

# TREHALOSE IN OSMOTIC STRESS TOLERANCE OF PLANTS

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## ABSTRACT

Any non-living or environmental factors which negatively affect living organisms, including plants, are categorized under the general term “abiotic stress” encompassing salinity, drought, temperature extremes, heavy metal toxicity, etc. These can cause oxidative damages due to osmotic stress. Plants have developed a variety of defense mechanisms to mitigate stress effects in the form of avoidance and adaptation. Compatible solutes are low-molecular weight compounds whose levels are elevated in order to lower cellular osmotic potential during stress conditions. This can facilitate dragging of more water within the cell and hence serve as an adaptive response against water deficit. Trehalose, a non-reducing disaccharide, is widely distributed in all biological systems like bacteria, yeast, fungi, lower and higher plants, as well as insects and invertebrates. Trehalose is one of the well-known compatible solutes whose function is associated with tolerance against multiple abiotic stresses in plants. Trehalose levels are relatively low in plants, but may alter during environmental stresses. Exogenous application of trehalose has been found to improve tolerance against different environmental stresses by stabilizing biological macromolecules and scavenging the reactive oxygen species. The biosynthetic pathway of trehalose is characterized in plants. Expression profiling of trehalose-6-phosphate synthases (TPSs) and trehalose-6-phosphate - phosphatases (TPPs) revealed that certain classes of TPS -

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and TPP exhibit differential regulation in response to osmotic stresses. Transgenic plants overexpressing trehalose biosynthesis genes have shown promising results in increasing the level of stress tolerance. In this review, the metabolism and biological functions of trehalose, along with their overall importance in boosting osmotic stress tolerance in plants have been presented.

## 1. INTRODUCTION

Cassells and Curry (2001) propose stress as “an unusual or usual factor of the biotic or abiotic environment modified in such a way that it has the capability of causing injury, disease or aberrant physiology.” Abiotic stress in the form of salinity, drought, high or low temperature, and heavy metals evoke severe osmotic stress by causing cellular water deficit. Water-deficit stress response is extremely complex, involving switching on/off several genes that regulate biochemical, cellular, and molecular mechanisms. During water shortage episodes, stomatal closure constitutes the immediate response for inhibiting severe water loss (Medrano *et al.* 2002). Long-term and recurrent unfavorable situations may lead to the development of various modes of tolerance mechanisms, ensuring protection against severe damages, for instance, by synthesizing compatible solutes or osmoprotectants (Wingler and Roitsch 2008). Compatible solutes or osmoprotectants constitute the compounds that are synthesized and accumulated in plants during osmotic stress. They are uncharged molecules with low-molecular weight and play vital roles by stabilizing cellular proteins and preventing damages of cellular membranes during environmental stresses. These molecules are called compatible, since they do not interfere with or disrupt the normal cellular metabolism, even if they accumulate at high concentrations. Compatible solutes are classified into three major groups; (i) amino acids (e.g.,

proline), (ii) quaternary amines (e.g., glycine betaine and polyamines), and (iii) polyol/sugars (e.g., mannitol, trehalose, fructans). The other functions of these solutes include scavenging of reactive oxygen species (ROS), stabilization of endogenous proteins and protein complexes and protection of cell membranes during different environmental stresses. Among the different classes of osmoprotectants, trehalose is one of the most potent protectants against hostile conditions (Roychoudhury and Chakraborty 2013).

## 2. DISCOVERY OF TREHALOSE AND CHARACTERISTICS

Trehalose was first discovered in 1832 by H.A. Wiggers, while in 1858, Mistcherlich isolated it from mushrooms and named it mycose. In the same year, Berthelot isolated a new type of sugar from an insect secretion named Trehala manna and named it “trehalic glucose” or trehalose. In the 1990s, the Hayashibara Company in Japan developed an inexpensive laboratory method for mass production of trehalose, using starch as a substrate and the enzymes, viz., maltooligosyl-trehalose trehalohydrolase (MTHase) and maltooligosyl-trehalose synthase (MTSase), derived from *Arthrobacter* sp. Trehalose is a non-reducing disaccharide (1, 1  $\alpha$ -D glucopyranosyl  $\alpha$ -D glucopyranoside), found in a wide range of organisms from unicellular bacteria to multicellular higher plants, as well as in various vertebrates and insects (Paul *et al.* 2008). Trehalose, a “minor” sugar and a canonical osmolyte, consists of two  $\alpha$ -glucose molecules, joined by a glycosidic bond. It has a more stable configuration, as compared to sucrose, since it stores lower energy levels. Trehalose has also been looked upon as a double-faced molecule, since it contributes, on one hand, to infectivity of many pathogens, while also triggering plant defense in response to biotic and abiotic -

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stresses, as a part of alleviation mechanism. Trehalose is found in millimolar amounts in limited plant species, viz., specialized resurrection species, where it confers protection against desiccation. It is present in only trace (low micromolar or even less) amounts in the vast majority of plants and is well-known for its protective ability, stability, and low reactivity (El-Bashiti *et al.*, 2005). The protective function of trehalose with regard to membranes and proteins is attributed to its ability of hydrogen bond formation with the polar residues. Trehalose forms an amorphous glass structure that limits the molecular motion, preventing free radical diffusion and protein aggregation during dehydration stress. The structural integrity of the lipid bilayer of the membrane is preserved during water stress, primarily due to  $\alpha$ -1, 1 configuration of trehalose. Trehalose can withstand high temperature (100°C) between pH 3.5-10 for 24 h (Almeida *et al.*, 2007). Because of its role in protecting membranes and proteins, engineering of plants with genes belonging to the trehalose biosynthetic pathway has been emphasized.

### 3. BIOSYNTHESIS OF TREHALOSE IN PLANTS

Trehalose biosynthesis in higher plants resembles the pathways operating for most of the other organisms (Iordachescu and Imai, 2008). The biosynthetic pathway consists of two steps: trehalose 6-phosphate (T6P) is first synthesized from glucose-6-phosphate and uridine diphosphate (UDP)-glucose. This step is catalyzed by trehalose 6-phosphate synthase (TPS). Trehalose-6-phosphate phosphatase (TPP) enzyme carries out a dephosphorylation reaction due to which trehalose, along with inorganic phosphate, is generated. The OtsA-OtsB pathway is the most widespread pathway involving the intermediate T6P. *Escherichia coli* genes, *otsA* and *otsB* encode TPS and TPP, respectively. Trehalose breakdown by trehalase to two -

glucose units is a universal reaction. In *Arabidopsis thaliana*, there are 11 TPS or TPS-like genes, 10 TPP genes, and one trehalase-encoding gene (TRE). All of the TPP genes originated from whole-genome duplication events. Extensive gene expression analyses using transgenic lines with promoter- $\beta$ -glucuronidase/green fluorescent protein unraveled cell- and tissue-specific expression patterns, conferring spatio-temporal control of trehalose metabolism (Vandesteene *et al.*, 2012). The numbers of classes of TPS, TPP and trehalase genes in plants are 2, 2 and 1, respectively. While TPS genes have eukaryotic origin, TPP genes are most closely related to bacteria. Trehalose and its metabolism are evolutionarily ancient, since TPS genes are represented in the genomes of non-flowering plants (*Selaginella moellendorffii* and *Physcomitrella patens*). Among the identified class I TPSs (TPS1-4 in *A. thaliana*), only one (TPS1) shows demonstrable TPS activity. Class II TPS (AtTPS5-11) enzymes show separate synthase domains and phosphatase domains. Class II TPSs are expressed in the whole plant, possessing parallel expression patterns of TPS8 and TPS11 (Paul *et al.*, 2008). Moreover, class II TPSs are extensively regulated by cytokinin, light, sugars, starvation and diurnal rhythms.

### 4. MECHANISM OF PROTECTION CONFERRED BY TREHALOSE

Trehalose protects biomolecules in three ways: water replacement, glass transformation, and chemical stability. These three mechanisms are not mutually exclusive, so that all three together confer stabilizing property to trehalose. According to the "water replacement" theory, hydrogen bond formation of water with biological macromolecules stabilizes the latter. Protein denaturation and membrane fusions are prevented during dehydration, since trehalose -

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replaces the water molecules. Substantial levels of trehalose were detected in two resurrection plants, *Myrothamnus flabellifolia* and *Sporobolus stapfianus* (Phillips *et al.*, 2002), which enabled these species to prevent structural damages during anhydrobiosis. Trehalose protects membranes against lipid peroxidation, interacts directly with the portions of the oxidation-sensitive unsaturated fatty acids, thereby preventing their auto-oxidation. Compared to other disaccharides like sucrose, trehalose exhibits greater flexibility in the glycosidic linkage between the two D-glucose molecules. This allows trehalose to conform to the irregular polar groups of those same macromolecules. According to the “glass transformation” theory, sugar solutions are transformed into a glassy state, instead of getting crystallized. The unique feature of trehalose is that it forms a non-hygroscopic glass, which is stable at high temperature or during desiccation. Such glass formation enables holding biomolecules in such a way that they can return to a native state and function upon rehydration (Luo *et al.*, 2008; Lun 2007). According to the third theory, viz., “chemical stabilization” hypothesis, trehalose remains stable and does not undergo Maillard browning with proteins (Wingler, 2002), even at high temperature and at low pH. The 1,1 glycosidic linkage makes trehalose non-reducing and confers chemical stability. It is highly resistant to hydrolysis and chemically inert in its interactions with proteins. The chemical stability and the water replacement theory are debatable, while the glassy state hypothesis is more widely accepted. An alternative anhydrobiosis mechanism of trehalose was proposed by Sussisich *et al.* (2001). This mechanism emphasizes on the nucleation and formation of layered epitaxial crystals of dihydrate trehalose on the surface of cellular membranes. The dihydrate trehalose is not involved in water replacement; rather, it captures the water molecules in the same hydrogen bonding -

network as in the solvated trehalose. With prolonged dehydration, more dihydrate formation occurs at the membrane surface without disrupting the cellular structures. Finally, slow dehydration of the layered crystals produces anhydrous trehalose. Such mechanisms can show potentiality in protecting cellular functions during water deficit, by preserving active molecular conformations and membrane structure. Trehalose also induces the activity of the antioxidant enzymes, thereby scavenging the ROS like hydrogen peroxide and superoxide anions.

## 5. ROLE IN OSMOTIC STRESS TOLERANCE OF PLANTS

### 5.1. SALINITY STRESS

Salt stress significantly induced most of the *TPS/TPP* genes in the leaves of maize, with the exception of *ZmTPSI.1.1*, *ZmTPSII.2.1* and *ZmTPPA.3* transcripts which were repressed under osmotic stress (Henry *et al.*, 2015). An increase in the level of trehalose-6-phosphate was observed in the leaf, cob and kernels of maize plants under salt stress. Another study by Mostofa *et al.* (2015a, b) in rice reported that pre-treatment with trehalose (10 mM) for two days lowered the salinity-induced damages by suppressing the ROS and malondialdehyde, whereas chlorophyll contents, ascorbate, reduced glutathione, relative water content and redox status were improved substantially. Trehalose-pretreated salt-stressed rice seedlings maintained catalase, glutathione-S-transferase, and glutathione reductase activities, along with activated glyoxalase under salt stress. Exogenously applied trehalose in salt (200 mM NaCl)-treated rice improved the plant growth; the effect was more pronounced in the salt-sensitive cultivar (Khao Dawk Mali 105), as compared to the salt-tolerant variety (Pokkali). Such tolerance was related to reduction in Na



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to K ratio and stimulated activity of the antioxidative enzyme, ascorbate peroxidase, particularly in the salt-sensitive rice cultivar (Nounjan and Theerakulpisut 2012). Garcia *et al.* (1997) reported reduced trehalose accumulation in rice roots, 3 days post salt treatment. External applications of low trehalose concentrations (up to 5 mM) reduced Na accumulation and growth inhibition, while higher concentrations (10 mM) preserved the root integrity, prevented the chlorophyll damages in leaf blades, and moderated the expression of the osmotic stress-responsive *salt* gene. However, trehalose did not prevent the accumulation of salt in plant cells. Actually, the low accumulation of endogenous trehalose upon salt stress (7 µg/100 mg fresh weight on day 3 during 1 M NaCl treatment) questions its role as osmoprotectant. It is uncertain whether the sugar remains stable for longer periods of time, since no data were available after 3 days of stress exposure. Trehalose application modulated the expression of abscisic acid (ABA)-metabolism related genes like *NCED1*, *NCED2*, *CYP707A1*, and *CYP707A2* for increasing the ABA content to alleviate salt stress (Feng *et al.*, 2019). El-Bashiti *et al.* (2005) observed accumulation of trehalose in wheat cultivars during salt and drought stress with sharp increase in TPS activity, especially in the roots during drought stress. Transgenic tomato plants, overexpressing *TPS1* during drought, salt and oxidative stress, showed improved tolerance than the wild type, suggesting that stress tolerance could be linked to carbohydrate alterations produced by trehalose biosynthesis. Dehydration stress in tomatoes was largely overcome due to the overexpression of *TPS1* gene, which allowed better vegetative and floral development. Trehalose-synthesising tomatoes, during salinity, also showed improved stress tolerance. Such observations indicated the feasibility of engineering tomato for increased tolerance to abiotic stress, without decreasing the productivity, under stress conditions, through enhanced trehalose biosynthesis -

(Cortina and Culianez-Macia`2005). Enhanced tolerance to salinity, drought and cold stresses in rice, without compromised plant growth, was observed due to higher trehalose production, when *TPS* and *TPP* genes were overexpressed under *ubiquitin (Ubi1)* promoter from maize (Jang *et al.*, 2003). Foliar treatment with trehalose (10 mM and 50 mM) during salinity stress (6.25 dS/m) proved to be effective in enhancing the growth parameters and membrane stability and significantly decreasing the lipid peroxidation, hydrogen peroxide contents, and lipoxygenase (LOX) activity due to increases in total phenolic contents and increased accumulations of glucose, sucrose and trehalose (Sadak 2019). Adding exogenous trehalose (30 mM) to the culture media of liquid-grown seeds of *Arabidopsis thaliana* provoked a transient increase in trehalose concentration (up to 300 mg/g fresh weight after 12 h, below 20 mg/g fresh weight after 24 h) and induced the expression of genes involved in detoxification and stress response within 6-12 h, as well as the synthesis of related proteins (Bae *et al.*, 2005). *Arabidopsis thaliana* AtTPPD is a chloroplast-localized trehalose-6-phosphate phosphatase enzyme. Plants deficient in AtTPPD were sensitive, whereas plants overexpressing AtTPPD were more tolerant to salinity stress (Krasensky *et al.*, 2014). ROS accumulation, ionic imbalance and programmed cell death (PCD) were significantly overcome in *Arabidopsis* when trehalose was applied exogenously at 0.5-5 mM concentrations during salt (150-250 mM NaCl) stress (Yang *et al.*, 2014). Trehalose also participates in the conservation of ion pumps, enabling effluxing out of sodium from the chloroplast. During salinity, the level of trehalose in plant organs could be equally regulated by degradation. Trehalose accumulates in the nodules of *Medicago truncatula* and the *trehalase* gene (*MtTRE1*) is repressed, suggesting that the increase in the trehalose level results, at least partially, from *MtTRE1* transcriptional repression (Lopez *et al.*, 2008).

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## 5.2. DROUGHT STRESS

During dehydration or freezing, membrane destabilization occurs due to lipid phase transitions and vesicle fusion. Even minute levels of trehalose can inhibit fusion of membrane vesicles as well as can lower the phase transition temperature of desiccated lipids during drought stress. Thus, the lipids are well maintained in a liquid state in presence of trehalose even during water scarcity. Trehalose remains highly hydrated even during drought or cold stress. Trehalose molecules replace surface-bound water, normally associated with biological structures, thereby stabilizing dry proteins and biological membranes. Owing to high hydration potential of trehalose, the polar groups of proteins and phosphate groups of membranes form hydrogen bonding with hydroxyl groups of trehalose, thereby stabilizing the dry biological membranes and proteins (Kawai *et al.*, 1992). Vitrification constitutes the second mechanism by which trehalose confers protection against desiccation. In this case, trehalose tends to form a protective hygroscopic glass-like structure, within which trehalose is extremely stable both at high temperature and when completely desiccated. The glassy state of trehalose is more stable than that of other disaccharides. This is due to the non-reducing character of trehalose and also because of the fact that addition of a small volume of water may form trehalose dihydrate on the outer surface of the glass (Richards *et al.*, 2002). A study of trehalose contents in the drought-tolerant crops cassava (*Manihot esculenta*), *Jatropha curcas*, and castor bean (*Ricinus communis*) showed far higher levels of trehalose (up to 3 mmol trehalose per g fresh weight), particularly in the leaves (Han *et al.*, 2016). Exogenous application of trehalose induced tolerance to water deficit in maize. Foliar treatment of trehalose with different concentrations (2.5, 5 and 7.5 mM) could alleviate the adverse effects of drought stress and increased significantly the growth parameters, yield quantity and quality of the -

two varieties of flax plant via enhancing indole acetic acid (IAA), photosynthetic pigments, total phenolics, total soluble sugars, free amino acids, proline and antioxidant enzyme systems, as compared with their corresponding untreated controls. 5 mM trehalose applied in the form of foliar treatment appeared to be the most effective concentration (Sadak *et al.*, 2019). Targeted overexpression of TPP to developing maize ears improved the allocation of carbon to the ear during water deficit (Ali and Ashraf, 2011; Nuccio *et al.*, 2015). Recently, Joshi *et al.* (2020) generated high-yielding transgenic IR-64 rice overexpressing a fusion gene from *E. coli* coding for trehalose-6-phosphate synthase/phosphatase (TPSP) under the control of an ABA-inducible promoter. These transgenic plants could tolerate severe drought (30-35% soil moisture content) and retained higher relative water content, chlorophyll content, K/Na ratio, stomatal conductance and photosynthetic efficiency due to increased trehalose production and other metabolic switches like changes in the levels of sugars, amino acids and organic acids. In case of *Arabidopsis thaliana*, the loss-of-function mutation of *TPPF1*, a member of the *TPP* gene family, resulted in a drought-sensitive phenotype with more hydrogen peroxide accumulation. Transgenic plants overexpressing *TPPF* showed increased drought tolerance with less hydrogen peroxide accumulation due to increased contents of trehalose, sucrose, and total soluble sugars, along with stimulated binding of the transcription factor DREB1A with the DRE/CRT of *TPPF* promoter (Lin *et al.*, 2019). In another work by Garg *et al.*, (2002), regulated overexpression of *Escherichia coli* trehalose biosynthetic genes (*otsA* and *otsB*) as a fusion gene led to the generation of transgenic rice, tolerant to abiotic stresses like salinity, drought, and low temperature due to increased trehalose levels that correlated with higher soluble carbohydrate levels and an elevated capacity for photosynthesis. This suggested the role of trehalose in modulating sugar sensing and -

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carbohydrate metabolism. The endogenous increase in trehalose level (3 mg/g fresh weight) in cassava plants under water-deficit conditions was reported by Han *et al.* (2016). Ibrahim and Abdellatif (2016) observed that exogenous application (10 mM as a foliar spray) of trehalose induced drought-stress tolerance in wheat plants. Drought tolerance in bananas was associated with exogenous application (foliar application at 20, 60 and 100 mM) of trehalose (Said *et al.*, 2015). Foliar spray of trehalose at 5 mM and 30 mM concentrations also improved drought tolerance in *Brassica* (Alam *et al.*, 2014), and maize (Ali and Ashraf, 2011), respectively. Photosynthesis and water retention capacity were also increased in maize.

### 5.3. LOW AND HIGH-TEMPERATURE STRESS

The expression of *OsTPP1* and *OsTPP2* genes were transiently induced during cold, salt and drought stress in rice and also triggered by external application of ABA (50  $\mu$ M). Transient enhancement in trehalose level, synchronized with the changes in glucose and fructose levels, was observed during chilling stress (Pramanik and Imai 2005). AtTPS5 in *Arabidopsis* interacts with MBF1c, a transcriptional activator that is a key regulator of thermotolerance (Suzuki and Bird 2008). *Arabidopsis* plants engineered with yeast *TPS1* gene under the control of *CaMV 35S* promoter or yeast *TPS1-TPS2* gene fusion under the control of a stress-regulated promoter showed trehalose accumulation at lower levels, along with tolerance towards freezing and heat stress. Low-temperature stress tolerance in chickpea was associated with high accumulation (2 mg/g fresh weight) of trehalose (Farooq *et al.*, 2017). Exogenous application of trehalose (0, 10, 15, 20, and 25 mM) as foliar sprays increased tolerance against heat stress in maize plants by decreasing malondialdehyde levels and ion leakage (Li *et al.*, 2014). Foliar spraying of trehalose (50 mmol) improved heat tolerance

in wheat plants, and strengthened the defense mechanism against oxidative stress. In *A. thaliana*, the trehalose level was doubled after 4 h of heat stress (40°C), while increased 8.0-fold, 4 days after cold exposure (4°C) (Kaplan *et al.*, 2004).

### 5.4. METAL STRESS

Foliar spray of duckweed with trehalose (0.5, 1, 2 and 5 mM) alleviated cadmium stress by reducing malondialdehyde and Cd accumulation and improving the activity of superoxide dismutase, catalase and ascorbate peroxidase (Duman *et al.*, 2011). Pre-treatment with trehalose (10 mM) alleviated copper toxicity in rice by increasing the concentration of ascorbate, proline, and antioxidants including glutathione, and decreased malondialdehyde and ROS accumulation, thereby limiting Cu absorption (Mostofa *et al.*, 2015a, b).

## 6. CONCLUSION AND FUTURE PERSPECTIVES

Being present in extremely small quantities, the disaccharide trehalose plays a significant role as an important osmoprotectant in controlling metabolic adjustments linked to osmotic stress tolerance. Trehalose accumulation and mobilization in response to different stress conditions are of utmost ecological relevance. It is noteworthy that externally applied trehalose may undergo at least partial degradation by the apoplastic trehalase enzyme (Almeida *et al.*, 2007). The protective roles of trehalose should be carefully investigated, since it is possible that trehalose might not play the leading role, but might act more as an elicitor, inducing specific signal transduction pathways and also triggering the endogenous trehalose biosynthesis pathway. With further advancements in high-throughput techniques, the complex regulatory network of trehalose metabolic pathways during normal and stress conditions will be better deciphered in the near future. Genetic engineering to -

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manipulate trehalose levels in plants via overexpression of plant-derived genes controlling trehalose biosynthesis have already shown encouraging results (Paul *et al.* 2008). An alternative strategy for trehalose accumulation appears to be the blocking of trehalase, the enzyme responsible for trehalose breakdown. High level of accumulation of trehalose is also possible through the use of trehalase inhibitors. However, there is still some room for further research with regard to the generation of abiotic stress tolerance in field conditions. Any claim that trehalose accumulation can enhance stress tolerance remains useless if its role cannot be translated in crop plants growing in the field. Field-evaluation of trehalose-accumulating transgenic plants has not yet been carried out. Moreover, there is a dearth of information about trehalose metabolism in the whole context of sugar metabolism. The knowledge of individual trehalose biosynthesis genes will help, not only in understanding the precise role of trehalose in abiotic stress, but also the overall sugar signaling mechanism. The specific signaling mechanism carried out by diverse microbial species in trehalose-induced abiotic stress response in plants requires further understanding. An exhaustive screening of plant varieties and breeding lines, having higher trehalose content during stress is quintessential. It is also imperative to screen the strains of rhizobia and arbuscular mycorrhizal fungi as starter cultures for breeding new lines, producing higher trehalose levels, and improving stress tolerance (McIntyre *et al.* 2007). Thus, there is scope for future research to exploit, in a much better way, the role of trehalose as a compatible solute to further strengthen plant tolerance and defense against diverse osmotic stresses.

## REFERENCES

Alam, M.M., Nahar, K., Hasanuzzaman, M., and Fujita, M. (2014). Trehalose-induced drought -

stress tolerance: a comparative study among different *Brassica* species. *Plant Omics J* 7, 271-283.

Ali, Q., and Ashraf, M. (2011). Induction of drought tolerance in maize (*Zea mays* L.) due to exogenous application of trehalose: growth, photosynthesis, water relations and oxidative defence mechanism. *Journal of Agronomy and Crop Science*, 197(4), 258-271.

Almeida, A. M., Cardoso, L. A., Santos, D. M., Torné, J. M., and Fevereiro, P. S. (2007). Trehalose and its applications in plant biotechnology. *In Vitro Cellular & Developmental Biology-Plant*, 43(3), 167-177.

Bae, H., Herman, E., and Sicher, R. (2005). Exogenous trehalose promotes non-structural carbohydrate accumulation and induces chemical detoxification and stress response proteins in *Arabidopsis thaliana* grown in liquid culture. *Plant Science*, 168(5), 1293-1301.

Cassells, A. C., and Curry, R. F. (2001). Oxidative stress and physiological, epigenetic and genetic variability in plant tissue culture: implications for micropropagators and genetic engineers. *Plant Cell, Tissue and Organ Culture*, 64(2), 145-157.

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

Duman, F., Aksoy, A., Aydin, Z., and Temizgul, R. (2011). Effects of exogenous glycinebetaine and trehalose on cadmium accumulation and biological responses of an aquatic plant (*Lemna gibba* L.). *Water, Air, & Soil Pollution*, 217(1), 545-556.

El-Bashiti, T., Hamamcı, H., Öktem, H. A., and Yücel, M. (2005). Biochemical analysis of -



- Zhao, J., Wang, M., Chen, X., and Kang, Z. (2016). Role of alternate hosts in epidemiology and pathogen variation of cereal rusts. *Annual Review of Phytopathology*, 54, 207-228.
- Farooq, M., Hussain, M., Nawaz, A., Lee, D. J., Alghamdi, S. S., and Siddique, K. H. (2017). Seed priming improves chilling tolerance in chickpea by modulating germination metabolism, trehalose accumulation and carbon assimilation. *Plant Physiol. Biochem.* 111, 274-283.
- Feng, Y., Chen, X., He, Y., Kou, X., and Xue, Z. (2019). Effects of exogenous trehalose on the metabolism of sugar and abscisic acid in tomato seedlings under salt stress. *Transactions of Tianjin University*, 25(5), 451-471.
- Garcia, A. B., Engler, J. D. A., Iyer, S., Gerats, T., Van Montagu, M., and Caplan, A. B. (1997). Effects of osmoprotectants upon NaCl stress in rice. *Plant physiology*, 115(1), 159-169.
- Garg, A. K., Kim, J. K., Owens, T. G., Ranwala, A. P., Do Choi, Y., Kochian, L. V., and Wu, R. J. (2002). Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses. *Proceedings of the National Academy of Sciences*, 99(25), 15898-15903.
- Han, B., Fu, L., Zhang, D., He, X., Chen, Q., Peng, M., and Zhang, J. (2016). Interspecies and intraspecies analysis of trehalose contents and the biosynthesis pathway gene family reveals crucial roles of trehalose in osmotic-stress tolerance in cassava. *International Journal of Molecular Sciences*, 17(7), 1077.
- Henry, C., Bledsoe, S. W., Griffiths, C. A., Kollman, A., Paul, M. J., Sakr, S., and Lagrimini, L. M. (2015). Differential role for trehalose metabolism in salt-stressed maize. *Plant physiology*, 169(2), 1072-1089.
- Ibrahim, H.A., and Abdellati, Y.M.R. (2016). Effect of maltose and trehalose on growth, yield and some biochemical components of wheat plant under water stress. *Annals of Agricultural Sciences* 61(2), 267-274.
- Iordachescu, M., and Imai, R. (2008). Trehalose biosynthesis in response to abiotic stresses. *Journal of Integrative Plant Biology*, 50(10), 1223-1229.
- Jang, I. C., Oh, S. J., Seo, J. S., Choi, W. B., Song, S. I., Kim, C. H., ... and Kim, J. K. (2003). Expression of a bifunctional fusion of the *Escherichia coli* genes for trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase in transgenic rice plants increases trehalose accumulation and abiotic stress tolerance without stunting growth. *Plant Physiology*, 131(2), 516-524.
- Joshi, R., Sahoo, K. K., Singh, A. K., Anwar, K., Pundir, P., Gautam, R. K., ... and Singla-Pareek, S. L. (2020). Enhancing trehalose biosynthesis improves yield potential in marker-free transgenic rice under drought, saline, and sodic conditions. *Journal of Experimental Botany*, 71(2), 653-668.
- Kaplan, F., Kopka, J., Haskell, D. W., Zhao, W., Schiller, K. C., Gatzke, N., ... and Guy, C. L. (2004). Exploring the temperature-stress metabolome of *Arabidopsis*. *Plant Physiology*, 136(4), 4159-4168.
- Kawai, H., Sakurai, M., Inoue, Y., Chujo, R., and Kobayashi, S. (1992). Hydration of oligosaccharides: anomalous hydration ability of trehalose. *Cryobiology* 29: 599-606
- Krasensky, J., Broyart, C., Rabanal, F. A., and Jonak, C. (2014). The redox sensitive chloroplast trehalose-6-phosphate phosphatase AtTPPD regulates salt stress tolerance. *Antioxid Redox Signal* 21(9):1289-1304

- Li, Z-G., Luo, L-J., and Zhu L-P. (2014). Involvement of trehalose in hydrogen sulfide donor sodium hydrosulfide-induced the acquisition of heat tolerance in maize (*Zea mays* L.) seedlings. *Botanical Studies* 55, 20.
- Lin, Q., Yang, J., Wang, Q., Zhu, H., Chen, Z., Dao, Y., and Wang, K. (2019). Overexpression of the trehalose-6-phosphate phosphatase family gene AtTPPF improves the drought tolerance of *Arabidopsis thaliana*. *BMC Plant Biol* 19:381
- López, M., Tejera, N. A., Iribarne, C., Lluch, C., and Herrera-Cervera, J. A. (2008). Trehalose and trehalase in root nodules of *Medicago truncatula* and *Phaseolus vulgaris* in response to salt stress. *Physiologia plantarum*, 134(4), 575-582.
- Lunn, J.E. (2007). Gene families and evolution of trehalose metabolism in plants. *Funct Plant Biol* 34: 550-563
- Luo, Y., Li, W. M., and Wang, W. (2008). Trehalose: protector of antioxidant enzymes or reactive oxygen species scavenger under heat stress? *Environmental and Experimental Botany*, 63(1-3), 378-384.
- McIntyre, H. J., Davies, H., Hore, T. A., Miller, S. H., Dufou, J. P., and Ronson, C. W. (2007). Trehalose biosynthesis in *Rhizobium leguminosarum* by Trifolii and its role in desiccation tolerance. *Appl Environ Microbiol* 73: 3984-3992
- Medrano, H., Escalona, J. M., Bota, J., Gulías, J., and Flexas, J. (2002). Regulation of photosynthesis of C3 plants in response to progressive drought: stomatal conductance as a reference parameter. *Ann Bot* 89: 895-905
- Mostofa, M. G., Hossain, M. A., and Fujita, M. (2015b). Trehalose pretreatment induces salt tolerance in rice (*Oryza sativa* L.) seedlings: oxidative damage and co-induction of antioxidant defense and glyoxalase systems *Protoplasma* 252(2):461-475
- Mostofa, M. G., Hossain, M. A., Fujita, M., and Tran, L. S. P. (2015a) Physiological and biochemical mechanisms associated with trehalose-induced copper-stress tolerance in rice. *Sci Rep* 5:1-16.
- Nounjan, N., and Theerakulpisut, P. (2012). Effects of exogenous proline and trehalose on physiological responses in rice seedlings during salt-stress and after recovery. *Plant Soil Environ* 58 (7): 309-315
- Nuccio, M. L., Wu, J., Mowers, R., Zhou, H. P., Meghji, M., Primavesi, L. F., ... and Lagrimini, L. M. (2015). Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nature Biotechnology*, 33(8), 862-869.
- Paul, M. J., Primavesi, L. F., Jhurreea, D., and Zhang, Y. (2008). Trehalose Metabolism and Signaling. *Annu Rev Plant Biol* 59:417-441
- Phillips, J. R., Oliver, M. J., and Bartels, D. (2002). Molecular Genetics of Desiccation and Tolerant Systems. In: *Desiccation and survival in plants: drying without dying* (Ed. Black, M., Pritchard, H.W.), CABI Publishers, Wallingford, UK, pp. 319-341.
- Pramanik, M. H. R., and Imai, R. (2005). Functional identification of a trehalose 6-phosphate phosphatase gene that is involved in transient induction of trehalose biosynthesis during chilling stress in rice. *Plant Molecular Biology*, 58(6), 751-762.
- Richards, A. B., Krakowka, S., Dexter, L. B., Schmid, H., Wolterbeek, A. P. M., Waalkens-Berendsen, D. H., ... and Kurimoto, M. (2002). Trehalose: a review of properties, history of use and human tolerance, and results of multiple safety studies. *Food and Chemical Toxicology*.

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40(7), 871-898.

Roychoudhury, A., and Chakraborty, M. (2013). Biochemical and Molecular Basis of Varietal Difference in Plant Salt Tolerance. Annual Review & Research in Biology 3: 422-454

Sadak, M. S. (2019). Physiological role of trehalose on enhancing salinity tolerance of wheat plant. Bulletin of the National Research Centre 43:53.

Sadak, M. S., Bakry, A. B., and M., H. Taha. (2019). Physiological role of trehalose on growth, some biochemical aspects and yield of two flax varieties grown under drought stress. Plant Archives 19: Supplement 2, 215-225

Said, E.M., Mahmoud, R. A., Al-Akshar, R., and Safwat, G. (2015). Drought stress tolerance and enhancement of banana plantlets in vitro. Austin J. Biotechnol. Bioeng. 2(2): 1040-1046.

Sussich, F., Skopec, C., Brady, J., and Cesàro, A. (2001). Reversible dehydration of trehalose and anhydrobiosis: from solution state to an exotic crystal? Carbohydr Res 334(3):165-176

Suzuki, N., Bajad, S., Shuman, J., Shulaev, V., and Mittler, R. (2008). The Transcriptional Coactivator MBF1c Is a Key Regulator of Thermotolerance in *Arabidopsis thaliana*. The Journal of Biological Chemistry 283: 9269-9275

Vandesteene, L., López-Galvis, L., Vanneste, K., Feil, R., Maere, S., Lammens, W., ... and Van Dijck, P. (2012). Expansive evolution of the trehalose-6-phosphate phosphatase gene family in *Arabidopsis*. Plant Physiology, 160(2), 884-896.

Wingler, A. (2002). The function of trehalose biosynthesis in plants. Phytochemistry 60: 437-440

Wingler, A., and Roitsch, T. (2008). Metabolic regulation of leaf senescence: interactions of -

sugar signaling with biotic and abiotic stress responses. Plant Biol 10: 50-62

Yang, L., Zhao, X., Zhu, H., Paul, M., Zu, Y., and Tang, Z. (2014). Exogenous trehalose largely alleviates ionic unbalance, ROS burst, and PCD occurrence induced by high salinity in *Arabidopsis* seedlings. Front Plant Sci 5:1-11

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