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RESEARCH ARTICLE

GENETIC ENGINEERING FOR OSMOLYTE OVERPRODUCTION FOR ABIOTIC STRESS TOLERANCE IN PLANTS

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ABSTRACT

Many plants accumulate organic osmolytes in response to the imposition of environmental stresses that cause cellular dehydration. Even though an adaptive involvement of these compounds in regulating osmotic adjustment and preventing subcellular structure from adverse conditions has vital role in stress physiology, the proof in favour of this proposition is largely correlative. Transgenic plants engineered to accumulate proline, mannitol, fructans, trehalose, glycine betaine or ononitol exhibit marginal enhancement in salt and/or drought tolerance. There is significant causative relationships which connects both osmolyte levels and stress tolerance, the complete osmolyte concentrations in these plants are improbable to mediate osmotic adjustment. Metabolic benefits of osmolyte accumulation may supplement the classically proved the roles of these compounds. In re-assessing the functional significance of compatible solute accumulation, it is suggested that proline and glycine betaine synthesis may buffer cellular redox potential. Instability in hexose sensing in transgenic plants which modify to produce trehalose, fructans or mannitol may be an important causative factor to the stress-tolerant phenotypes observed. Osmolyte transport between compartments of subcellular organs or different organs correspond to a bottleneck that confines stress tolerance at the whole-plant level is presently imprecise. None the less, if osmolyte metabolism interrupt on hexose or redox signalling, then it may be important in long-range signal transmission throughout the plant.

INTRODUCTION

Abiotic stresses are one of the major causes for crop losses throughout the world. Abiotic stresses such as drought, salinity, extreme temperature and ion imbalance are key limitations for growth and productivity of crop plants. Certain plants, marine algae, bacteria and other organism have developed a number of adaptations in responses to such abiotic stresses. Different approaches have been proposed for overcome from these adverse conditions. As abiotic stress is influenced by many factors, conventional breeding have resulted less success in getting stress tolerant plant. One such metabolic adaptation that is omnipresent in plants is the accumulation of certain organic solutes of low molecular weight that are collectively known as compatible solutes (known as osmoprotectants) (Bohnert *et al* 1995). Osmoprotectants or compatible solutes are small molecules that act as osmolytes and accumulate at high intracellular levels to help organisms to survive under extreme adverse conditions (Lang *et al* 2000). Osmolytes are naturally accumulated organic compounds, which represent different chemical classes including betaines (fully N - methylated amino acid derivatives) and related compounds such as dimethylsulfoniopropionate (DMSP) and choline-O-sulfate; certain amino acids like proline and ectoine; and polyols and nonreducing sugars such as trehalose. These compounds are non toxic even if accumulate in high concentrations they do not inhibit the activity of enzymes and increase the ability of cells to maintain osmotic balance under stress conditions. Some of osmolytes can also stabilize protein and membranes against deleterious effects of high salt concentrations such as Na+ and Cl- and others (especially polyols) protect against reactive oxygen species.

The osmoprotectants are commonly present in plants and differ in their taxonomic distribution. Gly betaine is widespread among both angiosperms and algae, whereas DMSP is common in algae and lacking in higher plants, while ectoine is occur only in bacteria. Certain crop plants such as rice, soybeans, and potatoes are deficient in considerable amounts of betaines or any other osmoprotectant. This deficiency is the basis for metabolic engineering technology to establish the osmolyte synthesis pathways in such crops for improving their tolerance to drought, salinity, and other stresses.

Biosynthetic Pathways

Ectoine

Ectoine occurs in halophilic bacteria mostly in *Halomonas elongate*, and it synthesized from aspartate semialdehyde in

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three steps (Ono *et al*., 1999). There are three *H. elongata* genes namely, ectA, ectB, and ectC which encodes enzymes responsible for ectoine synthesis were each placed and regulated by constitutive CaMV 35S promoter in a single gene construct, which was transformed into cultured tobacco cells (Nakayama *et al*., 2000). Tobacco transformed cell lines produced small amount of ectoine and showed a low level of resistance against osmotic stress under certain level of mannitol (Nakayama *et al*., 2000).

Glycine Betaine

Quaternary ammonium compounds such as glycine betaine, proline betaine, b-alanine betaine, choline O-sulphate and the tertiary sulphonium compound dimethylsulphoniopropionate are efficient osmoprotectants showed wide taxonomic distribution in various organism, marine algae, bacteria and many plants (Rhodes and Hanson, 1993; Gorham, 1995; Gage and Rathinasabapathi, 1999). Glycine betaine synthesis is a two-step oxidation reaction of choline via betaine aldehyde but different enzymes are involved. Choline is the precursor for phosphatidylcholine, a main constituent of cell membrane phospholipids in eukaryotes. In the enteric bacterium, *Escherichia coli*, the aldehyde, which in turn is oxidized to glycine betaine by a soluble, NAD-linked betaine aldehyde dehydrogenase (Andersen *et al*., 1988). In the plant pathway, choline oxidation via betaine aldehyde two different enzymes are involved. Choline oxidation to betaine aldehyde is catalysed by ferredoxin dependent choline monooxygenase (CMO), an iron-sulphur enzyme (Burnet *et al*., 1995; Rathinasabapathi *et al*., 1997). Betaine aldehyde oxidation to glycine betaine is catalysed by betaine aldehyde dehydrogenase (BADH). (Trossat *et al*., 1997; Vojtechova *et al*., 1997). Both these enzymes are chloroplastic stress-inducible enzymes (McCue and Hanson, 1992; Russell *et al*., 1998). All these enzymes have been used to transform plants that lacks Gly Bet synthesis pathways by introducing these genes under the control of CaMV 35S promoter to enhace stress tolerance.

Polyols Biosynthesis

Polyols such as glycerol, mannitol, sorbitol and sucrose are osmoprotectants occur in certain halophytic plants, algae and insects exposed to extreme low temperature (Yancey*et al*., 1982). Glucose-6-phosphate serves as a precursor to a number of metabolites for synthesis of Myo-inositol, which are related to membrane biogenesis, cell signalling and protection against several stresses (Loewus and Murthy, 1999). Mannitol synthesis is catalyzed by the action of NADPH-dependent mannitol 1-phosphate dehydrogenase from fructose 6-phosphate. When tobacco and Arabidopsis is transformed by a gene encoding mannitol 1-phosphate dehydrogenase (mtlD) from *Escherichia coli* causes mannitol production and a resistance against salinity stress (Tarczynski*et al*., 1993; Thomas *et al*., 1995).

Trehalose

Trehalose is a non-reducing disaccharide of glucose that accumulate as a osmoprotectant and play a vital role in the stabilization of biological structures under abiotic stress in bacteria, fungi, and few extremely desiccation tolerant lower plants (resurrection plants including the fern *Selaginella lepidophylla*, which tolerate complete dessication and upon rehydration spring back to life) (Goddijn and van Dun, 1999; Iturriaga *et al*., 2000). It is very rare compound, all members of the plant kingdom do not seem to accumulate trehalose. Therefore trehalose accumulation is one of the significant area of scientific research. The rationale for this is the ability of trehalose is to stabilize enzymes, proteins, and cell membranes during dehydration. However, homologous genes for biosynthesis of trehalose have been recently identified in several prokaryotes and eukaryotes (Goddijn and van Dun, 1999, Zentella *et al* 1999) which proved to be excellent candidates for genetic engineering for trehalose accumulation in plants not only to enhance stress resistance but also to produce trehalose at low cost for use as a stabilizing agent for pharmaceuticals and other products. Trehalose is synthesized by two steps reaction initiating from glucose-6-phosphate and uridine diphosphoglucose, via trehalose-6-phosphate. The first step is catalyzed by trehalose-6- phosphate synthase (TPS), and the second by trehalose-6-phosphate phosphatase (TPP).

Trehalose-6-phosphate synthase is a important enzyme for trehalose biosynthesis in *Saccharomyces cerevisiae*, encoded by the gene TPS1. The gene from yeast was expressed in transgenic potato plants (Yeo *et al* 2000). The transformants showed various morphological phenotypic alterations in vitro - from normal to severely anomalous growth and shapes. But after acclimatize in soil mixture, the plants improved to normal phenotype. The TPS1 transgenic potato plants showed significantly improved stress tolerance. The TPS1 gene was expressed in tobacco plants also (Romero *et al* 1997) and accumulated trehalose at very low levels in the transformants. They exhibited various morphological phenotypes, including less sucrose content and enhanced drought tolerance. These results showed that trehalose accumulation causes alteration of sugar metabolism, plant development and significantly improved stress tolerance, inspite of providing an osmoprotectant effect. Significantly, abiotic stress tolerance was showed in rice by overexpression of *Escherichia coli* trehalose biosynthetic genes (otsA and otsB) under the control of either tissue specific or stress inducible promoter resulted in high content of trehalose in comparison to control plants (Garg *et al* 2002). Compared with control rice plants, transgenic lines exhibited increased tolerance to salt, drought, and low-temperature stress conditions.

Increased trehalose accumulation directly related to the enhanced photosynthetic activity and with higher soluble carbohydrate levels. Recently trehalose synthase (TSase) gene from the edible wood fungi *Grifola frondosa* was transformed in tobacco (Zhang *et al* 2005). The transformants showed a increased trehalose accumulation ascompared to other transgenics plants. Various phenotypic alterations were reported, like dark coloured leaves, but growth is not inhibited. Trehalose accumulation in 35S-35S:TSase plants showed improved tolerance to dessication and salt stress. These results proposed that expression TSase gene in transgenic plants can cause higher accumulation of trehalose and have increased tolerance to drought and salt stresses.

Osmolytes	Gene source	Gene	Plant species engineered	Reference
Glycine betaine	*E.coli*	*CDH*	Tobacco	Lilius *et al.*,1996
	E.coli	*CDH+BADH*	Tobacco	Holmstrom *et al.*, 2000
	Arthrobacter	*codA*	*Arabidopsis*	Sakamoto and Murata 2000
	Arthrobacter	*codA*	Rice	Su *et al.*, 2006
	Arthrobacter	*COX*	Canola Tobacco	Huang *et al.*, 2000
	Arthrobacter	*codA*	Tomato	Park *et al.*, 2007
	A.halophytica	*GSMT+DMT*	*Arabidopsis*	Waditee *et al.*, 2005
	Beta vulgaris	*BvCMO*	Tobacco	Zhang *et al.*, 2008
	Spinacia oleracea	*BADH*	Tobacco	Yang *et al.*, 2008
		betaine aldehyde dehydrogenase.	Medicago sativa	G.B.; Zhang *et al.*, 2011
	Arthrobacter	*codA*	Eucalyptus globules	Yu, X. *et al.*,2009
	Arthrobacter	*codA*	*Brassica chinensis*	Wang, Q.B *et al.*, 2010
	Spinacia oleracea	*BADH*	potato	Zhang, N *et al.*, 2011
		codA	Rice	Kathuria, H *et al.*, 2009
	Spinacia oleracea	*BADH*	*Ipomoea batatas*	Fan, W *et al.*, 2012
	Oryza sativa	*BADH*	Tobacco	Hasthanasombut, S. *et al.*, 2010
	Arthrobacter	*codA*	Tomato	Bansal, K.C *et al.*, 2011
Proline	*Mothbean*	*P5CS*	Tobacco	Kishor *et al.*, 1995; Hong *et al.*, 2000
	Brassica juncea	*BjDREB1B*	Tobacco	Cong *et al.*, 2008
	Arabidopsis, Rice	*P5CS*	Petunia	Yamada *et al.*, 2005
	Arabidopsis	*P5CS1*	*Arabidopsis*	Mattioli *et al.*, 2008
	Barley	*hva1*	Mulberry	Lal *et al.*, 2008
	Arabidopsis	*SOS*	*Populus trichocarpa*	Zhou J;2014
Ectoine	*Halomonas*	*ectA+ectB+ ectC*	Tobacco cells	Nakayama *et al.*, 2000
	Halomonas Elongate	*ect. ABC*	Tobacco	Moghaieb *et al.*, 2006
Mannitol	*Escherichia coli*	*mtlD*	*Populus tomentosa*	Hu *et al.*, 2005
	E.coli	*MtlD*	Tobacco *Arabidopsis*	Tarczynski *et al.*, 1992; Thomas *et al.*, 1995
	E.coli	*MtlD*	Wheat	Abebe *et al.*, 2003
Sorbitol	Apple	*Stpd 1*	Tobacco	Tao *et al.*, 1995 Sheveleva *et al.*, 1998
		PmSDH1	*Plantago major*	Pommerrenig *et al.*, 2007
Polyamines	*D.stramonium*	*ADC*	Rice	Capell *et al.*, 2004
	Yeast	*ySAMdc*	Tomato	Mattoo *et al.*, 2007
	Apple	*MdSPDS1*	Pear	Wen *et al.*, 2008
Trehalose	Yeast	*TPS1*	Potato	Stiller *et al.*, 2008
	Yeast	*TPS1-TPS2*	*Arabidopsis*	Miranda *et al.*, 2007
	Yeast	*TPP*	Tobacco	Karim *et al.*, 2007
	Arabidopsis thaliana	*TPS*	Tobacco	Almeida *et al.*, 2007
	E.coli	*TPS or TPS+TPP*	Tobacco Potato tuber	Goddijn *et al.*, 1997 Pilon Smits *et al.*, 1998
	Yeast	*TPS 1*	Tobacco	Holmstrom *et al.*, 1996, Romero *et al.*, 1997
	E.coli	*TPSP (otsA + otsB)*	Rice	Garg *et al.*, 2002.
	S.cerevisiae	*TPS 1*	Tomato	Cortina *et al.*, 2005
	Grifola frondosa	TSase	Tobacco	Shu-Zhen Zhang *et al.*,2005
	Grifola frondosa	TSase	Sugarcane	Shu-Zhen Zhang 2006

Conclusion

There can be some uncertainty that transgenic plants will be important in assessing the role that osmolytes play in the functional network that involved in stress tolerance. Although biosynthetic pathways reactions causing osmolyte accumulation in bacteria, yeasts and plants have been characterized at the molecular level, the exact role of participants which are involved in the multifaceted regulation of osmolyte accumulation in plants remains to be identified. This will necessitate not only improved knowledge of the degradation and transport of compatible solutes, but also explanation of the molecular events involved in stress perception and stress-related signal transduction. Basic research leading to the recognition of strongly regulated stress-inducible promoters that are also responsive to suitable tissue-specific expression and endogenous developmental regulation is likely to be significant in increasing the overall physiological and metabolism performance of transgenic crops.

The up-and-coming view that the functional significance of osmolyte accumulation may not yet have been fully acceptable is mainly because of the known complexity of stress tolerance, which is a multifactorial product of the coordinated interplay of

various levels of cellular control, including hormonal regulation, nutrient status and redox potential as well as inter- and intracellular signalling and transport capacity. Irrespective of whether the definitive target of alleviating stress effects will be realized by efforts to increase osmolyte accumulation, there can be little misgiving that continued work in this area will not only be possible for the identification of rational approaches to improve crop productivity, but also enable appreciation of how plants coordinate growth and development in a constantly changing environment.

REFRENCES

Bohnert HJ, Nelson DE, Jensen RG. Adaptations to environmental stresses. The Plant Cell. 1995;7:1099–1111.

Lang F. Mechanisms and significance of cell volume regulation. *J. Am. Coll. Nutr*. 2007;26(5 Suppl):613S–623S.

Ono, H., Sawada, K.,Khunajakr, N., Tao, T., Yamamoto, M.,Hiramoto, M.,Shinmyo, A., Takano, M., and Murooka, Y. 1999. Characterization of biosynthetic enzymes for Ectoine as a compatible solute in a moderately halophilic eubacterium, Halomonas elongate . *J Bacteriol*. 181 , 91-99.

Nakayama, H., Yoshida, K., Ono, H., Murooka, Y., and Shinmyo, A. 2000. Ectoine, the compatible solute of Halomonas elongate, confers hyperosmotic tolerance in cultured tobacco cells. *Plant Physiol*. 122, 1239-1247.

Rhodes D and Hanson AD. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher plants. *Annual Review of Plant Physiology and Plant Molecular Biology* 44: 357-384.

Gorham J. 1995. Betaines in higher plants Ðbiosynthesis and role in stress metabolism. In: Wallsgrove RM, ed. Amino acids and their derivatives in higher plants. Cambridge: Cambridge University Press, 173 -203.

Gage DA, Rathinasabapathi B. 1999. Role of glycine betaine and dimethylsulfoniopropionate in water-stress tolerance. In: Shino zaki K, Yamaguchi-Shinozaki K, eds. Molecular responses to cold, drought, heat and salt stress in higher plants. Austin, TX: R.G. Landes Co., 125-152.

Andersen PA, Kaasen I, Styrvold OB, Boulnois G, Strin AR. 1988. Molecular cloning, physical mapping and expression of the bet genes governing the osmoregulatory choline-glycine betaine pathway of Escherichia coli. *Journal of General Microbiology* 134: 1737-1746.

Burnet M, Lafontaine PJ, Hanson AD. 1995. Assay, purification, and partial characterization of choline monooxygenase from spinach chloroplast stroma. *Plant Physiology* 90: 581-588.

Rathinasabapathi B, Burnet M, Russell BL, Gage DA, Liao P-C, Nye GJ, Scott P, Golbeck JH, Hanson AD. 1997. Choline monooxygenase, an unusual iron-sulfur enzyme catalyzing the ®rst step of glycine betaine synthesis in plants: Prosthetic group characterization and cDNA cloning. *Proceedings of the National Academy of Sciences*, USA 94: 3454-3458.

Trossat C, Rathinasabapathi B, Hanson AD. 1997. Transgenically expressed betaine aldehyde dehydrogenase e ciently catalyzes oxidation of dimethylsulfonio propionaldehyde and omega-aminoaldehydes. *Plant Physiology* 113: 1457-1461.

Vojtechova M, Hanson AD, Munoz-Clares RA. 1997. Betaine aldehyde dehydrogenase from amaranth leaves e ciently catalyzes the NAD-dependent oxidation of dimethyl-sulfoniopropionaldehyde to dimethylsulfoniopropionate. *Archives of Biochemistry and Biophysics* 337: 81-88.

McCue KF, Hanson AD. 1992. E€ects of soil salinity on the expression of betaine aldehyde dehydrogenase in leaves: investigation on hydraulic, ionic and biochemical signals. *Australian Journal of Plant Physiology* 19: 555-564.

Russell BL, Rathinasabapathi B, Hanson AD. 1998. Osmotic stress induces expression of choline monooxygenase in sugar beet and amaranth. *Plant Physiology* 116: 859±865.

Yancey PH, Clark ME, Hand SC, Bowlus RD, Somero GN. 1982.Living with water stress: *Evolution of osmolyte systems. Science* 217: 1214-1222.

Loewus FA and Murthy PPN. 1999. myo-Inositol metabolism in plants. *Plant Science* 150: 1-19.

Tarczynski MC, Jensen RG, Bohnert HJ. 1993. Stress protection of transgenic tobacco by production of the osmolyte mannitol. *Science* 259: 508-510.

Thomas JC, Sepahi M, Arendall B, Bohnert HJ. 1995. Enhancement of seed germination in high salinity by engineering mannitol expression in Arabidopsis thaliana. *Plant Cell and Environment* 18: 801-806.

Iturriaga, G., Gaff, D.F., and Zentella, R. 2000. New desiccation-tolerant plants, including a grass, in the central highlands of Mexico, accumulate trehalose. *Aust. J. Bot.* 48, 153-158.

Goddijn, O.J.M., and van Dun, K. 1999. Trehalose metabolism in plants. *Trends Plant Sci*. 4, 315-319.

Zentella R., Gallardo J.O.M., Van Dijck P., Mallol J.F., Bonini B., Van Vaeck C., Gaxiola R., Covarrubias A.A., Sotelo J.N., Thevelein J.M., Iturriaga G. 1999. A Selaginella lepidophylla Trehalose-6-Phosphate Synthase Complements Growth and Stress-Tolerance Defects in a Yeasttps1 Mutant. *Plant Physiol*., 119, 1473-1482.

Yeo E.T., Kwon H.B., Han S.E., Lee J.T., Ryu J.C., Byu M.O. 2000 Genetic engineering of drought resistant potato plants by introduction of the trehalose-6-phosphate synthase (TPS1) gene from Saccharomyces cerevisiae. Mol. Cells., 10(3), 263-268.

Romero C., BelleÂs J., JoseÂ L., VayaÂ, Serrano R., Francisco A., CuliaÂnÄez-Macia Á. 1997 Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. Planta, 201, 293-297.

Garg A.K., Kim J-K., Owens T.G., Ranwala A.P., Choi Y-D., Kochian L.V., Wu R.J. 2002 Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses PNAS, 99(25), 15898-15903.

Zhang S.-Z., Yang B.-P., Feng C.-I., Tang H.L. 2005 Genetic Transformation of Tobacco with the Trehalose Synthase Gene from Grifola frondosa Fr. Enhances the Resistance to Drought and Salt in Tobacco *J. Integrat. Plant Biol*., 47(5), 579 - 586.

Lilius, G., Holmberg, N. and Bulow, L. 1996. Enhanced NaCl Stress Tolerance in Transgenic Tobacco Expressing Bacterial Choline Dehydrogenase. Nat. Biotech. 14: 177–180.

Holmstrom, K-O., Somersalo, S., Mandal, A., Palva, E.T. and Welin, B. 2000. Improved tolerance to salinity and low

temperature in transgenic tobacco producing glycine betaine. *J Exp Bot*. 51: 177-185.

Sakamoto, A. and Murata, N. 2000. Genetic engineering of glycine betaine synthesis in plants: current status and implications for enhancement of stress tolerance. *J Exp Bot*. 51: 81–88.

Su, J., Hirji, R., Zhang, L., He, C., Selvaraj, G. and 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. *J Exp Bot*. 57(5): 1129-1135.

Huang, J., Hirji, R., Adam, L., Kevin, L.R., Joe, K.H., Keller, A. and Selvaraj, G. 2000. Genetic Engineering of Glycine betaine Production toward Enhancing Stress Tolerance in Plants: Metabolic Limitations. *Plant Physiol*. 122: 747-756.

Park, E.J., Jeknic, Z., Pino, M.T., Murata, N. and Chen, T.H. 2007. Glycinebetaine accumulation is more effective in chloroplasts than in the cytosol for protecting transgenic tomato plants against abiotic stress. *Plant Cell Environ*. 30(8): 994-1005.

Waditee, R., Bhuiyan, M.N.H., Rai, V., Aoki, K., Tanaka, Y., Hibino, T., Suzuki, S., Takano, J., Jagendorf, A.T., Takabe, T. and Takabe, T. 2005 Genes for direct methylation of glycine provide high levels of glycinebetaine and abiotic-stress tolerance in Synechococcus and Arabidopsis. Proc Natl Acad Sci USA. 102(5): 1318–1323.

Zhang, J., Tan, W., Yang, X.H. and Zhang, H.X. 2008. Plastid-expressed choline monooxygenase gene improves salt and drought tolerance through accumulation of glycine betaine in tobacco. *Plant Cell Rep*. 27(6): 1113-24.

Yang, X., Liang, Z., Wen, X. and Lu, C. 2008. Genetic engineering of the biosynthesis of glycine betaine leads to increased tolerance of photosynthesis to salt stress in transgenic tobacco plants. *Plant Mol Biol*. 66(1-2): 73-86.

G.B.; Zhang, Y.M. Enhancement of salt tolerance in alfalfa transformed with the gene encoding for betaine aldehyde dehydrogenase. Euphytica, 2011, 178: 363-372.

Yu, X.; Kikuchi, A.; Matsunaga, E.; Morishita, Y.; Nanto, K.; Sakurai, N.; Suzuki, H.; Shibata, D.; Shimada, T.; Watanabe, K.N. Establishment of the evaluation system of salt tolerance on transgenic woody plants in the special netted-house. *Plant Biotechnol*., 2009, 26: 135-141.

Wang, Q.B.; Xu, W.; Xue, Q.Z.; Su, W. Transgenic Brassica chinensis plants expressing a bacterial codA gene exhibit enhanced tolerance to extreme temperature and high salinity. *J. Zhejiang Univ. Sci*., 2010, 11: 851-861.

Zhang, N.; Si, N.H.; Wen, G.; Du, H.H.; Liu, B.L.; Wang, D. Enhanced drought and salinity tolerance in transgenic potato plants with a BADH gene from spinach. *Plant Biotechnol. Rep*., 2011, 5:71–77.

Kathuria, H.; Giri, J.; Nataraja, K.N.; Murata, N.; Udayakumar, M.; Tyagi, A.K. Glycinebetaine-induced water-stress tolerance in codA-expressing transgenic indica rice is associated with upregulation of several stress responsive genes. *Plant Biotechnol J*.,2009, 7: 512-26.

Fan, W.; Zhang, M.; Zhang, H.; Zhang, P. Improved Tolerance to Various Abiotic Stresses in Transgenic Sweet Potato (Ipomoea batatas) Expressing Spinach Betaine Aldehyde Dehydrogenase. PLoS One. 2012, 7: e37344.

Hasthanasombut, S.; Ntui, Valentine.; Supaibulwatana, K.; Mii, M.; Nakamura, I. Expression of Indica rice OsBADH1 gene under salinity stress in transgenic tobacco. Plant Biotechnol. Rep., 2010, 4: 75-83.

Bansal, K.C.; Goel, D.; Singh, A.K.; Yadav, V.; Babbar, S.B.; Murata, N. Transformation of tomato with a bacterial codA gene enhances tolerance to salt and water stresses. *J. Plant Physiol*., 2011, 168: 1286-1294.

Kishor, P.B.K., Hong, Z., Miao, G.H., Hu, C.A.A. and Verma, D.P.S. 1995. Overexpression of 1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol*. 108: 1387–1394.

Hong, Z., Lakkineni, K., Zhang, Z. and Verma, D.P. 2000. Removal of feedback inhibition of Δ1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol*. 122: 1129–1136.

Cong, L., Chai, T.Y. and Zhang, Y.X. 2008. Characterization of the novel gene BjDREB1B encoding a DRE-binding transcription factor from Brassica juncea L. Biochem Biophys Res Commun. 371(4): 702-6.

Yamada, M., Morishita, H., Urano, K., Shinozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K. and Yoshiba, Y. 2005. Effects of free proline accumulation in petunias under drought stress. *J Exp Bot*. 56(417): 1975-1981.

Mattioli, R., Marchese, D., D'Angeli, S., Altamura, M.M., Costantino, P. and Trovato, M. 2008. Modulation of intracellular proline levels affects flowering time and inflorescence architecture in Arabidopsis. *Plant Mol Biol*. 66(3): 277-88.

Lal, S., Gulyani, V. and Khurana, P. 2008. Overexpression of HVA1 gene from barley generates tolerance to salinity and water stress in transgenic mulberry (Morus indica). Transgenic Res. 17(4): 651-63.

Zhou J1, Wang J1, Bi Y1, Wang L1, Tang L1, Yu X2, Ohtani M2, Demura T2, Zhuge Q 2014 Overexpression of PtSOS2 Enhances Salt Tolerance in Transgenic Poplars. *Plant Mol Biol Rep*.;32:185-197.

Nakayama, H., Yoshida, K., Ono, H., Murooka, Y. and Shinmyo, A. 2000. Ectoine, the Compatible Solute of Halomonas elongata, Confers Hyperosmotic Tolerance in Cultured Tobacco Cells. *Plant Physiol*. 122: 1239-1248.

Moghaieb, R.E., Tanaka, N., Saneoka, H., Murooka, Y., Ono, H., Morikawa, H., Nakamura, A., Nguyen, N.T., Suwa, R. and Fujita, K. 2006. Characterization of salt tolerance in ectoine-transformed tobacco plants (Nicotiana tabaccum): photosynthesis, osmotic adjustment, and nitrogen partitioning. *Plant Cell Environ*. 29(2): 173-82.

Hu, L., Lu, H., Liu, Q., Chen, X. and Jiang, X. 2005. Overexpression of mtlD gene in transgenic Populus tomentosa improves salt tolerance through accumulation of mannitol. *Tree Physiol*. 25(10): 1273-81.

Tarczynski, M.C., Jensen, R.G. and Bohnert, H.J. 1992. Expression of a bacterial mtlD gene in transgenic tobacco leads to production and accumulation of mannitol. *Proc Natl Acad Sci USA*. 89: 2600-2604.

Thomas, J.C., Sepahi, M., Arendall, B. and Bohnert, H.J. 1995. "Enhancement of seed germination in high salinity by engineering mannitol expression in Arabidopsis thaliana". *Plant Cell Environ*. 18: 801-806.

Abebe, T., Arron, C., Martin, B. and Cushman, J.C. 2003. Tolerance of Mannitol-Accumulating Transgenic Wheat to Water Stress and Salinity. Plant Physiol. 131: 1748-1755.

Tao, R., Uratsu, S.L. and Dandekar, A.M. 1995. Sorbitol Synthesis in Transgenic Tobacco with Apple cDNA Encoding NADP-Dependent Sorbitol-6-Phosphate Dehydrogenase. Plant Cell Physiol. 36(3): 525-532.

Sheveleva, E.V., Marquez, S., Chmawa, W., Zegeer, A., Jensen, R.G. and Bohnert, H.J. 1998. Sorbitol-6-phosphate dehydrogenase expression in transgenic tobacco: high amounts of sorbitol lead to necrotic lesions. Plant Physiol. 117: 831–839.

Pommerrenig, B., Papini-Terzi, F.S. and Sauer, N. 2007. Differential regulation of sorbitol and sucrose loading into the phloem of Plantago major in response to salt stress. Plant Physiol. 144: 1029 – 1038.

Capell, T., Bassie, L. and Christou, P. 2004. Modulation of the polyamine biosynthetic pathway in transgenic rice confers tolerance to drought stress. Proc Natl Acad Sci USA. 101: 9909-9914.

Mattoo, A.K., Chung, S.H., Goyal, R.K., Fatima, T., Solomos, T., Srivastava, A. and Handa, A.K. 2007. Over accumulation of higher polyamines in ripening transgenic tomato fruit revives metabolic memory, upregulates anabolism-related genes, and positively impacts nutritional quality. J AOAC Int. 90(5): 1456-64

Wen, X.P., Pang, X.M., Matsuda, N., Kita, M., Inoue, H., Hao, Y.J., Honda, C. and Moriguchi, T. 2008. Over-expression of the apple spermidine synthase gene in pear confers multiple abiotic stress tolerance by altering polyamine titers. Transgenic Res. 17(2): 251-63.

Stiller, I., Dulai, S., Kondrak, M., Tarnai, R., Szabo, L., Toldi, O. and Banfalvi, Z. 2008. Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of Saccharomyces cerevisiae. Planta. 227(2): 299-308.

Miranda, J.A., Avonce, N., Suarez, R., Thevelein, J.M., Van Dijck, P. And Iturriaga, G. 2007. A bifunctional TPS-TPP enzyme from yeast confers tolerance to multiple and extreme abiotic-stress conditions in transgenic Arabidopsis. Planta. 226(6): 1411-21.

Karim, S., Aronsson, H., Ericson, H., Pirhonen, M., Leyman, B., Welin, B., Mantyla, E., Palva, E.T., Van Dijck, P. and Holmstrom, K.O. 2007. Improved drought tolerance without undesired side effects in transgenic plants producing trehalose. Plant Mol Biol. 64(4): 371-86.

Almeida, A.M., Santos, M., Villalobos, E., Araujo, S.S., Van Dijck, P., Leyman, B., Cardoso, L.A., Santos, D., Fevereiro, P.S. and Torne, J.M. 2007. Immunogold localization of trehalose-6-phosphate synthase in leaf segments of wild-type and transgenic tobacco plants expressing the AtTPS1 gene from Arabidopsis thaliana. Protoplasma. 230: 41-49.

Goddijn, O., Verwoerd, T.C., Voogd, E., Krutwagen, R.W.H.H., De Graaf, P.T.H.M., Poels, J.,van Dun, K., Ponstein, A.S., Damm, B. and Pen, J. 1997. Inhibition of trehalase activity enhances trehalose accumulation in transgenic plants. Plant Physiol. 113: 181-190.

Pilon-Smit, E.A.H., Terry, N., Sears, T., Kim, H., Zayed, A., Hwang, S., van Dun, K., Voogd, E., Verwoerd, T.C., Krutwagen, R.W.H.H. and Goddijn, O.J.M. 1998. Trehalose-producing transgenic tobacco plants show improved growth performance under drought stress. J Plant Physiol. 152: 525-532.

Holmstrom, K.O., Mantyla, E., Welin, B., Mandal, A. and Palva, E.T. 1996. Drought tolerance in tobacco. Nature. 379: 683-684.

Romero, C., Belles, J.M., Vaya, J.L., Serrano, R. and Culianez-Macia, F.A. 1997. Expression of the yeast trehalose-6-phosphate synthase gene in transgenic tobacco plants: pleiotropic phenotypes include drought tolerance. Planta. 201: 293-297.

Garg, A.K., Kim, J.K., Owens, T.G., Ranwala, A.P., Choi, Y.D., Kochian, L.V. and Wu, R.J. 2002. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. Proc Natl Acad Sci USA. 99: 15898-15903.

Cortina, C. and Culiáñez-Macià, A. 2005. Tomato abiotic stress enhanced tolerance by trehalose biosynthesis. Plant Sci. 169(1): 75-82

Shu-Zhen ZHANG, Ben-Peng YANG, Cui-Lian FENG, Huo-Long TANG. 2005. Genetic Transformation of Tobacco with the Trehalose Synthase Gene from Grifola frondosa Fr. Enhances the Resistance to Drought and Salt in Tobacco. Journal of Integrative Plant Biology. 47(5): 579–587

Shu-Zhen Zhang, Ben-Peng Yang, Cui-Lian Feng, Ru-Kai Chen, Jing-Ping Luo, Wen-Wei Cai, Fei-Hu Liu. 2006. Expression of the Grifola frondosa Trehalose Synthase Gene and Improvement of Drought-Tolerance in Sugarcane (Saccharum officinarum L.). Journal of Integrative Plant Biology . 49(4): 453–459

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