



Osmoprotection in plants under abiotic stresses: new insights into a classical phenomenon

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Abstract

Main conclusion Plant osmoprotectants protect against abiotic stresses. Introgression of osmoprotectant genes into crop plants via genetic engineering is an important strategy in developing more productive plants.

Abstract Plants employ adaptive mechanisms to survive various abiotic stresses. One mechanism, the osmoprotection system, utilizes various groups of low molecular weight compounds, collectively known as osmoprotectants, to mitigate the negative effect of abiotic stresses. Osmoprotectants may include amino acids, polyamines, quaternary ammonium compounds and sugars. These nontoxic compounds stabilize cellular structures and enzymes, act as metabolic signals, and scavenge reactive oxygen species produced under stressful conditions. The advent of recent drastic fluctuations in the global climate necessitates the development of plants better adapted to abiotic stresses. The introgression of genes related to osmoprotectant biosynthesis from one plant to another by genetic engineering is a unique strategy bypassing laborious conventional and classical breeding programs. Herein, we review recent literature related to osmoprotectants and transgenic plants engineered with specific osmoprotectant properties.

Keywords Abiotic stresses · Amino acids · Climate change · Glycine betaine · Osmoprotectants · Proline · Transgenic crops

Introduction

Plants being sessile in nature, have to simultaneously or sequentially endure changing environments including various prevalent abiotic stresses. These abiotic stresses include temperature extremes, high light intensity, salinity, water scarcity, flooding and nutrient deficiency/excess that individually or in combination pose negative effects on plant growth, development and productivity (Dutta et al. 2018; Khan et al. 2019). It is estimated that global yield reductions to abiotic stress approach 70 percent (Acquaah 2009). These stresses need to be managed to ensure optimum crop productivity under extreme environmental conditions. In recent

years, concerns have grown about the effects of climate change in reinforcing the intensity of abiotic stresses (Fedoroff et al. 2010; Songstad et al. 2017; Khan et al. 2019). It is therefore necessary to improve plant abiotic stress tolerance. However, improvement in plant traits can only be effectively accomplished with a complete knowledge of plant natural defense mechanisms. Thus, it is imperative to appraise plant physiological and biochemical strategies by which plants can protect their cellular machinery, e.g., proteins and cell structure under varying environmental conditions that are a serious threat for agriculture.

Plant cellular functions are modified differently upon exposure to a particular abiotic stress or under specific stress combinations (Wang et al. 2018). Among these modifications, production and accumulation of a myriad of organic, highly soluble, low molecular weight, electrically neutral and nontoxic compounds, generally known as osmolytes or osmoprotectants (Slama et al. 2015; Per et al. 2017; Riaz et al. 2019) are important because of their protective role against cellular machinery damage in response to a stressful environment. The occurrence of osmoprotectants in plants is a common phenomenon, however, their tissue/cellular levels

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depend on a number of factors such as tissue or cell types, developmental phase, and type and permanency of a stressful environment (Joshi et al. 2010).

The production and upregulation of these intracellular low molecular weight compatible solutes or osmoprotectants play a protective role against protein denaturation and disruption of cell structures without interfering with normal metabolism of the plant (Szabados et al. 2011; Nahar et al. 2016). In addition, nontoxic compatible solutes have been shown to stabilize the cell turgor pressure and oxidation–reduction phenomenon to counteract high levels of ROS and regain the redox balance to offset stressful environments (Cortleven et al. 2019). Based on their chemical properties, osmoprotectants are categorized into three types: amino acids (proline, ectoine, pipercolic acid, etc.), betaines (glycine betaine, choline-O-sulfate, β -alanine betaine, etc.), and sugar alcohols and non-reducing sugars (trehalose, sorbitol, inositol, mannitol, etc.) (Slama et al. 2015). Plants can produce 5–50 $\mu\text{mol g}^{-1}$ fresh weight of osmoprotectants naturally, which remain in different cell components such as chloroplast, cytosol and in different components of cytoplasm (Rhodes and Hanson 1993). However, their concentration and composition vary with plant species and environmental stress (Lugan et al. 2010). Contrary to inorganic ions, which pose negative effects on plant cells at high concentrations, osmoprotectants as compatible solutes, have the ability to maintain cell turgor pressure, alleviating ion toxicity and replacing inorganic salts (Szabados et al. 2011). Moreover, these osmoprotectants are considered to facilitate osmotic adjustment under limited water supply, in order to provide alternative water in biochemical reactions, and regulate the internal osmotic potential and macromolecule structures (Parida and Das 2005). Achieving food production goals for increasing human population demands effective strategies to cope with the unfavorable environmental conditions such as drought, and high or low temperature that drastically affect crop yields worldwide. Owing to variation in the levels of osmoprotectants, even between cultivars of one species, there exists an opportunity to increase the tolerance towards harsh conditions by utilizing the lines or cultivars with high osmoprotectant production (Li et al. 2019a). In this regard, many attempts have successfully used conventional breeding protocols to develop crop varieties with enhanced endurance against abiotic stresses. Among recent advances, knowledge on plant cellular functions and genome editing technologies have facilitated an increase in the ability of plants to accumulate increased concentrations of osmoprotectants making the crop plants better able to resist abiotic stresses and ensuring maximum food production (Ashraf and Foolad 2007; Marwein et al. 2019). Transcriptome analysis has made it possible to identify genes related to the biosynthesis of osmoprotectants under abiotic stress conditions (Suprasanna et al. 2016). This approach aims to develop transgenic plants

with enhanced osmoprotectant production, by introducing genes for abiotic stress tolerance along with those conveying the additional benefits of high yield and biomass production. Transgenic plants developed with enhanced accumulation of osmoprotectants include wheat (Sawahel and Hassan 2002), potato (Zhang et al. 2011), rice (Garg et al. 2002; Su and Wu 2004), maize (Quan et al. 2004; Bai et al. 2019), pigeon pea (Surekha et al. 2014), soybean (Zhang et al. 2015), and tobacco (Szabados and Savoure 2010). In the present review, the recent advances on the role of individual potential osmoprotectants playing a role in tolerating abiotic stress conditions are discussed. Moreover, it also elaborates the latest genetic engineering strategies used to develop transgenic plants with enhanced osmoprotectant production ability for improvement in plant abiotic stress tolerance.

Osmoprotectant functions in plants—an overview

A high accumulation of cellular osmoprotectants mediates diverse functions in plant defense mechanisms under varying environmental conditions. These small and highly soluble, organic and compatible compounds have low molecular weight with hydrophilic properties. Moreover, unlike inorganic compounds, osmoprotectants, at high cellular concentrations, are nontoxic to intracellular metabolisms operating in plants under harsh environmental conditions (Nahar et al. 2016). Under unfavorable conditions, osmoprotective compounds that accumulate in plants are proline, ectoine, fructan, pipercolic acid, trehalose, polyols, quaternary ammonium compounds including glycine betaine, alanine betaine, proline betaine, choline-O-sulfate, γ -aminobutyric acids, hydroxyproline, betaine, pipercolite betaine, polyamines, D-ononitol, fructan, raffinose, sorbitol, inositol, amino acids, mannitol, gamma amino butyric acid (GABA), and carbohydrate sugars (Ashraf and Foolad 2007) (Fig. 1). Generally, three distinct categories on the basis of chemical composition of osmoprotectants are well known that include: (1) the amino acids, (2) quaternary ammonium compounds, (3) polyols, sugars, and sugar alcohols (Nahar et al. 2016). The accumulation, concentration, structure and compartmentalization of osmoprotectants at cellular level in plants under abiotic stresses depend on factors including growth conditions, stress type, severity of stress and plant species (Kumar 2009; Evers et al. 2010, Ashraf and Foolad 2007). The main role of osmoprotectant accumulation under harsh conditions is regulation of osmotic balance in plants. Under harsh conditions, osmoprotectants maintain cell turgor pressure via osmoregulation, replace inorganic ions, protect cellular components, and alleviate ion toxicity. In brief, osmoprotectants play a diverse role in improving stress tolerance by protecting biological membranes, stabilizing protein structure and other cellular structures, detoxifying ROS, and maintaining cellular redox balance (Suprasanna et al. 2016). Moreover, osmoprotectants also control the regulation of protein folding

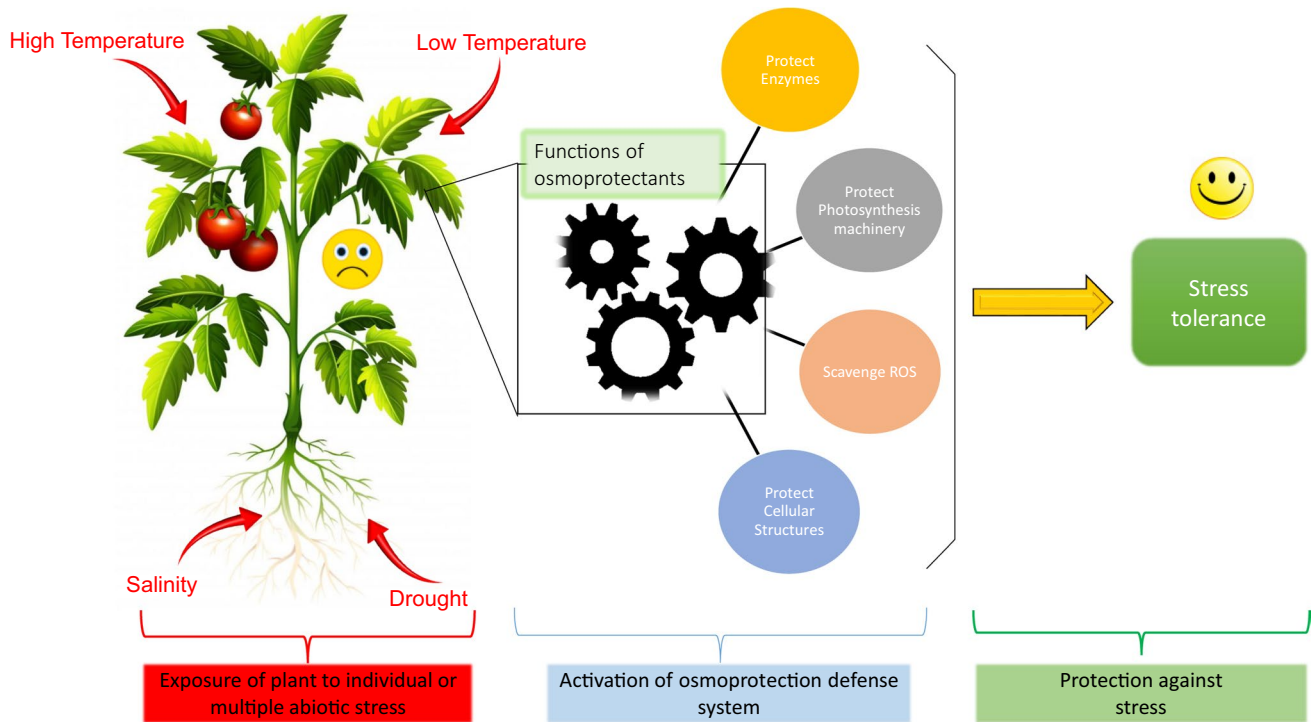


Fig. 1 A schematic representation describing the functions of osmoprotection in plants. On the onset of individual or multiple stresses (red part in the left side), plant activates its natural osmoprotection

system and ultimately acquires stress tolerance. Various functions performed by osmoprotectants to mitigate the negative effects of abiotic stresses are illustrated in middle step (blue portion)

that assists in mediating stress signaling (Rosgen 2007). These organic compounds also help to stabilize thylakoid membranes, resulting in upregulation of photosynthesis (Alam et al. 2014). These osmoprotective compounds improve the antioxidant defense system of plants by directly scavenging toxic ROS and protecting key antioxidant enzymes (Hasanuzzaman et al. 2014). Moreover, osmoprotectants play their role in the activation of defense related genes under various stresses (Wani et al. 2018). Thus, osmoprotectants in plants are an important and well-organized evolutionary strategy to survive under hostile environments. Owing to their importance in plant survival, this topic is considered as a central dogma in plant physiology and molecular biology. Understanding the diverse roles of individual osmoprotectants is therefore necessary to improve stress tolerance in crops. In the following sections, we briefly provide an update on different osmoprotectants, their accumulation, functions and the transgenic approaches adopted to achieve their increased production and accumulation in crops.

Group of osmoprotectants

Amino acids

Amino acid metabolism in plants under abiotic stress condition play a pivotal role in inducing stress tolerance in plants

(Joshi et al., 2010). On the exposure to abiotic stresses and resultant desiccation, the accumulation of different amino acids including proline, alanine, arginine, glycine, amides such as glutamine and asparagine and nonprotein amino acids such as gamma-aminobutyric acid (GABA), citrulline, pipercolic acid and ornithine, and minor amino acids and branched chain amino acids (isoleucine, leucine and valine) is well documented (Araújo et al. 2010; Carillo et al. 2005; Mansour 2000; Joshi et al. 2010; Woodrow et al. 2017). Among these, proline (Pro), a proteinogenic amino acid, is widely documented and plays a crucial role in both the metabolism and plant defense as an osmoprotectant (Kaur and Asthir 2015). As a molecular chaperone, it plays an important role in regulation of enzyme activities and protection of protein integrity (Suprasanna et al. 2016). It also acts as an antioxidant having singlet oxygen quenching and ROS scavenging abilities (Suprasanna et al. 2016). Several studies have reported the accumulation of Pro in response to different stresses including drought (Akram et al. 2007), salinity (Akram et al. 2012; Vives-Peris et al. 2017), metal toxicity (Zouari et al. 2016), and high temperature (Vives-Peris et al. 2017). On exposure to stress conditions, plants accumulate elevated levels of Pro in the cytoplasm and chloroplast (Rejeb et al. 2015). Pro is one of the abundantly distributed osmoprotectants in plants, however, the nature of its accumulation in stressed plants is still debatable (Carillo 2018).

It is not yet obvious whether Pro accumulation is an indication of stress, response of stress, or just an adaptive strategy. However, Pro can have diverse functions including: its putative role as an osmoprotectant, a stabilizing compound of membranes and proteins, buffer cellular redox potential, a scavenger of ROS, an inducer of expression of salt stress responsive genes, in particular genes with Pro-responsive elements (e.g., PRE, ACTCAT) in their promoters. However, Pro can be abruptly metabolized when it is no longer needed (Woodrow et al. 2017; Carillo 2018). However, the accumulation/concentration of Pro varies from species to species and can be a hundred times more under stressful conditions than that under control conditions (Verbruggen and Hermans 2008). Proline accumulation in response to stresses can occur due to its increased accumulation and/or reduced degradation (Verbruggen and Hermans 2008). Proline accumulation helps protect the cell from ROS (Kaur and Asthir 2015). Proline synthesis in plant cells can occur through two pathways: glutamate pathway and ornithine pathway (Liang et al. 2013; Rai and Penna 2013). Of these two, the glutamate pathway is considered the major source of Pro accumulation, while the ornithine pathway is activated in chloroplasts or cytoplasm, producing Pro from glutamic acid through an intermediate pyrroline-5-carboxylate (P5C) under nitrogen limiting or osmotic stress conditions (Delauney et al. 1993; Dar et al. 2016). The two main enzymes in the glutamate pathway are: (1) pyrroline 5-carboxylate synthetase (P5CS), and (2) pyrroline 5-carboxylate reductase (P5CR) (Sekhar et al. 2007). In most plants, P5CS and P5CR are encoded by two and one genes, respectively (Dar et al. 2016). Moreover, Pro synthesis in the chloroplast under stress conditions balances the low NADPH: NADP⁺ ratio, sustains the electron flow between photosynthetic excitation centers, regulates the redox balance, alleviates cytoplasmic acidosis, and protects from photoinhibition and damage of photosynthetic apparatus (Taiz and Zeiger 2010; Filippou et al. 2014).

After plant exposure to stress conditions, catabolism of Pro in the mitochondria contributes to oxidative respiration and produces energy for resuming plant growth (Kaur and Asthir 2015). It acts as a metabolic signal for stabilizing metabolite pools and, hence, causes a beneficial impact on growth and development (Verbruggen and Hermans 2008). However, there are species-specific differences in Pro accumulation in plants under stress conditions (Dar et al. 2016). Proline engineering of transgenic crop varieties with enhanced stress endurance ability is mediated through overexpression and accumulation of *P5CR*, *P5CS*, ornithine aminotransferase (*OAT*), or via domination of proline dehydrogenase (*ProDH*) (Kaur and Asthir 2015). In this regard, development of transgenic crop varieties harboring osmoprotectant genes from other organisms has been achieved worldwide. For instance, transgenic tobacco with *Vigna aconitifolia P5CS* expression produced more Pro than wild

tobacco (Kishor et al. 1995). Similarly, in transgenic rice and chickpea plants, expression of introduced *P5CS* gene from *V. aconitifolia* resulted in a Pro content five times greater than that in non-transformed plants (Karthikeyan et al. 2011). Surekha et al. (2014) reported a fourfold increase in Pro accumulation along with salt tolerance compared to its wild counterpart by transformation of pigeon pea (*Cajanus cajan*) with *V. aconitifolia P5CSF129A*. Li et al. (2019b) produced transgenic *Arabidopsis* expressing salt-tolerant sweet potato *IbRAP2-12* gene, which accumulated increased Pro and reduced ROS accumulation under salt and drought stresses compared with the wild type. Thus, further crops must be considered for the introduction of such beneficial genes to counteract the negative effect of abiotic stresses.

Quaternary amines: glycine betaine

Glycine betaine (GB) [(CH₃)₃N + CH₂COO⁻] is a major and efficient putative osmoprotectant that accumulates in different plant species. It is widely believed that GB can protect the plants against exposure to harsh environmental conditions such as drought, high temperature and salinity without causing cellular toxicity (Ashraf and Foolad 2007). Glycine betaine is a zwitterionic, quaternary ammonium compound which is a *N*-methylated derivative of glycine (Ashraf and Foolad 2007; Fariduddin et al. 2013) and accumulates abundantly in many plant species, especially halophytes, under a range of environmental stresses (Pardo-Domènech et al. 2016). Owing to its unique structure, GB tends to interact with both the hydrophilic and hydrophobic domains of plant cellular macromolecules such as enzymes and proteins (Ashraf and Foolad 2007; Kumar et al. 2017). The intrinsic levels of GB are believed to be ontogenetically regulated because it is found in young tissues during continued stress, while its degradation does not significantly occur in plants. The ability of plants to synthesize/accumulate excess levels of GB in young tissues under stressful environments does not depend on N availability. This supports the viewpoint that plant N allocation is required to safeguard the developing tissues, even under N deficit regimes (Annunziata et al. 2019). Since GB is not actively degraded/metabolized plant tissues, therefore its concentration depends on synthesis, transport and dilution in plants (Annunziata et al. 2019).

The extent of accumulation depends on the plant species and degree of their stress tolerance (Rhodes and Hanson 1993). Glycine betaine is a dipolar and electrically neutral osmoprotectant at physiological pH (Rhodes and Hanson 1993). It increases the cell osmolality in plants under abiotic stress conditions (Rhodes and Hanson 1993). In angiosperms, GB is synthesized widely in chloroplasts and protects membranes, enzymes, and proteins of the photosynthetic machinery (e.g., Rubisco and PSII) under harsh environmental conditions (Ashraf and Foolad 2007; Chen

and Murata 2011) (Fig. 2). It has been reported that GB accumulation in plants in response to abiotic stresses protects the reproductive organs resulting in high yields (Chen and Murata 2008).

Generally, GB biosynthesis in higher plants is via two substrates, choline and glycine. GB synthesis from choline in the chloroplast is a two-step process. Biosynthesis of GB occurs in the chloroplast from serine through ethanolamine, choline, and betaine aldehyde (Hanson and Scott 1980; Rhodes and Hanson 1993). Choline in plants is transformed to betaine aldehyde by the choline monooxygenase enzyme, the betaine aldehyde being subsequently converted to GB by the action betaine aldehyde dehydrogenase (BADH) (Ashraf and Foolad 2007). Although other pathways including direct N-methylation of glycine also exist, the choline to GB pathway has been detected in all GB-accumulating plant species (Weretilnyk et al. 1989).

Under normal conditions, plants accumulate low levels of GB which increase at the onset of abiotic stress (Chen and Murata 2011; Fariduddin et al. 2013). Annunziata et al. (2019) reviewed the spatial and temporal profile of GB in plants under abiotic stress conditions and inferred that the efficacy of GB metabolism transformation for field grown crop plants, has not been completely demonstrated. This could be due to the reason that although GB concentration in genetically modified plants increases considerably, its levels are still markedly lower than those in naturally high accumulator plant species. Moreover, even if GB is supplied exogenously to older parts, it is rapidly re-translocated to younger expanding tissues. However, despite spatial distribution of GB, its synthesis is temporally delayed compared to that of

other vital osmoprotectants like proline. It is believed to be due the reason that GB cannot be metabolized. As a matter of fact, it is synthesized/accumulated in young tissues/organs of plants exposed to stressful cues as well as at N deficit regimes. Thus, it can be inferred that GB plays a critical role in safeguarding young expanding tissues.

However, there are some species that are naturally non-accumulators of GB under both normal and stress conditions (Chen and Murata 2008). For instance, major cereals like maize, wheat or barley partially lack the natural ability of adequate GB accumulation under harsh conditions (Fariduddin et al. 2013; Kurepin et al. 2015). Additionally, rice, tomato, and tobacco are among those crops that completely lack the GB accumulation ability under normal or stress conditions (Fariduddin et al. 2013; Kurepin et al. 2015). Identification and transfer of GB biosynthesis-associated genes into non-betaine accumulator crops via genetic engineering has been targeted as a potentially effective strategy to improve abiotic stress tolerance (Chen and Murata 2011). This approach has been successfully applied in diverse crops to develop transgenic cultivars.

Quan et al. (2004) transformed maize with the *Escherichia coli* choline dehydrogenase *betA* gene, which improved maize tolerance to chilling stress by increasing the accumulation of GB compared with that in the untransformed plants. Similarly, Di et al. (2015) transferred the BADH gene from *Atriplex micrantha* into two maize inbred lines and the resultant maize plants demonstrated enhanced GB accumulation and tolerance to salt stress along with increased growth and biomass production. In a recent study, Song et al. (2018) developed transgenic cotton with co-expression of

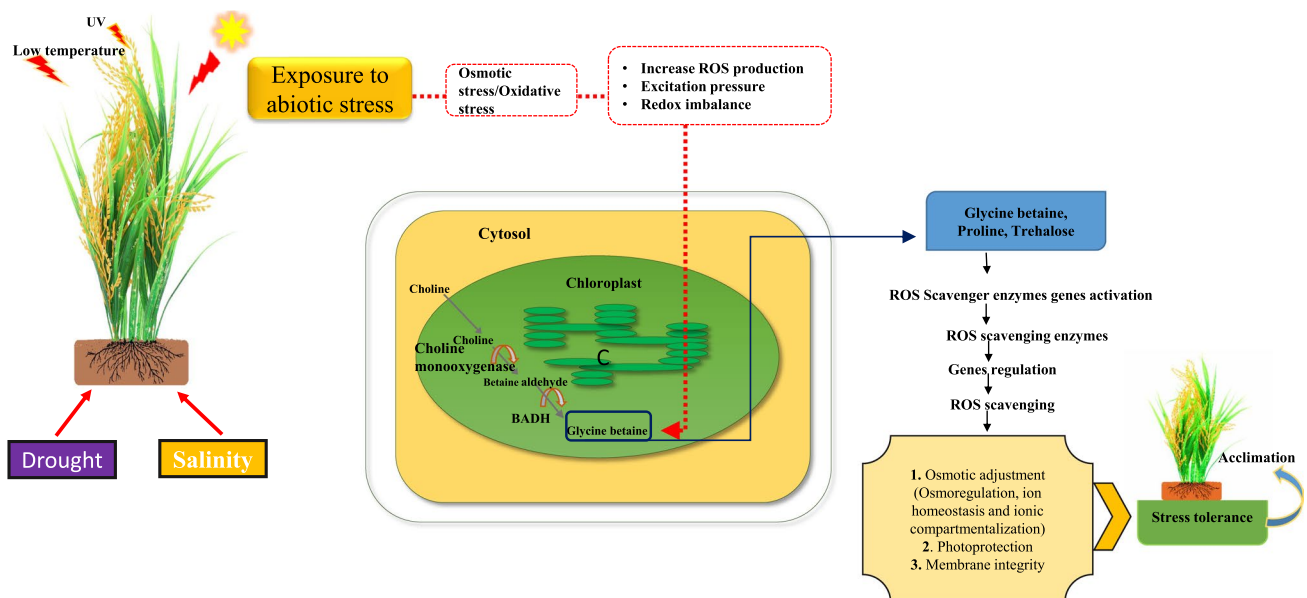


Fig. 2 Schematic example showing involvement of an osmoprotectant (glycine betaine) in abiotic stress tolerance

ApGSMT2g and *ApDMT2g* genes and observed increased GB accumulation under saline stress. Tian et al. (2017) transformed wheat with the *BADH* gene from *Atriplex hortensis* and the resultant wheat plants showed tolerance to salt stress which was found to be mainly associated with increased GB biosynthesis along with regulation of different physiological and biochemical attributes. Enhanced heat tolerance was observed in a transgenic tomato line possessing the *BADH* gene (Li et al. 2014), which accumulated high levels of GB compared with its non-transformed counterpart. While also working with tomato, Wei et al. 2017 developed a transgenic line by transferring the *Arthrobacter globiformis* choline oxidase gene *codA* and reported enhanced GB accumulation which resulted in improved salt tolerance. In a more recent study, Zhang et al. (2019) reported increased fruit size in transgenic tomato in response to enhanced GB biosynthesis.

Sugars: trehalose

Trehalose (Tre), a naturally occurring non-reducing disaccharide sugar, plays a critical role in plant metabolic processes as a reserve carbohydrate and an osmoprotectant (stress protector) in several living organisms including plants (Elbein et al. 2003; Almeida et al. 2007; Paul et al. 2008). Trehalose is composed of two glucose residues (D-glucopyranose units) joined by a very stable α - α - (1 \rightarrow 1) linkage (Richards et al. 2002; Fernandez et al. 2010). Trehalose is highly soluble in nature and chemically unreactive because of its non-reducing property that makes it a compatible solute or osmoprotectant even at its high concentrations (Lunn et al. 2014). It has been suggested that Tre has the ability to function as scavenging ROS, thus providing protection to protein synthesis machinery of plants (Luo et al. 2010; Koyro et al. 2012). Trehalose 6-phosphate (Tre6P), an intermediate of trehalose biosynthesis, is of paramount importance as a signal metabolite in plants, connecting growth and development to carbon status (Figuerola and Lunn, 2016). Tre is responsible for expression of genes and signaling pathways associated with stress response and detoxification (Abdallah et al. 2016; John et al. 2017). Trehalose possesses high hydrophilicity because of the absence of internal hydrogen bonding (Paul and Paul 2014; Abdallah et al. 2016). Owing to this unique property, Tre acts as a protective molecule for cellular, membranous and proteinaceous structures (López-Gómez and Lluch 2012; Abdallah et al. 2016). Under stress conditions, Tre protects the structures of membranes and proteins by making an amorphous glass structure and by acting with surrounding polar phospholipids head groups or with amino acids via hydrogen bonding (Crowe et al. 1984; Crowe 2007; Einfalt et al. 2013). This amorphous glassy structure formation saves the biomolecules from the adverse effects of abiotic stresses especially

dehydration and helps in recovery of their specific functions on the onset of normal non-stress environmental conditions (Fernandez et al. 2010; Kosar et al. 2018).

Trehalose biosynthesis in plants follows the OtsA–OtsB pathway which depends on two key molecules: uridine-diphospho-glucose (UDP-Glc) and glucose-6-phosphate (Glc-6-P) in a two-reaction process (Paul et al. 2008; Kosar et al. 2018). Initially, the enzyme trehalose phosphate synthetase (TPS) catalyzes UDP-Glc and Glc-6-P into trehalose-6-phosphate (T-6-P) and uridine diphosphate (UDP) (Zentella et al. 1999). Subsequently, the T-6-P is dephosphorylated into Tre by trehalose-6-phosphate phosphatase (TPP) (Vandesteenea et al. 2010). Additionally, the trehalase enzyme catalyzes the hydrolysis of Tre (Vandesteenea et al. 2010).

Various studies have reported enhanced levels of Tre in plants under different abiotic stress conditions (Iordachescu and Imai 2008; Henry et al. 2015; Akram et al. 2016; Shafiq et al. 2015). In view of the well-known stress mitigating properties of Tre, attempts are currently underway to generate transgenic plants with enhanced Tre biosynthesis. For instance, transgenic rice with bacterial Tre biosynthesis genes (*otsA* and *otsB*) resulted in an increased accumulation of Tre and ultimately improved tolerance to drought, salt, and cold (Garg et al. 2002). Likewise, in the same crop, Li et al. (2011) reported increased Tre and Pro accumulation along with salt, cold and drought tolerance via over-expression of Tre synthase gene (*OsTPS1*) compared with its wild counterpart. Nuccio et al. (2015) developed transgenic maize plants by introducing a gene encoding a rice trehalose-6-phosphate phosphatase (TPP) gene from rice. The transgenic maize plants produced 31–130% higher yield than that the non-transgenic controls. Thus, there is a need to further elaborate the genes related to Tre biosynthesis genes from different organisms and to evaluate their potential to enhance the abiotic stress tolerance when introduced into in crop plants.

Sugar alcohols: inositol and mannitol

Sugar alcohols are categorized into two groups, namely cyclic polyols (e.g., pinitol) and acyclic polyols (e.g., mannitol) (Slama et al. 2015). Their accumulation in plants is believed to perform multiple functions including facilitation of osmotic adjustment, and regulation of redox system (ROS scavengers) and molecular chaperons (Szabados et al. 2011; Upadhyay et al. 2015). Of the acyclic sugar alcohols, mannitol is the six-carbon liquor polyol (Upadhyay et al. 2015). It is the most important and common osmoprotectant that is widely accumulated in various plant species except halophytes (Slama et al. 2015) and has a critical role in photosynthesis and abiotic stress tolerance (Loescher et al. 1992). Mannitol possesses the capacity to act as an

osmoprotectant to counteract ROS, a repository of reducing power, and as a carbon stockpiling compound (Upadhyay et al. 2015). In plants, biosynthesis of mannitol starts from fructose-6-P by the actions of different enzymes including mannose-6-P isomerase (phosphomannose isomerase), mannose-6-phosphate reductase and mannose-1-phosphate phosphatase (Loescher et al. 1992). The enzyme mannitol dehydrogenase which then controls the catabolism of mannitol, produces mannose which on phosphorylation is converted into mannose 6-P. Further, the enzyme mannose 6-P isomerase converts mannose 6-P into fructose 6-P (Loescher 1987). Mannitol is not accumulated naturally in all plant species and thus introduction of mannitol into non-mannitol accumulators can improve their tolerance of harsh environmental conditions. For instance, Bhauso et al. (2014) transferred *MtID* gene from bacteria into a non-mannitol accumulator plant species peanut and the resulted transgenic plants showed increased accumulation of mannitol and hence improved drought tolerance. Rahnama et al. (2011) reported improved salinity tolerance in transgenic potato expressing the *mtID* gene, resulting over-accumulation of mannitol. Similarly, Patel et al. (2016) reported enhanced salinity stress tolerance in transgenic peanut manipulated with bacterial mannitol dehydrogenase *mtID* gene. Thus, incorporation of mannitol biosynthesis genes from different organisms into crop plants to enhance their tolerance to abiotic stresses can be an effective strategy to counter the adverse impacts of climate change.

Inositol

Myo-inositol (cyclohexane hexol) is a sugar-like unique carbohydrate, which is critically important for a myriad of plant cellular processes (Valluru and Van den Ende 2011; Nisa et al. 2016). Besides performing basic cellular functions, inositol is also found to contribute as an osmoprotectant for plant protection against abiotic stress (Sengupta et al. 2012). Moreover, this compound is reported to control the transport of plant hormones such as auxins, membrane biogenesis, phytic acid biosynthesis, signal transduction, plant immunity and programmed cell death (Hazra et al. 2019). In addition, myo-inositol also contributes to the synthesis of phosphoinositide, which aids in a well-defined signaling pathway (P1), especially under osmotic stress signaling (Munnik and Vermeer 2010). Besides inositol (myo-inositol), its derivatives such as pinitol, galactinol, and ononitol also accumulate in plants and perform diverse functions including osmoprotection (Valluru and Ende 2011; Handa et al. 2018). It has been observed that under salinity stress, inositol functions in two ways: as a protectant against ROS and as a controller of cell water potential. Of the seven isomers of inositol, myo-inositol is the most abundant (Sengupta et al. 2012). The glycosidic linkage present in inositol and its derivatives

(pinitol, galactinol, and ononitol) is not hydrolysis-labile and owing to this property, inositol derivatives are among the most stable compounds in the plant cell (Valluru and Ende 2011). The biosynthesis of inositol is a two-step biochemical pathway that involves enzymatic conversion of D-glucose-6-P into myo-inositol-1-P mediated by myo-inositol-1-P synthase (Majumder et al. 1997), followed by dephosphorylation of myo-inositol-1-P resulting in myo-inositol that further produces different inositol containing compounds such as phospholipids (Dastidar et al. 2006). There is great potential to use genetic engineering to increase production of myo-inositol and its derivatives in transgenic crop plants which could thrive well under adverse environmental conditions (Sengupta et al. 2012). For example, Nisa et al. (2016) transferred *GsMIPS2*, the myo-inositol-1-phosphate synthase biosynthesis gene from *Glycine soja* (wild soybean) into *Arabidopsis* and observed increased tolerance of salt stress.

Sambe et al. (2015) reported increased accumulation of inositol along with cold tolerance in tobacco plants via transformation with a myo-inositol transporter-like protein (MfINT-like) of *Medicago sativa* subsp. *Falcate* compared with its wild counterpart. Khurana et al. (2017) developed transgenic *Arabidopsis* plants with an introduced wheat *TaMIPS2* gene encoding the myo-inositol phosphate synthase biosynthesis gene and observed increased tolerance to heat stress. Developing transgenic plants with enhanced inositol biosynthesis may ameliorate the effects of abiotic stresses on crop plants.

GABA

A four carbon non-proteinogenic amino acid, γ -amino butyric acid (GABA), widely existing in uni- and multicellular organisms including plants, performs diverse functions in plant life cycles (Salah et al. 2019). GABA was first discovered during the 1940s in potato tubers (Steward et al. 1949). Since then it has received increased attention by plant physiologists investigating its role in plant metabolism. GABA is produced endogenously in plant cells and acts as a signaling molecule which rapidly accumulates under biotic stress conditions and provides osmoprotection to plants (Bown and Shelp 2016; Li et al. 2018; Podlešáková et al. 2018). Additionally, under harsh environmental conditions, GABA contributes to the regulation of redox status, osmotic pressure, maintenance of cytosolic pH, C and N fluxes and C–N metabolism (Suprasanna et al. 2016; Salah et al. 2019). It can have a scavenging activity against ROS exceeding those of proline and glycine betaine (Carillo, 2018). In a recent review, it was suggested that the synthesis of GABA by glutamate decarboxylation catalyzed by GAD could be conducive to the dissipation of excess energy and release of CO₂, enabling the Calvin cycle to function while employing

a lower influence on photosynthetic electron chain along with decreasing both ROS and photo-damage (Carillo 2018). GABA is metabolized in plants via a pathway known as the GABA shunt, which bypasses two steps of the tricarboxylic acid cycle (TCAC) (Shimajiri et al. 2013). In plant cells, GABA biosynthesis primarily occurs in the presence of the enzyme α -glutamate decarboxylase (GAD) via the decarboxylation of glutamate in the cytosol from where GABA is transported to mitochondria (Sağlam and Jan 2014; Seifikalhor et al. 2019). The GABA shunt is a feedback loop for the production and conservation of GABA (Seifikalhor et al. 2019). Information on transgenic plants modified with GABA biosynthesis genes is scarce. Research on transferring GABA biosynthesis-associated genes from different organisms via genetic engineering to crop plants to enhance plant tolerance to abiotic stresses should be considered.

AL-Quraan and Al-Share (2016), characterized *Arabidopsis thaliana pop2* mutant lines for their tolerance ability against different abiotic stresses including high temperature, low temperature and salinity. The transgenic lines transformed with γ -aminobutyric acid transaminase, subjected to different stresses showed variation in their response to stress. The authors reported a significant increase in GABA concentration in transgenic lines under low temperature and osmotic (mannitol) stress. However, only a slight increase was observed in both wild and mutant lines under salinity stress. It was concluded that the response of GABA transferred genes varies under different stress types and acts as an osmoprotectant in plants under some abiotic stress conditions (Al-Quraan and Al-Share 2016). There is a need to evaluate the impact of GABA genes introduced into different plant species to better understand their potential, especially under abiotic stresses.

Ammonium compound group: polyamines

Polyamines are small aliphatic nitrogenous compounds with hydrocarbon chains and amino groups that are reported to be important in plant growth and development (Pál et al. 2015). By virtue of their endogenous protonation (cationic nature) ability at cell physiological pH, polyamines regulate diverse biological activities such as cell division, differentiation, organogenesis, floral induction, root formation, pollination, tuber development, fruit ripening and programmed cell death (Saxena et al. 2013 and references therein). These ubiquitous low molecular weight compounds also function as anti-stress agents in plants (Pál et al. 2015). Polyamines are important for counterbalancing the excess ROS levels from the plant cell under abiotic stress conditions. These positively charged molecules also protect plant cells from oxidative damage via a direct or an indirect route. Directly, polyamines act as antioxidants and indirectly they regulate the enzymatic and non-enzymatic oxidants in the cell

environment correlating with the level of stress tolerance in plants. Among the various osmoprotectants, polyamines are contemplated as the most important for alleviating the negative effects of environmental stresses. In higher plants, the most abundant and studied polyamines are putrescine, spermidine, and spermine (Liu et al. 2015). Besides these major polyamines, others including cadaverine and homospermine also occur in living organisms including plants (Liu et al. 2015). In addition to their role as osmoprotectants, polyamines are known as nitrogen sinks in plants (Pál et al. 2015). In plants, the biosynthesis pathway and key enzymes involved are well documented (Tiburcio et al. 2014; Khare et al. 2018). The biosynthesis pathway involves decarboxylation of arginine or ornithine, catalyzed by arginine decarboxylase (ADC) or arginase to give rise putrescine. The agmatine resulting from arginine is then transformed to putrescine, by agmatine iminohydrolase (AIH) and *N*-carbamoylputrescine amidohydrolase (CPA). Spermine and spermidine are derived by the consecutive addition of aminopropyl groups to putrescine and spermidine from decarboxylated *S*-adenosylmethionine (SAM) by the action of SAM decarboxylase. Additionally, a large body of research suggests that transformation of plants with polyamine biosynthetic pathway genes, encoding arginine decarboxylase, ornithine decarboxylase, *S*-adenosylmethionine decarboxylase or Spd synthase, and their overexpression enhanced abiotic stress tolerance in several plant species (Gill and Tuteja 2010). There are many promising reports on the development of transgenic crop plants harboring polyamine biosynthetic genes with the aim to enhance abiotic stress tolerance. For example, overexpression of *ADC*, EC 4.1.1.19 genes from *Datura stramonium* and *Avena sativa* resulted in increased accumulation of putrescine in transgenic crop plants, which ultimately enhanced drought tolerance compared with that of the wild counterparts (Roy and Wu 2002; Capell et al. 2004). Espasandin et al. (2014) generated transgenic *Lotus tenuis* plants overexpressing oat *ADC* gene. The authors reported increased putrescine content accumulation in the transgenic plants under drought stress that ultimately improved water balance of the cells by enhancing drought tolerance. In another study, Duque et al. (2016) transferred the oat *Adc* gene of arginine decarboxylase (*ADC*), a key enzyme responsible for polyamine (PA) biosynthetic pathway to *Medicago truncatula* and reported increased drought tolerance in transgenic plants. In a recent study, Espasandin et al. (2018) developed *Lotus tenuis* transgenic plants expressing *ADC* (*pRD29A*: oat arginine) decarboxylase gene. Overexpression of *ADC* increased salinity tolerance by osmotic adjustment along with Pro accumulation and balanced Na^+/K^+ ratios compared with those in wild counterparts (Espasandin et al. 2018). Considering the diverse roles of polycationic amines, there is a need to further elucidate how polyamine biosynthesis genes can be engineered in crop

plants to trigger metabolism able to counteract the adverse effects of stresses.

Enhanced synthesis of osmoprotectants through genetic engineering

Global crop yields must increase to feed the growing population. To accomplish this, it is essential to develop practical and sustainable strategies to increase yields by protecting crops from biotic and abiotic stresses (Aquino et al. 2011). For many crops, there is limited or low genetic variability available, compatible for use in conventional breeding, from which to develop improved cultivars (Ashraf et al. 2008). Additionally, conventional breeding technologies are time consuming, laborious, costly and inefficient in developing stress-tolerant cultivars because abiotic stress tolerance is primarily multigenic, whereas biotic stress tolerance is often monogenic (Flowers 2004). Transgenic technology has the potential to improve plant abiotic stress tolerance by developing stress-resistant plants (Jain, 2015). Genome editing protocols can be employed in different organisms for achieving different processes such as targeted mutation, deletion, insertion, and exact sequence alteration by tailored nucleases. Transcriptional activator-like effector nucleases (TALENs), zinc finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 (CRISPR-associated nuclease 9) are contemplated to be promising genome editing tools (Kumar and Jain 2015; Jain 2015). Among these, CRISPR-Cas9 system has considerable potential to enable the appraisal of gene/genome function and engineering of abiotic stress tolerance in a variety of plants (Jain 2015). It is an inexpensive, simple, most user-friendly, easy and rapidly adopted genome editing tool for producing genome edited crops to fulfill the increasing food demands in the context of climate change (Khatodia et al. 2016). Identification and classification of the specific genes associated with the complex mechanisms of tolerance is essential to better understand the underlying metabolism. Success in developing stress-tolerant cultivars depends upon concerted efforts of multiple research domains including cell physiology, genetics and molecular biology. Biotechnology, genomics and plant molecular biology along with plant breeding are successfully contributing to the development of abiotic stress-tolerant crops. Abiotic stress results in upregulation of the genes to counter abiotic stress condition. These genes are of two types: directly involved genes including osmoprotectants, chaperones, antifreeze proteins; and the regulation genes for regulating the genes expression for upregulation and signal transduction including protein kinases or transcription factors (Kasuga et al. 1999). These genes can be altered and expressed in different species via transformation system and, on their introduction, cause molecular, biochemical and physiological changes that leads

to increase abiotic stress tolerance and enhanced growth and yield characters (Bhatnagar-Mathur et al. 2008). Many crops lack the ability to upregulate the production of osmoprotectants under abiotic stress conditions. Therefore, it was hypothesized that introduction of osmoprotectants pathways into such crops is a potential strategy to improve stress tolerance (Rathinasabapathi 2000; Wani et al. 2013). Osmoprotectants are not species-specific and, thus, can be engineered into various crop plants to create stress-tolerant cultivars (Bhatnagar-Mathur et al. 2008). In plants, natural accumulation of osmoprotectants ranges from 5 to 50 $\mu\text{mol g}^{-1}$ fresh weight and become higher under the exposure to harsh conditions (Rontein et al. 2002). Osmoprotectants are mainly confined to the chloroplast, cytosol and other cytoplasmic divisions of plant cells. However, the natural biosynthesis of osmoprotectants is lacking in many major crops. Many studies have been executed to evaluate the response of plants that have been genetically transformed to increase osmoprotectant production under abiotic stress conditions (Park et al. 2004; Yang et al. 2005; 2008; Cai et al. 2017; Wei et al. 2017; Zhang et al. 2019). However, the incorporation of transgenes into the host genome, though is quite often not stable, it is certainly of considerable public concern when it comes to edible plants (Stephens and Barakate 2017).

Conclusions and future perspectives

The increased food demand of the growing world human population presents a great challenge in the era of climate change where reliance on the sustainable production approaches with minimal additional resources is also desirable. Climate change has aggravated this problem as its associated abiotic stresses limit crop productivity by adversely affecting vital crop growth and developmental processes. Naturally, some plants possess the ability to cope with these harsh environmental stresses and this ability varies between species and between cultivars within species. In general, stress-tolerant plants accumulate low molecular weight compounds known as osmoprotectants under abiotic stress conditions. These important compounds perform vital adaptive functions in regulating osmotic adjustment and protecting structures at cellular and subcellular levels. However, non- or low-accumulator crops, cannot accumulate sufficient levels of these osmoprotectants under stress conditions with low yield resulting from their lack of this ability. Thus, in order to ensure food security under the situations described above, increasing osmoprotectant levels in non- or low-accumulators is considered an important endeavor. To increase accumulation, multiple strategies are being employed in an attempt to enhance the accumulation of osmoprotectants in important crop plants. These strategies include: conventional breeding, a time consuming process, or exogenous application of osmoprotectants, a costly and laborious task.

Table 1 Improving plant tolerance to abiotic stresses through engineering genes for enhanced accumulation of osmoprotectants

Gene engineered	Source species	Transgenic host species	Growth conditions for testing	Comments	Reference
<i>IbRAP2-12</i>	<i>Ipomoea batatas</i>	<i>Arabidopsis thaliana</i>	Laboratory and field conditions	Improved salt and drought tolerance; overexpression resulted in upregulation of genes related to abscisic acid and jasmonate signaling, Pro biosynthesis, ROS scavenging and plant–water relations under salt and drought stress conditions	Li et al. 2019b
<i>AtERF019</i>	<i>Arachis hypogea</i> L.	<i>A. thaliana</i>	Laboratory conditions	Transgenics had better tolerance to drought, heat, and salt stresses; overexpression resulted in improved vigor under abiotic stresses	Wan et al. 2014
<i>pRD29A::oatADC</i>	<i>A. thaliana</i>	<i>Lotus tenuis</i>	Laboratory conditions	Improved salt tolerance; transgenics showed improved vegetative, physiological and biochemical traits compared with wild counterparts	Espasandin et al. 2018
Oat ADC	<i>Avena sativa</i>	<i>Medicago truncatula</i>	Growth chamber	Improved drought tolerance; transgenics had improved physiological status and enhanced polyamines under water-deficit conditions	Duque et al. 2016
<i>TaMIPS2</i>	<i>Triticum aestivum</i>	<i>A. thaliana</i>	Laboratory conditions	Improved drought, heat, salinity and cold tolerance; overexpression resulted in tolerance to abiotic stresses by improving morphological, physiological and biochemical attributes, especially increased inositol	Khurana et al. 2017
<i>BADH</i>	<i>Atriplex hortensis</i> L	<i>T. aestivum</i>	Laboratory conditions	Improved salt tolerance; transgenics had high tolerance towards salinity via overexpression that enhanced GB accumulation. Resulted in protection of thylakoid membrane and ultimately enhanced photosynthesis	Tian et al. 2017

Table 1 (continued)

Gene engineered	Source species	Transgenic host species	Growth conditions for testing	Comments	Reference
<i>BADH</i>	<i>Atriplex micrantha</i>	<i>Zea mays</i>	Laboratory conditions and greenhouse	Enhanced salinity tolerance; maize transgenics exhibited enhanced betaine aldehyde dehydrogenase activity and had increased fresh weight, lower malondialdehyde content, lower relative electrical conductivity, higher chlorophyll content, taller plant height, and higher grain yield	Di et al. 2015
<i>MJNT-like</i>	<i>Medicago sativa</i> subsp. <i>falcata</i>	<i>Nicotiana tabacum</i>	Greenhouse	Transformants expressing <i>MJNT-like</i> expressed higher myo-inositol transport activity, lower ion leakage and higher maximal photochemical efficiency of photosystem II and thus survived better to freezing temperature	Sambe et al. 2015
<i>P5CSF129A</i>	<i>Vigna aconitifolia</i>	<i>Cajanus cajan</i> L.	Polyhouse	Overexpression resulted in enhanced Pro content and salt tolerance	Surekha et al. 2014
<i>GsMIPS2</i>	<i>Glycine soja</i>	<i>A. thaliana</i>	Laboratory condition	Overexpression in transgenics had enhanced salt tolerance	Nisa et al. 2016
<i>mtID</i>	<i>Escherichia coli</i>	<i>Solanum tuberosum</i> L.	Laboratory condition	Overexpression resulted in enhanced mannitol content and salt tolerance	Rahnama et al. 2011
<i>ApGSMT2 g</i> and <i>ApDMT2 g</i>	<i>Aphananthece halophytica</i>	<i>Gossypium hirsutum</i> L.	Field condition	Co-expression resulted in salt tolerance and yield enhancement	Song et al. 2018
<i>pRD29A::oat ADC</i>	<i>A. sativa</i>	<i>L. tenuis</i>	Field condition	Overexpression resulted in enhanced drought tolerance	Espasandin et al. 2014
<i>codA</i>	–	<i>Solanum lycopersicum</i>	Greenhouse	Overexpression resulted in enhanced GB accumulation and salinity tolerance	Wei et al. 2017
<i>SHMT3</i>	<i>Oryza sativa</i>	<i>A. thaliana</i>	Hydroponics	Overexpression resulted in salt tolerance	Mishra et al. 2019
<i>AtHDG11</i>	<i>A. thaliana</i>	<i>Arachis hypogea</i>	Field condition	Overexpression resulted in drought and salinity tolerance	Banavath et al. 2018
<i>codA</i>	–	<i>Populus alba</i> × <i>P. glandulosa</i>	Laboratory condition	Overexpression resulted in enhanced GB accumulation and tolerance against drought, salinity and low temperature stress	Ke et al. 2016
<i>PeCHYRI</i>	<i>Populus euphratica</i>	<i>P. alba</i> × <i>Populus glandulosa</i>	–	Overexpression enhanced drought tolerance via ABA-induced stomatal closure caused by hydrogen peroxide production	He et al. 2018

Table 1 (continued)

Gene engineered	Source species	Transgenic host species	Growth conditions for testing	Comments	Reference
<i>codA</i>	<i>Arthrobacter globiformis</i>	<i>Solanum tuberosum</i>	Greenhouse condition	Transformation enhanced drought tolerance	You et al. 2019
<i>IbLEA14</i>	<i>I. batatas</i>	<i>P. alba</i> × <i>P. glandulosa</i>	Laboratory condition	Transgenic exhibited increased tolerance to MV-mediated oxidative, drought, salt and heat stress	Ke et al. 2018

As an alternative to these strategies, new technologies for introgression of stress resistant or enhanced accumulator genes into the desired non- or low-accumulator crop plants in order to engineer transgenic plants with durable resistance are increasingly being used to overcome the challenges associated with the conventional breeding strategies. Researchers have successfully developed a number of transgenic crop plants (Table 1) with enhanced osmoprotectant accumulation that not only assists crops in enduring harsh environmental stresses but also enhances their yield. Future research needs to assess the performance of these genetically modified crops in farmers' fields in order to evaluate their practical and economic value. Furthermore, plants at the field level usually have to face more than one abiotic stress, thus introgression and overexpression of multiple genes must be the ultimate research objective.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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