Review article

Endogenous glycine betaine accumulation mediates abiotic stress tolerance in plants

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Abstract: Abiotic stresses like salinity, drought, cold, high temperature, etc. are largely responsible for a considerable degree of annual crop losses worldwide. Till date, several effectors have been reported which confer stress tolerance to the plants. Glycine betaine (GB) is one such important regulator which accumulates in the cell as a crucial osmolyte and alleviates the cell from damages due to salinity, drought, temperature and oxidative stresses. This has been verified through several investigations at the physiological, biochemical and molecular levels. Apart from acting as an important compatible solute, GB has a prominent role in maintaining cellular homeostasis and mediating chaperone activity to prohibit undesirable protein folding under stress. The protective role of exogenous applications of GB in crops is also a well-studied fact. In addition, several detailed literatures are available on the transgenic technology and on-field tests which have depicted the increasing stress tolerance developed in plants accumulating higher levels of endogenous GB. All these issues have been reviewed and documented in the present communication.

Keywords: Abiotic stresses - Stress tolerance - Glycine betaine - Transgenic technology.

INTRODUCTION

Environmental stresses, particularly salinity and drought, are the major constraints which limit the global distribution and production of crop plants. In response to such abiotic stresses, plants have evolutionarily developed a plethora of stress responsive cascades which aid them in developing stress tolerance. The accumulation of certain organic metabolites of low molecular weight, collectively called the compatible solutes, is one such ubiquitous mechanism in plants (Bohnert *et al.* 1995). These compatible solutes act as crucial osmoprotectants and help the plant system to survive severe osmotic stress. Accumulation of compatible osmolytes aid plant cells to regain turgor and resume growth during ionic stress. The osmotic potential of the cytosol is reduced in order to facilitate the uptake as well as the retention of water molecules. Being small organic molecules of low molecular weight, these compatible solutes have the tendency to accumulate to high levels without interfering with normal intra and intercellular homeostasis (Roychoudhury *et al.* 2008). Compatible solutes have been depicted to prevent ion entry into the sensitive plant parts and also to increase the ion exclusion from them. These metabolites are polar, highly soluble and typically hydrophilic and hence exhibit their protective functions by maintaining the hydration sphere of proteins under desiccating conditions. Compatible solutes at high concentrations prohibit misfolding of proteins, thus acting as low molecular weight chaperones, stabilize macromolecules or molecular assemblies, increase the thermal stability of enzymes and prevent dissociation of enzymatic complexes (Roychoudhury *et al.* 2013).

Compatible solutes often act as the scavengers of reactive oxygen species (ROS; extremely toxic, short lived active oxygen species) and thus preserve membrane and cellular integrity. Some common compatible solutes accumulated under desiccation stress are reducing sugar and major carbohydrates like sucrose, fructose, glucose; sugar alcohols (pinitol, ononitol, cyclitol); polyols (either straight chain compounds like adonitol, sorbitol and mannitol or cyclic polyols as myo-inositol); complex sugars (trehalose, raffinose and fructans); total free amino acids especially proline (Pro) and glycine betaine (GB); organic acids like lactate, malate, citrate, succinate,

fumarate, benzoate, salicylate, malonate and γ-amino butyric acid (GABA); free ammonia and quaternary ammonium compounds (β-alanine-betaine, proline-betaine and hydroxyprolinebetaine); tertiary sulfonium salts (dimethylsulfoniopropionate, choline o-sulfate) etc. (Roychoudhury & Chakraborty 2013, Roychoudhury *et al.* 2015).

GB (N, N, N-trimethylglycine) is an important member among the compatible solutes with widely ranging protective functions under salinity, drought and extreme temperature stresses (Chen & Murata 2008). GB is dipolar at physiological pH, but electrically neutral in nature. The essential role carried out by GB in plants exposed to salinity stress is the protection of plant tissues via osmotic adjustment (OA), stabilization of proteins like RuBisCO, photosynthetic apparatus protection and scavenging of ROS (Wani *et al.* 2013). In many halophytic plants and cyanobacteria, GB accumulates to an osmotically significant level (Rhodes & Hanson 1993). Natural accumulators of GB have shown the accumulation of betaine in response to salt, drought and cold. Water stress, induced by polyethylene glycol showed enhanced GB accumulation in rice; however, the level was much higher in the tolerant variety Pokkali, as compared to the sensitive varieties IR-29 and Pusa Basmati (Basu *et al.* 2010), showing that GB could act as a better osmoprotectant against dehydration stress in the tolerant variety. The taxonomically distant plant species referred to as the natural accumulators of GB, normally contain low levels of GB under control conditions. However, they accumulate considerable levels of GB on exposure to stress (Giri 2011).

It has been suggested that compatible solutes like GB at low levels protect macromolecules like nucleic acids, proteins and lipids and also act as reservoirs of carbon and nitrogen sources (Umezawa *et al.* 2006). The major crops like potato and tomato are unable to accumulate GB. Thus, these species have been regarded as the targets for engineering betaine biosynthesis (Wani *et al.* 2013). In these review, we present a picture of the thorough development of abiotic stress tolerance in plants mediated by GB accumulation.

BIOSYNTHESIS OF GLYCINE BETAINE (GB)

A brief discussion of the biosynthetic pathway of GB is essential, since these genes and hence enzymes can be cloned and expressed in crop plants which do not accumulate GB. GB can be synthesized via two pathways. The starting metabolites in these pathways are different. One is initiated by choline, while the other with glycine. The common pathway in higher plants starts with choline, which is catalyzed by choline monooxygenase (CMO) into the hydrated form of betaine aldehyde. Betaine aldehyde is acted upon by NAD^+ dependent betaine aldehyde dehydrogenase (BADH) to ultimately form GB. Both CMO and BADH are localized within the stroma of chloroplasts (Sakamoto & Murata 2002). Choline dehydrogenase (CDH) and BADH regulates the formation of GB in *Escherichia coli*, while choline oxidase A (codA) in the soil bacterium, *Arthrobacter globiformis* catalyses a single step conversion of choline to GB and hydrogen peroxide (Giri 2011).

The alternate pathway for GB biosynthesis has been reported in only two extreme halophilic microorganisms, *Ectothiorhodospira halochloris* and *Actinopolyspora halophilia*. In this recently reported pathway, glycine undergoes three successive N-methylations catalysed by glycine sarcosine methyltransferase (GSMT) and sarcosine dimethylglycine methyltransferase (SDMT). Both GSMT and SDMT are actually two S-adenosylmethionine-dependent methyl transferases (Takabe *et al.* 2006).

DEVELOPING STRESS-TOLERANT PLANTS BY EXOGENOUS APPLICATION OF GB: AGRONOMICAL ASPECTS

Exogenous treatment of GB has been reported as a potential and traditional approach for crop tolerance against multiple abiotic stresses. Thus, this strategy can be used as an easy method for generating abiotic stress-tolerant crops directly in the field by foliar spray of solutions containing GB at the optimum concentrations. The correlation between GB accumulation in plants and their economical productivity under stress has been indicated (Smirnoff & Stewart 1985). The natural accumulation of GB is widespread across plant families like Asteraceae, Chenopodiaceae, Poaceae and Solanaceae in response to stress (Jones & Storey 1981). Exogenous foliar treatment of soybean plants (low accumulator of GB with average accumulation of around 5 μmol g^{-1} dry weight) enhanced the GB accumulation by 12 folds to 60 μmol g^{-1} dry weight (DW). This led to an overall increased photosynthetic yield, nitrogen fixation, leaf area expansion and seed production (Makela *et al.* 1996). The effectiveness of exogenous foliar application of GB depends on plant species, developmental stage at the

time of application, concentration of GB used and the number of applications (Ashraf & Harris 2004). It is quintessential to determine an optimum GB concentration depending on the type of crop species to achieve the best possible stress tolerance. For instance, at higher concentrations, GB is more sensitive for the broadleaved species like bean, tomato and grape than for the cereals. Hence, the proper concentration of GB should be used with caution, so as to derive the maximum benefit of OA in different plant species (Muhammad *et al.* 2006, Wani *et al.* 2013).

Another recent instance of GB acting as a scavenger of ROS has been reported (Sui *et al.* 2012). Production of ROS as a result of wounding is enhanced when yeast antagonists are used as biocontrol agents in the wounds. It was found that pre-exposure of some yeast strains with GB ameliorated the ROS-mediated oxidative stress in the antagonistic yeast. The exogenous GB application to the antagonistic yeast strain *Candida oleophila* improved their adaptation to apple fruit wounds. The GB-treated yeasts also showed up regulation of major antioxidant genes like *peroxisomal catalase*, *peroxiredoxin TSA1* and *glutathione peroxidase*. Thus, such improved biocontrol efficacy can be achieved via the activation of antioxidant responses in the biocontrol yeasts.

GB as an important osmoprotectant has been reported in the perennial grasses *Holcus lanatus* and *Alopecurus pratensis* (Gargallo-Garriga *et al.* 2015). The role of GB in decreasing the level of malondialdehyde (MDA) to protect the membrane systems has been recently explained in the leaves of *Aegiceras corniculatum* and *Kandelia obovata* exposed to drought stress (Guan *et al.* 2015). Other positive effects of GB in developing drought tolerance have also been depicted in tobacco, wheat, barley, sorghum and soybean (Ashraf & Harris 2004). The foliar application of GB to the tomato plants under field condition at a dose of 3.36 Kg ha^{-1} during mid-flowering period increased fruit yield to 36% and 39% during salt and heat stress respectively, as compared to control (Makela *et al.* 1998). Generally, under normal field conditions, rice has been reported as a GB non-accumulator. However, some basal levels of GB have been found in rice cultivars like KDML105, Annapurna and Dongjin, exposed to salinity stress (Wani *et al.* 2013). A change in the transcript levels of *WCOR410* and *catalase* in wheat and tomato plants was observed after the exogenous application of GB. WCOR410 is an acidic dehydrin which improved the freezing tolerance of the tomato plants during cold acclimation. Catalase, on the other hand, exhibited imparted antioxidant potential by scavenging hydrogen peroxide, a member of the reactive oxygen species (Allard *et al.* 1998).

MAJOR TRANSGENIC APPROACHES IN DEVELOPING ABIOTIC STRESS TOLERANCE VIA INCREASED ACCUMULATION OF GB

Several transgenic approaches have been undertaken to emphasize the alleviation of salinity and drought stress through accumulation of GB. The most investigated among these transgenics in terms of the morphological and physiological aspects are those overexpressing the *codA* gene, which we have mentioned under the biosynthesis section. These transgenics exhibited stress tolerance at almost all stages of development along with improved photosynthetic activity and greater yield of fruits and seeds. The transgenic rice plants showed accumulation of 5.3 mmol g^{-1} fresh weight (FW), whereas the wild type plants were found to be non-accumulators of GB (Sakamoto *et al.* 1998). Maize, being a natural accumulator of GB, exhibited highly increased accumulation of 5.7 mmol g^{-1} FW of GB in the transgenic lines (Quan *et al.* 2004). The c*odA* gene from *Arthrobacter* spp. was also overexpressed in the model plant *Arabidopsis thaliana, Eucalyptus globulus*, Japanese persimmon (*Diospyros kaki*), *Brassica campestris* L. spp. *chinensis, Solanum tuberosum* and *Lycopersicon esculentum* to generate tolerance against salinity, drought, chilling and low relative humidity (Giri 2011). A G-protein named RabAc4 involved in membrane trafficking has been reported for mediating GB-mediated chilling tolerance in plants (Wani *et al.* 2013).

Both GB and Pro have the capability to destabilize the DNA double helix by lowering the helix melting point in vivo. As a result, under stress conditions, GB acts as an effective activator of replication and transcription by promoting the melting of DNA helices and thus the stress-responsive genes are easily accessed by the transcription machinery (Rajendrakumar *et al.* 1997). Transformation of *Arabidopsis* by *GSMT* and *SDMT* from *Aphanothece halophytica* resulted in higher GB accumulation than in the transgenic lines overexpressing the choline oxidizing enzymes. This obviously indicates that under stress conditions, choline oxidizing enzymes have limited applications in genetic engineering program, whereas transgenic approaches involving *GSMT* and *SDMT* in combination led to better and higher endogenous GB content. However, such

high endogenous GB accumulation was not recorded when the plants were exposed to 0.1 M NaCl stress, supplemented with 5 mM glycine (Waditee *et al.* 2005). The *choline oxidase* (*COX*) gene from *Arthrobacter pascens* fused to a chloroplast targeting sequence was expressed in rice under the control of a salt-inducible promoter and a constitutive ubiquitin promoter. Under salinity stress, the total GB accumulation was obviously higher in the lines expressing the transgene via the constitutive promoter. However, the inducible lines showed a swapping 89% increase in the GB content under salt stress, whereas the increase in the constitutively expressed lines was 44% (Su *et al.* 2006). Until recent times, it was unknown whether the ortholog of *CMO* was functional or a pseudogene in rice was functionally orthologous to *BADH*. When the *CMO* from sugarbeet was overexpressed in the chloroplast of tobacco, the transplastomic lines showed foliar accumulation of 0.2 to 0.5 mmol g^{-1} FW and the plants were tolerant to oxidative stress (Zhang *et al.* 2008). The *OsCMO* gene was isolated from *Oryza sativa* L. spp. *japonica* cv. Nipponbare using RT-PCR. The up regulation of *OsCMO* by salt stress was also corroborated by Northern Blot analyses. Transgenic tobacco plants overexpressing *OsCMO* exhibited increased GB content with enhanced salt tolerance. Immunoblotting analysis demonstrated that a functional OsCMO protein with correct size was present in transgenic tobacco, but rarely accumulated in wild-type rice plants. Surprisingly, a large amount of truncated proteins derived from *OsCMO* was induced in the rice seedlings in response to salt stresses. This suggested that it is presumably the non-functionality at the protein level of OsCMO which hinders the synthesis of GB in rice plants exposed to stress (Luo *et al.* 2014). On the other hand, the importance of *BADH1* in the accumulation of GB in rice has also been shown (Tang *et al.* 2014). The down regulation of *OsBADH1* by RNA interference (RNAi) exhibited much lower salinity, drought and cold tolerance in transgenic rice. The decrease of stress tolerance occurring in the *OsBADH1-RNAi* repression lines was associated with an elevated level of malondialdehyde content and hydrogen peroxide. The transgene-positive and transgene-negative lines derived from heterozygous transgenic T$_0$ plants did not show any accumulation of GB. Moreover, transgenic *OsBADH1-RNAi* repression lines showed significantly reduced seed set and yield. Hence, the down regulation of *OsBADH1* resulted in the reduction of ability to dehydrogenate the accumulating metabolism-derived aldehydes and subsequently gave rise to decreased stress tolerance and crop productivity, without changing the GB content. The authors suggested that *OsBADH1* possessed an enzymatic activity to catalyze other aldehydes in addition to betaine aldehyde (the precursor of GB) and thus alleviated their toxic effects under abiotic stresses (Tang *et al.* 2014).

The heterologous expression of *BADH* gene from the xerophytic leguminous plant *Ammopiptanthus nanus* in *E. coli* conferred salt and heat tolerance under the stress conditions of 700 mM NaCl and 55°C temperature (Yu *et al.* 2014). This report actually emphasizes the fact that *AnBADH* is a crucial mediator of abiotic stress tolerance in *A. nanus*. Hence, this gene can be engineered in heterologous stress-sensitive cultivars to increase their stress tolerance. The plant tolerance to various abiotic stresses is improved without any strong phenotypic changes (Fan *et al.* 2012). Thus, targeting the GB-synthesizing genes give a feasible correlation of the transgenic load and the crop yield. This view was justified in transgenic sweet potatoes (*Ipomoea batatas* cv. Sushu-2) overexpressing the chloroplastic *BADH* gene from *Spinacia oleracea* (*SoBADH*). The genetically engineered cultivars exhibited higher accumulation of GB which conferred tolerance against salinity, low temperature and oxidative stresses. Such tolerance was achieved via increased protection against cell damage by a steady maintenance of cell membrane integrity, higher photosynthetic activity and elevated rates of ROS scavenging. The transgenics also showed increased accumulation of Pro with a synchronized up regulation of ROS-scavenging genes, possibly through some unknown interconnecting cross-talk pathway. This experiment has also led to the development of a novel germplasm for sweet potato production on marginal lands under suboptimal conditions (Fan *et al.* 2012). When the *SoBADH* was overexpressed in the chloroplastic genome of carrot, the genetically modified carrots accumulated high levels of GB up to 100 mmol g^{-1} DW, which conferred high salinity tolerance to the transgenic lines (Kumar *et al.* 2004).

The scarcity of endogenous choline and the transport of choline across the chloroplast envelope are the two most significant hurdles for enhancing GB accumulation in the transgenic plants overexpressing various enzymes of the GB biosynthetic pathway. The enzymes oxidising choline into GB was targeted in *Arabidopsis*, *Brassica napus* and tobacco (Huang *et al.* 2000). Exogenous supply of choline in these plants led to a significant increase in GB content. A model was designed for the labelling kinetics of choline metabolites, which proved the importance of choline import into chloroplasts in the biosynthesis of GB (McNeil *et al.* 2000). The activity of phosphoethanolamine N-methyltransferase (PEAMT) was found to be 30 to 100 times higher in spinach than

in tobacco (Nuccio *et al.* 2000). This can also be a reason for the limitation in the endogenous choline supply of GB non-accumulators, like tobacco. So, it is likely that the targeting of PEAMT can increase the choline supply and hence promote GB accumulation in the GB non-accumulators.

CONCLUSION AND FUTURE PERSPECTIVES

The present review highlights quite thoroughly the roles and instances of GB, acting as an osmolyte in plants exposed to multiple abiotic stresses. The greatest advantage in targeting GB-associated genes is that the phenotypic characters, especially the crop productivity, is least affected. Thus, this approach can be extremely useful for generating crops under suboptimal climates showing the same production capacity. Recently, the accumulation of GB was reported in the plant growth-promoting bacteria, isolated from the rhizosphere of the plantation crops like coconut, cocoa and arecanut exposed to stress (Gupta *et al.* 2014). It was shown that the presence of GB, along with other essential anti-stress factors, ultimately enabled the bacteria to survive the abiotic stress. These obviously indicate that along with its capacity as a potent osmolyte, GB might also be involved in directly regulating transcriptomic changes during stress and also adjusting the cellular metabolic homeostasis. Further researches in these directions are required to establish the overall roles of GB in altering the chromatin architecture for initializing the predicted transcriptomic adjustments under stress (Gupta *et al.* 2014).

REFERENCES

Allard F, Houde FM, Krol M, Ivanov A, Huner NPA & Sarhan F (1998) Betaine improves freezing tolerance in wheat. *Plant and Cell Physiology* 39: 1194–1202.

Ashraf M & Harris PJC (2004) Potential biochemical indicators of salinity tolerance in plants. *Plant Science* 166: 3–16.

Basu S, Roychoudhury A, Saha PP & Sengupta DN (2010) Comparative analysis of some biochemical responses of three indica rice varieties during polyethylene glycol-mediated water stress exhibits distinct varietal differences. *Acta Physiologiae Plantarum* 32: 551–563.

Bohnert HJ, Nelson DE & Jensen RG (1995) Adaptations to environmental stresses. *Plant Cell* 7: 1099–1111.

Chen THH & Murata N (2008) Glycinebetaine: an effective protectant against abiotic stress in plants. *Trends in Plant Science* 13: 499–505.

Fan W, Zhang M, Zhang H & Zhang P (2012) Improved tolerance to various abiotic stresses in transgenic sweet potato (*Ipomoea batatas*) expressing spinach betaine aldehyde dehydrogenase. *PLoS ONE* 7: e37344.

Gargallo-Garriga A, Sardans J, Pérez-Trujillo M, Oravec M, Urban O, Jentsch A, Kreyling J, Beierkuhnlein C, Parella T & Peñuelas J (2015) Warming differentially influences the effects of drought on stoichiometry and metabolomics in shoots and roots. *New Phytologist* 207: 591–603.

Giri J (2011) Glycinebetaine and abiotic stress tolerance in plants. *Plant Signaling & Behavior* 6: 1746–1751.

Guan GF, Wang YS, Cheng H, Jiang ZY & Fei J (2015) Physiological and biochemical response to drought stress in the leaves of *Aegiceras corniculatum* and *Kandelia obovata*. *Ecotoxicology* 24: 1668–1676.

Gupta A, Gopal M, Thomas GV, Manikandan V, Gajewski J, Thomas G, Seshagiri S, Schuster SC, Rajesh P & Gupta R (2014) Whole genome sequencing and analysis of plant growth promoting bacteria isolated from the rhizosphere of plantation crops coconut, cocoa and arecanut. *PLoS One* 9: e104259.

Huang J, Hirji R, Adam L, Rozwadowski KL, Hammerlindl JK, Keller WA & Selvaraj G (2000) Genetic engineering of glycinebetaine production toward enhancing stress tolerance in plants: metabolic limitations. *Plant Physiology* 122: 747–756.

Jones RGW & Storey R (1981) Betaines. In: Paleg LG & Aspinal D (eds) *The physiology and biochemistry of drought resistance in plants*. Academic Press, New York, pp. 171–204.

Kumar S, Dhingra A & Daniell H (2004) Plastid-expressed betaine aldehyde dehydrogenase gene in carrot cultured cells, roots, and leaves confer enhanced salt tolerance. *Plant Physiology* 136: 2843–2854.

Luo D, Niu X, Yu J, Yan J, Gou X, Lu BR & Liu Y (2014) Rice choline monooxygenase (OsCMO) protein functions in enhancing glycine betaine biosynthesis in transgenic tobacco but does not accumulate in rice (*Oryza sativa* L. ssp. *japonica*). *Gene* 549: 77–84.

Makela P, Jokinen K, Kontturi M, Peltonen-Sainio P, Pehu E & Somersalo S (1998) Foliar application of glycine betaine - a novel product from sugar beet as an approach to increase tomato yield. *Industrial Crops and Products* 7: 139–148.

Makela P, Peltonen-Sainio P, Jokinen K, Pehu E, Setala H, Hinkkanen R & Somersalo S (1996) Uptake and translocation of foliar applied glycine betaine in crop plants. *Plant Science* 121: 221–230.

McNeil SD, Rhodes D, Russell BL, Nuccio ML, Shachar-Hill Y & Hanson AD (2000) Metabolic modeling identifies key constraints on an engineered glycine betaine synthesis pathway in tobacco. *Plant Physiology* 124: 153–162.

Muhammad I, Ambreen A & Nabeela K (2006) Four foliar application of glycine betaine did not alleviate advers effects of salt stress on growth of sunflower. *Pakistan Journal of Botany* 38: 1561–1570.

Nuccio ML, Ziemak MJ, Henry SA, Weretilnyk EA & Hanson AD (2000) cDNA cloning of phosphoethanolamine *N*-Methyltransferase from spinach by complementation in *Schizosaccharomyces pombe* and characterization of the recombinant enzyme. *The Journal of Biological Chemistry* 275: 14095–14101.

Quan R, Shang M, Zhang H, Zhao Y & Zhang J (2004) Improved chilling tolerance by transformation with *betA* gene for the enhancement of glycinebetaine synthesis in maize. *Plant Science* 166: 141–149.

Rajendrakumar CS, Suryanarayana T & Reddy AR (1997) DNA helix destabilization by proline and betaine: possible role in the salinity tolerance process. *FEBS Letters* 410: 201–205.

Rhodes D & Hanson AD (1993) Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology* 44: 357–384.

Roychoudhury A & Chakraborty M (2013) Biochemical and molecular basis of varietal difference in plant salt tolerance. *Annual Review & Research in Biology* 3: 422–454.

Roychoudhury A, Banerjee A & Lahiri V (2015) Metabolic and molecular-genetic regulation of proline signalling and its cross-talk with major effectors mediates abiotic stress tolerance in plants. *Turkish Journal of Botany* 39: 887–910.

Roychoudhury A, Basu S, Sarkar SN & Sengupta DN (2008) Comparative physiological and molecular responses of a common aromatic indica rice cultivar to high salinity with non-aromatic indica rice cultivars. *Plant Cell Reports* 27: 1395–1410.

Roychoudhury A, Paul S & Basu S (2013) Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. *Plant Cell Reports* 32: 985–1006.

Sakamoto A & Murata N (2002) The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. *Plant Cell & Environment* 25: 163–171.

Sakamoto A, Alia & Murata N (1998) Metabolic engineering of rice leading to biosynthesis of glycinebetaine and tolerance to salt and cold. *Plant Molecular Biology* 38: 1011–1019.

Smirnoff N & Stewart GR (1985) Stress metabolites and their role in coastal plains. In: Beeftink WG, Rozema J & Huiskes AHL (eds) Ecology of coastal vegetation. *Vegetatio* 61&62: 273–278.

Su J, Hirji R, Zhang L, He C, Selvaraj G & Wu R (2006) Evaluation of the stress-inducible production of choline oxidase in transgenic rice as a strategy for producing the stress-protectant glycine betaine. *Journal of Experimental Botany* 57: 1129–1135.

Sui Y, Liu J, Wisniewski M, Droby S, Norelli J & Hershkovitz V (2012) Pretreatment of the yeast antagonist, *Candida oleophila*, with glycine betaine increases oxidative stress tolerance in the microenvironment of apple wounds. *International Journal of Food Microbiology* 157: 45–51.

Takabe T, Rai V & Hibino T (2006) Metabolic engineering of glycinebetaine. In: Rai A & Takabe T (eds) *Abiotic stress tolerance in plants: toward the improvement of global environment and food*. Springer, Dordrecht, Netherlands, pp. 137–151.

Tang W, Sun J, Liu J, Liu F, Yan J, Gou X, Lu BR & Liu Y (2014) RNAi-directed down regulation of betaine aldehyde dehydrogenase 1 (OsBADH1) results in decreased stress tolerance and increased oxidative markers without affecting glycine betaine biosynthesis in rice (*Oryza sativa*). *Plant Molecular Biology* 86: 443–454.

Umezawa T, Fujita M, Yamaguchi-Shinozaki K & Shinozaki K (2006) Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology* 17: 113–122.

Waditee R, Bhuiyan MNH, Rai V, Aoki K, Tanaka Y, Hibino T, Suzukim S, Takanom J, Jagendorf AT, Takabe T & Takabe T (2005) Genes for direct methylation of glycine provide highlevels of glycinebetaine and abiotic-stress tolerance in *Synechococcus* and *Arabidopsis*. *Proceedings of the National Academy of Sciences USA* 102: 1318–1323.

Wani SH, Singh NB, Haribhushan A & Mir JI (2013) Compatible solute engineering in plants for abiotic stress tolerance - role of glycine betaine. *Current Genomics* 14: 157–165.

Yu HQ, Wang YG, Yong TM, She YH, Fu FL & Li WC (2014) Heterologous expression of betaine aldehyde dehydrogenase gene from *Ammopiptanthus nanus* confers high salt and heat tolerance to *Escherichia coli*. *Plant Molecular Biology* 86: 443–454.

Zhang J, Tan W, Yang XH & Zhang HX (2008) Plastid expressed choline mono-oxygenase gene improved salt and drought tolerance through accumulation of glycine betaine in tobacco. *Plant Cell Reports* 27: 1113–1124.