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Utilization of genes encoding osmoprotectants in transgenic plants for enhanced abiotic stress tolerance

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ABSTRACT

Global agriculture in the context of growing and expanding populations is under huge pressure to provide increased food, feed, and fiber. The recent phenomenon of climate change has further added fuel to the fire. It has been practically established now that the global temperature has been on the increase with associated fluctuations in annual rainfall regimes, and the resultant drought and flood events and increasing soil and water salinization. These challenges would be met with the introduction and utilization of new technologies coupled with conventional approaches. In recent years, transgenic technology has been proved very effective in terms of production of improved varieties of crop plants, resistant to biotic stresses. The abiotic stresses such as salt and drought are more complex traits, controlled by many genes. Transgenic plant development for these stresses has utilized many single genes. However, much emphasis has been placed on genes catalyzing the biosynthetic pathways of osmoprotectants. This review focuses on the current status of research on osmoprotectant genes and their role in abiotic stress tolerance in transgenic plants.

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1. Introduction

Plant adaptation to abiotic stresses is controlled by cascades of events at the molecular level. As a result, several defense mechanisms are triggered to re-establish homeostasis and protection of proteins and membranes. On the molecular level, several gene families are responsible for the induction of stress related defense pathways. These genes can be distributed into three groups: the first category contains those involved in the direct protection of important proteins and membranes such as osmoprotectants, free radical scavengers [1], late embryogenesis abundant (LEA) proteins, heat shock proteins and chaperons [2,3,4]. The second group includes membrane transporters and ion channels, involved in water and ion uptake [5]. The third group contains transcription factors, involved in transcriptional control of stress-related genes. Transcription factors are distributed in several gene families such as MYB/MYC, bZIP, NAC, CBF/DREB and ABF/ABAE [6,7,8,9].

Current efforts to improve abiotic stress tolerance of plants with genes working in the stress response pathways have resulted in significant achievements. However, due to the complex nature of abiotic stress tolerance, the present technologies have to overcome several limitations. In this review, we will overview the recent research progress in plant abiotic stress tolerance conferred by functional genes that belong to the osmoprotectant group. Much emphasis will be placed on transgenic plants with a biosynthetic accumulation of glycine betaine, proline and sugars and their role in abiotic stress tolerance.

2. Salt and drought stresses; plant responses

Salinity stress is a constant threat to crop production in many regions of the world. According to an estimate, more than 800 million hectares (Mha) of land are affected by both sodic (434 Mha) and saline (397 Mha) salts throughout the world [10]. The total irrigated land that supports agriculture has been reported as 275 million hectares in 2004, and around 20% of this land is salt affected [11]. Lack of fresh water resources and the use of brackish or saline water for irrigation are the limiting factors restricting plant growth and productivity. Crop production is practiced on saline soils in many countries, where alternative resources are not available [12]. Most of these salt affected areas are concentrated in the semi-arid and arid zones such as deserts of South America, the Mediterranean, parts of South Asia, Australia, China, Japan and South Korea [13]. Salt stress induces various biochemical and physiological responses in plants and affects almost all plant functions including photosynthesis and overall growth and development. Salinity imposes osmotic stress, ion toxicity (Na^+, Cl^- and SO_4^{2-}), nutrient (Ca, Fe, K, N, P, and Zn) deficiency and oxidative stresses on plants [14].

Drought stress is one of the major abiotic stresses that cause huge losses to world food production [15]. In the past, drought stress remained a major contributor to severe food shortages and famines. At present, nearly 70% of the world water reservoir is used for agriculture, resulting 40% of world food production in irrigated soils [16]. With increasing world population, pressure will be mounted on the already fixed water resources. The situation will be further aggravated by the predicted increase in temperature and decreased precipitation due to global warming [17].

In response to salt and drought stresses, plants undergo molecular, physiological and metabolic alterations. Plants produce low molecular weight compounds known as osmoprotectants, to cope with osmotic stress. The most important osmoprotectants that are rapidly accumulated in plants subjected to salt stress, include amino acid (*e.g.* proline), quaternary amines (*e.g.* glycine betaine and polyamines), and polyol/sugars (*e.g.* mannitol, trehalose). These compounds help plants re-establish osmotic homeostasis by increasing water potential. In addition, osmoprotectants protect cellular organelles and vital proteins and enzymes from salt-induced damage. For Na^+ ion specific stress, plants have a system of membrane proteins that regulate the uptake, influx, efflux, and upward movement and distribution of Na^+ ions. Specific cation channels in cellular membranes remove excess sodium out of the cell. In parallel, vacuolar membranes remove excess sodium from the cytosol and stores in the vacuoles. In response to oxidative stress-induced ROS production, plants produce ROS scavengers such as superoxide dismutase (SOD), catalase, ascorbate, glutathione, and peroxidase. In addition, the osmoprotectant, proline accumulation has also been suggested to have a ROS scavenging activity in plants during exposure to salt stress.

3. Transgenic plants with abiotic stress tolerance

The abundance of abiotic stress-related transcriptome analysis data generated in several plant species has revealed the importance of a number of genes catalyzing biosynthetic pathways of osmoprotectants. In many ways then, these osmoprotectants protect the plants against the damaging effects of secondary stresses such as osmotic and ionic stresses. Various plant species have been engineered, using these different genes to enhance their abiotic stress tolerance. Several review articles have been published that described the overall research endeavors on the role of osmoprotectants and confronting future challenges in plant stress tolerance [18,19,20]. The present review provides updates of the recent research breakthroughs of transgenic plants with osmoprotectant genes and discusses new approaches and technologies to develop better transgenic plants with enhanced stress tolerance and yield improvement. An overview of the biosynthetic pathways of significant osmoprotectants is given (Fig. 1). This figure highlights the major genes encoding enzymes for plant genetic engineering with enhanced abiotic stress tolerance.

4. Osmoprotectants

Compatible solutes or osmoprotectants are compounds produced in plants during osmotic stress condition. Chemically, these are small, electrically neutral molecules, which play important roles in the protection and stabilization of proteins and membranes against abiotic stresses without disrupting plant metabolism [21]. Compatible solutes are classified into three major groups; (1) amino acids (*e.g.* proline), (2) polyol/sugars (*e.g.* mannitol, trehalose, fructans), and (3) quaternary amines (*e.g.* glycine betaine and polyamines).

4.1. Proline

Proline accumulation has been reported in various plant species during a wide range of abiotic stresses [22]. In plants, proline accumulation has been reported during osmotic stress induced by salt and drought stresses [23]. The primary function of proline in plants is to counteract the osmotic effects by stabilizing protein structures and scavenging free radicals [24,25]. Apart from the above, proline also serves to store carbon and nitrogen [26]. The proline biosynthetic pathway starts from glutamate as the precursor molecule (Fig. 1b). Two enzymes catalyze the pathway, *i.e.*, P5C synthase (P5CS) that catalyzes the conversion of glutamate to P5C, and P5C reductase, which catalyzes the reduction of P5C to proline. An alternative precursor for proline biosynthesis is ornithine (Orn), which can be transaminated to P5C by Orn-D-aminotransferase (OAT), a mitochondrially located enzyme (Fig. 1b). However, glutamate pathway is the main pathway for proline biosynthesis during osmotic stress. Genes encoding proline biosynthetic pathway have been extensively studied in *Arabidopsis thaliana*. In *A. thaliana* and other plant species, the P5CS, which is a rate-limiting factor in proline biosynthesis, is encoded by two genes [27,28], while P5CR is encoded by a single gene [29].

4.1.1. Genetic manipulation of proline biosynthesis in plants

Proline accumulation in plants during salt, drought, and osmotic stress has indicated that it contributes a major part in plants

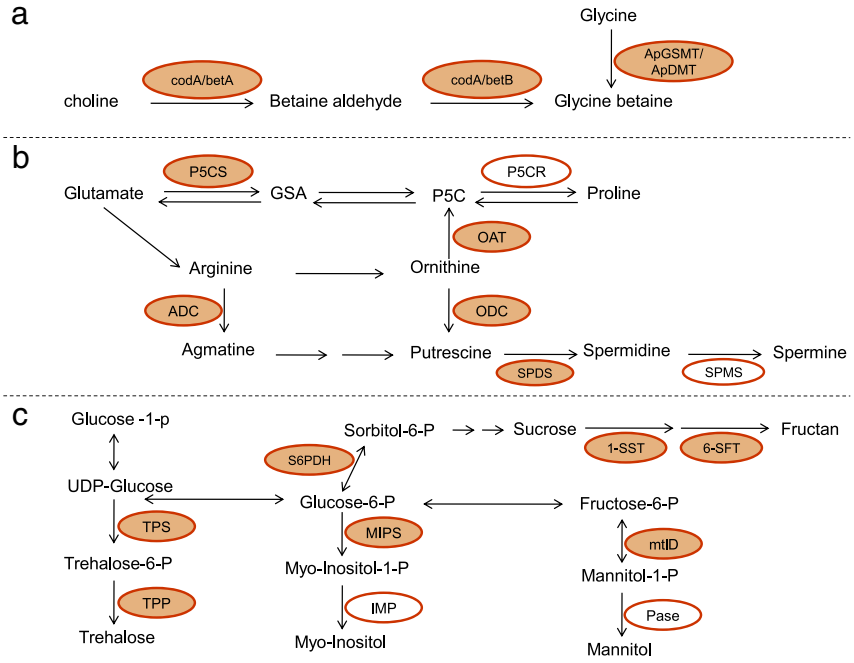

Fig. 1. Schematic overview of (a) glycine betaine, (b) proline, and (c) sugars metabolism. Expression of genes encoding the indicated enzymes (green circles) in transgenic plants, showed enhanced activities and abiotic stress tolerance. codA: choline oxidase; betA: choline dehydrogenase; betB: betaine aldehyde dehydrogenase; ApGSMT: Aphanothece halophytica glycine sarcosine methyltransferase; ApDMT: Aphanothece halophytica dimethylglycine methyltransferase; P5CS: 1-pyrroline-5-carboxylate synthetase; P5CR: pyrroline-5-carboxylate reductase; D-OAT: ornithine D-aminotransferase; ADC: arginine decarboxylase; ODC: ornithine decarboxylase; SPDS: spermidine synthase; SPMS: spermine synthase; TPS: trehalose-6-phosphate synthase; TPP: trehalose-6-phosphate phosphatase; MIPS: inositol-1-phosphate synthase; IMP: inositol-1-phosphate phosphatase; S6PDH: sorbitol-6-phosphate dehydrogenase; 1-SST: sucrose:sucrose 1-fructosyltransferase; 6-SFT: sucrose:fructan 6-fructosyltransferase; mtlD: mannitol-1-phosphate dehydrogenase; Pase: unspecific phosphatase.

adaptation to stress condition. Genetic engineering of plants with overexpression of proline biosynthetic genes has enabled to counteract the osmotic stress due to salt and drought stresses (Table 1).

Kishor et al. [30] reported overproduction of proline in transgenic tobacco and the transgenic plants produced enhanced root biomass under water stress. In one recent study, the *OsP5CS1* and *OsP5CS2* genes were co-expressed in tobacco that conferred transgenic plants increased proline accumulation and reduced oxidative damage to cells under abiotic stress conditions [31]. Similar proline production was also reported in P5CS-transgenic rice, wheat and carrot plants that showed tolerance to salt stress [32,33,34]. Transgenic *Arabidopsis* plants that expressed P5CS antisense gene were found with morphological abnormalities, and the plants were hypersensitive to osmotic stress [35]. In addition to its role in protecting vital proteins, it was proposed that proline would play a possible role in ROS scavenging [24]. In transgenic *Arabidopsis p5cs* mutant lines, it was reported that the ROS scavenging enzymes showed significantly lower activities. This evidence suggested that proline either protects enzymes of the glutathione–ascorbate cycle or enhances their activities during osmotic stress [28].

In order to enhance salt stress tolerance, the Arabidopsis *P5CS* gene was transferred to potato under a stress inducible promoter. The effect of its expression was observed in plant growth, tuber morphology and yield [36]. Transgenic potato plants accumulated high proline content compared to control under high salt stress (100 mM NaCl) and, in turn, showed improved salt tolerance by reduced tuber yield and weight compared to that of non-transgenic control. In addition, some other studies were conducted on transgenic petunias and pigeon pea (*Cajanus cajan*) with the *P5CS* gene that conferred these plants drought and salt tolerance, respectively [37,38] (Table 1). Petunia was transformed with pyrroline-5-carboxylate synthetase genes (*AtP5CS* from *A. thaliana* L. or *OsP5CS* from *Oryza sativa* L.). Transgenic plants accumulated more proline that resulted in drought tolerance for a period of 14 d. Surekha et al. [38] transformed pigeon pea with the mutagenized version (*P5CSF129A*) of wild *P5CS* gene from *Vigna aconitifolia*. The resultant transgenic plants accumulated more proline content than their non-transgenic plants. About four times higher proline content was observed in the T1 transgenic plants compared to that of non-transgenic under 200 mM NaCl stress. As a result of comparatively high proline accumulation, the transgenic plants exhibited better growth, more chlorophyll and relative water content and lower levels of lipid peroxidation under salt stress. These findings suggest the important role of proline biosynthesis in transgenic plants against osmotic stress induced by salt and drought stresses.

Table 1
Proline biosynthesis in transgenic plants confers abiotic stress tolerance.

Target	Transgene	Stress tolerance	Reference
A. thaliana	Antisense *ProDH* cDNA	Freezing, salt	[139]
C. cajan	*P5CSF129A*	Salt	[38]
Daucus carota	*P5CS*	Salt	[34]
N. tabacum	*P5CS*	Drought	[30]
Petunia	*P5CS*	Drought	[37]
N. tabacum	*P5CSF129A* cDNA	Drought	[140]
N. tabacum	*OsP5CS1*and *OsP5CS2*	Abiotic stress tolerance	[31]
O. sativa	P5CS	Salt	[32]
S. tuberosum	*P5CS*	Salt	[36]
Triticum aestivum	*P5CS*	Salt	[33]

4.2. Polyols/sugars

Accumulation of polyols in various plant species is related to high tolerance to salt and drought stress [39]. Polyols protect membranes and enzyme complexes from reactive oxygen species mainly by interacting with enzymes of the glutathione–ascorbate cycle. This group of compounds includes mannitol, D-ononitol, trehalose, sucrose, and fructane.

4.2.1. Mannitol/sorbitol/D-ononitol

Mannitol, an important member of the polyols was studied in model plants such as *Arabidopsis* and tobacco. When a bacterial gene encoding

mannitol-1-phosphatedehydrogenase (*mt1D*), engineered for expression in tobacco plants, mannitol concentrations exceeded 6 μmol/g (fresh weight) in the leaves and the roots of some transformants. Whereas this sugar alcohol was not detected in these organs of wild-type tobacco plants that underwent the same regeneration scheme [40]. Plants containing mannitol had an increased ability to tolerate high salinity [41]. The expression of *mtlD* and mannitol accumulation in the chloroplast conferred transgenic plants enhanced tolerance to osmotic stress [42]. Transgenic *Arabidopsis* expressing *mtlD* showed higher seed germination under salt stress [43].

Several transgenic plants were produced with genes of sorbitol biosynthesis. Transgenic tobacco plants expressing apple cDNA for sorbitol-6-phosphate-dehydrogenase accumulated sorbitol in varying concentrations. Transgenic plants with lower sorbitol content showed normal phenotypes; however plants with higher content were found with growth retardation [44]. *Diospyros kaki* transformed with sorbitol-6-phosphate dehydrogenase accumulated sorbitol and showed higher photosynthetic activity than that of the wild control plants [45].

Genes catalyzing the ononitol biosynthetic pathway have got little attention so far. Tobacco plants were transformed with *imtl* gene encoding myo-inositol-omethyl-transferase, an enzyme that works in the ononitol biosynthetic pathway [46]. Transgenic plants showed more tolerance to salt and drought stress than wild type control.

In addition to the expression of sugar biosynthetic genes in transgenic plants, the role of kinases on sugar metabolism was demonstrated. Kempa et al. [47] investigated the role of glycogen synthase kinase 3 (GSK-3), a regulator of glycogen synthesis on sugar metabolism and salt stress. The MsK4, a novel *Medicago sativa* GSK-3 like kinase connects stress signaling with carbon metabolism. The transgenic lines with over-expression of MsK4 revealed changes in sugar metabolism, accumulation of higher levels of starch, glucose and G6P compared to wild type plants. The MsK4 lines also showed salt tolerance suggesting a possible role of MsK4 on sugar metabolism under salinity stress.

4.2.2. Trehalose/fructans

Trehalose, a non-reducing disaccharide of glucose has been proposed to play a major role in abiotic stress tolerance of bacteria, fungi, and invertebrates [48]. In plants, this compound has not been thoroughly investigated in relation to abiotic stress tolerance [49]. In recent years, due to the discovery of its role in protecting membranes and proteins [50] emphasis was placed on genetic engineering of plants with genes of trehalose biosynthetic pathway. Various genes catalyzing the trehalose biosynthetic pathway were isolated from prokaryotes and crop plants [51,52]. The expression of these genes conferred enhanced tolerance to various abiotic stresses in transgenic plants [53]. The yeast (*Saccharomyces cerevisiae*) gene trehalose-6-phosphate synthase (*TPSI*) was expressed in tobacco plants [54]. Transgenic tobacco plants showed drought tolerance along with various developmental changes including lower sucrose content. The same gene was constitutively expressed in potato [55]. Initially, the *in vitro* plants showed some phenotypic aberrations. However, the normal growth was restored when plants were acclimatized to the soil. Transgenic plants showed significantly enhanced drought tolerance.

The *Escherichia coli otsA* and *otsB* genes were introduced into potato and tobacco under the control of either a constitutive *CaMV35S* promoter or the tuber specific patatine promoter [56]. However, very low level of trehalose accumulation was observed in the leaves of the obtained transgenic tobacco plants, whereas transgenic potato tubers showed no trehalose accumulation. Same genes were expressed in rice under both tissue-specific and stress-inducible promoters [57]. Compared to control plants, the transgenic rice plants showed vigorous growth under salt, drought, temperature and osmotic stress.

The TPS1 gene was expressed in potato under a drought responsive StDS2 promoter [58]. In transgenic plants, the TPS1 expression was not induced; however the very low level of constitutive expression was sufficient to alter their response to drought stress. The transgenic accumulation of trehalose seems to be species specific as variable concentrations were found with genes of different origins. Recently a trehalose biosynthetic gene, trehalose synthase (*TSase*), from *Grifola frondosa* was expressed in tobacco, and the transgenic plants accumulated significantly higher trehalose content than previously reported for *E. coli TPS*, *TPP*, *TPSP*, and yeast *TPS1* gene expressed in tobacco and rice plants. Transgenic tobacco plants also showed enhanced tolerance to salt and drought stress [59]. In some other studies, expression of the trehalose biosynthetic genes conferred tolerance to abiotic stresses in transgenic *Arabidopsis* [60] and rice plants [61,62].

Fructans are polymers of fructose and in many plant species serve as storage of carbohydrates [63]. Plants accumulate fructans in vacuoles, where these play a role in abiotic stress tolerance [64]. Fructans biosynthetic genes were isolated from both bacteria and higher plants. A fructan biosynthetic related gene *SacB* from *Bacillus subtilis*, encoding levansucrase enzyme was expressed in tobacco and potato under constitutive promoter [65,66]. Transgenic tobacco plants showed increased tolerance to salt, drought and chilling stress [67]. Similarly, sugar beet were transformed with the same *SacB* gene and the resultant transgenic plants showed enhanced biomass irrespective of lower fructans accumulation than that accumulated in transgenic tobacco and potato [68]. Also the expression of fructan biosynthetic genes in tobacco and rice conferred enhanced tolerance to low temperature stress [69,70]. So far, it is not clear about the tolerance mechanism conferred by fructans accumulation. However, it is speculated that like mannitol and trehalose, fructans work in connection with either glutathione–ascorbate cycle or cell signaling pathways during stress [71].

4.3. Osmotin-like proteins

Osmotin and osmotin-like proteins are a group of stress proteins that have been classified as members of the pathogenesis-related (PR type-5) proteins. The tobacco osmotin gene expression was activated by NaCl, ABA, wounding, viral infection and ethylene [72]. Moreover, several authors have reported the dual function of osmotin and osmotin-like proteins in plant defense and osmotic stress [73,74].

In the wild potato, *Solanum commersonii*, at least six genes have been identified as members of a multigene family. Zhu et al. [72] isolated two osmotin-like protein genes from *S. commersonii* and overexpressed in the same species. Treatments with abscisic acid (ABA), low temperature, and NaCl and fungal infection increased the accumulation of three mRNAs encoding osmotin-like proteins in potato (*S. commersonii*). However, accumulation of osmotin-like proteins was detected only in fungi-infected tissues but not in plants treated with ABA, NaCl, or low temperature. Evers et al. [75] transformed potato (*Solanum tuberosum* L. cv. Bintje) with the *Arabidopsis* cDNA clone encoding an osmotin-like protein (Table 2). The proline content was only slightly increased in transformed plants compared to non-transformed ones that had marked proline accumulation upon salt stress. Irrespective of low proline accumulation, the transgenic plants showed comparatively more salt tolerance than that of non-transgenic plants. Transgenic soybean with the osmotin-like protein (SnOLP), from *Solanum nigrum* had a higher leaf water potential at predawn, net CO_2 assimilation rate, stomatal conductance, transpiration rate and 100-grain weight than non-transgenic plants under water deficit condition [76].

4.4. Glyceraldehyde-3 phosphate dehydrogenase (GPD)

The role of GPD expression in a range of organisms including plants, fungi and mammals was established under environmental stresses, such as heat shock and anaerobiosis. Salt stress-induced the GPD expression in *A. nidulans* [77,78]. A GPD gene, isolated from oyster mushroom (*Pleurotus sajor-caju*) was overexpressed in yeast cells, and

Table 2
Transgenic plants for abiotic stress tolerance with genes encoding functional proteins.

Target	Transgene	Stress tolerance	Reference
D. kaki	*Stpd1*		[45]
G. max	Osmotin-like protein	Drought	[76]
Lycopersicum esculentum	Osmotin-like protein	Cold	[141]
N. tabacum	*EctA*	–	[142]
N. tabacum	*Yeast invertase*	–	[143]
O. sativa	*Glutamine synthetase*	–	[144]
O. sativa	*TPSP*	–	[57]
O. sativa	*OsTPP1*	Salt, cold	[61]
O. sativa	*OsTPS1*	Abiotic stress tolerance	[62]
Pinus taeda	*MtlD/GutD*	–	[145]
S. tuberosum	*Arabidopsis* osmotin-like protein	–	[75]
S. tuberosum	*GPD*	–	[80]
S. tuberosum	*Oxalate oxidase*	–	[146]
S. tuberosum	*AtNDPK2*	–	[147]
V. mungo	*Glyoxalase1*	–	[148]

its expression was induced under various environmental stress conditions including salt, drought and heat [79]. The GPD gene was overexpressed in potato, to further investigate its role in osmotic stress tolerance, that conferred transgenic plants improved tolerance against salt loading [80] (Table 2).

4.5. Oxalate oxidase

Oxalate oxidase is an enzyme that catabolizes oxalic acid. This enzyme is a structural and functional homolog of the germin protein in wheat [81]. Germin proteins have a protective role during osmotic stress, also involved in cell wall expansion in cereals [82,83] (Table 2). Potato was transformed with barley oxalate oxidase, o further verify its role in salt and osmotic stress, and the effect was determined on plant growth and tuber morphology under salt stress. Transgenic plants showed relatively higher salt tolerance and superior tuber yield than the non-transgenic control.

4.6. Glycine betaine

Glycine betaine (GB), a quaternary ammonium compound, is an important osmoprotectant in bacteria, animals, and angiosperms [84]. GB accumulation has been reported in plants when exposed to environmental stresses such as salt, drought, and extreme temperatures [85]. In plants, the accumulated GB confers protection by acting as an osmolyte, adjusts osmotic balance [86], stabilizes membranes and protects macromolecules, photosystem II from dehydration, high salt concentration and oxidative damage [87,88]. In nature, some plants such as sugar beet, maize, spinach, and barley accumulate GB in response to abiotic stresses [89]. However, despite GB accumulation in natural accumulators, some economically important crops are non-accumulators and are thus potential targets for genetic engineering with GB biosynthetic genes [90].

4.6.1. Transgenic plants with GB

The GB biosynthetic genes in transgenic plants proved very effective in conferring stress tolerance compared to that of other osmoprotectant genes. Several studies have reviewed the important roles of GB in transgenic plants under various abiotic stresses [91,92]. A number of transgenic plants with GB biosynthetic genes have been tested for GB accumulation and the resultant salt, drought and temperature tolerance (see Table 3). Transgenic plants such as Arabidopsis, eucalyptus, tobacco, rice, tomato, potato and wheat with GB biosynthetic genes have showed increased GB accumulation and stress tolerance [93,94,95,96,97,98,99,100]. The GB accumulation was targeted in the chloroplast, in most of these transgenic plants, where its increased concentration conferred protection against various abiotic stresses, particularly salt, and drought stresses. Overall, in transgenic plants the accumulated GB content and the resultant stress tolerance is believed to be influenced by three factors: choline (precursor for GB) availability [101], type of transgene of the GB biosynthetic pathway [102,92], and the type of promoter (constitutive and stress-inducible) [103].

In some GB-transgenic plants, it was observed that the accumulated GB not only conferred stress tolerance but also improved reproductive and yield components such as flowers and fruits [97,104]. For example, the *codA*-transgenic tomato showed protective effects of GB on reproductive organs under chilling stress [104,105]. Transgenic plants accumulated 2.8–3.8 times higher GB in reproductive organs than in the leaves. The increased GB content not only conferred chilling tolerance but also increased fruit set by 10–30% under chilling stress. The effect of the *codA* gene on reproductive organs was further investigated in transgenic tomato plants [106]. The constitutive expression of *codA* resulted in the improved growth of flowers and fruits. Transgenic plants produced large flowers and 54% heavier fruits compared to non-transgenic control. These results indicate that the use of constitutive promoters to express GB biosynthetic genes has no adverse effects and is rather beneficial to have positive effects on reproductive organs under both stresses and non-stress conditions.

4.7. Polyamines

Polyamines have been identified as small molecules that are highly responsive to various abiotic stresses such as salt, drought and low temperatures [107,108]. Polyamines are present in higher plants in the form of putrescine, spermine, and spermidine. Although the specific role of polyamines in stress tolerance is still unclear, it is believed that these protect membranes from the damaging effects of oxidative stress [108,109].

Table 3
Metabolic engineering of plants for GB biosynthesis confers tolerance to various abiotic stresses.

Species	Gene	GB accumulation (organ)	Targeted organelle	Tolerance	Reference
A. thaliana	*GSMT/SDMT*	0.8–1.7 μmoL g^{-1} fw (seeds)	N.A.	Salt	[99]
Eucalyptus globulus	*codA*	0.17–0.29 μmoL g^{-1} fw (leaves)	N.A.	Salt	[100]
Gossypium hirsutum	*betA* (CDH)	130.2–142.0 μmoL g^{-1} dw (leaves)	N.A.	Drought	[135]
N. tabacum	BADH (spinach)	0.44–4.92 μmoL g^{-1} fw (leaves)	Chloroplast	Salt stress	[136]
N. tabacum	BADH/*SeNHX1*	4.7–7.0 μmoL g^{-1} dw (leaves)	N.A.	Salt	[137]
N. tabacum	*codA*	–	N.A.	Salt	[96]
O. sativa	COX	2.6–3.12 μmoL g^{-1} fw (leaves)	Chloroplast	Salt	[103]
O. sativa	CMO (spinach)	0.29–0.43 μmoL g^{-1} dw (leaves)	Chloroplast	Salt	[98]
S. lycopersicum	*codA*	0.1–0.3 μmoL g^{-1} fw (leaves)	Chloroplast	Chilling	[104]
S. lycopersicum	*codA*	–	–	Salt, drought	[94]
S. tuberosum	*codA*	–	Chloroplast	Salt, drought	[93]
S. tuberosum	*codA*	–	Chloroplast	Drought	[138]
T. aestivum	*beta*	–	Chloroplast	Salt	[95]

N.A: not available; dw: dry weight; fw: fresh weight.

A number of transgenic plants engineered with genes catalyzing the biosynthetic pathways of various polyamines have shown a positive correlation between the accumulated levels of polyamines and stress tolerance [110,111]. Arabidopsis that over-expressed the SPDS gene produced higher spermidine content and showed a high tolerance to drought, salt and cold stress [112]. Furthermore, the transformation of tobacco with the mouse ornithine decarboxylase (ODC) resulted in high polyamine accumulation and salt tolerance [113]. The role of polyamines in salt stress tolerance was further investigated in 18 rice cultivars [114]. This study explored the possible correlations between the polyamine content, expression levels of polyamine synthesizing genes, physiological parameters and sensitivity to salt stress of the rice cultivars. They also compared the salt stress-induced changes in polyamine content and gene expression levels with results obtained under drought conditions for the tested rice cultivars. Microarray analysis confirmed five genes (*CPA1*, *CPA2*, *CPA4*, *SAMDC1*, and *SPD*/*SPM1*) as induced by salt stress and *AlH* was confirmed as specifically drought-induced in the salt tolerant and sensitive rice cultivars. These genes were previously classified in drought stress experiments [115].

4.8. Transcription control of metabolic alterations

The important role of polyamines in stress tolerance has further been demonstrated in transgenic Arabidopsis with *alx8* as a mutation in the *SAL1*, an enzyme that dephosphorylate dinucleotide phosphates or inositol phosphates [116]. Interestingly, the mutant plants showed increased drought tolerance when soil-grown intact plants were subjected to water stress. Microarray analysis demonstrated altered the expression of more than 1800 genes in the *alx8* mutant and *fry1-1* (previously reported mutant). Metabolomic analysis showed that both mutants exhibited metabolomic reprogramming, including increased levels of polyamine putrescine. They suggested that SAL1 acts as a negative regulator of drought tolerance and its inactivation resulted in altered metabolism including increased putrescine levels and subsequent drought tolerance.

Transcription factors play an important role in plant stress adaptation by regulating the downstream genes involved in stress tolerance [117]. These stress-related genes and pathways may also include expression of genes encoding osmoprotectants. In one study, Wu et al. [118] transformed rice with the *OsWRKY11* gene under the control of *HSP11* promoter. They reported enhanced heat and drought tolerance in transgenic rice seedlings. Further studies were conducted to examine the contents of sucrose, glucose, fructose and raffinose, and their relationship to desiccation tolerance in the *OsWRKY11*-transgenic plants [119]. They reported up-regulation of the expression of two genes, Os07g0209100 encoding raffinose synthase and Os07g0687900 encoding galactinol synthase. They reported that the expression of *OsWRKY11* induced enhanced raffinose accumulation and desiccation tolerance in transgenic plants. These studies suggest a transcriptional control of the stress-induced metabolic alterations.

5. Availability of the precursors of osmoprotectant pathways

In addition to engineering plants with individual osmoprotectant genes to alter the levels of metabolites under stress, the levels and proper placement of precursors of osmoprotectants, need proper attention [101,120]. For example, choline acts as a precursor for glycine betaine biosynthesis in *E. coli* and plants. Choline biosynthesis occurs in the cytosol and is then transported to chloroplast for GB production in higher plants [92]. In transgenic plants, choline availability is one of the main factors that limit GB accumulation [101]. Several studies have demonstrated variable levels of GB in the cytosol and chloroplast suggesting different capacities of plant species in either choline biosynthesis in the cytosol or its transport to the chloroplast [105,121]. Based on these findings, choline availability in the chloroplast is crucial to GB biosynthesis and subsequent stress tolerance.

The other major osmoprotectant proline is considered to be synthesized in either cytosol or chloroplast or both [28,122]. The availability of precursor glutamate for proline synthesis may be a limiting factor. The source of glutamate may vary with nitrogen source and stress condition. The proline metabolism, various enzymes working in its biosynthetic pathway, cellular localization of these enzymes and proline translocation between cellular organelles has been reviewed [122].

6. Conclusion and future prospects

So far, much progress has been made towards engineering transgenic plants with abiotic stress tolerance that utilized genes encoding osmoprotectants, and other stress-related functional proteins. In comparison to other genes, biosynthetic accumulation of glycine betaine, proline and other osmoprotectant genes in several transgenic crop plants have shown some improvement in abiotic stress tolerance. However, the success of these genes has been limited in the sense that most of the developed transgenic plants have been tested under controlled laboratory conditions. In such conditions, transgenic plants are evaluated at the early growth stage and are exposed to stress condition for a short period. Such short exposure experiments at early growth stages may not predict the response of the plant from seedling to mature and reproductive stage under realistic field conditions. In addition, under field conditions, transgenic plants may experience multiple stresses such as salt, drought, and extreme temperatures. These stresses may suppress the protective effects of the inserted gene. Abiotic stress tolerance in plants is a multigenic response controlled by many genes working in different stress response pathways [19]. However, the level of abiotic stress tolerance that was initially anticipated has not been fully achieved in many transgenic plants, transformed with single genes [123,124].

The future research should focus on combining different strategies such as the multigenic approach to simultaneously incorporate more than one gene in transgenic plants (Fig. 2). In this context, the osmoprotectants synthesizing genes should be co-expressed with other stress-related genes such as transcription factors, ion transporters, and other functional genes. On the other hand, these genes such as the glycine betaine synthesizing *codA* and other genes should be co-expressed with molecular determinants of blue, green algae and cyanobacteria. These microbes have very efficient carbon concentrating mechanisms (CCM). The combining of these different approaches would result in enhanced CO_2 assimilation, photosynthesis, stress tolerance and improved yield in transgenic plants. In addition to this, studies should be progressed to analyze and discover the various molecular components involved in stress response adaptation at the biochemical, physiological and molecular levels in plants.

Moreover, research on transgenic plants with osmoprotectant genes should be progressed under controlled laboratory conditions to the more realistic field conditions where the effects may be evaluated in terms of the ultimate target that is yield improvement under stress condition. Plant growth and yield components such as improved water uptake through roots, enhanced CO_2 uptake and photosynthesis and water use efficiency should be targeted coupled with transgenic technology. On one side, the osmoprotectant genes insertion in transgenic plants should be targeted to achieve maximum adaptation to stress condition. While on the other side, efforts should be done to cointegrate these genes with other molecular determinants controlling the several growth and yield parameters.

The use of individual genes, targeting various mechanisms involved in ABA biosynthesis and stomatal regulation to achieve improved water use efficiency, enhanced CO_2 assimilation and ultimately yield increase has a great promise for future transgenic

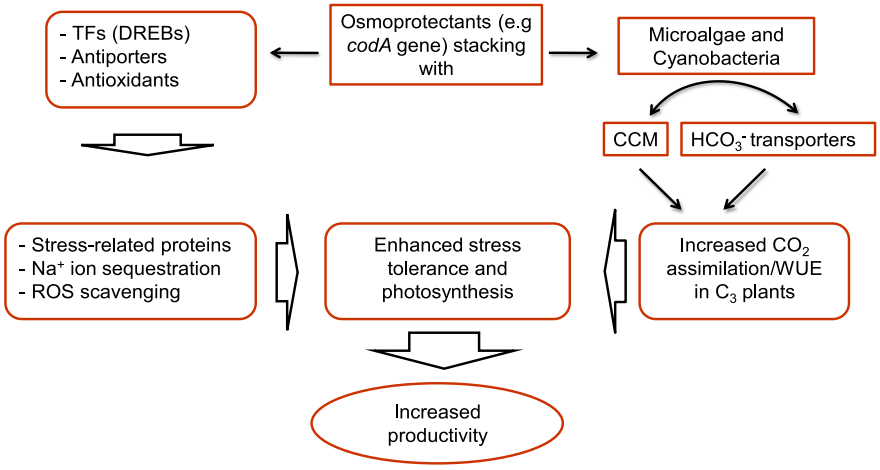

Fig. 2. Alternative strategies to combine osmoprotectant accumulation with other abiotic stress responsive genes in transgenic plants. Gene stacking of osmoprotectants with other suitable genes such as DREBs, antiporters and antioxidants will improve the underlying mechanisms resulting enhanced stress tolerance and productivity.

development. Some of the target genes such as aquaporins, *A. thaliana HARDY* (*HRD*), *A. thaliana GT-2 Like 1* (*AtGTL1*), *A. thaliana LOS5* (*AtLOS5*) that encodes molybdenum co-factor, and *Lycopersicon esculentum 9-cis-epoxycarotenoid dioxygenase* (*LeNCED*) should be stacked with stress-inducible expression of *DREB* genes in transgenic plants to achieve increased water use efficiency (WUE), CO_2 assimilation and the resultant higher yield. In past studies, these individual genes were used in the transgenic development. For example, aquaporins have been proven very effective in increasing CO_2 and water permeability under stress resulting increased water use efficiency in transgenic plants. It has been demonstrated in tobacco and tomato with *NtAQP1* [125]. In addition, other important genes involved in stomatal regulation and ABA biosynthesis should be used in transgenic plants to improve WUE. The ABA biosynthesis optimization will certainly improve stomatal regulation, water and CO_2 permeability and photosynthesis. ABA biosynthetic genes such as *AtLOS5* and *LeNCED* were used in transgenic rice and tomato plants that showed drought tolerance, increased WUE, high spikelet fertility, high yield in rice and improved WUE in tomato [126,127]. Transgenic plants with high osmoprotectant accumulation and the resultant improved photosynthesis and water-use efficiency under abiotic stress conditions may enable farmers to overcome enormous losses due to these stresses.

So far, research on the transcriptomic and proteomic analysis of plant genomes to identify osmoprotectant-related gene expression under stress conditions is limited. Only a few notable studies focused on expression assays and searches in EST databases, targeting a single osmoprotectant gene [128,129]. Evaluation of transcriptomic databases generated under stress by SuperSAGE in association with Next Generation Sequencing (NGS) has emerged as a powerful tool to identify individual genes related to stress tolerance in plants. Kido et al. [130] conducted the transcriptomic evaluation with NGS of osmoprotectant related genes in soybean under water deficit and biotic stress. This study focused on seven prominent genes related to the biosynthesis of proline (*P5CS* & *P5CR*), glycine betaine (*BADH* & *CMO*), trehalose (*TPS1* & *TPPB*) and myo-inositol (*INPS1*). This novel approach identified 36 differentially expressed genes related to the biosynthesis of the four important osmoprotectants. These genes were found mapped on 25 loci in the soybean genome.

In addition to the above example, the transcriptomic analysis was carried out in sugarcane to identify the expression of genes related to osmoprotectant biosynthesis under drought stress [131]. Sugarcane transcriptome under drought stress was analyzed, using a combination of high throughout transcriptome profiling by SuperSAGE and next generation sequencing technology. Four drought tolerant and four drought sensitive genotypes were used to generate differentially expressed stress-responsive genes. The SuperSAGE libraries produced 205,975 unitags. Several unitags were identified related to major osmoprotectant genes such as *BADH*, *P5CS*, *P5CR*, *MIPS*, *TPS*, and *TPP*.

Such studies should be extended to other crop plants to identify the differential expression of osmoprotectant-related genes under various abiotic stress conditions. The genes identified in these studies will be helpful to unravel the underlying mechanism of stress tolerance and may be used in the future breeding programs and transgenic development of stress tolerant crop plants in a stress-specific and plant-specific manner. Moreover, new breeding approaches should be adopted to develop stress tolerant plant genotypes. The use of stress-related QTLs and map-based cloning methods in association with new approaches such as microarray-based expression profiling of differential gene expression should be used. These individual methods have already been applied for identification of stress-related genes [132,133,134]. More advanced techniques such as the “omics” tools and next generation sequencing have a promising role in exploring gene function and plant responses to stress. In addition, the more time saving and highly accurate techniques of SuperSAGE, Multi-SNP analysis, ligation free cloning, Phylo-CSF, and glycol-proteomics should be used in combination with the genetic engineering approaches for development of abiotic stress tolerant plants.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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References

[1] Bohnert HJ, Sheveleva E. Plant stress adaptations — Making metabolism move. Curr Opin Plant Biol 1998;1:267–74. http://dx.doi.org/10.1016/S1369-5266(98)80115-5.
[2] Bray EA, Bailey-Serres J, Weretilnyk E. Responses to abiotic stresses. In: Gruissem W, Buchannan B, Jones R, editors. Biochemistry and molecular biology of plants. American Society of Plant PhysiologistsRockville, MD: Wiley & Sons; 2000. p. 1158–249 [ISBN 0-943088-39-9].
[3] Ingram J, Bartels D. The molecular basis of dehydration tolerance in plants. Annu Rev Plant Biol 1996;47:377–403. http://dx.doi.org/10.1146/annurev.arplant.47.1.377.
[4] Vierling E. The roles of heat-shock proteins in plants. Annu Rev Plant Biol 1991;42: 579–620. http://dx.doi.org/10.1146/annurev.pp. 42.060191.003051.

[5] Blumwald E. Sodium transport and salt tolerance in plants. Curr Opin Cell Biol 2000;12:431–4. http://dx.doi.org/10.1016/S0955-0674(00)00112-5.

[6] Frank SR, Schroeder M, Fernández P, Taubert S, Amati B. Binding of c-Myc to chromatin mediates mitogen-induced acetylation of histone H4 and gene activation. Genes Dev 2001;15:2069–82. http://dx.doi.org/10.1101/gad.906601.

[7] Munnik T, Ligterink W, Meskiene I, Calderini O, Beyerly J, Musgrave A, et al. Distinct osmo-sensing protein kinase pathways are involved in signaling moderate and severe hyper-osmotic stress. Plant J 1999;20:381–8. http://dx.doi.org/10.1046/j.1365-313x.1999.00610.x.

[8] Shinozaki K, Yamaguchi-Shinozaki K. Gene expression and signal transduction in water-stress response. Plant Physiol 1997;115:327–34. http://dx.doi.org/10.1104/pp. 115.2.327.

[9] Shinozaki K, Yamaguchi-Shinozaki K, Seki M. Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 2003;6:410–7. http://dx.doi.org/10.1016/S1369-5266(03)00092-X.

[10] Munns R. Genes and salt tolerance: Bringing them together. New Phytol 2005;167: 645–63. http://dx.doi.org/10.1111/j.1469-8137.2005.01487.x.

[11] Flowers TJ, Flowers SA. Why does salinity pose such a difficult problem for plant breeders? Agric Water Manag 2005;78:15–24. http://dx.doi.org/10.1016/j.agwat.2005.04.015.

[12] Bustan A, Sagi M, Malach YD, Pasternak D. Effects of saline irrigation water and heat waves on potato production in an arid environment. Field Crop Res 2004;90: 275–85. http://dx.doi.org/10.1016/j.fcr.2004.03.007.

[13] Levy D, Veilleux RE. Adaptation of potato to high temperatures and salinity—A review. Am J Potato Res 2007;84:437–56. http://dx.doi.org/10.1007/BF02987885.

[14] Chinnusamy V, Zhu J, Zhu JK. Salt stress signaling and mechanisms of plant salt tolerance. Genet Eng 2006;27:141–77. http://dx.doi.org/10.1007/0-387-25856-6_9.

[15] Boyer JS. Plant productivity and environment. Science 1982;218:443–8. http://dx.doi.org/10.1126/science.218.4571.443.

[16] Chaves MM, Oliveira MM. Mechanisms underlying plant resilience to water deficits: Prospects for water-saving agriculture. J Exp Bot 2004;55:2365–84. http://dx.doi.org/10.1093/jxb/erh269.

[17] Mannion AM. Biotechnology and environmental quality. Prog Phys Geogr 1995;19: 192–215. http://dx.doi.org/10.1177/030913339501900203.

[18] Flowers TJ. Improving crop salt tolerance. J Exp Bot 2004;55:307–19. http://dx.doi.org/10.1093/jxb/erh003.

[19] Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: Achievements and limitations. Curr Opin Biotechnol 2005;16:123–32. http://dx.doi.org/10.1016/j.copbio.2005.02.001.

[20] Szábados L, Kovacs H, Zilberstein A, Bouchereau A. Plants in extreme environments: Importance of protective compounds in stress tolerance. Adv Bot Res 2011;57:105–50. http://dx.doi.org/10.1016/B978-0-12-387692-8.00004-7.

[21] Yancey PH. Compatible and counteracting solutes. In: Strange SK, editor. Cellular and molecular physiology of cell volume regulation. Boca Raton: CRC Press. ISBN 0849344484; 1994. p. 81–109.

[22] Hayat S, Hayat Q, Alyemeni MN, Wani AS, Pichtel J, Ahmad A. Role of proline under changing environments: A review. Plant Signal Behav 2012;7:1456–66. http://dx.doi.org/10.4161/psb.21949.

[23] Delaney AJ, Verma DP. Proline biosynthesis and osmoregulation in plants. Plant J 1993;4:215–23. http://dx.doi.org/10.1046/j.1365-313X.1993.04020215.x.

[24] Smirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 1989;28:1057–60. http://dx.doi.org/10.1016/0031-9422(89)80182-7.

[25] Biedermannova L, Riley KE, Berka K, Hobza P, Vondrasek J. Another role of proline: Stabilization interactions in proteins and protein complexes concerning proline and tryptophan. Phys Chem Chem Phys 2008;10:6350–9. http://dx.doi.org/10.1039/b805087b.

[26] Hare PD, Cress WA. Metabolic implications of stress-induced proline accumulation in plants. Plant Growth Regul 1997;21:79–102. http://dx.doi.org/10.1023/A:1005703923347.

[27] Fujita T, Maggio A, Garcia-Rios M, Bressan RA, Csonka LN. Comparative analysis of the regulation of expression and structures of two evolutionarily divergent genes for Δ^1-pyrroline-5-carboxylate synthetase from tomato. Plant Physiol 1998;118: 661–74. http://dx.doi.org/10.1104/pp. 118.2.661.

[28] Székely G, Abrahám E, Cséplö A, Rigó G, Zsigmond L, Csiszár J, et al. Duplicated *P5CS* genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J 2008;53:11–28. http://dx.doi.org/10.1111/j.1365-313X.2007.03318.x.

[29] Verbruggen N, Villarroel R, Van Montagu M. Osmoregulation of a pyrroline-5-carboxylate reductase gene in *Arabidopsis thaliana*. Plant Physiol 1993;103:771–81. http://dx.doi.org/10.1104/pp. 103.3.771.

[30] Kishor KPB, Hong Z, Miao GH, Hu CAA, Verma DPS. Overexpression of [delta]-pyrroline-5-carboxylate synthetase increase proline production and confers osmotolerance in transgenic plants. Plant Physiol 1995;108:1387–94.

[31] Zhang X, Tang W, Liu J, Liu Y. Co-expression of rice *OsP5CS1* and *OsP5CS2* genes in transgenic tobacco resulted in elevated proline biosynthesis and enhanced abiotic stress tolerance. Chin J Appl Environ Biol 2014:717–22.

[32] Zhu B, Su J, Chang M, Verma DPS, Fan YL, Wu R. Overexpression of a Δ^1-pyrroline-5-carboxylate synthase gene and analysis of tolerance to water and salt stress in transgenic rice. Plant Sci 1998;139:41–8. http://dx.doi.org/10.1016/S0168-9452(98)00175-7.

[33] Sawahel WA, Hassan AH. Generation of transgenic wheat plants producing high levels of the osmoprotectant proline. Biotechnol Lett 2002;24:721–5. http://dx.doi.org/10.1023/A:1015294319114.

[34] Han KH, Hwang CH. Salt tolerance enhanced by transformation of a *P5CS* gene in carrot. J Plant Biotechnol 2003;5:149–53.

[35] Nanjo T, Kobayashi M, Yoshiba Y, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K. Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. FEBS Lett 1999;461:205–10. http://dx.doi.org/10.1016/S0014-5793(99)01451-9.

[36] Hmida-Sayari A, Gargouri-Bouzid R, Bidani A, Jaoua L, Savouré A, Jaoua S. Overexpression of Δ^1-pyrroline-5-carboxylate synthetase increases proline production and confers salt tolerance in transgenic potato plants. Plant Sci 2005;169:746–52. http://dx.doi.org/10.1016/j.plantsci.2005.05.025.

[37] Yamada M, Morishita H, Urano K, Shiozaki N, Yamaguchi-Shinozaki K, Shinozaki K, et al. Effects of free proline accumulation in petunias under drought stress. J Exp Bot 2005;56:1975–81. http://dx.doi.org/10.1093/jxb/eri195.

[38] Surekha CH, Nirmala Kumari K, Aruna LV, Suneetha G, Arundhati A, Kavi Kishor PB. Expression of the *Vigna aconitifolia P5CSF129A* gene in transgenic pigeon pea enhances proline accumulation and salt tolerance. Plant Cell Tissue Organ Cult 2013;116:27–36. http://dx.doi.org/10.1007/s11240-013-0378-z.

[39] Bohnert HJ, Jensen RG. Strategies for engineering water-stress tolerance in plants. Trends Biotechnol 1996;14:89–97. http://dx.doi.org/10.1016/0167-7799(96)80929-2.

[40] Tarczynski MC, Jensen RG, Bohnert HJ. Expression of a bacterial mtlD gene in transgenic tobacco leads to production and accumulation of mannitol. Proc Natl Acad Sci U S A 1992;89:2600–4.

[41] Tarczynski MC, Jensen RG, Bohnert HJ. Stress protection of transgenic tobacco by production of the osmolyte mannitol. Science 1993;259:508–10. http://dx.doi.org/10.1126/science.259.5094.508.

[42] Shen B, Jensen RG, Bohnert HJ. Mannitol protects against oxidation by hydroxyl radicals. Plant Physiol 1997;115:527–32.

[43] Thomas JC, Sepahi M, Arendall B, Bohnert HJ. Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana*. Plant Cell Environ 1995;18:801–6. http://dx.doi.org/10.1111/j.1365-3040.1995.tb00584.x.

[44] Sheveleva EV, Marquez S, Chmara W, Zegeer A, Jensen RG, Bohnert HJ. Sorbitol-6-phosphate dehydrogenase expression in transgenic tobacco. Plant Physiol 1998; 117:831–9. http://dx.doi.org/10.1104/pp. 117.3.831.

[45] Gao M, Tao R, Miura K, Dandekar AM, Sugiura A. Transformation of Japanese persimmon (*Diospyros kaki* Thunb.) with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase. Plant Sci 2001;160:837–45. http://dx.doi.org/10.1016/S0168-9452(00)00458-1.

[46] Sheveleva E, Chmara W, Bohnert HJ, Jensen RG. Increased salt and drought tolerance by D-ononitol production in transgenic *Nicotiana tabacum* L. Plant Physiol 1997;115:1211–9. http://dx.doi.org/10.1104/pp. 115.3.1211.

[47] Kempa S, Rozhon W, Samaj J, Erban A, Baluska F, Becker T, et al. A plastid-localized glycogen synthase kinase 3 modulates stress tolerance and carbohydrate metabolism. Plant J 2007;49:1076–90. http://dx.doi.org/10.1111/j.1365-313X.2006.03025.x.

[48] Djilianov D, Georgieva T, Moyankova D, Atanassov A, Shinozaki K, Smeeken SCM, et al. Improved abiotic stress tolerance in plants by accumulation of osmoprotectants-gene transfer approach. Biotechnol Biotechnol Equip 2005; 19:63–70. http://dx.doi.org/10.1080/13102818.2005.10817287.

[49] Crowe JH, Hoekstra FA, Crowe LM. Anhydrobiosis. Annu Rev Physiol 1992;54: 579–99. http://dx.doi.org/10.1146/annurev.ph.54.030192.003051.

[50] Paul MJ, Primavesi LF, Jhurreea D, Zhang YH. Trehalose metabolism and signaling. Annu Rev Plant Biol 2008;59:417–41. http://dx.doi.org/10.1146/annurev.arplant.59.032607.092945.

[51] Goddijn OJM, Van Dun K. Trehalose metabolism in plants. Trends Plant Sci 1999;4: 315–9. http://dx.doi.org/10.1016/S1360-1385(99)01446-6.

[52] Zentella R, Mascorro-Gallardo JO, Van Dijck P, Folch-Mallol J, Bonini B, Van Vaeck C, et al. A *Selaginella lepidophylla* trehalose-6-phosphate synthase complements growth and stress-tolerance defects in a yeast*tps1* mutant. Plant Physiol 1999; 119:1473–82. http://dx.doi.org/10.1104/pp. 119.4.1473.

[53] Iordachescu M, Imai R. Trehalose biosynthesis in response to abiotic stresses. J Integr Plant Biol 2008;50:1223–9. http://dx.doi.org/10.1111/j.1744-7909.2008.00736.x.

[54] Romero C, Bellés JM, Vayá JL, Serrano R, Culiánez-Macia FA. Expression of the yeast *trehalose-6-phosphate synthase* gene in transgenic tobacco plants: Pleiotropic phenotypes include drought tolerance. Planta 1997;201:293–7.

[55] Yeo ET, Kwon HB, Han SE, Lee JT, Ryu JC, Byun MO. Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphate synthase (*TPS1*) gene from *Saccharomyces cerevisiae*. Mol Cells 2000;10:263–8.

[56] Goddijn OJ, Verwoerd TC, Voogd E, Krutwagen RW, de Graaf PT, Van Dun K. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol 1997;113:181–90. http://dx.doi.org/10.1104/pp. 113.1.181.

[57] Garg AK, Kim JK, Owens TG, Ranwala AP, Choi YD, Kochian LV, et al. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci U S A 2002;99:15898–903. http://dx.doi.org/10.1073/pnas.252637799.

[58] Stiller I, Dulai S, Kondrák M, Tarnai R, Szabó L, Toldi O, et al. Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of *Saccharomyces cerevisiae*. Planta 2008;227:299–308. http://dx.doi.org/10.1007/s00425-007-0617-9.

[59] Zhang SH, Yang BP, Feng CL, Tang HL. Genetic transformation of tobacco with the trehalose synthase gene from *Grifola frondosa* Fr. enhances the resistance to drought and salt in tobacco. J Integr Plant Biol 2005;47:579–87. http://dx.doi.org/10.1111/j.1744-7909.2005.00046.x.

[60] Avonce N, Leyman B, Mascorro-Gallardo JO, Van Dijck P, Thevelein JM, Iturriaga G. The *Arabidopsis* trehalose-6-P synthase *AtTPS1* gene is a regulator of glucose, abscisic acid, and stress signaling. Plant Physiol 2004;136:3649–59. http://dx.doi.org/10.1104/pp. 104.052084.

[61] Ge LF, Chao DY, Shi M, Zhu MZ, Gao JP, Lin HX. Overexpression of the trehalose-6-phosphate phosphatase gene *OsTPP1* confers stress tolerance in rice and results in the activation of stress responsive genes. Planta 2008;228:191–201. http://dx.doi.org/10.1007/s00425-008-0729-x.

[62] Li HW, Zang BS, Deng XW, Wang XP. Overexpression of the trehalose-6-phosphate synthase gene *OsTPS1* enhances abiotic stress tolerance in rice. Planta 2011;234: 1007–18. http://dx.doi.org/10.1007/s00425-011-1458-0.
[63] Vijn I, Smeekens S. Fructan: More than a reserve carbohydrate? Plant Physiol 1999;120:351–60. http://dx.doi.org/10.1104/pp. 120.2.351.
[64] Konstantinova T, Parvanova D, Atanassov A, Djilianov D. Freezing tolerant tobacco, transformed to accumulate osmoprotectants. Plant Sci 2002;163:157–64. http://dx.doi.org/10.1016/S0168-9452(02)00090-0.
[65] Ebskamp MJM, Van der Meer IM, Spronk BA, Weisbeek PJ, Smeekens SCM. Accumulation of fructose polymers in transgenic tobacco. Nat Biotechnol 1994;12:272–5. http://dx.doi.org/10.1038/nbt0394-272.
[66] Van der Meer IM, Ebskamp MJM, Visser RGF, Weisbeek PJ, Smeekens SCM. Fructan as a new carbohydrate sink in transgenic potato plants. Plant Cell 1994;6:561–70. http://dx.doi.org/10.1105/tpc.6.4.561.
[67] Pilon-Smits EAH, Ebskamp MJM, Paul MJ, Jeuken MJW, Weisbeek PJ, Smeekens SCM. Improved performance of transgenic fructan-accumulating tobacco under drought stress. Plant Physiol 1995;107:125–30.
[68] Pilon-Smits EAH, Terry N, Sears T, Van Dun K. Enhanced drought resistance in fructan-producing sugar beet. Plant Physiol Biochem 1999;37:313–7. http://dx.doi.org/10.1104/pp. 107.1.125.
[69] Li HJ, Yang AF, Zhang XC, Gao F, Zhang JR. Improving freezing tolerance of transgenic tobacco expressing sucrose: Sucrose 1-fructosyltransferase gene from *Lactuca sativa*. Plant Cell Tissue Organ Cult 2007;89:37–48. http://dx.doi.org/10.1007/s11240-007-9213-8.
[70] Kawakami A, Sato Y, Yoshida M. Genetic engineering of rice capable of synthesizing fructans and enhancing chilling tolerance. J Exp Bot 2008;59:793–802. http://dx.doi.org/10.1093/jxb/erm367.
[71] Shen B, Jensen RG, Bohnert HJ. Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts. Plant Physiol 1997;113: 1177–83. http://dx.doi.org/10.1104/pp. 113.4.1177.
[72] Zhu B, Chen THH, Li PH. Expression of three osmotin-like protein genes in response to osmotic stress and fungal infection in potato. Plant Mol Biol 1995;28:17–26. http://dx.doi.org/10.1007/BF00042034.
[73] LaRosa PC, Chen Z, Nelson DE, Singh NK, Hasegawa PM, Bressan RA. Osmotin gene expression is posttranscriptionally regulated. Plant Physiol 1992;100:409–15. http://dx.doi.org/10.1104/pp. 100.1.409.
[74] Nelson DE, Raghothama KG, Singh NK, Hasegawa PM, Bressan RA. Analysis of structure and transcriptional activation of an osmotin gene. Plant Mol Biol 1992;19:577–88. http://dx.doi.org/10.1007/BF00026784.
[75] Evers D, Overney S, Simon P, Greppin H, Hausman JF. Salt tolerance of *Solanum tuberosum L.*: Overexpressing an heterologous osmotin-like protein. Biol Plant 1999;42:105–12. http://dx.doi.org/10.1023/A:1002131812340.
[76] Weber RLM, Strohm BW, Bredemeier C, Pinheiro MM, de Brito GG, Rechenmacher C, et al. Expression of an osmotin-like protein from *Solanum nigrum* confers drought tolerance in transgenic soybean. BMC Plant Biol 2014;14:343. http://dx.doi.org/10.1186/s12870-014-0343-y.
[77] Redkar PJ, Lemke PA, Singh NK. Isolation of differentially expressed cDNA clones from salt-adapted *Aspergillus nidulans*. Curr Genet 1996;29:130–5. http://dx.doi.org/10.1007/s002940050027.
[78] Redkar RJ, Herzog RW, Singh NN. Transcriptional activation of the *Aspergillus nidulans gpdA* promoter by osmotic signals. Appl Environ Microbiol 1998;64: 2229–31.
[79] Jeong MJ, Park SC, Kwon HB, Byun MO. Isolation and characterization of the gene encoding glyceraldehyde-3-phosphate dehydrogenase. Biochem Biophys Res Commun 2000;278:192–6. http://dx.doi.org/10.1006/bbrc.2000.3732.
[80] Jeong MJ, Park SC, Byun MO. Improvement of salt tolerance in transgenic potato plants by glyceraldehyde-3 phosphate dehydrogenase gene transfer. Mol Cells 2001;12:185–9.
[81] Lane BG, Dunwell JM, Ray JA, Schmitt MR, Cuming AC. Germin, a protein marker of early plant development, is an oxalate oxidase. J Biol Chem 1993;268: 12239–42.
[82] Hurkman WJ, Tao HP, Tanaka CK. Germin-like polypeptides increase in barley roots during salt stress. Plant Physiol 1991;97:366–74. http://dx.doi.org/10.1104/pp. 97.1.366.
[83] Jaikaran ASI, Kennedy TD, Dratewka-Kos E, Lane BG. Covalently bonded and adventitious glycans in germin. J Biol Chem 1990;265:12503–12.
[84] Chen THH, Murata N. Enhancement of tolerance of abiotic stress by metabolic engineering of betaines and other compatible solutes. Curr Opin Plant Biol 2002;5: 250–7. http://dx.doi.org/10.1016/S1369-5266(02)00255-8.
[85] Bohnert HJ, Nelson DE, Jensen RG. Adaptations to environmental stresses. Plant Cell 1995;7:1099-11. http://dx.doi.org/10.1105/tpc.7.7.1099.
[86] Robinson SP, Jones GP. Accumulation of glycine betaine in chloroplasts provides osmotic adjustment during salt stress. Aust J Plant Physiol 1986;13:659–68. http://dx.doi.org/10.1071/PP9860659.
[87] Papageorgiou GC, Murata N. The unusually strong stabilizing effects of glycine betaine on the structure and function of the oxygen-evolving Photosystem II complex. Photosynth Res 1995;44:243–52. http://dx.doi.org/10.1007/BF00048597.
[88] Chen TH, Murata N. Glycinebetaine protects plants against abiotic stress: Mechanisms and biotechnological applications. Plant Cell Environ 2011;34:1–20. http://dx.doi.org/10.1111/j.1365-3040.2010.02232.x.
[89] Kishitani S, Watanabe K, Yasuda S, Arakawa K, Takabe T. Accumulation of glycinebetaine during cold acclimation and freezing tolerance in leaves of winter and spring barley plants. Plant Cell Environ 1994;17:89–95. http://dx.doi.org/10.1111/j.1365-3040.1994.tb00269.x.
[90] McCue KF, Hanson AD. Drought and salt tolerance: Towards understanding and application. Trends Biotechnol 1990;8:358–62. http://dx.doi.org/10.1016/0167-7799(90)90225-M.
[91] Chen THH, Murata N. Glycinebetaine: An effective protectant against abiotic stress in plants. Trends Plant Sci 2008;13:499–505. http://dx.doi.org/10.1016/j.tplants.2008.06.007.
[92] Khan MS, Yu X, Kikuchi A, Asahina M, Watanabe KN. Genetic engineering of glycine betaine biosynthesis to enhance abiotic stress tolerance in plants. Plant Biotechnol 2009;26:125–34. http://dx.doi.org/10.5511/plantbiotechnology.26.125.
[93] Ahmad R, Kim MD, Back KH, Kim HS, Lee HS, Kwon SY, et al. Stress-induced expression of choline oxidase in potato plant chloroplasts confers enhanced tolerance to oxidative, salt, and drought stresses. Plant Cell Rep 2008;27:687–98. http://dx.doi.org/10.1007/s00299-007-0479-4.
[94] Goel D, Singh AK, Yadav V, Babbar SB, Murata N, Bansal KC. Transformation of tomato with a bacterial *codA* gene enhances tolerance to salt and water stresses. J Plant Physiol 2011;168:1286–94. http://dx.doi.org/10.1016/j.jplph.2011.01.010.
[95] He C, Yang A, Zhang W, Gao Q, Zhang J. Improved salt tolerance of transgenic wheat by introducing *betA* gene for glycine betaine synthesis. Plant Cell Tiss Organ Cult 2010;101:65–78. http://dx.doi.org/10.1007/s11240-009-9665-0.
[96] Jing J, Li H, He G, Yin Y, Liu M, Liu B, et al. Over-expression of the codA gene by *Rd29A* promoter improves salt tolerance in *Nicotiana tabacum*. Pak J Bot 2013;45:821–7.
[97] Sulpice R, Tsukaya H, Nonaka H, Mustardy L, Chen TH, Murata N. Enhanced formation of flowers in salt-stressed *Arabidopsis* after genetic engineering of the synthesis of glycine betaine. Plant J 2003;36:165–76. http://dx.doi.org/10.1046/j.1365-313X.2003.01873.x.
[98] Shirasawa K, Takabe T, Takabe T, Kishitani S. Accumulation of glycinebetaine in rice plants that overexpress choline monooxygenase from spinach and evaluation of their tolerance to abiotic stress. Ann Bot 2006;98:565–71. http://dx.doi.org/10.1093/aob/mcl126.
[99] Waditee R, Bhuiyan MN, Rai V, Aoki K, Tanaka Y, Hibino T, et al. Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis*. Proc Natl Acad Sci U S A 2005;102: 1318–23. http://dx.doi.org/10.1073/pnas.0409017102.
[100] Yu X, Kikuchi A, Matsunaga E, Morishita Y, Nanto K, Sakurai N, et al. Establishment of the evaluation system of salt tolerance on transgenic woody plants in the special netted-house. Plant Biotechnol 2009;26:135–41. http://dx.doi.org/10.5511/plantbiotechnology.26.135.
[101] Huang J, Hirji R, Adam L, Rozwadowski KL, Hammerlindl JK, Keller WA, et al. Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: Metabolic limitations. Plant Physiol 2000;122:747–56. http://dx.doi.org/10.1104/pp. 122.3.747.
[102] Hibino T, Waditee R, Araki E, Ishikawa H, Aoki K, Tanaka Y, et al. Functional characterization of choline monooxygenase, an enzyme for betaine synthesis in plants. J Biol Chem 2002;277:41352–60. http://dx.doi.org/10.1074/jbc.M205965200.
[103] Su J, Hirji R, Zhang L, He C, Selvaraj G, Wu R. Evaluation of the stress-inducible production of choline oxidase in transgenic rice as a strategy for producing the stress-protectant glycine betaine. J Exp Bot 2006;57:1129–35. http://dx.doi.org/10.1093/jxb/erj133.
[104] Park EJ, Jeknic Z, Sakamoto A, DeNoma J, Yuwansiri R, Murata N, et al. Genetic engineering of glycinebetaine synthesis in tomato protects seeds, plants, and flowers from chilling damage. Plant J 2004;40:474–87. http://dx.doi.org/10.1111/j.1365-313X.2004.02237.x.
[105] Park EJ, Jeknić Z, Pino MT, Murata N, Chen TH. Glycinebetaine accumulation is more effective in chloroplasts than in the cytosol for protecting transgenic tomato plants against abiotic stress. Plant Cell Environ 2007;30:994–1005. http://dx.doi.org/10.1111/j.1365-3040.2007.01694.x.
[106] Park EJ, Jeknic Z, Chen THH, Murata N. The *codA* transgene for glycinebetaine synthesis increases the size of flowers and fruits in tomato. Plant Biotechnol J 2007;5: 422–30. http://dx.doi.org/10.1111/j.1467-7652.2007.00251.x.
[107] Quinet M, Ndayiragije A, Lefevre I, Lambillotte B, Dupont-Gillain CC, Lutts S. Putrescine differently influences the effect of salt stress on polyamine metabolism and ethylene synthesis in rice cultivars differing in salt resistance. J Exp Bot 2010;61: 2719–33. http://dx.doi.org/10.1093/jxb/erq118.
[108] Alcazar R, Cuevas JC, Planas J, Zarza X, Bortolotti C, Carrasco P, et al. Integration of polyamines in the cold acclimation response. Plant Sci 2011;180:31–8. http://dx.doi.org/10.1016/j.plantsci.2010.07.022.
[109] Hussain SS, Ali M, Ahmad M, Siddique KHM. Polyamines: Natural and engineered abiotic and biotic stress tolerance in plants. Biotechnol Adv 2011;29:300–11. http://dx.doi.org/10.1016/j.biotechadv.2011.01.003.
[110] Alcazar R, Planas J, Saxena T, Zarza X, Bortolotti C, Cuevas J, et al. Putrescine accumulation confers drought tolerance in transgenic *Arabidopsis* plants over-expressing the homologous *Arginine decarboxylase 2* gene. Plant Physiol Biochem 2010;48:547–52. http://dx.doi.org/10.1016/j.plaphy.2010.02.002.
[111] Alet AI, Sánchez DH, Cuevas JC, Del Valle S, Altabella T, Tiburcio AF, et al. Putrescine accumulation in *Arabidopsis thaliana* transgenic lines enhances tolerance to dehydration and freezing stress. Plant Signal Behav 2011;6:278–86. http://dx.doi.org/10.4161/psb.6.2.14702.
[112] Kasukabe Y, He L, Nada K, Misawa S, Ihara I, Tachibana S. Overexpression of spermidine synthase enhances tolerance to multiple environmental stresses and up-regulates the expression of various stress-regulated genes in transgenic *Arabidopsis thaliana*. Plant Cell Physiol 2004;45:712–22. http://dx.doi.org/10.1093/pcp/pch083.
[113] Kumriaa R, Rajam MV. Ornithine decarboxylase transgene in tobacco affects polyamines, *in vitro*-morphogenesis and response to salt stress. J Plant Physiol 2002; 159:933–90. http://dx.doi.org/10.1078/0176-1617-00822.
[114] Do PT, Drechsel O, Heyer AG, Hincha DK, Zuther E. Changes in free polyamine levels, expression of polyamine biosynthesis genes, and performance of rice cultivars under salt stress: A comparison with responses to drought. Front Plant Sci 2014;5:182. http://dx.doi.org/10.3389/fpls.2014.00182.
[115] Do PT, Degenkolbe T, Erban A, Heyer AG, Kopka J, Köhl KI, et al. Dissecting rice polyamine metabolism under controlled long-term drought stress. PLoS ONE 2013;8. http://dx.doi.org/10.1371/journal.pone.0060325.

[116] Wilson PB, Estavillo GM, Field KJ, Pornsiriwong W, Carroll AJ, Howell KA, et al. The nucleotidase/phosphatase SAL1 is a negative regulator of drought tolerance in Arabidopsis. Plant J 2009;58:299–317. http://dx.doi.org/10.1111/j.1365-313X.2008.03780.x.
[117] Lata C, Yadav A, Prasad M. Role of plant transcription factors in abiotic stress tolerance. In: Shanker A, editor. Abiotic stress response in plants — Physiological, biochemical and genetic perspectives. InTech; 2011. http://dx.doi.org/10.5772/23172.
[118] Wu X, Shiroto Y, Kishitani S, Ito Y, Toriyama K. Enhanced heat and drought tolerance in transgenic rice seedlings overexpressing *OsWRKY11* under the control of *HSP101* promoter. Plant Cell Rep 2009;28:21–30. http://dx.doi.org/10.1007/s00299-008-0614-x.
[119] Wu X, Kishitani S, Ito Y, Toriyama K. Accumulation of raffinose in rice seedlings overexpressing *OsWRKY11* in relation to desiccation tolerance. Plant Biotechnol 2009;26:431–4. http://dx.doi.org/10.5511/plantbiotechnology.26.431.
[120] Díaz P, Betti M, Sánchez DH, Udvardi MK, Monza J, Márquez AJ. Deficiency in plastidic glutamine synthetase alters proline metabolism and transcriptomic response in *Lotus japonicas* under drought stress. New Phytol 2010;188:1001–13. http://dx.doi.org/10.1111/j.1469-8137.2010.03440.x.
[121] McNeil SD, Nuccio ML, Ziemak MJ, Hanson AD. Enhanced synthesis of choline and glycine betaine in transgenic tobacco plants that overexpress phosphoethanolamine *N*-methyltransferase. Proc Natl Acad Sci U S A 2001;98:10001–5. http://dx.doi.org/10.1073/pnas.171228998.
[122] Verslues PE, Sharma S. Proline metabolism and its implications for plant–environment interaction. Arabidopsis book, 8; 2010. http://dx.doi.org/10.1199/tab.0140.
[123] Tayal D, Srivastava PS, Bansal KC. Transgenic crops for abiotic stress tolerance. In: Srivastava PS, Narula A, Srivastava S, editors. Plant biotechnology and molecular markers. New Delhi: Anamaya Publishers; 2004. p. 346–65.
[124] Ashraf M, Akram NA. Improving salinity tolerance of plants through conventional breeding and genetic engineering: An analytical comparison. Biotechnol Adv 2009;27:744–52. http://dx.doi.org/10.1016/j.biotechadv.2009.05.026.
[125] Sade N, Gebretsadik M, Seligmann R, Schwartz A, Wallach R, Moshelion M. The role of tobacco aquaporin1 in improving water use efficiency, hydraulic conductivity, and yield production under salt stress. Plant Physiol 2010;152:245–54. http://dx.doi.org/10.1104/pp. 109.145854.
[126] Tung SA, Smeeton R, White CA, Black CR, Taylor IB, Hilton HW, et al. Overexpression of *LeNCED1* in tomato (*Solanum lycopersicum* L.) with the *rbcS3C* promoter allows recovery of lines that accumulate very high levels of abscisic acid and exhibit severe phenotypes. Plant Cell Environ 2008;31:968–81. http://dx.doi.org/10.1111/j.1365-3040.2008.01812.x.
[127] Xiao BZ, Chen X, Xiang CB, Tang N, Zhang QF, Xiong LZ. Evaluation of seven function-known candidate genes for their effects on improving drought resistance of transgenic rice under field conditions. Mol Plant 2009;2:73–83. http://dx.doi.org/10.1093/mp/ssn068.
[128] Barros PDS, Soares-Cavalcanti NM, Vieira-Mello GS, Wanderley-Nogueira AC, Calsa-Junior T, Benko-Iseppon AM. *In silico* evaluation of osmoprotectants in *eucalyptus* transcriptome. Lect Notes Comput Sci 2009;5488:66–77. http://dx.doi.org/10.1007/978-3-642-02504-4_6.
[129] Chen S, Gollop N, Heuer B. Proteomic analysis of salt-stressed tomato (*Solanum lycopersicum*) seedlings: Effect of genotype and exogenous application of glycinebetaine. J Exp Bot 2009;60:2005–19. http://dx.doi.org/10.1093/jxb/erp075.
[130] Kido EA, Ferreira Neto JR, Silva RL, Belarmino LC, Bezerra Neto JP, Soares-Cavalcanti NM, et al. Expression dynamics and genome distribution of osmoprotectants in soybean: Identifying important components to face abiotic stress. BMC Bioinforma 2013;14. http://dx.doi.org/10.1186/1471-2105-14-S1-S7.
[131] Silva RLO, Ferreira Neto JRC, Pandolfi V, Chabregas SM, Burnquist WL, Benko-Iseppon AM, et al. Transcriptomics of sugarcane osmoprotectants under drought. In: Vasanthaiah H, editor. Plants and environment. InTech; 2011. http://dx.doi.org/10.5772/23726.
[132] Salvi S, Tuberosa R. To clone or not to clone plant QTLs: Present and future challenges. Trends Plant Sci 2005;10:297–304. http://dx.doi.org/10.1016/j.tplants.2005.04.008.
[133] Walia H, Wilson C, Zeng L, Ismail AM, Condamine P, Close TJ. Genome-wide transcriptional analysis of salinity stressed *japonica* and *indica* rice genotypes during panicle initiation stage. Plant Mol Biol 2007;63:609–23. http://dx.doi.org/10.1007/s11103-006-9112-0.
[134] Pandit A, Rai V, Bal S, Sinha S, Kumar V, Chauhan M, et al. Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.). Mol Genet Genomics 2010; 284:121–36. http://dx.doi.org/10.1007/s00438-010-0551-6.
[135] Lv S, Yang A, Zhang K, Wang L, Zhang J. Increase of glycinebetaine synthesis improves drought tolerance in cotton. Mol Breed 2007;20:233–48. http://dx.doi.org/10.1007/s11032-007-9086-x.
[136] Yang X, Liang Z, Wen X, Lu C. Genetic engineering of the biosynthesis of glycinebetaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. Plant Mol Biol 2008;66:73–86. http://dx.doi.org/10.1007/s11103-007-9253-9.
[137] Zhou S, Chen X, Zhang X, Li Y. Improved salt tolerance in tobacco plants by co-transformation of a betaine synthesis gene *BADH* and a vacuolar Na^+/H^+ antiporter gene *SeNHX1*. Biotechnol Lett 2008;30:369–76. http://dx.doi.org/10.1007/s10529-007-9548-6.
[138] Cheng YJ, Deng XP, Kwak SS, Chen W, Eneji AE. Enhanced tolerance of transgenic potato plants expressing choline oxidase in chloroplasts against water stress. Bot Stud 2013;54:30. http://dx.doi.org/10.1186/1999-3110-54-30.
[139] Nanjo T, Fujita M, Seki M, Kato T, Tabata S, Shinozaki K. Toxicity of free proline revealed in an *Arabidopsis* T-DNA-tagged mutant deficient in proline dehydrogenase. Plant Cell Physiol 2003;44:541–8. http://dx.doi.org/10.1093/pcp/pcg066.
[140] Gubis J, Vanková R, Cervená V, Dragunová M, Hudcovicová M, Lichtnerová H, et al. Transformed tobacco plants with increased tolerance to drought. S Afr J Bot 2007; 73:505–11. http://dx.doi.org/10.1016/j.sajb.2007.03.011.
[141] Patade VY, Khatri D, Kumari M, Grover A, Gupta SM, Ahmed Z. Cold tolerance in *Osmotin* transgenic tomato (*Solanum lycopersicum* L.) is associated with modulation in transcript abundance of stress responsive genes. SpringerPlus 2013;2:117. http://dx.doi.org/10.1186/2193-1801-2-117.
[142] Nakayama H, Yoshida K, Ono H, Murooka Y, Shinmyo A. Ectoine, the compatible solute of *Halomonas elongata*, confers hyperosmotic tolerance in cultured tobacco cells. Plant Physiol 2000;122:1239–48. http://dx.doi.org/10.1104/pp. 122.4.1239.
[143] Fukushima E, Arata Y, Endo T, Sonnewald U, Sato F. Improved salt tolerance of transgenic tobacco expressing apoplastic yeast-derived invertase. Plant Cell Physiol 2001;42:245–9. http://dx.doi.org/10.1093/pcp/pce027.
[144] Hoshida H, Tanaka Y, Hibino T, Hayashi Y, Tanaka A, Takabe T, et al. Enhanced tolerance to salt stress in transgenic rice that overexpresses chloroplast glutamine synthetase. Plant Mol Biol 2000;43:103–11. http://dx.doi.org/10.1023/A:1006408712416.
[145] Tang W, Peng X, Newton RJ. Enhanced tolerance to salt stress in transgenic loblolly pine simultaneously expressing two genes encoding mannitol-1-phosphate dehydrogenase and glucitol-6-phosphate dehydrogenase. Plant Physiol Biochem 2005;43:139–46. http://dx.doi.org/10.1016/j.plaphy.2005.01.009.
[146] Turhan H. Salinity response of transgenic potato genotypes expressing the oxalate oxidase gene. Turk J Agric For 2005;29:187–95.
[147] Tang L, Kim MD, Yang KS, Kwon SY, Kim SH, Kim JS, et al. Enhanced tolerance of transgenic potato plants overexpressing nucleoside diphosphate kinase 2 against multiple environmental stresses. Transgenic Res 2008;17:705–17. http://dx.doi.org/10.1007/s11248-007-9155-2.
[148] Bhomkar P, Upadhyay CP, Saxena M, Muthusamy MA, Prakash NS, Pooggin M, et al. Salt stress alleviation in transgenic *Vigna mungo* L. Hepper (blackgram) by overexpression of the glyoxalase I gene using a novel Cestrum yellow leaf curling virus (CmYLCV) promoter. Mol Breed 2008;22:169–81. http://dx.doi.org/10.1007/s11032-008-9164-8.