (from Perennial Ryegrass (*Lolium Perenne L.*)), which are expressed in different sites, revealed that the expression of these genes depends on the locations as well as developmental stages of plants (Lasseur et al. 2006). Lasseur et al.'s (2011) studies revealed that the biosynthesis of the distinct class of the fructans highly correlated with enzyme activity along with substrates diversity, such as the formation of structurally diverse fructans in the two species *L. perenne* and *H. vulgare*. However, the 6-SFT enzymes from these two species have almost similar catalytic properties. The expression of three genes that encode among them; one sucrose-sucrose 1-fructosyltransferase (1-SST) and the other two genes encode fructan-fructan 6G-fructosyltransferase (6G-FFT); studies revealed that the expression of the gene depends on the accumulation of the end product in the response of the cold stress (Hisano et al. 2008).

Fructan is an essential sugar that protects plants from abiotic stress. The biosynthesis of the fructan has been taking place from the sucrose molecules. The modulation of enzyme activity that participates in the biosynthetic pathway and metabolism leads to the accumulation of structurally diverse fructan molecules (Fig. 6.1; Ritsema and Smeekens, 2003). A list of genes related to the fructans biosynthesis and their role in abiotic stress are given in Table 6.2.

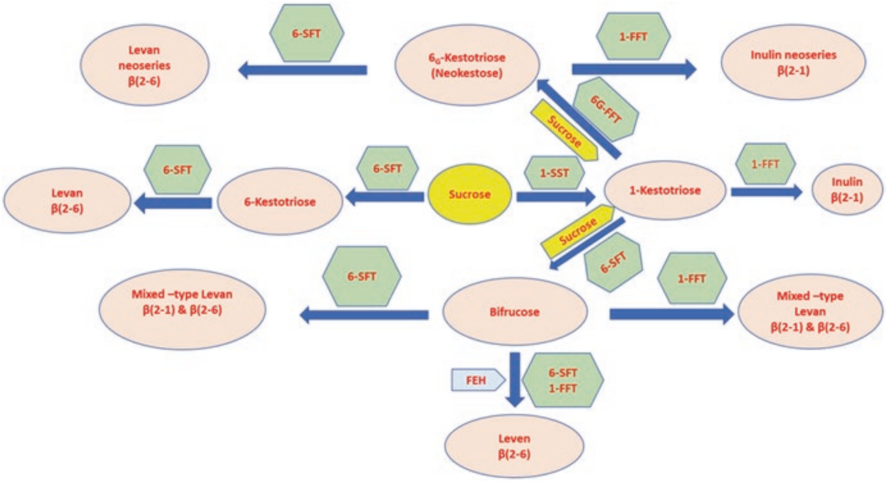

Fig. 6.1 Proposed pathway of structurally diverse fructan (Modified by using Livingston et al. 2009)

Table 6.2 A list of genes related to fructans biosynthesis and their role in abiotic stress

S. No.	Plant species	Gene (s)	Abiotic stress	Impact of gene (s) on fructans biosynthesis	References
1.	Tobacco	*1-FFT, Ht1-FFT*	Drought	+	Sun et al. (2020)
2.	Ryegrass	*Lp1-FFT*	Cold	+	Abeynayake et al. (2015)
3.	Blue grass	*Pp6-SFT, PpFEH, PpCAP*	Cold	+	Rao et al. 2011
4.	Rice	*wtf2, 1-SST*	Cold	+	Kawakami et al. (2008)
5.	Barley and ryegrass	*1-SST, 6G-FFT*	Cold	+	Hisano et al. (2008)
6.	Tobacco	*1-SST*	Cold	+	Li et al. (2007)
7.	Tobacco	*sacB*	Cold	+	Suzuki et al. (2006)
8.	Wheat	*1-SST, 6-SFT*	Drought	+	Xue et al. (2008)
9.	Tobaco	*6-SFT*	Drought	+	Li et al. (2007)
10.	Potato	*SST/FFT*	Drought	+	Knipp and Honermeier, (2006)
11.	Beet	*sacB*	Drought	+	Pilon-Smits et al. (1999)
12.	Tobacco	*sacB*	Drought	+	Pilon-Smits et al. (1996)

+ = Increase fructans accumulation

6.7 Breeding Approaches

Although we have strong evidences of significant role that Fructans play in aboitic stress tolerance in crop plants, breeding approaches focussing on this aspect have not been sufficiently exploited. Scientific studies have identified that crop plants like wheat, onion, garlic and chicory have high fructan content. According to the available literature, the genes or QTLs related to the high fructan content have not been used in the breeding programs using marker-assisted selection (MAS). It has also been shown that fructan content is profoundly affected by the environment, and no single dominant gene is responsible for the high content of fructan except few reports. To the characterization of high grain fructan content in plants, some QTL mappings have been performed in few crop species, like wheat, ryegrass, onion, etc. (McCallum et al. 2006; Turner et al. 2008; Zeng et al. 2020). The fructan content is adversely affected by light intensity, temperature, and agronomic practices. It has been seen that genotype-by-environment (G × E) interaction is also associated with the synthesis of fructans (Huynh et al. 2008). The research work conducted previously was mainly related to the physiological and biochemical aspects (Savitch et al. 2000; Hisano et al. 2004) and also the health benefits (Guo et al. 2015; Veenstra et al. 2017). The QTLs related to the high fructan content in wheat have been mapped on the chromosome 2B, 2D, 3B, 5A, 6D, and 7A in which two QTLs located on 6D and 7A were important and explained 17% and 27% of phenotypic variation, respectively (Huynh et al. 2008; Dong et al. 2016).

The decisive role of high fructan content in drought stress tolerance has been observed in ryegrass (Lolium perenne) using the F_2 mapping population that segregates for carbohydrate metabolism. In this study, 25 QTLs were identified and explained 8.9% to 34.1% of the total phenotypic variation (Turner et al. 2008). A QTL located on chromosome 7 was responsible for drought tolerance and that provided the 28% phenotypic variation to fructan content in barley. (Hayes et al. 1993). The pleiotropic effect of QTL has also been observed for frost tolerance and high fructan content in wheat (Roberts et al. 1993).

6.8 Examples of the Utilization of Genes in the Crop Improvement Program

The genetic fitness of the plants plays a vital role in tolerance from the abiotic stress. The regulation of the gene positively and negatively leads to the lifespan enhancement of the plant while facing abiotic stress. Several methods have been used for the expression of the desired gene (Singla-Pareek et al. 2001). SacB gene from the Bacillus subtilis has been applied in the tobacco plants that lead to accumulation of the fructans, which assists in the combat with drought stress (Pilon-Smits et al. 1995).

Another important sugar Trehalose acts as an osmoprotectant, principally from the drought stress. The expression of Trehalose-6-phosphate synthase leads to the accumulation of trehalose biosynthesis in the plants, which leads to the protection from the drought stress by the loss of detached leaves that slows down the water loss (Romero et al. 1997). The overexpression of the fructan biosynthesis gene leads to the accumulation of the gene-specific product in the plants (Vijn et al. 1998).

6.9 Transgenic Approaches

Plants those have modified gene are considered as transgenic plants (genetically modified organism). The preparation of transgenic plants is a popular way of improvement to the crop. The transgenic approach has been used for the improvement of the overall fitness of the plants, such as abiotic stress and biotic stress (Table 6.3). Several crops have been commercialized successfully in the market, such as Bt-cotton, Bt-brinjal, and golden rice. The upregulation of genes related to the synthesis of the osmoprotectant, such as biocompatible alcohol, sugar, and other class of osmoprotectant, leads to the protection from the abiotic stress (Hussain et al. 2012). The expression/overexpression of genes related to sugar accumulation

Table 6.3 Effects of fructan biosynthetic genes in transgenic plants

Transformed genes	Source plant	Host plant	Effect	References
Sucrose:6-fructosyltransferase gene (6-SFT)	American dune grass (*Leymus mollis*)	*Arabidopsis thaliana*	Positively regulates salinity tolerance and enhances fructan levels	Li et al. (2019)
Fructan 1-fructosyl-transferase (1-FFT) gene (Ht1-FFT)	Jerusalem artichoke (*Helianthus tuberosus*)	Tobacco (*Nicotiana tabacum*)	Increased the PEG-simulated drought stress tolerance	Sun et al. (2020)
Sucrose:sucrose 1-fructosyltransferase (1-SST) enzyme gene (Ht1-SST) and fructan:fructan 1-fructosyltransferase (1-FFT) enzyme gene (Ht1-FFT)	Jerusalem artichoke (*Helianthus tuberosus*)	Potato (*Solanum tuberosum*)	Resistance against abiotic stress by cold to drought	Moon et al. (2019)
Fructan-6-fructosyltransferase gene (6-SFT)	*Dasypyrum villosum*	Tobacco (*Nicotiana tabacum*)	Enhances abiotic tolerance	He et al. (2017)
wft2 (sucrose:sucrose 1-fructosyltransferase) and wft1 (sucrose:fructan 6-fructosyltransferase)	Wheat (*Triticum aestivum*)	Rice (*Oryza sativa*)	Enhancing chilling tolerance	Kawakami et al. (2008)
Ta1-SST, Ta6-SFT, and Ta1-FFT	Wheat (*Triticum aestivum*)	Tobacco (*Nicotiana tabacum*)	Increased tolerance to drought, low temperature, and high salinity	Bie et al. (2012)

enhances plant fitness in abiotic stress. Several genes have been genetically engineered, which helps in the upregulation of biosynthesis of compounds that leads to the protection from abiotic stress such as mannitol, glycine betaine, and heat shock proteins. The expression of a heterologous gene such as sucrose: sucrose 1-fructosyltransferase and fructan: fructan 6G-fructosyltransferase genes from onion (*Allium cepa*) leads to the elevation of three-fold fructan biosynthesis in perennial ryegrass lines (Gadegaard et al. 2008). Genetic engineering tools, which are recently in the trends such as CRISPR/CAS9 and antisense, RNAi, and CRISPR/Cas9 technologies, have been playing leading roles in the protection of the crops from the abiotic stress via the modulation of genes related to plant growth and development (Parmar et al. 2017).

6.10 Conclusion and Prospects

The roles of fructans in plants and human health have been well established, with the help of some techniques, the fructans' contents in crop plants have been measured, but it is time consuming and labor intensive. Although these methods are sufficient for the quantification of fructan content, some sensor-based techniques or equipment should be developed for speeding up the breeding programs. In spite of roles in health and tolerance, the high grain fructan may play a decisive role in early vigor to seedlings by supplying nonstarch carbohydrate stores during germination and initial growth stages. The role of fructans in abiotic stresses should also be aligned with yield traits. The development of crop varieties with high fructan content can provide more resilient crop plants with higher adaptation to abiotic stress conditions. Using the genetic engineering, the fructan level can be enhanced in crop plants, and transgenic crops should be utilized in the mainstream breeding programs. Wheat fructans also exhibit many desirable physiological impacts on humans and plants. The heritability of fructan content in wheat grains was revealed to be moderate to high (h2 = 0.64–0.94) (Huynh et al. 2008). On the basis of this observation, it has been concluded that breeding programs for the high fructan content should be taken at priority. Some QTLs related to high grain fructan content have been identified in few crop species but not utilized in breeding programs, so far. A molecular technique called MAS should be used along with the conventional breeding approaches for the selection of plants having high level of grain fructan content. For this purpose, robust QTLs are necessary, but most of the QTLs are not found in all the genetic backgrounds of particular plant species, and the effects of QTLs are not consistent. To overcome the problem, some breeding methods such as genomic selection (GS) and genetic engineering can also be utilized in breeding for the enhancement of fructan content. The cost of phenotypic screening of desirable traits within the plant population can be decreased using the GS. This approach can accelerate breeding cycles and the selection of plants based on the whole genome (Heffner et al. 2009). Using GE, the fructan metabolism is changed during the growth of the plant, and grain development is another breeding option (Verspreet et al. 2015). Any change in the fructan synthetic enzymes (1-SST, 1-SFT, 6-SFT, and 6G-FFT) can promote the upregulation of fructan synthesis genes and downregulate the fructan hydrolytic enzymes (1-FEH and 6-FEH), so that genetic engineering can also be increased, the fructan content in crop plants could be an efficient method along with other traditional plant breeding methods. The molecular mechanism for the synthesis of high fructan content and the existence of linkage drag or pleiotropy is not well known. The genotype-by-environment (G × E) interaction adversely affects the fructan content in plants, and allows a planned design for the breeding of high fructan content in plants. Despite a large number of challenges associated with the enhancement of fructan content in crop plants, it is still a desirable trait for the breeding of high yielding and abiotic stress-tolerant varieties.

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Chapter 7
The γ-Aminobutyric Acid (GABA) Towards Abiotic Stress Tolerance

Vikas Srivastava, Sonal Mishra, Aksar Ali Chowdhary, Skalzang Lhamo, and Shakti Mehrotra

Abstract The frequent exposure of plants to several environmental constraints (abiotic and biotic stress) limit plant growth and yield globally. These constraints are managed by plants through morphological, physiological and molecular alteration to resume the loss underway. Amongst, the priming with chemicals including GABA is a promising approach. GABA (γ-aminobutyric acid) is a non-protein amino acid produced through GABA-shunt, and usually accumulates in several plant species when subjected to any environmental stress (salt, drought, heat, etc.). Its accumulation or exogenous application leads to adjustments at many levels under stress conditions, to maintain the usual plant growth and development and therefore may provide better outcome in terms of productivity. The present chapter offers understanding of GABA metabolism and its significance towards promoting abiotic stress tolerance in plants.

Keywords Abiotic stress · Cold · Drought · GABA · Heavy Metal · Heat · Metabolism

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7.1 Introduction

The plants being sessile are frequently exposed to various environmental constraints both naturally or under agricultural set-up. These limitations are majorly unpredictable and lead to significant loss in crop productivity. Amongst such constraints, the abiotic factors/stress (salinity, drought, flooding, heat, cold, freezing, excess light, UV radiation, and heavy metal toxicity) play higher participation and majorly limit the global crop yields (Wang et al. 2003; Jain 2013). This majorly involves oxidative stress mediated by ROS, which may impact the plant by regulating several developmental features (Mishra et al. 2017). In certain instances, abiotic stress also provides ways to invite several biotic stress (Vijayakumari et al. 2016), thereby further promotes crop losses. To cope with these limitations, plants have developed methods that mainly involve participation of stress-related genes. Such genes remain silent during the normal course of plants' life and get up-regulated during stress conditions (Gao et al. 2007, Atkinson and Urwin 2012). Despite their direct relevance in stress tolerance, the transgenic for such genes many times exhibited compromise of desired traits; therefore, the development of methodologies that can better address the plant stress tolerance along with maintaining plant productivity is essentially required.

Plant priming (sensitization or hardening) is considered as one of the effective strategies which activate faster and/or stronger protective responses, when stress (biotic or abiotic) pressure is encountered. The strategy is originally devised for biotic stress, where a non-pathogenic microbe can boost plant immunity, thereby providing a strong defence, when the plant encounters any pathogenic agent in subsequent life. In recent past, the exogenous application of chemicals that can mitigate stress conditions (chemical priming) was found effective to better manage plants encountered to stress. In the 'primed' state, plants attain a unique physiological condition, which generates mild stress, and thus let plants to withstand further strong stress conditions (Savvides et al. 2016). Some of the important priming agents are sodium nitroprusside, hydrogen peroxide, sodium hydrosulfide, melatonin, polyamines, GABA, BABA, etc. (Vijayakumari et al. 2016; Savvides et al. 2016).

The γ-aminobutyric acid (4-aminobutyric acid, GABA), is a non-protein amino acid, which possesses an amino group at γ-carbon rather than α-carbon, therefore does not integrate into proteins. It contributes significantly to the free amino acid pool and exists in unbound form (Shelp et al. 1999). It exhibits zwitterionic features at physiological pH, high solubility in water and can attain many conformations in solution, including cyclic structure like proline (Christensen et al. 1994). The metabolism behind GABA is known as GABA shunt which bypasses two steps of the tricarboxylic acid cycle (Bouche and Fromm 2004). GABA has a wide distribution in prokaryotes and eukaryotes. The discovery of GABA in plants was reported by Steward et al. (1949); however, its occurrence is conserved from bacteria to plants and to vertebrates (Bouche and Fromm 2004). In animals, the study mainly focuses GABA as a signalling molecule and found to be accumulated in brain and functions in neurotransmission (Kinnersley and Turano 2000; Bouche and Fromm 2004). In crayfish stretch receptor neurons, it was reported to suppress impulses (Awapara et al. 1950; Elliott and Jasper 1959).

Though the contribution of GABA as a signalling molecule was considered quite early in animals, in plants, until recently, it was generally considered as a carbon-nitrogen metabolite (Shelp et al. 1999; Bouche et al. 2003; Bouche and Fromm 2004). In plants, the recent outlook suggested it as an endogenous signalling molecule for plant growth regulation and plant development (Carillo 2018). In plants, the accumulation of GABA is noticed under environmental stress conditions which facilitate plant chemical response to mitigate stress. Apart, it also has multiple functions under non-stressed conditions. It serves as a key metabolite of nitrogen metabolism and involves both primary and secondary metabolic pathways. Besides, it also provides source of carbon skeleton through GABA shunt for further down-stream metabolism (Ramos-Ruiz et al. 2019). Two mechanisms have been noticed for stress-induced GABA accumulation in plants viz. cytosolic acidification and Ca^{2+} rise. It has been noticed that stress resulted in cytosolic acidification which induces low pH-dependent activation on glutamate decarboxylase and therefore GABA biosynthesis and accumulation. Such acidic conditions are predominantly found under oxygen-deprived conditions as generated during flood stress. Further, other stresses (viz, cold, heat, salt, etc.) subsequently increase Ca^{2+} level in the cellular environment. These high cytosolic Ca^{2+} levels activate calmodulin-dependent glutamate decarboxylase activity and GABA biosynthesis (Kinnersley and Turano 2000).

The early decades of GABA research mostly considered it as metabolite, though argue exist regarding its functioning as signalling molecules (Bouche and Fromm 2004; Bown and Shelp 2016). However, there are certain observations that backed the signalling feature of GABA as highlighted in a review (Ramesh et al. 2015), which include features like rapid increase in response to biotic and abiotic stress, gradient of GABA concentration across plant tissues, inter- and intracellular compartmentation of GABA metabolism, discovery of GABA-binding site in plant cell membrane, and GABA-regulated ion channels in plants. Under several abiotic (hypoxia, heat, cold, drought, mechanical wounding) or biotic stress (wounding due to herbivory and infection), the quick accumulation of GABA was noticed (Shelp et al. 1999; Shelp et al. 2012). The accumulation promotes plant growth and can alleviate stress by up-regulating the anti-oxidant defence systems. Its metabolism is also involved in the nitrogen recycling and reallocation during leaf senescence in response to abiotic stress (Jalil et al. 2017; Jalil and Ansari 2020). Considering the diverse aspects of GABA in plant stress management, present chapter will provide judicial compilation on the representative studies of GABA as a stress alleviator and promises to combat the adverse situation.

7.2 GABA Shunt and GABA Metabolism

The biogenesis or metabolism of GABA involves a short pathway referred as GABA shunt which bypasses two steps of tricarboxylic (citric) acid cycle as mentioned in Fig. 7.1. Compared to TCA cycle, the shunt provides less energy as it directly converts α-ketoglutarate to succinate, and therefore does not produce high-energy

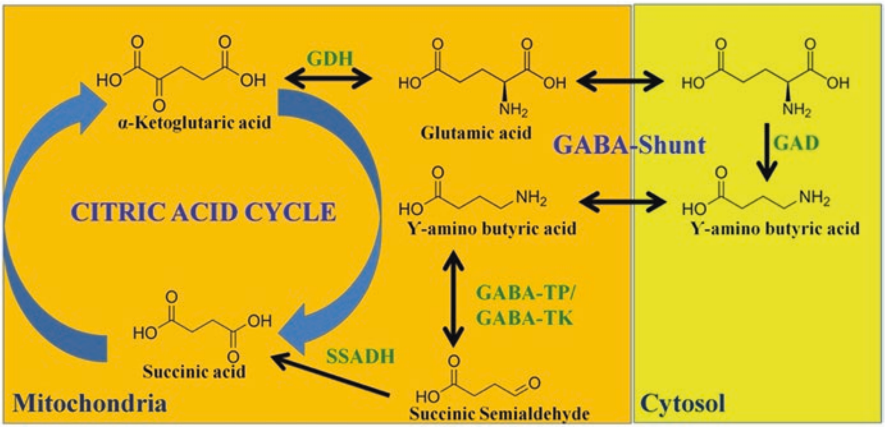

Fig. 7.1 Schematic representation of the metabolism of GABA via GABA-shunt and citric acid cycle regulation; enzymes involved are indicated in green bold, reversible reactions in arrow with both heads. Other abbreviations include GDH, glutamate dehydrogenase; GAD, glutamate decarboxylase; GABA-TP, GABA: pyruvate transaminase; GABA-TK, GABA: α-ketoglutarate transaminase; SSADH, succinic semi-aldehyde dehydrogenase

molecule viz., reduced nicotinamide adenine dinucleotide phosphate (NADH) molecule and guanosine triphosphate (GTP) molecule (Singh and Roychoudhary 2020). The precursor molecule of this shunt is α-ketoglutarate, which in mitochondria undergoes oxidative deamination and is converted to glutamate by glutamate dehydrogenase (GDH). Glutamate is then transported to cytosol and subsequently processed by GABA shunt enzymes.

The metabolism further involves participation of enzymes, comprising of one cytosolic (Glutamate decarboxylase, GAD) and two mitochondrial (GABA transaminase, GABA-T and Succinic semi-aldehyde dehydrogenase, SSADH) enzymes. The first enzyme (GAD) converts glutamate to GABA, along with a co-product carbon dioxide. In *Arabidopsis,* it was reported that GAD isoforms are expressed in tissue-dependent manner and their activity is modulated by Ca^{2+}–CaM (Zik et al. 1998a, b). However, this feature of Ca^{2+}–CaM is not mandatory as in rice, here GAD isoform lacks CaM-binding domain (Akama et al. 2001).

The GABA is then transported (through mediation of gamma-aminobutyric acid permease, GABAP; Michaeli et al. 2011) and metabolized in mitochondria to produce succinic semi-aldehyde (SSA). The GABA-T (GABA transaminase) facilitates this conversion, using either α-ketoglutarate (GABA-TK) or pyruvate (GABA-TP) as amino acid acceptors in plants, producing glutamate or alanine, respectively (Shelp et al. 1999). The succinic semi-aldehyde is then converted to succinic acid by the activity of succinic semi-aldehyde dehydrogenase (SSADH) (Ham et al. 2012). The association of GABA shunt with tricarboxylic acid cycle links carbon and nitrogen metabolism in plants. Beside this, SSA can also be transported to cytosol, where it produces γ-hydroxybutyric acid (GHB), through the activity of succinic semi-aldehyde reductase (SSR) (Singh and Roychoudhary 2020). Addition to this polyamine (spermidine and putresscine) catabolism and proline under oxidative stress can also produce GABA (Fait et al. 2008; Signorelli et al. 2015).

7.3 GABA: Significance Under Abiotic Environmental Constraints

During environment stress such as drought (Hanower and Brzozowsak 1975), heat (Mayer et al. 1990), cold (Wallace et al. 1984), there is high production of GABA; the intensity of increase is so high, as the cellular level of this non-protein amino acid exceeds the amino acid involved in protein synthesis (Shelp et al. 1999; Kinnersley and Turano 2000). It was observed that large amount of GABA is produced during biotic and abiotic stress and its metabolism was found connected with many features viz., C:N balance, regulation of cytosolic pH, osmoregulation, defence against oxidative stress, etc. (Bouche and Fromm 2004; Ham et al. 2012).

The endogenous production of GABA or its exogenous application has very high implication on plant stress mitigation. During this, the GABA is proposed to function as signalling molecule and found associated with nitrogen metabolism, cytosolic pH regulation, and offer protection against oxidative damage generated in response to several abiotic stresses (AL-Quraan et al. 2013). The priming using different chemicals has been observed as an effective strategy for the enhancement of abiotic stress tolerance. Vijayakumari et al. (2016) has recently reviewed the priming potential for non-protein amino acid-GABA along with BABA (β-amino butyric acid). Rapid γ-Aminobutyric acid (GABA) accumulation was observed, when plants are exposed to stress, which either exhibit the metabolism regulation in response to stress or an adaptive response for stress mitigation (Bown and Shelp 2016).

Further, the exogenous GABA application bestows stress tolerance by modulation of the genes associated with plant signalling, transcriptional regulation, hormone biosynthesis, reactive oxygen species production, and polyamine metabolism (Podlešáková et al. 2019). The accumulation and metabolism of GABA itself are regulated by several other biological molecules. Many evidence has also suggested the cross-talk between GABA and polyamines/ hormones (viz. abscisic acid, cytokinins, auxins, gibberellins and ethylene), where one affects the signalling pathway and metabolism of others (Podlešáková et al. 2019). Recently, the contribution of GABA in alleviation of salts stress, salinity-alkalinity stress, drought stress, heat stress, chromium stress etc., has been reported in a number of studies using different plants (Table 7.1). Few of the relevant stress studies are as follows:

Table 7.1 Response of exogenously supplied GABA to various abiotic stresses

S. No	Crop	Concentration	Response to abiotic stress	References
1	*Agrostis stolonifera*	0.5 mM	Induced tolerance to heat stress	Li et al. (2016a)
2	*A. stolonifera*	0.5μmol	Enhanced tolerance against heat stress	Li et al. (2019a)
3	*A. stolonifera*	0.5 mM	Induced drought tolerance	Li et al. (2019b)
4	*A. stolonifera*	0.5 mM	Improved tolerance to drought and heat stress	Li et al. (2018)
5	*A. stolonifera*	0.5 mM	Improved tolerance to drought/heat stress	Li et al. (2016)
6	*Brassica juncea L.*	125μM	Confers tolerance to chromium stress	Mahmud et al. (2017)
7	*Camellia sinensis L.*	5.0 mM	Improved tolerance to cold	Zhu et al. (2019a, b)
8	*Cucumis melo L.*	50 mM	Enhanced resistance to salinity-alkalinity stress	Xiang et al. (2016)
9	*C. melo L.*	50 mM	Increased tolerance to salinity-alkalinity	Chen et al. (2018)
10	*C. melo L.*	50 mM	Enhanced tolerance to salinity alkalinity	Jin et al. (2019)
11	*C. melo L .*	50 mM	Enhanced tolerance to calcium nitrate stress	Hu et al. (2015)
12	*H. vulgare L.*	0.5 mM	Increased tolerance against salinity stress	Ma et al. (2018)
13	*Lactuca sativa L.*	25μM	Enhanced salt tolerance	Kalhor et al. (2018)
14	*Lolium perenne*	50 mM	Promote tolerance against drought stress	Krishnan et al. (2013)
15	*Nigella sativa L.*	2.0 mg L^{-1}	Improved tolerance to drought stress	Rezaei-Chiyaneh et al. (2018)
16	*Oryza sativa L.*	0.5 mM	Enhance tolerance to arsenite stress	Kumar et al. (2019)
17	*O. sativa L.*	1 mM	Partially enhanced tolerance against heat stress	Nayyar et al. (2014)
18	*Solanum lycopersicum L.*	5 mM	Improved resistance against salinity stress	Wu et al. (2020)
19	*Trifolium repens L.*	1μM	Increased resistance against salinity stress	Cheng et al. (2018)
20	*Triticum aestivum L.*	0.5 mM	Increased tolerance against salinity stress	Li et al. (2016c)
21	*T. aestivum L.*	50 mg/L	Promoted tolerance to salinity stress	Wang et al. (2017a)

(continued)

Table 7.1 (continued)

S. No	Crop	Concentration	Response to abiotic stress	References
22	*Vigna radiate L.*	1 mM	Improved tolerance against heat stress	Priya et al. (2019)
23	*Zea mays L.*	25 and 50µM	Confered tolerance against cadmium stress	Seifikalhor et al. (2020)
24	*Z. mays L.*	0.5 mM	Improved tolerance against salinity stress	Wang et al. (2017a, b)

7.3.1 Salt Stress

The salinity in soil has been reported to severely devastate plant growth, thereby crop productivity, due to its negative impact on several metabolic and physiological processes. This impact mostly includes photosynthesis inhibition, ROS, and other metabolism. It has been reported that in salt-sensitive variety, there is inhibition of growth and development, reduction in respiration, photosynthesis, and protein synthesis (Parida and Das 2005; Munns and Tester 2008; Hussain et al. 2013). In addition to the above effects, the salt stress also causes generation of reactive oxygen species in chloroplast and mitochondria that lead to oxidative damage in plants (Masood et al. 2006). To deal with the stress, plant produces various metabolites that offer stress tolerance including GABA. Like other stresses, the GABA accumulates during salt stress, which maintains the pH and carbon/nitrogen metabolism for Kreb's cycle and scavenges free radicals through anti-oxidant systems (Jalil and Ansari 2020). Jalil and Ansari (2020) have described the salt tolerance mechanism by GABA, which includes promotion of photosynthesis and chlorophyll fluorescence, regulation of oxidative stress, and up-regulation of anti-oxidant activity and osmotic regulation through osmolyte accumulation.

The exogenous GABA application exhibited improvement in photosynthetic capacity and anti-oxidant enzyme activities and decreased MDA content and electrolyte conductivity, thereby mitigating the impact of salinity on the wheat seedlings (Li et al. 2016). The exogenous root-drenching-based GABA application to salt-stressed (moderate-150 mM to severe- 300 mM) maize seedlings exhibited an increase in endogenous GABA concentration, improves seedling growth, and demonstrates reduced GAD activity (Wang et al. 2017a, b). The exogenous applications offered attributes such as alleviation of membrane damage, accumulation of proline and soluble sugars in leaves, reduction in water loss, along with reduced oxidative damage (particularly of superoxide anion ($O_2^{\cdot-}$) and malondialdehyde content) and increased anti-oxidant enzyme activity. Thus, the exogenous application of GABA enhanced the salt tolerance of maize seedlings (Wang et al. 2017a, b). Under saline condition, GABA improves seed germination and plant growth in lettuce, by reducing the effects of salt stress on photosynthetic performance and salt stress–induced oxidative stress (Kalhor et al. 2018). In *Triticum aestivum* and *Hordeum vulgare*, the abundance of GABA shunt metabolites (GABA, glutamate, and alanine) was observed along with the increasing concentration of salt (Al-Quraan et al. 2019) which signify its importance during stress.

Many times, salt along with other stresses were also studied and this part of the chapter also describes some of few such instances. The NaCl treatment in *Arabidopsis* up-regulates GABA metabolism. Precisely, GABA-T (first step of its catabolism) was found to play a crucial function in salt tolerance, as the loss-of-function of its corresponding gene mutant (*pop2–1* mutant) was oversensitive to ionic stress but not to osmotic stress (Renault et al. 2010). In a study, Al-Quraan et al. (2013) have reported a significant increase in GABA, MDA, and GAD mRNA levels under salt and osmotic stress in the five wheat cultivars (Hurani 75, Sham I, Acsad 65, Um Qayes and Nodsieh). The study suggested key contribution of GABA shunt, which permits salt stress management in wheat. Sheteiwy et al. (2019) have demonstrated that the high tolerance to OS+S (PEG + NaCl) involves the GABA potentiality to regulate ROS level, secondary metabolism, and transcription. Jin et al. (2019), while working on salinity-alkalinity stress in muskmelon, have observed that the stress increased MDA content, relative electrical conductivity, and the activities of many anti-oxidant enzymes including SOD, APX, and DHAR. Along with that, it decreases shoot weight (dry and fresh), leaf area, and antioxidant metabolites. The pre-treatment with GABA, H_2O_2, GSH, or AsA was found effective and reduces the salinity-alkalinity-induced effects. Further, the mitigation effect of GABA involves the surplus accumulation of chl and its precursors to evade photo-oxidation injury. In this study, it was also demonstrated that GABA stimulates the free polyamine contents (PAs), which in turn reduces Na^+/K^+ ratio and alleviates membrane lipid peroxidation, under salinity-alkalinity stress in muskmelon (Xu et al. 2019).

7.3.2 *Drought Stress*

In nature, the combined interplay of less rainfall, reduced groundwater table, and insufficient water availability lead to drought stress (Singh and Laxmi 2015; Singh et al. 2015). The drought stress affects different physiological and metabolic processes of plant and significantly reduced growth, water content, chlorophyll content, and change in different fluorescence parameters (Levitt 1980; Ahmed et al. 2017). It also results in the production of reactive oxygen species, which in turn causes damage to cellular constituent such as lipids, nucleic acids, carbohydrates, and proteins (Waraich et al. 2011). Besides, the drought stresses also regulate genes associated with stress response (Mishra et al. 2015).

The possible contribution of hypoxia and drought stress on GAD in vivo activity was reported by Serraj et al. (1998). The analysis of GABA suggested its six-fold accumulation after 6 h hypoxia exposure to soybean nodules and no changes after PEG treatment. The GABA application in drought-stressed perennial ryegrass (*Lolium perenne*) demonstrated higher relative water content, turf quality, and peroxidise activity. However, lowering of wilt rating, canopy temperature depression, electrolyte leakage, and lipid peroxidation were also observed as compared to untreated plants. No significant impact of GABA application was observed in the activity of superoxide dismutase and catalase in this study (Krishnan et al. 2013). Vijayakumari and Puthur (2016) have reported the contribution of GABA priming for the enhancement of osmotic stress tolerance in *Piper nigrum* Linn, stressed with PEG. In leaves of white clover (*Trifolium repens*), the drought (15% PEG)-induced damage can be effectively managed by exogenous application of GABA. The physiological features include behind this alleviation are higher relative water content, lower electrolyte leakage, lipid peroxidation, and leaf wilting. This improvement of GABA-mediated drought tolerance was observed in white clover, which involves participation of GABA-shunt, polyamines, and proline metabolism (Yong et al. 2017). In order to further understand the specificity and GABA functioning, Mekonnen et al. (2016) have investigated the *gad1/2* (with GABA depletion) to drought stress. The GAD (glutamate decarboxylase) performs primary function of GABA biosynthesis and decarboxylate glutamate. In *A. thaliana*, five copies of GAD are available in genome. Amongst these, *GAD1* and *GAD2* are highly expressed and responsible for significant reduction of GABA content in knockouts. The *gad1/2* mutant phenotypically exhibited reduced shoot growth and early wilting after prolonged drought stress, mainly because of defect in stomata functioning (closure). This over-sensitivity can be reversed by functional complementation, which increases GABA content in leaves (Mekonnen et al. 2016).

7.3.3 Temperature Stress

Any variation to plants’ optima temperature is considered as ‘Temperature stress’, which can be of two types: heat stress (above optima) and chilling/cold (Below optima) stress. The application of GABA in mitigation of temperature has been demonstrated in both cold and heat stress (Nayyar et al. 2014; Li et al. 2016a; Wang et al. 2016). High temperature or heat stress (HT) is one of the major environmental stresses that limit the growth, development, and productivity of plant globally (Hasanuzzaman et al. 2013). Plant responses to HT vary with type of plant, duration, and degree of high temperature. Plants are generally susceptible to high temperature in almost every development stage, but reproductive stage is most sensitive stage (Hasanuzzaman et al. 2013). Heat-triggered morphological, physiological, biochemical, and molecular changes in plants limit plant growth and development. HT inhibits seed germination, scoring of stems and leaves, root and shoot growth inhibition, senescence, and abscission of leaf (Vollenweider and Gunthardt-Goerg 2005; Wassie et al., 2019). It has also been reported that HT reduces relative water content, impaired photosynthesis, and increases accumulation of reactive oxygen species which in turn results in oxidative stress. It is responsible for lipid peroxidation, disturbing the stability of membrane and enzymes and nucleic acid denaturation (Hasanuzzaman et al. 2013; Wassie et al. 2019). The heat stress was also demonstrated to regulate the transcript of stress-related genes including heat shock transcription factors and heat shock proteins, specifically characterised for heat stress, though they may also have some other applications (Mishra et al. 2019). Further, it has been reported that application of exogenous osmolytes, signalling molecules, polyamines, and phytohormones, can alleviate heat stress (Mishra et al. 2020).

In rice seedlings, with the rise in temperature the length of roots and shoots were found inhibited and caused decrease in survival, especially at 42/37 °C (Nayyar et al. 2014). The contrasting fluctuation in endogenous GABA content was observed under moderately (35/25 °C) and severe stress (42/37 °C) conditions, with more than two-fold increase and seven-fold decrease as compared to moderately stressed plants (possible reason for reduction in growth and survival), respectively. With exogenous GABA application, the growth of survival of heat-stressed plants improved, which found associated with lowering of membrane damage, better cellular reducing feature, chlorophyll content, and shoots photochemical efficiency. Further, improvement in relative water content and stomatal content was also noticed, which was found associated with increase in osmolyte (proline and trehalose) accumulation. Moreover, the damage caused in anti-oxidant system (enzymatic: superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase and metabolite /non-enzymatic: ascorbate and glutamate) under heat stressed condition was also improved with GABA application in rice (Nayyar et al. 2014).

In another study, Li et al. (2016) have observed that pre-treatment of GABA (0.5 mM), before heat stress, improved heat tolerance in creeping bentgrass (*Agrostis stolonifera*). The metabolic analysis suggested that GABA application leads to higher accumulation of amino acids (glutamic acid, aspartic acid, alanine, threonine, serine, and valine), organic acids (aconitic acid, malic acid, succinic acid, oxalic acid, and threonic acid), sugars (sucrose, fructose, glucose, galactose, and maltose), and sugar alcohols (mannitol and myo-inositol). The study suggested the involvement of photosynthesis and ascorbate-glutathione cycle enhancement, osmotic adjustment, and GABA shunt in GABA-induced heat tolerance. Further studies suggested that during heat stress, the exogenous GABA application enhances expression of stress genes viz., *ABF3*, *POD*, *APX*, *HSP90*, *DHN3*, and *MT1,* which could be the possible reason to offer heat tolerance (Li et al. 2018). The global investigation of mRNAs and miRNAs suggested about the important adaptive responses, triggered by GABA, which includes HSFs pathway regulation, increased carbon metabolism, and biosynthesis of amino acids and plant hormone signalling (Li et al. 2019a, b). This study also reported the close association of heat-survival with significant changes of *miR398s*, *cca-miR156b*, *aly-miR159c-3p*, and *ata-miR408-3p*. Along with the involvement of *vvi-miR845c*, *ama-miR156*, and other novel *miRNAs* such as *novel-24,223*, *novel-2964*, and *novel-10,098* was also observed in GABA-regulated heat tolerance. Over all, the GABA-mediated heat tolerance in creeping bentgrass involved physiological, transcriptional, and post-transcriptional regulation.

Besides, the contribution of GABA towards cold-stress, another variant of temperature stress was also observed, which also has many negative impacts on plant growth and development. The nitric oxide–mediated chilling tolerance in cold-stored banana involves GABA metabolism and increased glutamate decarboxylase and decreased GABA transaminase activity, resulting into GABA accumulation (Wang et al. 2016). The investigation of amino acid homeostasis in frost-resistant and sensitive barley and wheat seedling under cold or freezing stress conditions revealed the significant conversion of glutamate to GABA, which was proportional to stress severity. Cold acclimation resulted into amino acid pool and GABA shunt gene induction, suggesting their contribution in frost tolerance (Mazzucotelli et al. 2006). The Isobaric tags for relative and absolute quantification (iTRAQ) based analysis suggested the association of the GABA-induced physiological effects with cold tolerance in tea plants. Further protein-protein interaction studies suggested the endogenous GABA alteration and stress response factors during improvement of cold tolerance and were linked with stimulated interactions among photosynthesis, amino acid biosynthesis, and carbon and nitrogen metabolism (Zhu et al. 2019).

7.3.4 Heavy Metal Stress

From a long time, heavy metals such as Mn, Fe, Cu, Cd, Zn, Hg, As, and Ni have been accumulated in the soil through industrial waste and sewage disposal. Though, some of these metals are essential micronutrient for growth and development of plant, however, their excess concentrations in the soil have detrimental effect on growth and development of plant (Ghori et al. 2019). Heavy metal stress cause denaturation or inactivation of enzymes which are important for many basic plant metabolic reaction associated with homeostasis, respiration, and photosynthesis (Hossain et al. 2012). It also triggers the production of reactive oxygen species such as hydrogen peroxide, superoxide radical, and hydroxyl radical which in turn cause oxidative damage to plant (Ahmad et al. 2012).

The contribution of GABA in reversing the negative effect of heavy metals (Cd, Cadmium; Cr, Chromium; As, Arsenic) on plant growth and physiological parameters has been shown (Mahmud et al. 2017; Kumar et al. 2017, 2019; Maryam et al. 2020). The improvement of physiological response in Cr-treated mustard seedlings was conferred by GABA (Mahmud et al. 2017). The supplementation of GABA on Cr-treated seedlings reversed most of the effect, which involves reduction of Cr uptake and reduction of oxidative damage by up-regulating non-enzymatic (ascorbate, AsA; glutathione, GSH) and enzymatic (ascorbate peroxidise, APX; monodehydroascorbate reductase, MDHAR; dehydroascorbate reductase, DHAR; glutathione reductase, GR etc.), anti-oxidant systems. Further, the application of GABA also increased leaf RWC and chl content and decreased proline and phytochelatin content. The GABA accumulation lowered the expression of *Lsi-1* and *Lsi-2* transporter expression, and hence reduced the As accumulation, along with reduction of oxidative stress marker TBARS and H_2O_2 with GABA accumulation.

The GABA-mediated modulation of physiological responses was noted, which exhibited better tolerance of *Oryza sativa* for As stress (Kumar et al. 2017). Kumar et al. (2019) have observed that the exogenous application of GABA (0.5 mM) against As(III) stressed rice seedlings, reduced the levels of H_2O_2 and TBARS, and improved the growth parameters. Further, its application along with As(III) enhanced level of unsaturated fatty acids (USFAs, particularly linolenic acid) as compared to saturated fatty acids (SFAs). The similar enhancement was also observed in case of amino acids (AA, proline, methionine, glutamic acid, and cysteine) and expression of genes associated with polyamine (PA) biosynthesis (*arginine decarboxylase, spermine* and *spermidine synthase*). This study cumulatively demonstrated that GABA at low concentrations provides solution for managing As(III) tolerance in rice, due to its positive effect on biosynthesis of USFA, AA, and PA (mainly putrescine) and negative effect on TBARS and H_2O_2. The application of GABA on Cd-treated maize plants demonstrated improvement of growth parameters. Further, its application conferred reduction in Cd uptake, increased activities of antioxidant enzymes (catalase and superoxide dismutase), along with induction of polyamine biosynthesis-responsive genes (*ornithine decarboxylase* and *spermidine synthase*), and suppression of *polyamine oxidase*, responsible for polyamine catabolism (Maryam et al. 2020). The contribution of GABA has also been observed during Cd stress in *Monoraphidium* sp. QLY-1, which resulted in higher protein, glutathione content, up-regulation of α-amylase activity, and reduction of starch, ROS, and Cd^{2+}. Further, GABA under Cd stress positively increases lipid production in this alga (Zhao et al. 2020).

7.4 Conclusion and Prospects

The present chapter addresses the significance of GABA in various types of environmental constraints mainly salt, drought, heat, cold, and heavy metals. The endogenous (one of the survival strategy) or exogenous GABA attributes advantages viz., reduction of photosynthesis inhibition, polyamine accumulation, reduction of ROS etc., and assists plant to maintain their normal growth and development. Though the investigations suggested its contribution in offering stress tolerance feature in plants, still the knowledge of its association in the management of several stress-related traits is not well understood at molecular level. Along with the transcriptional regulation of GABA-shunt genes under diverse conditions (developmental, hormonal, pathogenic, etc.) also would be an interesting area to work on. Further, their relations with transcriptionally modulated genes during stress, particularly transcription factors, also need attention, which will give better understanding to address combinatorial methods for stress management in crop plants to meet desired production.

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Chapter 8
Sugar Alcohols and Osmotic Stress Adaptation in Plants

Ramachandra Reddy Pamuru, Chandra Obul Reddy Puli, Deepu Pandita, and Shabir Hussain Wani

Abstract Sugar alcohols or polyols are derivatives of sugars produced in high amounts during salt or drought (abiotic) stress in plants. A number of sugar alcohols identified in plants include manitol, sorbitol, D-ononitol, pinitol, adonitol, ribitol, glucitol etc., which are classified as cyclic and non-cyclic polyols. Osmoregulation is a phenomenon of regulating constant fluid osmotic pressure in cells through salt and water concentrations during abiotic stress conditions. In plants, abiotic stress induces water loss, and at the same time, they release osmolytes including sugar alcohols which maintains osmoregulation. Sugar alcohols play a crucial role in osmotic adaptations and exert tolerance to salt and drought stress in plants. Due to the importance of sugar alcohols in crop improvement its metaolism, osmotic adaptations and role of transformational studies are discussed in the present chapter.

Keywords Sugar alcohols or polyols · Drought · Salt stress · Osmoregulation · Transformation

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8.1 Introduction

Osmotic stress, an important reaction of any living system with its surroundings, influences physiology, growth and development of organisms. In plants, osmotic stress plays a major role in germination, growth and other physiological activities. The alteration in the growth and physiology of plant ultimately reflects its effect on the productivity of crops. Abiotic stress is one cause of osmotic stress in plants. The stress components such as high salt, high temperatures (heat), low temperatures (cold), dehydration and intolerant osmotic pressure come under plant abiotic stress. However, the growth of any organism is under control with its own metabolic activations and develops anti-stress mechanisms for survival. There are many mechanism identified against stress in plants (Bohnert et al. 1995).

Osmoregulation during drought and salt stress accomplished by formation of osmotically active compounds also called osmoprotectants or compatible solutes (Ranganayakulu et al. 2013; Shahbaz et al. 2013; Talat et al. 2013; Filippou et al. 2014; Regnat et al. 2018; Carly et al. 2017). Osmoprotectants are neutral, non-toxic, highly soluble, low molecular weight molecules reported in plants and algae (Ahn et al. 2011). A number of molecules are reported under compatible solutes: sugars (trehalose and sucrose), amino acids (ectoine and proline), ammonia derivatives (choline-o-sulfate, glycinebetaine, polyamines and betaine) and sugar alcohols (mannitol, sorbitol, fructon and D-ononitol) (Madhulika et al. 2015).

The plant adaptation mechanisms against various stress conditions are well specified with the release of different organic solute molecules like sugars, sugar alcohols (cyclic and acyclic polyols or commonly called polyols), proline and betaines by modulating its own metabolic pathways (Slama et al. 2015). In germinating seeds, sugars produced from starch and lipids play a crucial role in seed germination in nourishing the developing embryo. Besides this, they play a key role in osmotic regulation during seed germination (Bolarin et al. 1995) through gene regulatory mechanism (Yu et al. 1996). Enhanced sugar deposition against various stress conditions in different parts of plants is well known (Prado et al. 2000; Gill et al. 2001). Sugars are responsible for cell vitrification, where cell damage is avoided by crystallization of intracellular water during cold stress. Proline, an amino acid, protects the plants from extreme low and high temperatures and salt and metal stress (Hayat et al. 2013; Ranganayakulu et al. 2013). Among all types of stress-releasing molecules, sugar alcohols are in focus to determine its role in osmotic stress adaptation in plants.

Sugar alcohols are polyhydric, straight-chain organic compounds formed due to reduction reaction at aldehyde or keto group of sugar with hydroxyl group. The best examples for sugar alcohols are glucitol, sorbitol, adonitol, ribitol, etc., and they are named based on the backbone sugar molecule. These molecules are considered under special cateogry of carbohydrates or sugars due to their resemblance with the sugar from which they derived. Initially the polyols were not identified in regular metabolic cascades and were identified only after the invention of gas and high-performance liquid chromatography, in which they easily recognized and identified in the plant extracts (Wayne 1987).

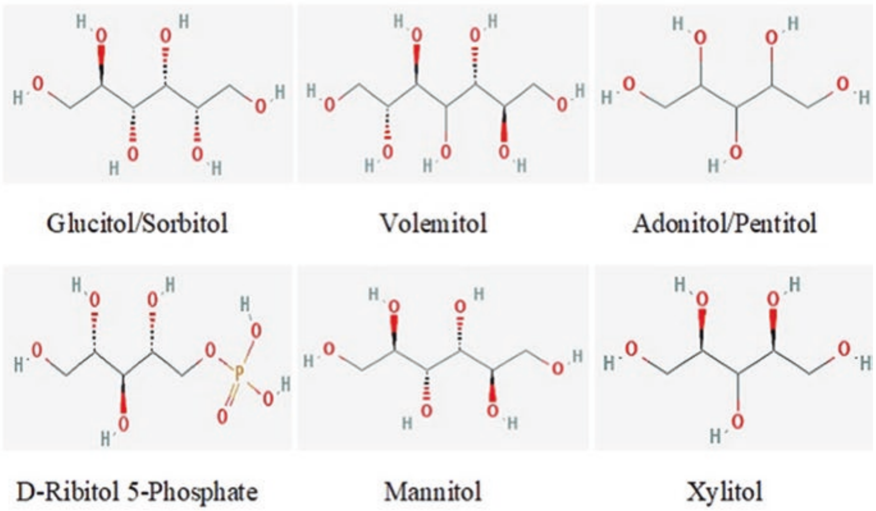
The compatible solutes, storage of carbon (reduced) and reducing power, coenzymes regulation and osmoregulation are primarily identified physiological functions of sugar alcohols (Lewis 1984). The polyols physiology in macroalgae and some higher plants were identified as phloem translocated sugars. The sorbitol and mannitol play important role in transporting up to 90% of leaf carbon dioxide to other parts of the plants for photosynthesis (Bieleski 1982). Polyols also act as plant storage compounds for carbohydrates and their derivatives. The seasonal variation in the occurrence and amount of sorbitol or mannitol was reported, where high amounts of these compounds were found in frost hardiness. The osmoprotectant role of polyols in plants can withstand the cellular activity from unfavourable (stress) conditions (Conde et al. 2011).

The importance of polyols as plant osmoprotectants is still on premature level, and presenting the sugar alcohols on osmotic stress adaptation in plants with available literature motivates the researchers for in-depth findings in future. Hence, the authors made an attempt to explain various aspects involved in plant osmotic stress adaptation in relation to polyols in further sections of the present chapter.

8.2 Sugar Alcohols (Polyols)

Sugar alcohols, popularly known as polyols, are produced against salinity and draught stress (abiotic stress) in plants and are primarily of two types based on their structure (Bohnert and Jensen 1996). They are linear polyols and cyclic polyols. Glucitol, sorbitol, adonitol, ribitol, mannitol, xylitol, and their derivatives come under linear polyols, whereas examples for cyclic polyols are pinitol, myo-inositol, etc. Polyols are the oxidative products of aldoses and their phosphate esters. These molecules are water soluble and regulate osmotic balance by interacting with scavenging reactive oxygen species on cell membranes, enzymes or protein complexes. During this process, polyols protect membranes of apoplast or vacuole through release of sodium ions (Li et al. 2011; Kanayama et al. 2006). Many studies have found the osmoprotectant role of polyols (mannitol, sorbitol and inositol) during abiotic stress (Williamson et al. 2002). Salt and drought stress tolerance by mannitol in *Zea mays* and *Fraxinus exvelsior* was reported (Patonnier et al. 1999; Kaya et al. 2013). In the same way, accumulation of D-ononitol was observed in response to salt and drought stress in *Arabidopsis thaliana* (Ahn et al. 2011). Because of osmoprotectant role, polyols become striking molecules for plant transformation. The structures of different sugar alcohols identified in the plants are presented in Fig. 8.1.

A. Non-cyclic polyols



B. Cyclic polyols

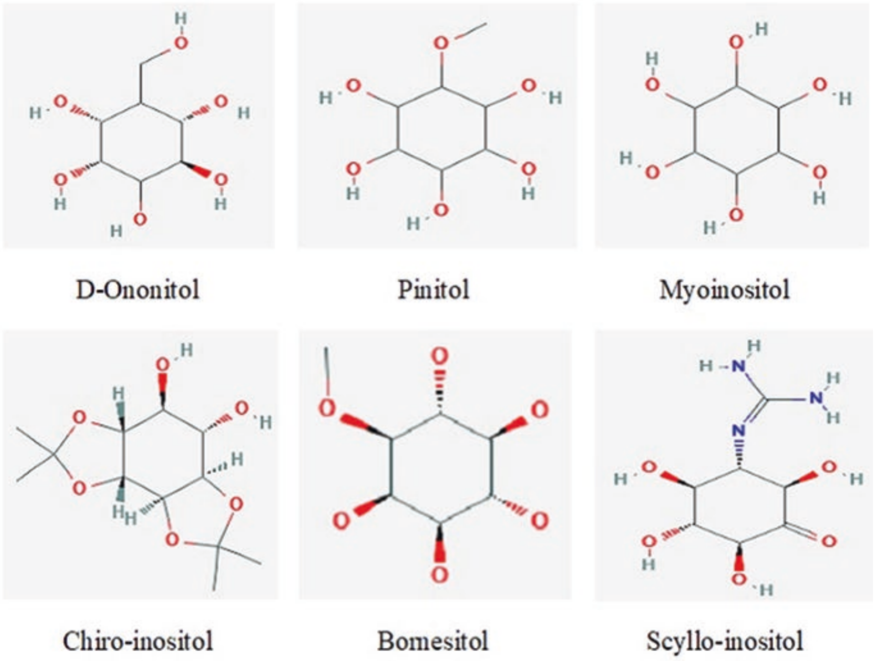


Fig. 8.1 Structures of different non-cyclic (**a**) and cyclic (**b**) polyols

8.2.1 Mannitol or Mannitol

A well-distributed sugar alcohol in plants is mannitol; its presence is reported in many species, including *Oleaceae, Apiaceae, Scrophulariaceae* and *Rubiaceae* members (Bieleski 1982). Being a most frequently identified polyol, mannitol was reported in more than 70 families of higher plants. Mannitol in plants, synthesized from the glycolysis intermediate i.e., glucose-6-phosphate (G-6-P) the precursor for manitol biosynthesis and is converted into manose-6-phosphate (M-6-P). Manitol-1-phosphate (M-1-P) is derived from M-6-P by the action of NADPH-dependent M-6-P reductase (Fig. 8.2). Phosphatase converts the M-1-P into mannitol through dephosphorylation (Rumpho et al. 1983).

Loescher et al. (1992) demonstrated the role of M-6-P reductase in mannitol synthesis during photosynthesis in *Aplum graveolens* and *Ligustrum vulgare*. They found the importance of NADPH-dependent M-6-P reductase in mannitol biosynthesis in vivo using ^{14}C-radiolabeling study. It is found to be produced in high amounts and quenches the $OH^{-}\cdot$ (hydroxyl) radical originated due to cellular electrochemical changes during stress conditions in plants (Mitoi et al. 2009; Gill and Tuteja 2010). Mannitol biosynthesis in some plants is linked to sucrose or rahenose synthesis. Vacuoles of celery leaves hold three parts of total mannitol (Nadwodnik and Lohaus 2008). The same amount of mannitol was found in vacuole, stroma and cytosol of from the total leaf content in parsley and snapdragon (Voitsekhovskaja et al. 2006; Moore et al. 1997). Varied vacuolar amounts of mannitol in leaf and petioles of celery were reported. It is found that tonoplast mediates the transportation of mannitol from its synthesizing site (cytosol).

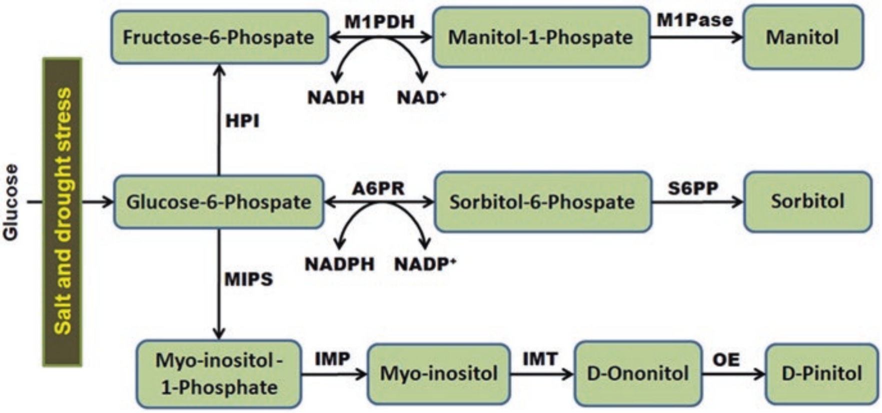


Fig. 8.2 Biosynthesis of various sugar alcohols under abiotic stress
HPI hexose phosphate isomerase, *M1PDH* manitol-1-phosphate dehydrogenase, *M1Pase* mannitol-1-phosphate phosphatase, *A6PR* aldose-6-phosphate reductase, *S6PP* sorbitol-6-phosphate phosphatase, *MIPS* myo-inositol-1-phosphate synthase, *IMP* inositol monophosphatase, *IMT* inositol methyltransferase, *OE* ononitol epimerase

8.2.2 *Sorbitol*

Sorbitol, another polyol of higher plants, is a major photoassimilate identified during phloem translocation in woody plants along with sucrose (Moing et al. 1997). Normal plants show low levels of sorbitol. Biologically sorbitol is synthesized from G-6-P. By the action of aldose-6-phosphate reductase G-6-P converts into sorbitol-6-phosphate (S-6-P). Further, S-6-P converted to sorbitol through phosphatase action. It is found that sorbitol content is showing high correlation with salt content (sodium concentration). Vacuoles of tonoplast mesophyll cells of sea plantain showed high sorbitol content, which is seven-fold higher than cytosol content, suggesting more functions including salt stress tolerance in this species (Nadwodnik and Lohaus 2008). Similarly, Moore et al. (1997) found 100% sorbitol in cacuols as its subcellular distribution in snapdragon. The sanpadragon cells holding low amounts of sorbitol, this is due to transportation of sorbitol to vacuoles after its synthesis in the cytosol. However, no sorbitol transporter is reported yet in mesophyll cells.

8.2.3 *Inositol or Myo-Inositol*

Another sugar alcohol, inositol, produced from G-6-P plays a crucial role in growth and development of plants along with acting as a secondary messenger for signal transduction and membrane biogenesis (Jain et al. 2010), and in storage of cellular carbohydrates (Bohnert et al. 1995). Besides these functions, inositol and its derivatives pinitol and galactinol are found as stress-related molecules and exhibit osmoprotectant functions (Taji et al. 2002). High amounts of myo-inositol were found in stroma of chloroplast (Voitsekhovskaja et al. 2006), and it is in contrast to sorbitol content (Nadwodnik and Lohaus 2008). Stress response, cell wall synthesis, hormone responses and seed germination are some of the physiological roles of inositol and its derivatives. These molecules were identified in peach, celery, common plantain and sea plantain.

8.3 Metabolism of Sugar Alcohols

The metabolic pathway identification of sugar alcohols started in the 1970s. Sorbitol biosynthesis in the leaves of apricot was discovered by Redgwell and Bieleski (1978). Later, enzymes involved in polyols biosynthesis were reported in many plants. Hirai (1981), in leaves and fruits of peach, apple, aprieot and pear, identified the enzyme aldose-6-phosphate reductase which is a key enzyme for sorbitol biosynthesis. Similarly, Rumpho et al. (1983) identified manose-6-phosphate reductase involved in mannitol synthesis in the leaves of celery and privet. The metabolic pathway of sugar alcohols synthesis from glucose and its derivatives formed during the glycolysis is presented in Fig. 8.2.

8.4 Osmotic Stress in Plants

Osmoregulation is a process which maintains the intact conditions of any cell and is linked to the regulation of water content. During the process of osmoregulation in plants, osmotically active compounds are produced to lower the cellular osmotic potential (turgor pressure). The cellular metabolism and physiological activities are associated with turgor pressure (Sharp et al. 1990). Cellular deposition of various organic and inorganic osmolytes plays an important role in regulating osmotic potential. However, the involvement of these osmolytes in overall osmoregulations varies with plant species and abiotic factors (Marek et al. 2016). In cereal crops, lowered osmotic potential is maintained by inorganic ions, especially potassium ions (K^+) (Morgan 1992). The organic solutes including sugar alcohols are another type of osmolytes that significantly contributes to lowering of osmotic potential in many plants.

Cell growth and elongation of plant cells is detrimental to water absorption through xylem (Nonami 1998). In plant cells, regulation of water relations and osmotic balance is maintained essentially by solute ions and its transporters, and major membrane intrinsic proteins called aquaporins. Aquaporins facilitate the membrane transport of water, urea and glycerol (Johansson et al. 2000). The protein abundance or transcript upregulation of aquaporins under stress (drought and salt) conditions suggests its importance in osmotic stress regulation (Kirch et al. 2000). Moreover, artificial drought stress is found to increase the membrane mercury-sensitive water channels for water transport in roots of rice plants (Lu and Neumann 1999) Johansson et al. (1998) demonstrated the low membrane permeability and minimized loss of water under drought stress with aquaporins through lowering its phosphorylation, whose reaction is essential during normal condition for water transport. This was supported Kirch et al. (2000) by studying the water flux modulated levels of aquaporins and their distribution and trafficking in endomembranes during osmotic stress. The above reports emphasizing the role of aquaporins or water channels in the regulation of osmotic stress in plants and are the potential targets for the transgenic drought-resistant plant production. Nevertheless, no reports are in focus to elucidate the combinational effect of aquaporins and osmolytes on osmoregulation in plants. In light of this, further studies are necessary in this direction.

8.5 Sugar Alcohols in Osmotic Stress Adaptation

Sugar alcohols play a crucial role in plants against abiotic stress. They compensate reduced cellular water potential by accumulating in high concentrations (Popp and Smirnoff 1995) (Table 8.1). A hydroxyl group of polyols effectively establishes hydrogen bonds to compensate the water loss and maintains the intactness of cellular membrane and enzymes (Nathalie et al. 2001). High osmotic potential due to

Table 8.1 Different sugar alcohols and their functions under abiotic (salt and drought) stress in various plants

Sugar alcohol	Source	Stress condition	Function	References
Mannitol	Maize (*Fraxinus excelsior*)	Drought and salt stress	Scavenger of stress-induced oxygen radicals and osmotic adjustment	Patonnier et al. (1999) and Abebe et al. (2003)
	Maize (*Zea mays*)	Salt stress	Stress alleviation	Rattan et al. (2012)
		Salinity stress	Osmoregulation by elevating K^+, Ca^{2+} and lowering Na^+	Kaya et al. (2013)
	Olive *(Olea japonica)*	Salt and osmotic stress	Increased mannitol transport activity and OeMaT1 expression	Conde et al. (2011)
	Tobacco	Water, light and temperature stress	No osmoprotection, but cell damage is protected by free radical scavenging	Macaluso et al. (2007)
D-Ononitol	*Arabidopsis thaliana*	Drought and salt stress	Osmolyte protects plants from water loss	Ahn et al. (2011)
Sorbitol	Rice plant	Salt stress	Catalyzes the reaction sorbitol to fructose through oxidation and acts as osmoprotectant	Wu et al. (2010) and Theerakulpisut and Gunnula (2012)
	Common bean (*Phaseolus vulgaris*)	Moderate water deficit	Tolerance to a lower oxidative state	Ramalho et al. (2014)
	Wheat (*Triticum aestivum*)	Seed priming	Osmopriming	Ahmed et al. (2016)
	Tomato	Salt stress	Increasing aldose reductase activity	Simon (2010)
	Rice seedlings	Salt stress	Stress alleviation	Theerakulpisut and Gunnula (2012)
	Sugar beet seedlings	Sodium chloride stress	Osmoregulation alleviation	Wu et al. (2015)
	Tomato	Abiotic stress	Increased production of sorbitol and ribitol	Afaf (2019)

exposure to drought or salt stress on the cell membranes is reduced by osmoprotectants, which leads to stabilization of membrane and proteins, and prevention of dehydration (Wani et al. 2013). It means osmolytic function of polyols ameliorates the reactive oxygen species (free radicals) and maintains the intactness of cellular macromolecules (Llanes et al. 2013). Simply the osmoprotective action of polyols protects the cells from its surroundings by balancing the osmotic difference. Usually osmoprotectants increase the cytoplasmic osmotic pressure as an adaptation to unfavourable conditions (Ranganayakulu et al. 2013; Jagesh et al. 2010).

The major polyols such as mannitol, inositol and sorbitol are adopted to enhance the tolerance potential in plants in response to drought and salinity. Stressed plants through Fenton reaction releases toxic $OH^{-}\cdot$ radicals, which creates membrane instability and further causes damage. Quenching of $OH^{-}\cdot$ radicals is done by release of great amounts of mannitol in plants under stress (Gill and Tuteja 2010). Polyols permit the sodium ion (Na^+) flux into the apoplast or vacuole and maintain osmotic potential to avoid the disability in cellular structures under osmotic stress (Li et al. 2011; Kanayama et al. 2006). High tolerance to drought and salt stress in plants with the accumulation of polyols is through protecting membranes from reactive oxygen species via glutathione-ascorbate cycle (Bohnert and Jensen 1996). This cycle appears in mitochondria, plastids and peroxidase of plant cell (Meyer 2009). Mannitol and D-ononitol are the identified molecules which reduces stress through glutathione-ascorbate cycle (Fig. 8.3).

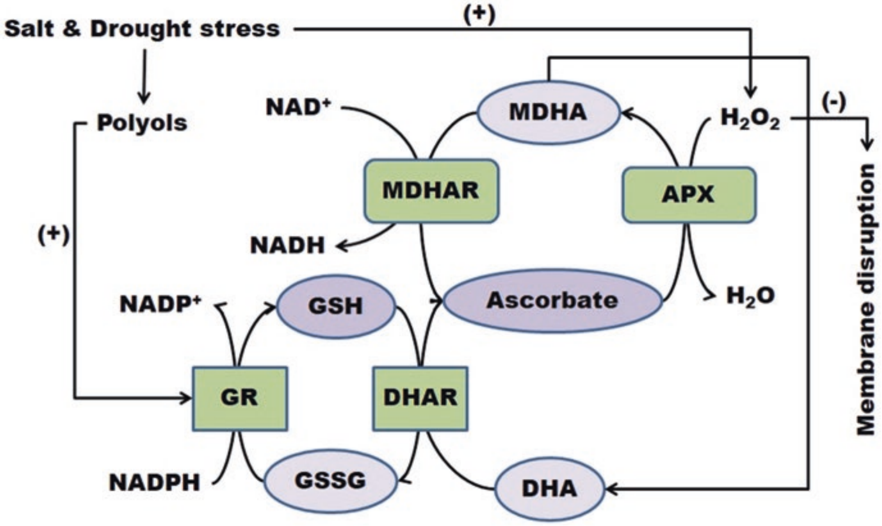


Fig. 8.3 Detoxification of hydrogen peroxide (H_2O_2) through ascorbate-glutathione cycle via polyols mediation. A series of reduction and oxidation reactions is present in the cycle
***GSH* glutathione reduced, *GSSG* glutathione oxidised, *APX* peroxidase, *DHAR* dehydroascorbate reductase, *MDHAR* monodehydroascorbate reductase, and *GR* glutathione reductase, *DHA* dehydroascorbate; '+': stimulation; '–': inhibition**

8.6 Sugar Alcohols in Transformation Studies

Osmoprotectants or polyols have identified molecules for crop improvement during drought and salt stress conditions. Using genetic engineering tools, the manipulation of stress tolerance is induced by introducing the polyol-responsive genes in crop plants (Reguera et al. 2012; Jain 2013). Tobacco and *Arabidopsis* are model test species for studying mannitol transformational studies. Oxidative stress tolerance was reported in transgenic tobacco-holding manitol-1-phosphate dehydrogenase (mt-1-D) gene in chloroplast (Shen et al. 1997). Same way, under salt stress, transgenic mt-1-D tobacco showed enhanced biomass and high amounts of cytosolic mannitol (Tarczynski et al. 1993). The overexpression of DREB1A/CBF3 and its transcription factors were recorded at low temperatures, which is regulated by sugar alcohols and other carbohydrates in transgenic *Arabidopsis* (Maruyama et al. 2009; Cook et al. 2004). Everard et al. (1997) cloned the major enzyme mannose-6-phosphate reductase responsible for mannitol biosynthesis. Transgenic mannitol producing *Arabidopsis thaliana* for salt tolerance was raised by Chan et al. (2011) and is an indication for establishing the changes in genes responsible for abiotic and biotic stress in the crop plants. Similarly, dononitol transgenic *Arabidopsis thaliana* exhibited salt and drought tolerance (Ahn et al. 2011). Sami et al. (2016) reviewed the role of polyols in plant growth and development, its physiological role in photosynthesis, senescence, seed germination, flowering and hypocotyl growth, and mechanism of these molecules in regulating these processes in plants. Reviews are empasized to explain the mechanism of abiotic stress by other than polyols response in the recent past Salt stress, cold stress and drought stress and its relation with sugar alcohols in plants are also cleared in this review.

Osmotic stress adaptation of transgenics introduced with various regulatory genes of sugar alcohols are well presented in the literature. High amounts of sorbitol levels were reported in transgenic tobacco carrying sorbitol-6-phosphte dehydrogenase cDNA isolated from apple (Sheveleva et al. 1998). It is identified that the transgenic plants have shown more tolerance than wild type to salt stress. Sheveleva et al. (1997) cloned myo-inositol-o-methyltransferase-encoding gene and found high amounts of D-ononitol under salt and drought stress. However, seeds of transformants showed delayed growth in normal conditions when compared to wild type plant seeds.

Moreover, significant seed germination at high salt conditions is found in seeds of transgenic *Arabidopsis* (Thomas et al. 1995). Transgenic sugar beet exhibited resistance to fungal pathogens with bacterial manitol-1-phosphate dehydrogenase gene (Goudarzi et al. 2015). In contrast to this, transgenic mt-l-D tobacco did not show significant osmotic protection with increased photosynthesis, but protected cells from damage with free radical–scavenging system (Macaluso et al. 2007).

8.7 Conclusion and Future Outlook

Polyols play very important role in protecting plants from unfavourable environmental (draught and salt stress) conditions called abiotic stress. The indirect effects of abiotic stress injure the plant cellular structures, which is protected from release of polyols (Li et al. 2011). It is emphasized that different polyols produced during abiotic stress can reduce risk of cell damage and increase the growth of the plant. This chapter described various types of sugar alcohols and their metabolic synthesis, osmotic stress and its tolerance by the action of sugar alcohols and role of polyol transgenics tolerance to salt and drought stress. A new unexplored direction for the elucidation and characterization of novel polyols and further transformational studies would be beneficial for the agricultural scientific community to move forward in developing crop transgenics using polyols metabolic regulatory genes. This may help to develop salt- and drought-resistant crops that give good yield. Nevertheless, focus has to be directed to know clear mechanism of sugar alcohols on osmotic adaptation in higher plants and more specifically crop plants.

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Conflict of Interest Authors have no difference of opinion on this chapter and declare no conflict of interest.

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Chapter 9
Cross-talk of Compatible Solutes with Other Signalling Pathways in Plants

Monika Bansal and Shabir Hussain Wani

Abstract Plants when exposed to varying level of abiotic stress conditions undergo a large number of changes at the physiological and molecular level that considerably affects their growth and development. Abiotic stresses affect cellular homeostasis and enhance generation of reactive oxygen species which ultimately leads to earlier senescence in plants. In response to stress conditions, plants accumulate enhanced level of osmolytes in coordination with different stress signalling pathways. This chapter discusses interaction of osmolytes and different signalling molecules and their role for survival of plants under stress conditions. Studies related to biosynthesis pathways of osmolytes have helped to identify novel candidate genes that regulate the stress response in plants.

Keywords Stress · Osmolytes · Signalling · Accumulation · Pathways

9.1 Introduction

Plants are exposed to tough environmental stress of temperature, drought, salinity and heavy metals that generates reactive oxygen species which hampers cellular and developmental processes. To save plants from damage caused due to oxidative stress, accumulation of compatible solutes occurs to maintain normal structure and functions of cellular machinery (Fig. 9.1). Most common osmolytes are proline, glycine betaine, polyamines and sugars, which protect plant cells by interfering with the generation of ROS like hydroxyl and superoxide ions, free radicals and peroxides. Initially, stress signals are sensed by definite receptors proteins present

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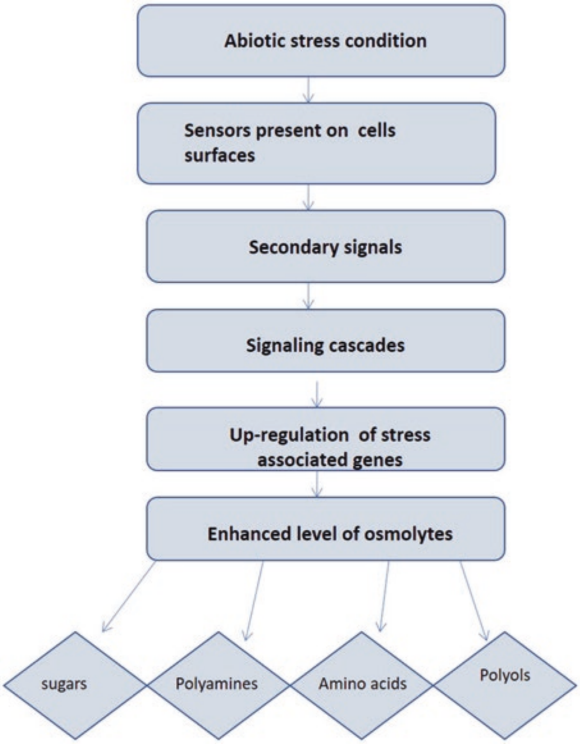

Fig. 9.1 Schematic mechanism of abiotic stress response on plants leads to enhanced accumulation of osmolytes. Stress signal activates signalling cascade, which up-regulates stress-responsive genes related to accumulation of osmolytes to make stress-tolerant plants

on the plant cell surfaces. These receptors further transmit received signals to nucleus where transcription factors up- or down-regulate the level of expression of genes associated with stress tolerance, which codes for various defensive and adaptive proteins. Common signalling agents in plants are Ca^{2+}, inositol phospholipids-proteins, cyclic nucleotides, kinases and protein phosphatases. The signalling pathway in plants involves the organization and harmonization of more than one signalling molecule in diverse cellular compartments and regulates different biochemical and physiological responses. There exist highly efficient cross-talk between various signalling pathways to connect different network cascades for signal transduction. Each pathway in these cascades involves multiple proteins: numbers may vary between few hundred to thousands. Earlier, signal transduction pathways were thought to be linear cascades, but with recent advances in database accessibility based on genomics and bioinformatics information gathered from transcriptome profiling, mutagenesis data, and plant 'omics,' it is now possible to understand interactomics between these molecules. This mechanism of coordination can be described as "cross-talk" and "network." Signalling molecules or intermediates when shared between two or more signalling pathway are said to be a case of direct cross-talk. Indirect cross-talk is a case of chronological reactions, where the function of first pathway is used for the activity of second pathway. During signalling cascades, critical components which make this machinery are ion channels, signalling proteins and second messengers that regulate early events in this pathway.

9.2 Signalling Cascades for Osmolytes Production

Drought, salinity and low temperature stress are complex phenomena; their individual effects on plants had many different attributes, but these stresses still have few related properties and cause similar kind of disturbances inside the cells. For example low temperature changes activities of some macromolecules and also reduces osmotic potential inside the cells. Similarly salinity stress also disturbs ionic and osmotic factors inside the plant cells. Under normal conditions, sensor present on plant cell surfaces detects stress-related change and initiates a cascade or stepwise reactions inside the cells. There may be a single and sometimes multiple primary sensors which sense initial signals induced under different stress conditions. Stress signal was initially received by primary sensors like histidine kinases and later transferred to transcriptional factors like MYC/MYB, AREB/ABF, DREB, etc. which regulate expression of genes involved for osmolyte biosynthesis. Secondary signals include hormones and messengers that trigger or activate the next series of events during this signalling cascade. Their receptors may be embedded at some locations within the cells which were different from that of primary sensors. Secondary signals involve interaction or cross-talk among different signalling pathways in response to different stresses. In plants, molecules which act as secondary messenger include mainly Ca^{2+}, IP_3 and cyclic GMP (cGMP). Ca^{2+} ion is a crucial molecule during signalling process and a common point for different signalling pathways (Tuteja and Mahajan 2007). In all eukaryotic cells, Mitogen-activated protein kinase (MAPK) works during transduction of stress signals by phosphorylation of other targets molecules like kinases, enzymes and transcription factors on serine or threonine amino acid. Plant hormones like auxins, abscisic acid, jasmonic acid, salicylic acid, ethylene and brassinosteroids influence signalling process by MAPK cascades (Hettenhausen et al. 2014; Lu et al. 2015).

MAPK cascade consists of three stepwise protein phosphorylation working on component serine/threonine kinases, a MAP kinase kinase kinase (MAPKKK), a MAP kinase kinase (MAPKK) and finally, the MAP kinase (MAPK), by inducing activation and phosphorylation of subsequent kinases in a cascade. The MAPK pathways get triggered by various receptors like tyrosine kinases, G-protein–coupled and two-component histidine kinases kind of receptors. Several putative sensory kinases will helps us to identify intermediates involved which will determine if the kinase signalling involved is specific or there are cross-talks between these pathways. Some members of MAPK cascades are activated by multiple stresses which suggests working of MAPK cascades as points of common junction during stress signalling.

The hormones or growth regulators in plants affect numerous physiological and developmental processes, beside this hormone affects plants nutritional status and response of the target cell to different environmental changes. Enhanced levels of osmolytes protect plants by minimizing damages and rescue them from oxidative damage caused by stress conditions.

9.3 Compatible Solutes

During stress, plants accumulate osmolytes like L-proline, glycine betaine (GB), glycerol, mannitol, sorbitol, etc. (Sharma et al. 2019, Rasool et al. 2013; Gupta and Huang 2014).Compatible solutes are soluble organic compounds with low molecular weight, which accumulate in plants cells cytosols. During stress conditions, these osmolytes change interaction chemistry of proteins with DNA by linking themselves with the nucleic acid (Kurz 2008). Most common classes of compatible solutes are betaines, sugars (glucose, fructose, fructan, etc.), sulfonium compounds, polyols (mannitol, glycerol, sorbitol), polyamines (spermine, spermidine and putrescine) and amino acids like proline and glutamine (Slama et al. 2015; Pathak and Wani 2015; Sah et al. 2016).

Plants accumulate compatible solute to stabilize proteins and cellular organelles by making osmotic adjustment and regulating redox metabolism for removal of excess ROS level and maintain redox balance in plant cells (Chinnusamy and Zhu 2009; Krasensky and Jonak 2012).The osmolytes accumulation is regulated by various hormones like abscisic acid, brassinosteroids, cytokinins, ethylene, jasmonates and salicylic acid. In this chapter, we will try to discuss mechanisms and understand the role of phytohormones for the osmolytes accumulation in response to abiotic stresses in plants.

9.4 Molecular Mechanism to Understand Cross-Talk

9.4.1 Glycine Betaine Biosynthesis

GB accumulates in response to abiotic stresses in plants. Glycine betaine is soluble quaternary ammonium component that does not cause toxicity inside cells (Cleland et al. 2004). Glycine betaine had ability for membrane stabilization, protects photosystem II and alleviates oxidative damage caused due to stress conditions (Chen and Murata, 2011). Glycine betaine bio-synthesis can be divided into two types on the basis of initial precursor used and enzymes involved during the reactions (Sakamoto and Murata 2000). GB synthesis takes place by using two different substrates: choline and glycine. Glycine betaine is synthesized by the oxidation of choline in two-step process catalyzed by separate enzymes. The enzyme responsible for catalysis of first step is choline monooxygenase (CMO) (Rathinasabapathi et al. 1997). Catalysis of other step is controlled by betaine aldehyde dehydrogenase (BADH) (Wood et al. 1996; Fitzgerald et al. 2009). The CMO and BADH expression was triggered by various stress conditions like salt (McCue and Hanson, 1992), drought (Li et al. 2016), cold (Xing and Rajashekar 2001) and heat (Mitsuya et al. 2011). ClCMO and ClBADH genes have a CGTCA-motif in their promoters which is responsible for response to hormonal signals like methyl jasmonate, ethephon, Abscisic acid and Salicylic acid which is further effective in inducing the expression of ClCMO and ClBADH genes, and glycine betaine accumulation in plants.

9.5 Glycine Betaine and Hormone Response

Enhanced level of ethylene synthesis is associated with abiotic stress responses in plants (Morgan and Drew 1997). First important step during ethylene synthesis is synthesis of S-adenosylmethionine from methionine. The SAM created during this reaction also works as precursor during biosynthesis of glycine betaine. Cross-talk between two pathways was experimentally proved by Khan et al. in 2014. They described that when salicylic acid is applied exogenously in *Vigna radiata* plants exposed to salt stress, it showed increased methionine concentration and accumulated GB. Enhanced concentration of glycine betaine and lower ethylene level during salt stress increased the glutathione (GSH) levels which resulted in lower oxidative stress damage. In tobacco and tomato, ethylene level was enhanced in response to cold stress, without any accumulation of GB (Park et al. 2004). Whereas in bean and wheat, Glycine betaine accumulates, while ethylene production decreased during cold stress (Wang et al. 2010). This type of reverse behaviour between ethylene and glycine betaine accumulation can be explained by the mechanism of donation of methyl group from SAM to choline, common link between the two biosynthesis pathways (Kurepin et al. 2015). Direct involvement of ethylene in mitigating light stress is less, however endogenous ethylene controls the synthesis of GB, which alleviates light stress-induced responses in plants. Brassinosteroids, when provided exogenously, show increased accumulation of glycine betaine and help in tolerance to stress as shown in Table 9.1 for some important crop plants. Studies using seven-day-old plants of *Raphanus sativus,* when exposed to Cr stress and treated with 24-epibrassinolide, exhibit enhanced glycine betaine level and expressed increased tolerance for Cr stress (Choudhary et al. 2011). BR-stimulated glycine betaine accumulation might be attributed to increased catalytic activity of betaine aldehyde dehydrogenase (BADH) (Rattan et al. 2014).

9.6 Proline Biosynthesis

Proline is an amino acid and a molecular chaperone that scavenges free radicals generated during abiotic stress in plants. In control conditions, proline plays important role in developmental process of seeds and embryo (Mattioli et al. 2009). Proline stabilizes secondary structures of proteins (Funck et al. 2012) and also serves as a reservoir for storing cellular carbon and nitrogen during the plant recovery phase (Kavi Kishor et al. 2005).

Table 9.1 Showing effect of different signalling molecules on osmolytes accumulation

Plant species	Stress	Signalling hormone	Response	References
Prunus persica fruits	Chilling injury(cold storage)	24-epibrassinolide	Increased proline level and also of Δ1-pyrroline-5-carboxylate synthetase (P5CS)activity	Delauney et al. (1993)
Raphanus sativus	Cu and Cr stress	24-epibrassinolide	Significant increase in proline content	Sharma et al. (2011) and Choudhary et al. (2010)
Salvia miltiorrhiza	Drought stress	Foliar spray of BR	Enhanced proline content	Zhu et al. (2014)
Cucumis sativus	Cu stress	24-epibrassinolide	Enhanced proline content	Fariduddin et al. (2013)
Capsicum annuum var. *frutescens*	Water stress	Foliar spray of BR	Significant increase in proline content	Khamsuk et al. (2018)
Brassica juncea	Copper stress	Synthetic BR	Enhanced proline level	Fariduddin et al. (2009)
Raphanus sativus	Cr stress	24-epibrassinolide	Enhanced level of glycine betaine	Choudhary et al. (2011)
Pisum sativum	Salt stress	24-epibrassinolide	Enhanced glycine betaine content	Shahid et al. (2014)
Tomato plants	Salt stress	24-epibrassinolide	Increased level of glycine betaine	Ahmad et al. (2018)
Pisum sativum	Cd stress	24-epibrassinolide	Enhanced glycine betaine content	Jan et al. (2018)
Triticum aestivum	Heat stress	SA (0.5 mM)	Increasing the content of proline	Khan et al. (2013)
Rauvolfia serpentina	Salt stress	Salicyclic acid	Increasing the content of proline	Misra and Misra(2012)
Torreya grandis	Salt stress	Salicyclic acid	Enhanced proline level	Li et al. (2014)
Brassica juncea	Heat stress	SA treatment	Enhanced proline level	Hayat et al. (2009)
Dianthus superbus	Salinity stress	SA treatment	Elevation in proline content	La et al. (2019)
Vigna radiata	Salt stress	SA	Glycine betaine accumulation	Misra and Misra (2012)
Triticum aestivum	Water deficit	GB and SA	Glycine betaine accumulation	Aldesuquy et al. (2012)
Helianthus annuus	Drought stress	GB and SA	Glycine betaine accumulation	Hussain et al. (2009)
Glycine max	Salinity stress	Salicyclic acid	Elevation in level of methionine, tyrosine and phenylalanine	Farhangi-Abriz and Ghassemi-Golezani (2016)

(continued)

Table 9.1 (continued)

Plant species	Stress	Signalling hormone	Response	References
Abelmoschus esculentus	Water deficit	Salicyclic acid	Elevation in amino acid	Sankar et al. (2007)
Sorghum bicolor	Water deficit,	Salicyclic acid	Elevation in amino acid	Yadav et al. (2005)
Helianthus annuus	Cu metal stress	Salicyclic acid	Elevation in amino acid	El-Tayeb et al. (2006)
Zea mays	Salinity	Salicycic acid	Proline and GB content	Hussein et al. (2007)
Quinoa	Drought	Salicycic acid	Enhancement in amino acid content	Abd Allah et al. (2015)
Brassica napus	Cold stress	Abscisic acid	Enhancement in the level of proline	Burbulis et al. (2010)
Cicer arietinum	Cold stress	Abscisic acid	Increased level of proline	Kumar et al. (2008)
Cynodon dactylon	Cold	Abscisic acid	Increased proline content	Cheng et al. (2016)
Medicago sativa	Drought	Abscisic acid	Enhancement in the level of proline	An et al. (2014)

Synthesis of proline occurs from either glutamate or ornithine (Fichman et al. 2014) in higher plants. Proline during synthesis from glutamate needs bifunctional enzyme $^1\Delta$-pyrroline-5-carboxylate (P5C) and synthetase (P5CS) for reactions that uses ATP and NADPH for generation of glutamate-γ-semialdehyde. Increased proline levels is a strong indication of stress tolerance because of their dynamic role for chlorophylls reconstruction and participation for establishment of Kreb's cycles intermediates (Ashraf and Foolad 2007; Ahmad et al. 2015).

9.7 Proline and Hormone Response

During drought conditions, *P5CS1* (pyrroline-5-carboxylate synthetase 1) expression level was increased, when exposed to drought stress the level of *P5CS1* was enhanced in control plants but it remains unaffected in case of *ein* mutants (Cui et al. 2015). Recently, Iqbal et al. (2015) explained interrelationship between proline and ethylene to provide salinity tolerance in *Brassica juncea*. Iqbal et al. (2015) reported that N regulates proline production and ethylene formation to alleviate salinity stress. Chrominski et al. in 1989 described increased change of ACC into ethylene on exposure to salt and water stress in *Allenrolfea occidentalis* and explained that under such conditions, exogenously applied proline balances level of ethylene in these plants.

Salicyclic acid is reported to be engaged for enhancing proline content under abiotic stress in plants (Khan et al. 2013). *Lens esculenta* plants when treated with

0.5 mM of Salicyclic acid under salt stress showed improved activity of proline synthesis enzymes. Exogenously applied proline causes enhanced accumulation of salicyclic acid endogenously (induced by NDR1-dependent pathways) and regulates Ca-mediated oxidative stress for plant defence (Chen and Murata 2011).

Jasmonic acid (JA) regulates water potential in plant cells under various abiotic stress conditions. Shan et al. (2015) described that JA alleviates drought stress by the proline biosynthesis. JA is responsible for up-regulation of key genes involved during adaptation response by increasing synthesis of secondary metabolites and stress-responsive proteins along with enahnced proline level (Abdelgawad et al. 2014). Jasmonic acid–induced changes in the proline level had been explained in several stress-based studies like heavy metal (Farooq et al. 2016; Poonam et al. 2013) and salt stress (Yoon et al. 2009). Jasmonic acid when applied exogenously increases level of organic intermediates formed during kreb cycle which include citrate, succinate, fumarate and malate which are responsible for providing abiotic stress tolerance in plants (Sharma et al. 2018).

Abscisic acid contributes significantly to control proline metabolism in plants during abiotic stress (Kumar et al. 2012). ABA enhances proline content in plants to protect from stress conditions (Sarafraz et al. 2014; Karimi and Ershadi 2015; Sarafraz-Ardakani et al. 2014). ABA increases the genes transcript levels which code for enzymes of proline biosynthetic pathways (Sripinyowanich et al. 2013).In *Medicago truncatula*, proline synthesis during drought stress was regulated by ABA signals (Planchet et al. 2014). ABA signalling controls proline biosynthesis in abiotic stress in plants (Pal et al. 2018; Verslues and Bray 2006).

9.8 Ethylene Role in Stress Signalling

Ethylene, a gaseous phytohormone, affects germination of seeds, developmental process in plants, senescence in case of leaf and flower, ripening of fruits and impacts abiotic stress tolerance (Osborne 1993). Iqbal et al. (2015) reported role of ethylene to regulate abiotic stress response by showing its effect on accumulation of osmolytes. Studies involving mutant of *ein2–5* and *ein3–1* (ethylene insensitive) confirmed role of ethylene during biosynthesis of osmolytes (Cui et al. 2015). Expression of pyrroline-5-carboxylate synthetase, during proline biosynthesis, was enhanced in the plants which were exposed to drought stress in comparison to control plants, but remained unaffected in *ein* mutants.

9.9 Carbohydrates

In plants, sugars work as chief energy source, but also perform other regulatory functions like signalling for regulating development and metabolic activity in plants. There is a tight link between sugar signalling and hormone signalling, which controls growth, development and stress reaction in plants. Sugars play important role during stress tolerance as powerful compatible solutes that help plants for conservation of water inside the cells, thus decreasing water access for nucleation in the apoplast (Ruelland et al. 2009). Disaccharides like sucrose, trehalose, raffinose family oligosaccharides and fructans actively participate in stress tolerance in plants (Keunen et al. 2013). Miranda et al. in 2007 studied how during cold acclimation in *Arabidopsis*, level of soluble sugars (trehalose) enhanced noticeably. Initial reaction in response to drought stress is observed with enhanced levels of monosaccharides, while delayed response is connected with increase in the fructans level (Kerepesi and Galiba 2000). Trehalose accumulation was reported in some desiccation-tolerant plants, since it has unique tendency to acts as substitute of water on the surface of macromolecules. Flowers (2004) detailed its role in ROS scavenging and signal cascades. Fructans have high water solubility and resistance to crystallization at subzero temperatures and the ability of the synthesis pathway of fructan to work normally even at low temperature conditions (Livingston et al. 2009). During drought stress, glucose provides desiccation stress tolerance by inducing closure of stomata (Osakabe et al. 2014). During water deficiency, sugar accumulation protects oxidation of cell membranes (Arabzadeh 2012).

Sugars function as osmoprotectant to reduce the harmful effects of salt stress on plants (Almodares et al. 2008). Rosa et al. in 2009 proved that noticeable augmentation in the level of glucose, sucrose and fructose occurs during salinity which is responsible in maintaining osmotic balance inside the cells. In case of wheat studies have shown that application of even low glucode level during seedling stage resulted in considerable enhanced germination even when the plants are exposed to high level of salinity.

Glucose when applied exogenously during salt stress results in increased dry weight, helps to maintain ionic balance, helps in accumulation of proline and prevents water loss (Hu et al. 2012). In higher plants, different soluble sugars, like glucose, sucrose, raffinose and stachyose, provide tolerance to freezing temperature (Yuanyuan et al. 2009). Fructans because of higher water solubility prevent crystallization of water during chilling stress (Livingston et al. 2009). In rice, trehalose content is enhanced during cold stress (Garg et al. 2002). Soluble sugars are connected with anabolism and catabolism of ROS; pentose phosphate pathway that is involved in NADPH production is involved in ROS scavenging (Hu et al. 2012). Exogenous application of glucose at lower level prevents peroxidation of lipids during NaCl treatment (Hu et al. 2012). Lower level of sugars like glucose and sucrose activates antioxidant enzymes during salt stress (Boriboonkaset et al. 2012). When applied exogenously, low nitrogen along with glucose up-regulates senescence-related gene expression level to about hundred-folds. Accelerated leaf senescence occurs due to hexokinase-based signalling mechanism (Wingler et al. 2006). In *Arabidopsis*, trehalose-6-phosphate synthase activity results in enhanced flowering (Gibson 2005).

9.10 Sugar as a Signalling Molecule

Plant growth regulators like ABA, gibberellins and kinins regulate sugar metabolism and its translocation to different locations during stress conditions (Gibson 2004). During germination, glucose works as powerful modulator for regulating expression of genes related with ABA synthesis (Price et al. 2003; Xu et al. 2010) and also suppresses ABA catabolism genes (Zhu et al. 2009). Application of glucose in *Arabidopsis* induced increased expression of ABA, like ABA2, ABI1 and ABI4 genes (Price et al. 2003). Cheng et al. in 2002 examined that higher glucose concentration up-regulates level of ABA biosynthesis genes like ABA2 and NCED3 (Cheng et al. 2002). In rice, OsNCED genes which are five in number and three ABA8'-hydroxylase (OsABA8ox) genes are involved in both synthesis and catabolism of ABA (Zhu et al. 2009). In higher plants, higher glucose level and ABA are known to arrest development of seedlings (Dekkers et al. 2008). A complex relationship exists between glucose and different hormones, like abscisic acid and ethylene (Cheng et al. 2002; León and Sheen 2003). During germination of seeds, ethylene antagonizes the negative effect of higher level of glucose. Ethylene shows opposite role in comparison with ABA during germination (Beaudoin et al. 2000). Negative type of correlation was observed with the exogenous application of sucrose (Xu et al. 1998). In barley and rice, exogenously applied glucose decreased gibberellins level (Gibson 2005). Cytokinins are responsible for causing decrease in glucose concentration by activating invertase enzyme that helps in utilization of sugars and is responsible for causing delay in senescence.

9.11 Amino Acids

In plants, amino acid concentration increased during abiotic stress (Lugan et al. 2010). Amino acids involved in stress tolerance include proline, alanine, glycine, glutamate, aspargine, γ-aminobutyric acid (GABA), citrulline, ornithine, etc. Three major types of polyamines are putrescine (Put), spermidine (Spd) and spermine (Spm), but some other forms of PAs, like cadaverine, may also be there in some cases. Polyamines have strong affinity to bind with nucleic acid and proteins via electrostatic linkages. These amino acids function as osmolytes and sometimes act as precursors also. In plants, PAs regulate very important physiological processes like senescence, developmental process, cellular proliferation, signal transduction and control gene expression for traits which cause different types of responses under stress conditions (Alcázar et al. 2006).

In some cases, three most common polyamines, Put, Spd and Spm, show considerable increase after exposure to abiotic stress (Yang et al. 2007). In majority of cases during abiotic stress only single type of polyamines showed considerable increase in the level of expression. Callus formed during Apple tissue culture on treatment with salt stress exhibited enhanced Put levels, while very minor changes were observed in the level of Spd and Spm (Liu et al. 2006). Sweet orange callus shows enhanced level of Spd after exposure to salt and cold stress for a brief duration (Wang and Liu 2009). In *Vitis vinifera* also sharp enhancement of Spd and Spm occurred during salt stress (Ikbal et al. 2014). Increased heat stress tolerance in alfalafa was attributed to enhanced level of spd and decreased level of put and spm amino acids (Shao et al. 2015). Biosyntheses of polyamines and ethylene are interconnected by SAM, which is a common precursor for both the reactions. Metabolism of polyamines affects nitric oxide generation also (Yamasaki and Cohen 2006). Polyamines induce formation of NO which works as common linking point for polyamine-mediated stress response and intermediaries involved during different stress mechanisms (Tun et al. 2006).

9.12 Gamma-Aminobutyric Acid (GABA)

GABA is a non-protein type of amino acid; it is produced during different abiotic stress conditions. The α-ketoglutarate is the precursor for GABA biosynthesis, which gets converted with the help of glutamate dehydrogenase to glutamate; another enzyme, glutamate decarboxylase, converts glutamate to GABA. During unfavourable conditions, GABA helps to maintain osmotic balance and regulates pH, nitrogen metabolism and prevents ROS build-up in plant cells (Barbosa et al. 2010; Renault et al. 2010).

GABA accumulates during high salinity level (Zhang et al. 2011; Renault et al. 2010) in plants. Flood stress causes reduction of cytosolic pH and oxygen deficiency, which causes increased GABA accumulation (Kinnersley and Turano 2000). Stress conditions like cold, heat, salt and other environmental stresses increase Ca^{2+} levels inside the cells, which can activate calmodulin-dependent GABA synthesis (Kinnersley and Turano 2000). Exogenous application of GABA in case of *Caragana intermedia* changes gene expression in roots which are exposed to NaCl stress and activates different pathways which have a role to play during signalling cascades.

9.13 Conclusions

Abiotic stress is one of the main restrictive factors responsible for decline in crop production. Plants have devised new strategies to overcome these environmental stresses. Technological advancement during the last three decades by use of molecular and genomic approaches provides stronger means to study molecular, and physiological, aspects regarding stress signalling in depth. This chapter briefly covers the knowledge of different intermediates involved during signalling metabolism, the signals which activate osmolyte accumulation and maintain their distribution during stress and also discusses the role of enzymes responsible for osmolytes synthesis and degradation. a Comprehensive scenario of functions of different osmolytes and their interaction networks in different environmental conditions was also discussed. Future focus of researchers will now be to explore and establish benefits of osmolytes to regulate crop improvement. To achieve this goal, recent developments in plant genetic engineering using genome editing will prove useful for generating genetically superior crops, with improved ability to accumulate osmolytes during stress conditions.

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Chapter 10
Effect and Importance of Compatible Solutes in Plant Growth Promotion Under Different Stress Conditions

Ashutosh Paliwal, Abhishek Verma, Harshita Tiwari, Manoj Kumar Singh, Jalaj Kumar Gour, Ashwini Kumar Nigam, Rohit Kumar, and Vimlendu Bhushan Sinha

Abstract A plant requires favorable conditions for their growth. When minerals, temperature, light, pH, and water are present in their optimum concentration, plant grows well, but any change in their level affects the growth of the plant. A situation that does not promote plant growth is called stress. For optimum growth of plants in stress conditions, the plant accumulates compatible solutes, an organic compound that is nontoxic and nonreactive and manages osmotic pressure in plants. Plants synthesize different types of compatible solutes in different kinds of biotic as well as abiotic stress. Compatible solutes also scavenge reactive oxygen species (ROS), and in this way, it could protect plants from oxidative stress. In this chapter, we review different types of compatible solutes and their impact on stress conditions. We also summarize the role of compatible solutes in plant growth promotion.

Keywords Stress · Glycine betaine · Plant growth · Compatible solutes

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10.1 Introduction

A compatible solute is an organic compound that accumulates in the cytoplasm of a plant for balancing the external osmotic pressure in stress conditions. Compatible solutes are low-molecular-weight complexes that are nonhazardous, nonlethal, and have high solubility. This accumulation of the solutes helps in maintaining the osmotic pressure, electrolytic concentration, cell volume, and mostly for cell proliferation and its viability (Anton 2015). Different advanced scientific protocols have also examined the production of compatible solutes in bacterial genera (Galinski 1995; Ventosa et al. 1998) and archaea (Martins et al. 1997). A combination of different techniques can also be used for the detection of the osmolytes: e.g., a combination of pulse amperometric detection and anion-exchange chromatography is a useful technology for the detection of ecotine (1,4,5,6-tetrahydro-2-methyl-4--pyrimidinecarboxylic acid) post hydrolysis of pyrimidine ring (Riis et al. 2003). The spectrum of these compatible solutes, which have been used by microbes, contains various compounds like sugars, polyols, free amino acids, sulfate esters, etc. (Kempf and Bremer 1998).

Microorganisms demonstrate two different types of strategies against osmotic stress, viz., high salt, freezing, and desiccation. The first one is the “salt-in” strategy that responds to the high salt concentration present in the external environment. This salt-in strategy is shown by a large number of microbes, including archaea, anaerobic bacteria of order Haloanaerobiales, and some Bacteroides (i.e., *Salinibacter ruber*) in which they accumulate a large quantity of inorganic ions (mainly potassium) in their cytoplasm. Secondly, the microbes accumulate inorganic ions in the intracellular environment either through adsorption or de novo synthesis of compatible solutes (known as osmolytes) for balancing the external osmotic pressure (Empadinhas and da Costa 2008). This solute accumulation allows cells to tolerate the osmotic changes, protects microbes from the stressful environment and promotes them to colonize ecological niches, which was otherwise inhibitory for their propagation. The best example of such solute is glycine betaine, a compatible solute accumulated in a food-borne pathogen *Listeria monocytogenes,* making it cold tolerant (Ko et al. 1994). Recently, some genes have been characterized by zwitterion compatible solutes found in halobacteria that show phenomenal protection of protein function (Galinski 1993; Louis and Galinski 1997). Compatible solutes never restrict the protein’s organization or function, but it mitigates the inhibitory effect of high ion concentration on enzymatic activity (Bohnert and Shen 1998). Compatible solutes increase the enzymatic thermal stability and inhibit the dissociation of the PS II oxygen-evolving complex (Papageorgiou and Murata 1995).

Some solutes have the potential to counter osmotic stress when present in higher concentrations (e.g., trehalose) but are protective in nature when they are present in their lower amount (Mackenzie et al. 1988; Holmström et al. 1996). There are two theoretical models proposed for explaining the defensive and alleviating role of compatible solutes on the organization and function of the proteins:

1. Preferential exclusion model
2. Preferential interaction model

In the preferential exclusion model, compatible solute omits from protein hydration case and provides stability to protein, promoting or maintaining protein-protein interaction by stabilizing their structure without disturbing the indigenous hydration water and interaction with the cytosolic water phase (Arakawa and Timasheff 1985). The second model emphasizes the interaction between the protein and the compatible solutes (Schobert 1977). During the water loss conditions, solutes directly combine with the hydrophobic part of the protein and prevent it from destabilization. The two models seem to be mutually restrictive, and the functions of compatible solutes can easily be understood by these two models (Bohnert and Shen 1998).

Some studies on fungal pathogen interactions illustrate that compatible solutes have the potential to scavenge free radicals, and mannitol's secretion protects these pathogens. The pathogenic infestation of plants and animals results in an intracellular free radical generation. The infection developed in the host by the pathogen can be restricted through speedy production and accretion of ROS that ultimately promotes cell death (Tenhaken et al. 1995). For example, *Cladosporium fulvum*, a tomato pathogen, starts production of mannitol at the time of infection, and mannitol production protects against fungal infection through ROS production (Joosten et al. 1990). ROS is not only limited to free radicals, viz., H_2O_2 and singlet oxygen; rather, it also expands toward superoxide and hydroxyl radicals. Mannitol, proline, ononitol, glycerol, and pinitol are reported as dynamic scavengers in a concentration-dependent manner in vitro, whereas glycine betaine did not show scavenging potential (Smirnoff and Cumbes 1989; Orthen et al. 1994). Relative scavenging efficacy of these compounds depends on the coefficient of reaction with hydroxyl radicals (OH^-), e.g., the coefficient of reaction of proline is one-fourth to that of mannitol, indicating better hydroxyl radical scavenging efficiency of mannitol than proline (Buxton et al. 1988).

10.2 Plant Growth and Stress

Important stress factors affecting growth and production of plants are cold, heat, and salt stresses. Common symptoms of salt stress are growth inhibition due to which the productivity of the plant decreases. Salt stress accelerates abscisic acid (ABA) synthesis, due to which the stomata become closed when it is transported to guard cells. Photosynthesis gets hampered due to closing of stomata, and therefore, photo-inhibition and oxidative stress predominate during salt stress. Sodium ions show a strong inhibition of potassium uptake by the roots, and due to excessive sodium ion, potassium ion is not absorbed by the plants. Potassium deficiency leads to the inhibition of plants' growth, because it is a lavish cellular cation and plays a crucial role in processes like keeping turgor pressure, membrane potential, and enzymatic activities inside the cell. A high level of calcium plays a protective role in sodium stress conditions. Calcium is facilitated by an intracellular signalling pathway that controls the expression and movement of potassium and sodium transporters (Jouyban 2012).

Plants exhibit various mechanisms against heat stress (Fig. 10.1), like rolling or senescence of leaves, inhibition of root and/or shoot growth, reduced seed germination, and decreased crop yield (Hasanuzzaman et al. 2013). Thermo-stability of cell membrane, photosynthesis rate, pollens' viability, and fruit setting serve as major factors to measure heat tolerance. As an example, the membrane permeability is varied by high temperature, promoting membrane fluidity and escalation of electrolyte loss, indicating the reduced cell membrane stability (Wahid et al. 2007). Inhibition of photosynthesis due to high temperature in the surrounding is also known as "photo-inhibition."

Damage from chilling creates following metabolic and physiological dysfunctions in plants:

- Interruption in the transformation of starch to sugar, i.e., amylase activity.
- Reduced carbon dioxide exchange and rate of photosynthesis.
- Destruction of chlorophyll pigment.
- Reduced size and decrease in the number of starch grain.
- Intensive dilation and vesiculation of smooth ER cisternae.
- Rough ER disappears and ribosomes are lost from the membrane surface.
- Mitochondria with reduced cristae and transparent matrix.

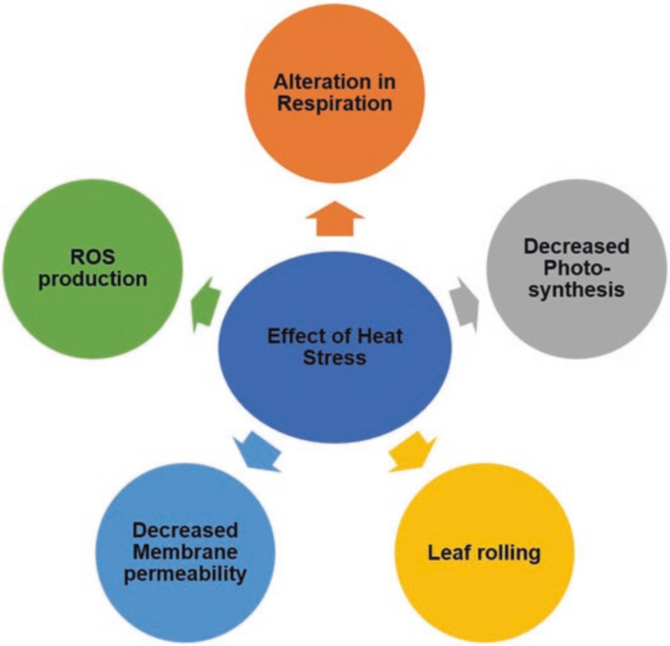

Fig. 10.1 Various effects of heat stress on plant growth

10.3 Stress and Its Types

Stress could be explained by any external factor that skeptically impacts the plant's productivity, progress, reproductive ability, or survivability. Stress generates many plant responses like metabolic dysfunction, altered gene expression, altered crop yield, growth, etc. (Verma et al. 2013). Stress can be classified into two main groups based on various factors:

(a) Abiotic or environmental stress factors
(b) Biotic or biological stress factors

Abiotic stress instigated by the environment may be physical or chemical (e.g., gaseous pollutants, heavy metals, and xenobiotics) in nature. In contrast, biotic stress includes pathogenic microorganisms such as bacteria, fungi and viruses, and herbivorous animals. Recovery of the plants from the stress is easy if the effect is mild or temporary; however, if the impact of stress is very harsh, stress can induce the plants' death by averting flowering or seed development, etc. (Verma et al. 2013).

10.3.1 Abiotic Stressors of Plants

Plant experiences various abiotic stresses like drought, salinity, temperature alterations, which show the negative effect on plant growth and limit the crop yield and the productivity of the plants worldwide. Growth rate and production efficiency are affected by the reaction, which is triggered by the set of genes that changes the pattern of expression in the plants. So it is necessary to identify genes that are associated with abiotic stress reaction mechanism in plants. There are some phytohormones, like abscisic acid, associated with plant adaptation besides the abiotic stress (Sah et al. 2016). The abiotic stresses occurring in plants include high salinity, drought, low temperature (cold, freezing), mechanical wounding, etc.

10.3.2 Cold or Freezing

Cold stress is the main abiotic stress that drastically lowers down plants' productivity, ultimately reducing agricultural crop yield. Motile organisms can escape the stress condition by moving from stressful situations to none or less stressed conditions, but plants cannot move from their natural position and face various stressful conditions. Due to this, plants acquire some tolerance known as acclimation against the stressful condition of chilling or freezing (Gull et al. 2019). In some important crops, which are incompetent to the cold acclimation, many cellular pathways are affected due to extreme cold conditions. Cold stress can be transduced by various signalling/transduction pathways (Fig. 10.2) e.g. ABA, protein phosphate, protein

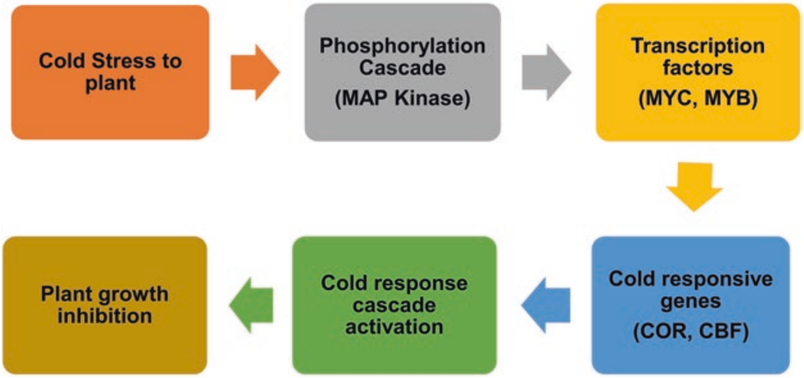

Fig. 10.2 Transduction pathway for cold stress

kinase, components of ROS, and Ca^{2+}, etc., and in all these, abscisic acid is the best one to be used.

10.3.3 Drought Stress

Rainfall is an essential abiotic component that is responsible for better productivity and yield of the crop. Climate changes continuously due to a tremendous increase in the level of CO_2 and temperature in the atmosphere. These climatic changes influence rainfall distribution and divide the land into two parts where one area benefited with higher rainfall, while the other one face low or no rainfall. This uneven rainfall distribution where the plants do not get a threshold amount of water according to their physiological demand is called drought, making the land unfavorable for plant growth. The plant reduces its shoot growth and metabolic requirements under drought conditions and synthesized some protective compounds under drought conditions for their osmotic adjustments.

10.3.4 Salt Stress

Soil salinity plays an important role in defining productivity and crop yield. Salt stress after freezing tremendously reduces the growth and yield of the growing plant. Salt stress influences the plant yield in two ways, viz., osmotic stress and toxicity. Under salinity stress, the soil's osmotic pressure is much higher than the osmotic pressure of plant cells that limits the uptake of water and minerals. Due to the limited or less absorption of water and minerals, plants show associated secondary effects such as suboptimal cell expansion, reduced cell membrane function, and condensed cytosolic metabolism.

10.3.5 Heat Stress

As we know, the temperature has a crucial role in the development of plants and plays a significant role in the plant's productivity and growth. We know that the earth's temperature is continuously increasing due to universe expansion and dependency on the products, which increases the level of greenhouse gases. This increase in the temperature largely affects the growth of the plant. Plant productivity rapidly declines due to heat stress as it directly affects the major processes of plants like photosynthetic efficiency, seed germination, and period of reproductive growth. Heat stress also disturbs the reproductive growth cycle due to which the tapetal cell's operation vanishes and anthers of the plant become dysplastic.

10.3.6 Plant Under Biotic Stress

There are various biotic stresses caused by fungi, viruses, bacteria, nematodes, insects, etc., making plant growth challenges. These biotic stress agents cause many diseases and result in a huge loss to the crop that ultimately lowers plants' yield. The researchers have developed various techniques to overcome these biotic stresses by studying the genetic mechanism behind the stresses. The development of genetically modified plants has proved a prodigious attempt against these biotic stresses by evolving resistant strains of crops, e.g., production of Bt-cotton, Bt-brinjal, etc.

10.4 Role of Compatible Solute on the Growth of the Plant in Stress

In response to various stressed conditions, the plant develops several physiological, morphological, and molecular mechanisms to cope with stress. The plant increases the production of compatible solutes and maintains the homeostasis inside the cell against various stresses. Compatible solutes protect the plants from stresses by maintaining cellular osmotic pressure by decreasing the detrimental risk by ROS, repairing membrane injury, and alleviating the stress-responsive proteins and enzymes (Singh et al. 2015). Osmoprotectants or compatible solutes are found in the cytoplasm by which these molecules provide tolerance under the stressed conditions (Ramanjulu and Bartels 2002). These compatible solutes offer osmotic balance by reducing cells' osmotic potential and maintaining turgor pressure under low water potential conditions (Pathan et al. 2004). Compatible solutes protect cellular apparatus from injury but are deprived of intrusive normal metabolic practices. Different osmoprotectants protect the cellular components from various stressors (Saxena et al. 2013):

- Mannitol reduces the size of the cell membrane and protects hydroxyl radical (OH^-) mediated damage to cellular structures.
- Glycine betaine protects protein complex involved in photosynthetic, reduces oxidative metabolism of lipid, and functions as a chaperon.
- Proline helps in the adjustment of cellular redox state.
- Ectoine, trehalose, and fructan help stabilizing the membranes from oxidative damage.
- Polyols such as myo-inositol, d-ononitol, and d-pinitol function as hydroxyl radicals scavengers, and regulate stomata closure.

Polyamines reported very powerfully against severe environmental issues. Polyamines are organic nitrogenous compounds comprising more than two amino groups and majorly positively charged at physiological pH. Rhizobacteria regulate water potential in plants, exaggerating osmolytes' production in drought situations (Jaleel et al. 2009). *Pseudomonas* species and their various substrains, e.g., *P. putida*, *P. syringae*, and *P. montelli*, upgraded drought stress in maize crop. Some aliphatic amine phytohormones containing aliphatic nitrogen such as putrescine (Put), spermidine (Spd), and spermine (Spm) have been characterized in plants and bacteria. Plants incline to collect a large volume of polyamines against various biotic and abiotic stresses (Singh et al. 2015).

10.5 Types of Compatible Solutes

Based on their chemical structures, organic osmolytes, or compatible solutes fall into four general categories (da Costa et al. 1998):

A. Amino acids: Glycine betaine, Proline
B. Sugars: Sucrose, Trehalose
C. Phosphodiesters: Mannosylglycerate
D. Polyols: Sorbitol, Mannitol

10.5.1 Amino Acid

Amino acids are commonly distributed compatible solutes among prokaryotes. Intracellular potassium ions play a very imperative role by sustaining the osmotic equilibrium across the membrane and stabilize cellular turgor pressure (da Costa et al. 1998). The rapid influx of potassium ions is directly proportional to the saline concentration in the growth medium. Neutralization of the sear riving K^+ is done by adding few organic ions (instead of Cl^-), which are the derivatives of amino acids like α-Glutamate, which can be synthesized from the medium (da Costa et al. 1998). During low saline conditions, K^+ is neutralized only by the glutamate accumulation (such as in *Petrotogamiotherma*). Still, during high salinity, they require their isomers or combinations of different amino acids to neutralize and maintain the osmotic balance (as observed in *P. mobilis*) to accumulate both forms of glutamates during high saline conditions (Jorge et al. 2007). Two different forms of glutamate viz., α- and β-glutamate have been reported in the hyperthermophilic bacterium, *Aquifex pyrophilus.* These glutamates are associated with other negatively charged phosphodiester compatible solutes in salinity conditions (Lamosa et al. 2006).

Alanine, glutamine, and proline are common forms of compatible solutes found abundantly in numerous plants (Fig. 10.3). Various gram-positive bacteria accrue low levels of alanine and glutamine; however, the proline level is always high in all prokaryotic organisms. Glutamine detected in the genus *Corynebacterium* members also comprises two forms, i.e., α- and β-glutamine, where the former is less soluble than the latter (Galinski 1995). β-glutamine is also present in the halophilic methanogens and acts as a competent compatible solute in osmoadaptation due to its high solubility rate (Robertson et al. 1990).

Glycine betaine is a universal compatible solute present in bacteria in a saline environment and recognized as an eco-physiological characteristic of halotolerant plants as well as algal species (Imhoff and Rodriguez-Valera 1984). Glycine betaine is a versatile solute and efficiently absorbed as an osmolyte to overcome the salt stress; therefore, it is also used as the regular component of the culture media. Few bacterial species and single methanogen, i.e., *Methanohalophilus portucalensis*, can synthesize betaine in two ways: either by methylating the glycine or by oxidizing the choline, which is also known as “biosynthesis of glycine” (Canovas et al. 1998; Nyyssola et al. 2000).

Positively charged amino acids like ornithine and lysine are converted into neutral zwitterion after its *N*-acetylation to form *N*-δ-acetyl ornithine and *N*-ε-acetyl-β-lysine, which helps in osmoadaptation during salt stress condition. *N*-δ-acetyl ornithine is accumulated by *Planococcus citreus, Sporosarcina halophila*, and *Bacillus* spp., while *N*-ε-acetyl-β-lysine is inimitable in methanogenic archaea during salt stress (Wohlfarth et al. 1993). Two genes *ablA* and *ablB* found in genomes of different methanogenic archaea express in salinity condition that encodes lysine-2,3-aminomutase and β-lysine acetyltransferase, respectively (Pfluger et al. 2003). Ectoine, a cyclic tetra-hydropyrimidone (1,4,5,6-tetrahydro-2-methyl-4-pyrimidinecarboxylic acid) (Fig. 10.4), and hydroxy-ectoine (a derivative of ectoine) were first identified in *Ectothiorhodospira halochloris,* a phototrophic sulfur bacterium and act as the marker for halophilic bacteria (Galinski et al. 1985). In *Halobacillus halophilus*, ectoine with proline is produced at very high salinity, but the ratio of ectione to proline up-regulated later during the stationary phase of the cell cycle (Saum and Muller 2008). Cells of *Vibrio cholera* grow well in

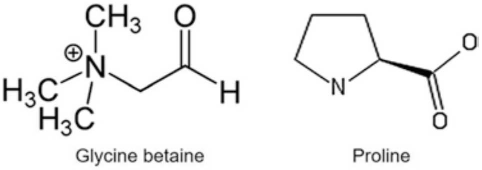

Fig. 10.3 General structures of amino acid–based compatible solutes

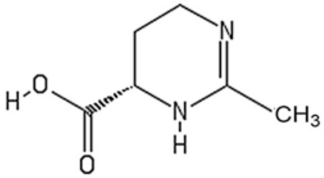

Fig. 10.4 General structure of Ectoine

high-osmolarity, gathered ectoine or betaine, and expand nonadapted cells (Pflughoeft et al. 2003).

10.5.2 Sugars

There are two types of sugars in nature – reducing or nonreducing sugars. Trehalose is a nonreducing disaccharide of glucose often used by various entities during drought conditions. However, it could also play an osmolyte role. Trehalose is found in multiple organisms ranging from bacteria, fungi, plants, and invertebrates. It protects plants from different stresses, e.g., oxidative stress, heat, dehydration, freezing, desiccation, and hyperosmotic conditions. Trehalose acts as a key solute in *Chromohalobacter israelensis* when the cells are exposed to salt stress (<0.6 MNaCl) (Regev et al. 1990). Trehalose acts as a signaling molecule in different metabolic pathways along with a source of energy and carbon for the cell (Elbein et al. 2003). There are five enzymatic systems reported to involve in the biosynthesis of trehalose (Avonce et al. 2006):

1. TPS/TPP enzymatic systems
2. TreSenzymatic systems
3. TreY-TreZenzymatic systems
4. TreP enzymatic systems
5. TreT enzymatic systems

The majority of the microbes depend only on a single biosynthesis pathway, but few others like *T. termophilus*, *Mycobacterium tuberculosis,* and *Corynebacterium glutamicum* have two to three pathways for trehalose synthesis (Silva et al. 2003; Avonce et al. 2006). A thermophile *Rubrobacter xylanophilus* contains genes for four pathways: TPS/TPP, TreS, TreY/TreZ, and TreT to synthesize trehalose. The bacterium is the most radiation-resistant organism by accumulating the major organic solute under all conditions (Empadinhas et al. 2007).

Sucrose is another type of nonreducing disaccharide of glucose and fructose. Sucrose is extensively disseminated in the plants and acts as energy storage, while in some halotolerant and halophilic organisms, it improves the growth at high salinity. Sucrose production is also reported in cyanobacteria and proteobacteria (Mackay et al. 1984), where it is generally accompanied by lower salt tolerance strains. Sucrose also acts as a compatible solute in osmotic stress, and its protective accretion is reported in freshwater and marine cyanobacteria and some in proteobacteria. The sucrose synthesis pathway was first described in higher plants and later in cyanobacteria and green algae. There are two distinct pathways involved in the production of sucrose. Sucrose synthesis starts from fructose-6-phosphate and UDP glucose and follows two intermediate steps through sucrose phosphate synthase (SPS) and sucrose-phosphate phosphatase in freshwater and marine cyanobacteria (Lunn 2002).

Another pathway is present in filamentous cyanobacteria (e.g., *Anabaena* sp.), and higher plants comprising sucrose synthase (SuS) enzyme, which reversibly catalyzes the condensation of fructose and ADP-glucose (UDP-glucose) to sucrose. As the sucrose synthase (SuS) activity is reversible, it is also involved in sucrose catabolism. Interestingly, enzymes associated with sucrose synthesis could not be recognized in other bacteria or archaea. In eukaryotes, sucrose synthesis is assumed to be assimilated by endosymbiotic cyanobacteria that may have chloroplasts as an ancestor (Curatti et al. 2000). Two different forms of SPS have been characterized by the strains of *Anabaena* and *Synechocystis,* in which both the enzymes are active; however, the reason behind this duplication remains unclear.

10.5.3 Phosphodiester

This category of compatible solutes is generally found in the microorganism with an optimum temperature of 80 °C or present in mesophilic strains as an abundant organic solute in the form of di-*myo*inositol phosphate (DIP) (Martins et al. 1997; Santos and da Costa 2002). Initially discovered in *Pyrococcus woesei*, DIP accumulation was later identified in response to supraoptimal growth temperatures in hyperthermophiles belonging to genera *Aeropyrum*, *Archaeoglobus*, *Pyrodictium*, *Pyrolobus*, *Thermococcus*, and *Thermotoga* (Scholz et al. 1992). DIP is considered to facilitate the protective function in extremely high temperatures. Polar head lipid groups of archaea and bacteria lack *myo*inositol-phosphate, and DIP is viewed as a by-product of the synthesis of inositol-containing phospholipids. These inositol-comprising phospholipids are found in bacteria, reached high levels in *Pyrococcus* and *Thermococcus,* and most abundantly in crenarchaeotes. *Pyrolobus fumarii*, the most thermophilic adaptive species of all known *Archaea* comprises DIP as the major organic osmolyte (Goncalves et al. 2008). Rodionov and collaborators elucidated the biosynthetic pathway and genes involved in DIP production (Rodionov et al. 2007).The genes for cytidine triphosphate (CTP): L-*myo*-inositol-1-phosphate cytidylyltransferase and fordi-myo-inositol-1, 3′-phosphate-1′-phosphate (DIPP) synthase have been identified in several hyperthermophiles with *Rubrobacter xylanophilus,* which accumulates DIP. DIPP synthase is a bifunctional enzyme that catalyzes the condensation of CTP and l-*myo*-inositol-1-phosphate into CDP-l-*myo*-inositol and also uses CDP-l-*myo*-inositol and l-*myo*-inositol-1-phosphate to synthesize DIPP (Rodrigues et al. 2007).

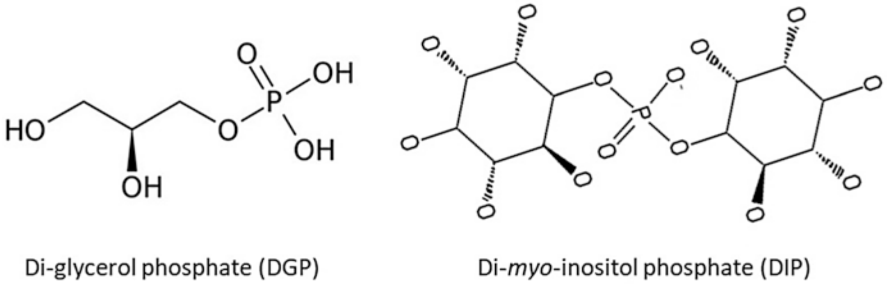

Fig. 10.5 General chemical structures of phosphodiester compatible solutes

The members of genus *Archaeoglobus* contain di-glycerol phosphate (DGP) collected in response to salinity, while glyceryl-*myo*inositol phosphate (GIP) (Fig. 10.5) has been reported in *Aquifex pyrophilus* and *Archaeoglobus fulgidus,* which is the structural chimera of DIP and DGP (Martins et al. 1997; Lamosa et al. 2000). GIP may have a role in both osmo- and thermoprotective activity, additionally its level upregulates against heat and osmotic stress. *Rubrobacter xylanophilus* gathers di-*N*-acetyl-glucosamine phosphate (DAGAP), having a similar structure to phosphodiester compatible solutes like di-*myo*inositol phosphate (DIP), di-glycerol phosphate (DGP), glyceryl-*myo*inositol phosphate (GIP), and di-2-O-β-mannosyl-di-myo-inositol-1,1′(3,3′)-phosphate (DMDIP) present in hyperthermophiles. Mostly phosphodiesters present in hyperthermophiles are polyol derivatives; on the contrary, DAGAP possesses a phosphate group linked to two sugar moieties (Empadinhas et al. 2007).

Cyclic-2,3-bisphosphoglycerate (cBPG) , a compatible solute to date, is only detected in methanogenic archaea with a different range of prime temperatures for its growth (Seely and Fahrney 1983; Gorkovenko and Roberts 1993; Shima et al. 1998). The cBPG has a diverse role in thermal protection and acts as an intermediate in the gluconeogenic pathway as well as a phosphate reservoir for ATP synthesis. However, its role in osmoadaptation remains unclear (Gorkovenko and Roberts 1993; Shima et al. 1998). The genes for cBPG synthesis have been reported in different methanogenic archaea, such as *Methanothermus fervidus* (Lehmacher et al. 1990), whereas the genome of hyperthermophilic archaeans belonging to genus *Pyrococcus* and *Rubrobacter* does not contain the genes for synthesis.

10.5.4 Polyols

It is a hardly used compatible solute and is usually present in prokaryotes. Polyols such as glycerol, arabitol, sorbitol, mannitol, and inositol are archea-specific compatible solutes but are also present in halotolerant fungi, few algae, and plants (Grant 2004). Except for *Zymomonas mobilis* and *Pseudomonas putida*, polyols are rarely used by the bacteria as compatible solutes (Lehmacher et al. 1990; Kets et al. 1996). *Pseudomonas putida* gathers mannitol when exposed to osmotic stress, while *Zymomonas mobilis* can convert sucrose into sorbitol and glucose, while sorbitol is used as a compatible solute. This accumulation of compatible solutes by the latter reflects the convergent evolution for osmoadaption, e.g., the yeast *Z. mobilis* developed an approach to modify the available and abundant sugar into compatible solute. During low water availability, cyclitol D-pinitol gathering is done in wide ranges of crop and woody plants such as *Glycine max, Cicer arietinum, Pisum sativum*, and transgenic tobacco, which exhibit prospective for this group of compounds to impact physiology among a range of herbaceous and woody plant species (Merchant and Richter 2011). Cyanobacteria and some halotolerant probacteria *Pseudomonas mendocina* (Mikkat et al. 2000) and *Stenotrophomonas rhizophila* (Roder et al. 2005) produce glucosyl glycerol and use it as compatible solute during high salinity. Glucosyl glycerol, an uncommon compatible solute, is a derivative of polyol and organizationally associated with galactosyl glycerol, which is found in red algae (Karsten et al. 2003). Compatible solute collection occurs in *Archaea* concern by applying polyol-phosphodiesters viz., diglycerol phosphate, and di-myo-inositol phosphate (Santos and da Costa 2002).

10.6 Conclusion

Crop production directly gets affected by its surroundings and environment and report a prodigious impact on it. As the world population is increasing day by day, crop production must be increased in the same proportion to nourish everyone. Adverse environmental conditions may affect the crop yield, which may create a global concern about food security. Stress negatively impacts plant growth and may change the physiology and morphology of plants. Unfavorable climatic conditions enforce various types of stress like heat, cold, salinity, heavy metal, nutrient, and drought stress and restrict plant growth and production capacity. To counter this, plants adapt stress conditions through morphological, biochemical, or molecular changes. Plants synthesized organic compounds known as compatible solutes, which help to maintain a balance between stress conditions and plant survival. Compatible solutes found in cytoplasm help to keep the osmotic pressure. By synthesis and accumulating compatible solutes, plants maintain homeostasis in a stressed environment. Compatible solutes have a protective role against cold, heat, drought, salt stress, and biotic stress as well.

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Chapter 11
Compatible Solute Engineering: An Approach for Plant Growth Under Climate Change

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Abstract Agriculture is not only a livelihood source but also fulfils the nutritional requirement of humans. To feed the world population increasing at an alarming rate, crop production must be increased. However, crop production is adversely affected by various abiotic factors or changes in climatic conditions worldwide. Climate exhibits a direct impact on the yield of the crop. In stress condition, the physiological state of plants gets altered that ultimately affects the overall growth of plants. Plants exhibit different mechanisms to survive these stress conditions. Plants synthesize and accumulated non-toxic, non-reactive organic compounds to counter the unfavourable conditions, and these compounds are known as compatible solutes. Compatible solutes exhibit beneficial characteristics by which plants could survive in hostile environments. Hereby, we discussed the role of few important compatible compounds and their role in plant growth as well as in different stress conditions. We have also summarized the effect of inducible changes in the compatible solutes on plant growth under different climatic conditions.

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Keywords Compatible solute · Osmoprotectants · Polyols · Climate change · Plant growth

11.1 Introduction

The human population is increasing rapidly worldwide, and demographic studies predicted that it would touch 9.1 billion by 2050 (Sah et al. 2016). For this fastest-growing population, cultivated crop production should also be increased accordingly or in a parallel manner, but regrettably, it is not happening. As agriculture is dependent on environmental conditions, they also play a key role in crop production (Kumar et al. 2019). Besides, stress is also a key factor behind the decline of crop yields. Additionally, variation in climatic conditions, delays in seasonal change, and unequal distribution of rainfall also account for the reduction in productivity, which ultimately creates a threat for global food security. Harvest efficiency of plants is extremely influenced by ecological pressures like the variation in temperature, salinity, freezing, and drought. These factors not only influence plant growth but are also risky for sustainable agriculture. In a report by FAO (2010), experts emphasized the need for acceleration in the production of different agricultural crops like rice, maize, and wheat by 60% for maintaining a balance between crop production with population eruption. Climate change is a major factor behind loss in crop production and significantly affects the efficiency of important cereal crops, viz. wheat, maize, and barley (Jaleel et al. 2009). Crops and various plants are regularly exposed to different sets of stresses. Most of the plants cannot sustain this pressure that decreases the harvest yields over the world. They do not have any defensive mechanism to battle this unfavourable condition; however, a few plants have some exceptional components by which they shield themselves from adverse or stressed conditions (Bohnert et al. 1995). They have some neutral molecules which get accumulated during the unfavourable condition in plants and help them during adverse situations. These molecules are known as compatible solutes. The compatible solutes are non-hazardous molecules that help the plants to survive the ominous or any stress condition (Wani et al. 2020). These molecules are also present in certain prokaryotes, making them stress-tolerant living beings like thermophiles and halophiles (Lang 2007). Compatible solutes are likewise known for their role in balancing out plant cells' structure in stress conditions (Flowers and Colmer 2008).

There are some reports about the accumulation of osmoprotectants by bacteria. Generally, bacteria could accumulate sugars and their derivatives, amino acids, and by-products of polyols and their derivatives either directly from their niche or through *de novo* synthesis (Paul 2013). Glycine betaine or trimethylglycine is a type of osmoprotectant mostly acquired by bacteria (Paul 2013). During the condition of salt stress, bacteria commonly assimilate and collect carnitine, a trimethyl amino acid, and a potent osmoprotectant (Jebbar et al. 1998). Microorganisms and plants also accumulate a high amount of proline when exposed to salt stress (Yoshiba et al. 1997; Kempf and Bremer 1998).

Moreover, gram-positive bacteria aggregate proline by up-regulation of their biosynthesis, whereas gram-negative bacteria escalate its absorption by uptake from the medium (Paul 2013). When microorganisms are exposed to salt stress, they also gather sugar osmoprotectants, preferably sucrose and trehalose. Similarly, gentiobiose, melibiose, maltose, turanose, raffinose, stachyose, verbascose, altrose, palatinose, and cellobiose are generally considered under infrequent sugar compatible solutes repeatedly reported in plants (Gouffi and Blanco 2000; Panikulangara et al. 2004).

11.2 Compatible Solutes

A low molecular weight, organic, highly soluble, and non-toxic compound accumulated against osmotic stress in various taxa, including plants, is known as osmoprotectant (Yancey et al. 1982). Osmoprotectants, also known as compatible solutes, usually comprise the following characteristics (Fig. 11.1):

(a) Neutral at physiological pH
(b) Highly soluble
(c) Non-toxic

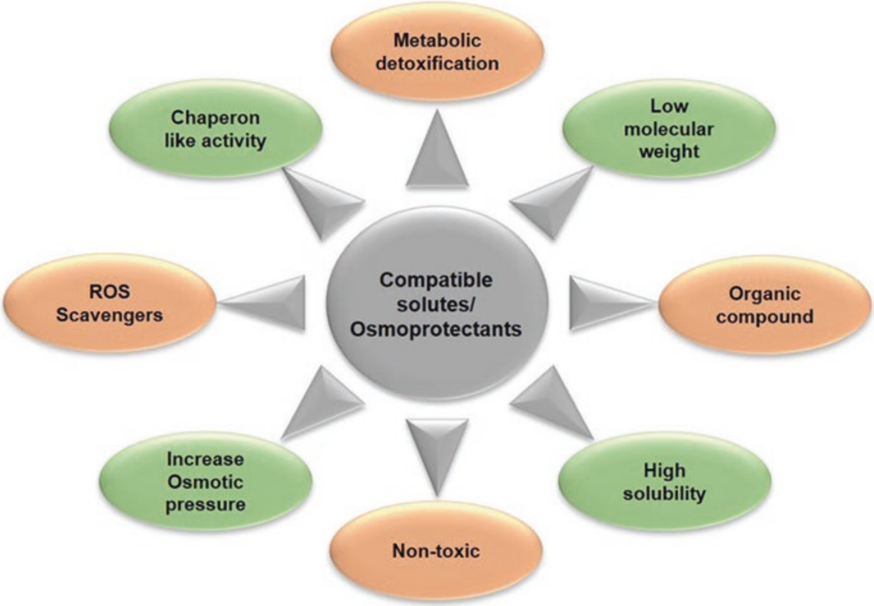

Fig. 11.1 General characteristics of compatible solutes

(d) Reactive oxygen species (ROS) scavenger
(e) Chaperon-like activity
(f) Metabolic cleansing
(g) Increased osmotic pressure (Serraj and Sinclair 2002)

Intracellular compatible solutes are accumulated through natural environments, e.g. osmolarity and temperature specific transcription factors and transporters regulation. It has been predicted that plants adopt a cautious mechanism by possessing compound action through freeze-defrost phases and at higher temperatures. At present, it is accepted that protein structure stabilized by evolving special prohibition from layers of water on the outside of hydrated proteins. This favours indigenous acquiescence and dislodging of inorganic components that would, in other ways, may favour misfolding (Ashraf and Harris 2004). The compatible solutes are majorly divided into five classes: Polysaccharides, Sugar alcohols, Amino acids, Betaines, and derivatives of sulphate, as given in Table 11.1 (Ashraf and Harris 2004).

The crops that cannot accumulate the uncommon compatible solutes normally amassed by stress-tolerant living organisms can have the option to introduce these molecules with the assistance of biotechnology. In this way, the novel biotechnological approaches are utilized for designing the compatible solutes biosynthesis pathways which is a latent system that refines the stress tolerance mechanism of crop plants. Various genes are altered to create a transgenic plant that gathers compatible solutes and upgrades their resilience to stress conditions (Ashraf and Harris 2004; Khan et al. 2009). The biochemical pathways, including the synthesis of compatible solutes, might be used to create a stress-tolerant plant. Certain plants may not involve in acquiring genes from stress-tolerant species: over-co-expressing or changing regulatory genes or a gene from an alternate, however stress-intolerant, species may be adequate. Significant models were designed for over-expression of genes for enzymes that increase putative compatible solutes, e.g. proline, polyols, or fructans (Tarczynski et al. 1993; Delauney and Verma 2002). A strong correlation between the number of compatible solutes and tolerance level has also been documented earlier (Bohnert et al. 1995). In this regard, the next step will be studying plants' engineering to express enzymes that lead to the synthesis of compatible

Table 11.1 The major classes of compatible solutes and their subclasses

S. No.	Class of compatible solutes	Subclasses
1.	Polysaccharides	Glucose, fructose, sucrose, trehalose, raffinose, and fructans
2.	Sugar alcohols	Polyols, e.g. sorbitol, mannitol, glycerol, inositol, and methylated inositols
3.	Amino acids	Proline, pipecolic corrosive; methylated proline-related mixes, e.g. methyl-proline, proline betaine, and hydroxyproline betaine.
4.	Betaines	Glycine betaine, b-alanine betaine
5.	Choline O-sulphate and tertiary sulphonium mixes	Dimethylsulphoniopropionate (DMSP)

solute and subsequent physiological analysis of these plants. This chapter will focus on polyols and its derivatives and review the engineered incorporation of polyols into the plants that are less tolerant to salinity and their ultimate effect in different stress conditions, especially drought or salinity.

11.3 Polyols

The polyols accumulate in many different forms, like straight-chain metabolites, e.g. mannitol and sorbitol (Bieleski 1982), and cyclic polyols, e.g. Myo-inositol and its methylated by-products (Fig. 11.2). These metabolites are accumulated by different living organisms, including bacteria, yeasts, marine algae, higher plants, and animals, and are directly related to their drought and saline tolerance affinity. Certain plants' polyols with sucrose and raffinose are considered the direct yield of photosynthesis responsible for translocation of carbon skeletons and energy transfer between source and energy-deficient organs. Amongst all, mannitol is considered as the most abundant polyol in nature, perceived in more than 100 vascular plant species of numerous families viz. apiaceae (celery, carrot, and parsley), rubiaceae (coffee), and Oleaceae (olive and privet) (Lewis 1986). After mannitol, sorbitol is the

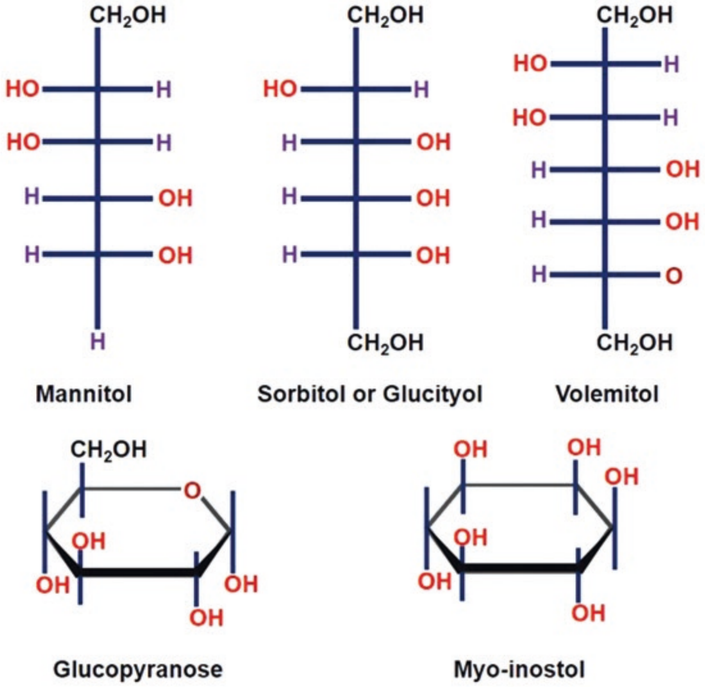

Fig. 11.2 Different structural forms of polyols present in plants

second most abundant polyol in nature, foremost translocated photoassimilate, found in the phloem of all economically important plant genera like *Pyrus* (pear), *Malus* (apple), and *Prunus* stone fruits such as peach, cherry, plum, and apricot (Bieleski 1982; Moing et al. 2006).

Generally, polyols help water retention in the cytoplasm and allow sodium translocation to the vacuole, balancing osmotic pressure. Additionally, compatible solutes provide a defence to several cellular organelles by scavenging reactive species. Polyols such as non-reducing sugars can also accumulate surplus carbon in different climatic stress situations (Kobayashi et al. 2015). Few other plants such as grape berry, tobacco generally produce polyols when encountered with saline and drought stress.

Polyols also provide efficient protection against different pathogens (Clark et al. 2003). The accumulated arabitol is likely to serve as osmoregulant by the pathogen, because water acquisition is important in pathogenicity. Polyol accumulation during infection has also been reported in cucumber and sunflower (Al-Janabi and Lösel 2003; Jobic et al. 2007). Abiotic stress tolerance in plants is an intricate mechanism and is intervened by numerous ecological adjustments that could include various genes. A single procedure can't introduce stress tolerance in the plants. The issue could be comprehended by following an encompassing methodology presenting genetic obstruction, coordinating genotypes to situations, following techniques of better preservation and use of soil dampness. Additionally, endeavours, including customary and biotechnological practices for a hereditary upgrade, can surely vow to incite and improve the required abiotic stress tolerance in these significant yield plants.

Biotechnology offers new dimensions and strategies pertinent to farming. It utilizes a reasonable system and specialized plant tissue culture and molecular biology methodologies for creating business procedures and items. These strategies empower choice of fruitful genotypes, improved disengagement, and cloning of required traits, production of transgenic plants with improved yield. This capacity has stimulated horticulture from an asset-centred to a science-based industry (Sharma et al. 2002). Collectively, these non-exclusive methods are both an expansion and a fundamental piece of old-style reproducing, backing effectively to shortening rearing and determination cycles (Sharma et al. 2002). Conventional breeding produces numerous varieties and hybrids, which eventually support the production of the harvests which feed the world (Borlaug 2002). Biotechnological products create ethical issues, whilst its focus should be on food security (Sharma et al. 2002). The use of biotechnological instruments provides quick and effective advancement in the quality and quantity of plants. It resulted in the form of increasing area of transgenic crops. The wise use of biotechnological instruments holds extraordinary potential in reducing a portion of the vital requirements to the proficiency of yields developed in abiotic stress.

11.4 Different Approaches Involved in Engineering

Two or three gene exchange approaches are applied to improve plants' stress tolerance (Holmberg and Bülow 1998). These are immediate consequences of a single gene or effects of numerous genes that drive the entire course of different qualities products inside the plant in the event of abiotic stress. Genetically improved plants for single protein product either result in biosynthesis of stress-tolerant solute (Tarczynski et al. 1993; Kishor et al. 1995), or change the property of lipid layer (Kodama et al. 1994) or LEA protein (Xu et al. 1996) that results in plant protection from abiotic stressors.

Basically, there are two methodologies that have demonstrated more significant returns to deal with the compatible solute-deficient crop turning them into a stress-tolerant crop. One method depends on biochemical pathways in which the genes modify the downstream response during signal transduction pathways. This procedure is acquired by either over-expression of individual proteins or anti-sense suppression of proteins. Proteins are mostly utilized for the desired biochemical response, and most transgenic experiments exploit this methodology (Sheveleva et al. 1998). Another method is to design the stress tolerant crop by modifying stress recognition and signalling. This technique uses the endogenous stress-relieving mechanism in the target species. Modification in this approach could be adopted for the species that exhibited sensitivity towards stress due to miscoordination between the signal and response modules. Response against stress could also be engineered to hyper-induce the tolerant compound. Plant species have gene types to respond in stress conditions or in other words, the plant responses can be utilized to identify the cause of stress. In this context, over-expression of a signal transduction pathway intermediate would be adequate to persuade global stress reactions. The presentation and over-expression of an *Escherichia coli* mannitol-1-phosphate dehydrogenase gene in plants, e.g. tobacco, *Arabidopsis*, wheat, and *Populus tomentosa,* resulted in aggregation of mannitol that provides tolerance to water deficiency or saltiness (Abebe et al. 2003; Hu et al. 2005). The fundamental result of mannitol oxidation in grapevine is fructose which is an NAD^+-subordinate response catalyzed by the oxidoreductase mannitol dehydrogenases as indicated by KEGG pathway analysis (Kyoto Encyclopaedia of Genes and Genomes) (Ogata et al. 1999).

If there is a requirement of Sorbitol aggregation in plants, the Sorbitol 6-phosphate dehydrogenase (S6PDH) gene is presented in sorbitol-deficient plants. Sorbitol 6-phosphate dehydrogenase is an essential photosynthate and a significant solvent starch in the phloem of rosaceous natural product trees (Moing et al. 2006). S6PDH is an important enzyme in the pathway of sorbitol biosynthesis that is ultimately accepted by sorbitol dehydrogenase (SDH) in the presence of NAD^+ and catalyzes the oxidation of sorbitol to fructose. Other significant segments are the sorbitol transporters, which assume a job in layer transport of sorbitol. Sorbitol digestion and transportation are significant in sugar amassing into the natural product as well as in holding high photosynthetic capacity in leaves (Zhou and Cheng 2008). Sorbitol additionally encourages phloem boron transport by the development of boron-sorbitol buildings in rosaceous natural product trees (Brown and Hu 1996; Hu et al. 1997). A job for sorbitol in boron versatility has been accounted in tobacco plants which are changed with S6PDH (Brown et al. 1999) translocation in rosaceous organic product trees. These transporters likely go about as proton/sorbitol co-transporters for dynamic take-up over the plasma film with the proton siphon. Watari et al. (2004) discovered transporters of sorbitol communicated in phloem tissues in apple source leaves, recommending apoplastic phloem stacking. A blend of apoplastic and symplastic phloem stacking in peach plants by estimating sorbitol's subcellular centralizations has also been reported (Nadwodnik and Lohaus 2008). Apoplastic emptying could likewise be significant in the natural product. Gao et al. (2003) separated cDNAs encoding sorbitol transporters from harsh cherry and associated their function in sink movement in leafy food leaves. Zhang et al. (2004) gave proof of a useful monosaccharide transporter's spatial restriction for apoplastic phloem emptying in creating natural apple products.

The cyclic polyol D-(+) onotinol can be presented in plants by gene exchange or over-expression of glucose-6-phosphate. These compatible solutes are commonly found in sugar pine and many ice plants like *Mesembryanthemum crystallinum* (Vernon and Bohnert 1992). Inositol phosphate synthase catalyzes the change of glucose-6-phosphate into inositol-1-P which is then dephosphorylated by inositol monophosphate. In *M. crystallinum,* the pressure initiated protein D-Myo-inositol methyltransferase catalyzes the methylation of Myo-inositol to frame D-ononitol (methyl-myoinositol) (Vernon & Bohnert, 1992). Myo-inositol and its subsidiaries are ordinarily known for their job in cell flagging and film biogenesis; they likewise take an interest in osmotic pressure reaction.

11.5 Role of Compatible Solutes in Plant Growth

Accumulation of compatible solutes provides defence against climatic change or abiotic stress and promotes plants' growth in adverse conditions. To start with, the low measures of fructan, mannitol, and trehalose appear to demonstrate that good solutes have a particular defensive function compared to compatible solutes. Aggregation probably won't be vital. Additionally, extraordinary compatible solutes appear to have changed capacities. Mannitol is reported as a hydroxyl radical scavenger (Shen et al. 1997) and gives specific defence besides impacts once found in transgenic plants (Hayashi et al. 1997). In this way, one proposal is that we must move genes prompting the amalgamation of various compatible solutes at little or adequate sums. Compatible solute engineering ought to be strengthened by designing rummaging frameworks for reactive oxygen species. So, the presentation of mannitol in mannitol deficient plants can shield the plant from ROS's impact and goes about as a radical forager. The amassing of D-ononitol reassures sodium sequestration and shields the photosynthetic mechanical assembly from the stress-actuated increment in ROS (Nelson et al. 1998). The mannitol also serves as a cell reinforcement component in plants; it has been proposed that the accumulation of mannitol in the chlorophyll of tobacco plant makes the plant impervious to methyl viologen, inciting oxidative pressure. Within sight of methyl viologen, disengaged mesophyll cells of transgenic tobacco showed expanded maintenance of chlorophyll just as higher CO_2 obsession rates than the wild type.

The new research recommends that polyol of microbial infection may contribute to plant-pathogen interaction. Investigation of transgenic plants while comparing to plant mannitol catabolism and transport affirms the role of mannitol during contamination and improved ailment opposition. The over-expression of celery Mannitol dehydrogenase, an important enzyme in mannitol catabolism in tobacco plants, decreased susceptibility to different plant diseases (Jennings et al. 2002). At the point when the celery mannitol transporter is communicated in tobacco plants, affectability to mannitol-emitting pathogenic parasites is diminished, indicating the role of polyol transporters in defence components (Juchaux-Cachau et al. 2007). Altogether, perfect solute makes the crop resistant to various climatic changes and helps them overcome troublesome conditions.

The function of polyols is likewise found in those plants which have a boron deficiency. Some plants are confined in the transfer of boron in a different part of the plant; however, they are ready to aggregate boron in leaves yet couldn't transfer it to different parts like root, shoot, etc. which impact plant growth and development, but polyols like sorbitol, mannitol, or galactitol also help plants to transfer boron in different parts. Such plants like tobacco, which were limited in the portability of boron, can have the option to amass boron and move it rapidly as different plants do with the assistance of these polyols (Dell and Huang 1997; Brown et al. 1999). In addition, if we consider those solutes which are not able to produce nitrogen, including Betain and proline, and fail to help plants to survive in stress conditions like unavailability of nitrogen from the environment, so in that condition, cyclic polyols like mannitol, sorbitol are also playing the major key role to help the plants to survive in this situation, the member plant species like Plantaginace and Mangrove accumulate these cyclic polyols to survive in salinity and water-deficient stress (Ahmad et al. 1979; Briens and Larher 1982).

11.6 Effect of Climate Change on Plant Growth

Changes in climatic conditions like increase in temperature, change in rainfall pattern, drought, waterlogging, etc., affects the agro-ecosystem and ultimately the plant growth. These changes exhibit a direct influence on crop yield by affecting plant growth and reduce crop quality. It is a big factor and should be addressed globally. It could disturb the availability of food, access, and stability of food supply (Wheeler and von Braun 2013). DaMatta et al. (2010) reported that climate change is associated with deteriorated food quality as climate change disturbs or reduces protein, micronutrient content, and modifies lipid composition. Climate change has also raised a great concern for global food security, where a fast-growing population also requires increased crop production.

Most importantly, new studies claimed that further rise to one degree Celsius (°C) in temperature could result in a drastic drop of ~5% in crop yield (Lobell et al. 2011). Climatic changes influence each component of the physiology and metabolism of the plant. Albeit a portion of the progressions is versatile, several may just be pathological results of injury due to stress. Crop Science Society of America suggested that new varieties/species of crops could offer an opportunity for more crop production in changed climatic conditions (Boote et al. 2011). Two approaches are there for acclimatization of crops to changed climatic conditions: one is developing a new crop, whereas the second one is the introduction of desired characters into existing strain by use of genetic engineering.

Moreover, crop improvement is not an easy task, because new cultivars exhibited several properties like increase crop production, competent in inputs, personalized to the rigorous requirement of the market, sustain stability even in inconstant climate, and potentially improvisation to climate mitigation. Information on some biotic stress like salt and drought is similarly significant, because they symbolize preferable aims for genetic dominance to expand salt and dry season stress tolerance. Naturally, for a plant to forfeit a part of its structure establishes a versatile procedure to endure a pressure scene. Signalling in stresses mainly drought and salt could separate into the following three components (Zhu et al. 1998):

1. Ionic and osmotic stress signalling for the refurbishment of cell homeostasis in stress
2. Decontamination signalling for regulating and repairing of injuries by stress
3. Signalling to synchronize cell divisions and development to levels appropriate for specific stress

Homeostasis signalling contrarily manages decontamination reactions, because when cell homeostasis is restored, stress damage would be decreased and the regain of homeostasis enabled, which would infuriate stress damage. Homeostasis and detoxification signalling lead to stress resistance and are required to adversely direct the development restraint reaction, i.e. to release development hindrance. It is not sufficient to know just the elements or components of signalling pathways. Great cognizance needs information on exact data sources and yields of pathways. Signalling in stress created by the water is utmost inadequately comprehended. During the treatment of drought, definite alterations were reported, which neither give assurance regarding direct input signal of drought nor adaptive response.

For the ionic part of salt stress, signal pathway dependents on the SOS (Salt Overly Sensitive) genes. The contribution of the SOS pathway is probably overabundance of intracellular or extracellular Na^+, in one way or another, prompts a cytoplasmic Ca^{2+} signal (Zhu 2000).

The plant growth is generally inhibited by water stress also. There is direct evidence that relates dynamic signalling to cell division and development mechanism (Zhu 2001). The cyclin-dependent kinase is mostly reduced under water stress, which is a cause of slower cell division (Schuppler et al. 1998). It is believed that the reduction in CDK activity is due to the combined effect of CDK inhibitors and transcription suppression of cyclins. The direct input signal(s) for the CDK regulation is not clear but could be a creation of stress damage or any of the primary or intermediary signals intricated in the homeostasis and detoxification pathways.

11.7 Role of Compatible Solutes Engineering in Plant Growth under Climatic Stress

Engineering of a polyol cycle describes an approach that eludes inhibition of polyol-induced development by introducing the transgene. Engineering does not promote the accretion of excess polyols. Many reports on polyols and other osmolytes have shown that the marginal collection of these solutes increases stress tolerance (Abebe et al. 2003; Chiang et al. 2005; Yu-Jen et al. 2005; Macaluso et al. 2007). In such incidents, osmotic regulations don't encourage stress tolerance, whereas ROS scavenging and stabilization of organizations of macromolecules are thought to be driving force behind stress tolerance; hence, engineering in polyol cycle is a useful technique for obtaining of plants which could gather an average amount of polyols and grow normally. Previous studies have shown that osmolytes, including polyols, demonstrated free radical scavenging potential (Shen et al. 1997, 1999). Cuin and Shabala (2007) found evidence of an in situ mitigating effect of low concentrations of compatible solutes, such as mannitol, on ROS by monitoring ROS-induced ion fluxes through the plasma membrane. Recent studies have shown that polyols protect proteins against denaturation. Jaindl and Popp (2006) measured the activity and circular dichroism spectra of glutamine synthase and malate dehydrogenase and showed that cyclitols protect these enzymes from thermally induced denaturation and deactivation. Anderson (2007) determined structure-function relationships between polyols and the thermal permanence of protein from pepper leaf, finding that the thermal permanence of proteins amplified with accumulative numbers of OH groups and the polarity of polyols. Since combinations of stabilizing and destabilizing compounds negate each other, the identification and elimination of destabilizing compounds should have a similar effect on increasing stabilizers to protect protein conformation.

11.8 Disadvantages

Various experimental studies revealed moderate differences between transgenic plants and wild types, which are seasonal and constrained to slight developmental phases. Bowler et al. (1991) reported a defensive role of enzymes such as superoxide dismutase, which is involved in H_2O_2 detoxification, and enzymes involved in the ascorbate glutathione cycle. Moreover, mannitol appears to act in hydroxyl radical scavenging but probably not in chloroplasts (Shen et al. 1997). Expression of bacterial levansucrase controlled accretion of fructan (Pilon-Smits et al. 1995); on the other hand, expression of a subunit of yeast trehalose synthase outcome in the form of low amounts of trehalose (Holmström et al. 1996). In the above cases, including mannitol accretion in the cytosol defensive potential of compatible solutes could not be elucidated by osmotic balance because of the low accumulation of metabolites (Thomas et al. 1995).

Similarly, Sheveleva et al. (1998) reported the accumulation of the methylated inositol, D-ononitol against drought stress, which showed low protective potential. These findings also have some contradictions, like Karakas et al. (1997) reported that the accumulation of mannitol slows down the growth of transgenic plants without any stress. Subsequently, claimed stress protection had been inferred as an effect of slower growth leading to less sodium uptake under stress. It was also documented in some studies that a high amount of mannitol or sorbitol caused the delay in plant growth in a concentration-dependent manner (Sheveleva et al. 1998) where the concentration of mannitol is reported less than 50 mM in total cell water, growth doesn't affect significantly while a minor defensive outcome can still be demonstrated.

11.9 Future Prospects

There are so many approaches by which we can treat the plants and protect them from the unfavourable conditions in which they are unable to survive. However, there is still a possibility of facing difficulties in these approaches. So, there is further need to study and analyse more genes involved in increasing stress tolerance in plants, because the genes that are found to be proved as prone for plants are not that much effective, because there is some disadvantage that cannot be neglected. Another fact is that there is a lack of information regarding the stress tolerance genes, which leaves the question mark for us whether we should use them or not. The difficulties are like unknown genes involved, a lack of models (or the use of too many models), the lack of information, and the inability to separate development and stress responses. So, there is a necessity to resolve these issues before any protective measures are being followed. In previous years, studies were conducted by using only two or three models like *Saccharomyces cerevisiae* and *Arabidopsis thaliana*. Hence, we should use other models, too, like the wheat plant, *C. richardii,* etc., which can give us a better understanding and additional information regarding the effective inherited system.

We need to focus on the fact that only ice plants like *Mesembryanthemum crystallinum* are used for genetic analysis and plants' physiological responses in unfavourable climatic conditions. In contrast, other plants like xerophytes and halophytic plant models must be used in the biological study and as a gene source. As much as we study more about the stress response, it will enhance our knowledge of basic plant biology and plant cell metabolism. There are so many tools that can be utilized for gene mapping, distinguish and recognize the genes. With the analysis of variant strains, we can easily identify the specific gene location on the chromosome. As we move toward the more basic information of physiological response at the genetic and molecular level, we begin to understand the biological means and application of genes that are helpful in climatic stress response. It was found that mechanisms of stress resistance in some plants are the same as the stress resistance responses in glycophyte, halophyte, and xerophytes are alike and have evolved that are based on a limited number of principles. The relative importance of these mechanisms, their biological details, and their symbiosis can be tested in transgenic plants. So the focus of future tasks must emphasize on biological studies of transgenic models. Likewise, a superior plan of inducible and cell, tissue, and stage-explicit articulation of transgenes must advance.

11.10 Conclusion

Several environmental constraints restrict plant growth and production, which causes a threat to global food security. As the population grows rapidly worldwide, it will be quite difficult to provide food security to every individual in low productivity, which is certainly a matter of global concern. The growth of plants depends on several factors, including the availability of nutrients, light, water, air, and many more. Stress affects the metabolism and physiology of the plant, and it ultimately results in low production. There is a huge need to develop an advanced strategy that could counter stress and could also improve crop yield. Compatible solutes, non-hazardous or non reactive organic compounds which synthesized by plants in stress conditions. There are biotechnological approaches implemented for improving the tolerance of plants against stress like salinity and drought. Biotechnological approaches include the introduction of the desired gene(s) to change their biochemical pathways. In the way of resistance, plants have developed adaptive mechanisms that could be coupled as imperative resources for the development of crops tolerant to extremities. Some advanced array of omics technologies like genomics, proteomics, and metabolomics currently assists researchers in recognizing the genetics behind the mechanism of plant tolerance. Bearing in mind the impact of osmoprotectants in many plant functions at a physiological and biochemical level under salinity and drought stresses, further research dealing with endogenous regulation of osmoprotectants metabolism may contribute much to deal in such confrontational conditions.

Increased biosynthesis and accretion of compatible solutes is an effective strategy evolved in plants in response to unfavourable effects of abiotic stresses. Due to their multifunctional roles during stress and recovery phases, compatible solutes present potential candidates for genetic engineering aimed to impart abiotic stress tolerance in resultant transgenics. Several successful attempts have been made to overexpress various consistent solute pathway gene(s) for abiotic stress tolerance. However, efforts are needed to produce transgenic plants with enhanced tolerance against combined abiotic stress and plant growth to commercialize transgenic technologies.

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