The Role of Proline, Glycinebetaine, and Trehalose in Stress-Responsive Gene Expression

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1 Introduction

Biosynthesis of osmoprotectant molecules such as proline, glycinebetaine, trehalose, polyols, poliamines, and sugars are among the most common protective mechanisms against stresses which affect the osmotic potential of the cells. Introducing or increasing the expression of genes related to the biosynthesis of osmoprotectant molecules was reported to be promising for accelerating stress tolerance in plants. There has been a huge amount of information regarding the contribution of these solutes to tolerance against drought and any other types of stress that cause osmotic effect; however, we still lack the knowledge on their exact mode of action. For instance, since the first resurrection plant was discovered, high concentration and rapid accumulation of trehalose were demonstrated to be a unique feature of these plants. However, drought-sensitive *Selaginella* sp. accumulated more trehalose than drought-tolerant *Selaginella* sp. (Pampurova and Van Dijck 2014; Bledsoe et al. 2017) which indicated that even in the drought-tolerant plants, the role and contribution of trehalose or other osmoprotectant molecules to stress tolerance was not completely deciphered.

Protective function of proline, glycinebetaine, and trehalose has been known since the 1990s with confirmation from transgenic studies in *A. thaliana*, tobacco, rice, and wheat (Liu and Zhu 1997; Sakamoto and Murata 1998, Bor and Ozdemir 2018). Several crop plants have been genetically engineered for proline-, glycinebetaine-, and trehalose-related genes which were reported to have improved tolerance to several environmental constraints. Among the pioneer investigations, overexpression of proline biosynthetic geneΔ-pyrroline-5-carboxylate synthase in *A. thaliana* and tobacco plants (Liu and Zhu 1997) and overexpression of choline oxidase

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(glycinebetaine biosynthesis-related gene) in rice (Sakamoto and Murata 1998) might be given as examples of which resulted in increased salinity tolerance in relation to proline and glycinebetaine accumulation, respectively. A more recent transgenic approach was reported by Nuccio et al. (2015). Maize plants overexpressing a rice trehalose-6-phosphate phosphatase gene had better yield performance under drought stress conditions at field trials (Nuccio et al. 2015).

The amino acid proline, is reported to be accumulated to high levels when plants encounter different type of stress conditions. Besides its function in growth and development, it acts as an osmoprotectant and redox-buffering agent with an antioxidant characteristic under abiotic stresses (Kishor and Sreenivasulu 2014). On the other hand, high levels of proline have detrimental effects in plant cells leading to cell death; therefore, keeping cellular proline content in balance was reported to be critical for plant survival (Szabados and Savoure 2010; Kishor and Sreenivasulu 2014). The second well-known osmoprotectant molecule, glycinebetaine (GB), is an *N*-methyl-substituted glycine derivative found in microorganisms, animals, and plants such as sugar beet, wheat, and spinach (Sakamoto and Murata 2002; Ahmad et al. 2013). Besides osmotic adjustment capacity, stabilization of macromolecules, protection of membrane integrity, and contribution to regulating reactive oxygen species (ROS) are among the major roles for GB under stress conditions (Chen and Murata 2011; Ahmad et al. 2013). The third and most studied osmoprotectant molecule, trehalose, is a non-reducing sugar which was reported to be responsible for osmoregulation and protection against environmental stresses in different organisms including plants (Houtte et al. 2013). Unlike other osmotic solutes, trehalose concentrations in wild-type and genetically engineered plants were reported to be low, and cellular compartmentalization was important. Therefore, trehalose-mediated improvement in abiotic stress responses was suggested to be related to the activation of stress-responsive genes and transcription factors rather than being as an osmoprotectant molecule (Lunn et al. 2006; Zhang et al. 2009; Houtte et al. 2013).

Contribution of proline, glycinebetaine, and trehalose to stress-responsive gene expression for increased tolerance has been investigated extensively (Table 1). Understanding which genes and especially which transcription factors are up- or downregulated by these molecules would be important not only for a better understanding of stress-coping mechanisms in plants but also for maintenance of better crop performance and yield through manipulation of these genes in cultivated plants. In this chapter, we summarized the latest information regarding the effects of proline, glycinebetaine, and trehalose on the expression of stress-responsive genes in plants. Among these three molecules, only glycinebetaine was reported to be compatible which had no toxic effects even at high levels. Keeping the balance in proline and trehalose contents of the cells need to be tightly regulated. Therefore, for these two molecules, we have given information both for the genes that they regulate and the genes which are related to their biosynthesis and hydrolysis.

Table 1 Proline-, glycinebetaine-, and trehalose-induced transcription factors and their roles in plant development and stress responses

	Transcription Factors	Function in Plants
Proline	bZIP11 bZIP53 bZIP44 bZIP2 MYBCORE WRKY MYC2 AP2/ERF TSRF1 JERF1 JERF3 SpERF1 DREB21 ERF71	ProDH-related sugar signaling (Verslues and Sharma 2010) Proline catabolism (Satoh et al. 2004) Upregulation of P5CS1 and P5CS2 (Su et al. 2011) Regulation of P5CS (Gao et al.2008; Zhang et al. 2013; Yang et al. 2016) ABA and proline signaling (Li et al. 2018)
Glycinebetaine	DREB2A NAC5 WRKY bZIP53 IAA9 bHLH-FRO2 NDPK2	CMO gene expression (Khattab et al. 2014) BADH gene expression (Liang et al. 2017). Chilling tolerance (Einset et al. 2007) Fruit development (Zhang et al. 2019) Dehydration response (Ahmad et al. 2013)
Trehalose	bZIP11 bZIP12 bZIP53 bZIP44 bZIP2 WRINKL1 HY5 ABI5 EEL KNOTTED1 LEAFY WUSCHEL ATAF1 MYBS1 CIPK15 SUSIBA2 WRYK6 AGL4 RNA polymerase σ 70-type initiation factor JUMANJI	Fine-tuning of carbon and nitrogen metabolism (Garapati et al. 2015; Chen et al. 2016; Laser and Weiste 2018) Development and growth responses (Garapati et al. 2015; Tsai and Gazzattini 2014; Chen et al. 2016; Laser and Weiste 2018) Low energy signaling (Garapati et al. 2015; Laser and Weiste 2018) Sugar signaling (Sun et al. 2003; Bae et al. 2005; Kretzshmer et al. 2015; Zhai et al. 2018) Fatty acid signaling (Zhai et al. 2018) ABA signaling (Bae et al. 2005; Tsai and Gazzattini 2014) Meristem identity function (Tsai and Gazzattini 2014; Coelho et al. 2018) Autophagy (Garapati et al. 2015) Anaerobic germination tolerance (Kretzschmer et al. 2015) Starch mobilization (Kretzschmer et al. 2015) Leaf senescence (Bae et al. 2005) Floral morphogenesis (Bae et al. 2005; Coelho et al. 2018) Plastid genome transcription, chromatin modification, transcriptional repression (Kondrak et al. 2012) Floral transition and shoot development (Coelho et al. 2018)

2 Proline

Proline is an amino acid which serves as an osmoprotectant and protective molecule at drought, salt, and other stress conditions. Although the accumulation of proline is a well-known response in stress-tolerant plants, the mode of action is still unclear (Ghars et al. 2012). Different roles have been attributed to proline such as scavenging of the hydroxyl radical, interacting with enzymes responsible for stress tolerance, protecting protein structure and enzyme activity, maintaining pH and redox balance, and supplementation of carbon, nitrogen, and energy (Hare et al. 1999; Szabados and Savoure 2010; Ghars et al. 2012). Biosynthesis of proline occurs in two different pathways which include glutamate and ornithine. Glutamate pathway is the predominant route with two steps: first, glutamate is phosphorylated and reduced to Δ-pyrroline-5-carboxylate (P5C) by PC5 synthase enzyme (P5CS), and then it is reduced to proline by P5C reductase (P5CR) enzyme (Kim and Nam 2013). The second pathway is related to the activity of ornithine δ-aminotransferase (OAT) which also produces P5C that contributes to proline (Szabados and Savoure 2010; Liang et al. 2013).

Recent findings have proved that proline has a significant role in osmotic adjustment, stabilization of cellular structures, and protection of photosynthetic apparatus. The translation start site of proline metabolism-related genes has putative *cis*-regulatory elements (CREs) site which interacts with several general transcription factors such as HD-HOX, AP2/EREBP, MYB, WRKY, and bZIP (Fichman et al. 2015). Therefore, regulation of proline content might be important not only for proline biosynthesis and catabolism but also for the control of the expression of different stress-responsive transcription factors and genes (Table 1). Accordingly, proline inhibited stomatal closure while promoted Ca^{+2} uptake in contrast to other amino acids such as histidine, methionine, aspartic acid, glutamic acid, and alanine (Rai and Sharma.1991; Rana and Rai 1996; Hayat et al. 2012). However, high levels of proline lead to impairment in the destabilization of DNA helix and susceptibility to S1 nuclease activity (Rajendrakumar et al. 1997; Szabados and Savoure 2010).

To date, all of the defined stress response and tolerance processes in plants are regulated by complex signaling networks and have multigenic characteristics. Since proline is a common stress-responsive and adaptive molecule, it would be a good candidate for manipulating stress responses and tolerance mechanisms. Understanding which genes and especially which transcription factors are induced by proline will be beneficial for providing solutions to agricultural practices under changing environmental conditions. Enhanced proline accumulation at stress conditions was reported to be parallel to increased transcriptional activation of P5CS and P5CR genes while ornithine route seemed to have a less impact (Fig. 1). There are two P5CS enzymes in *A. thaliana*; one is chloroplastic and the other is cytosolic (Liang et al. 2013). P5CS1 is reported to be responsible for stress-induced proline biosynthesis, while the second one is required for developmental processes (Strizhov et al. 1997; Mattioli et al. 2009). P5CS1 transcription and proline accumulation are induced by cooperation of Ca^{+2}-dependent calmodulin with MYB2

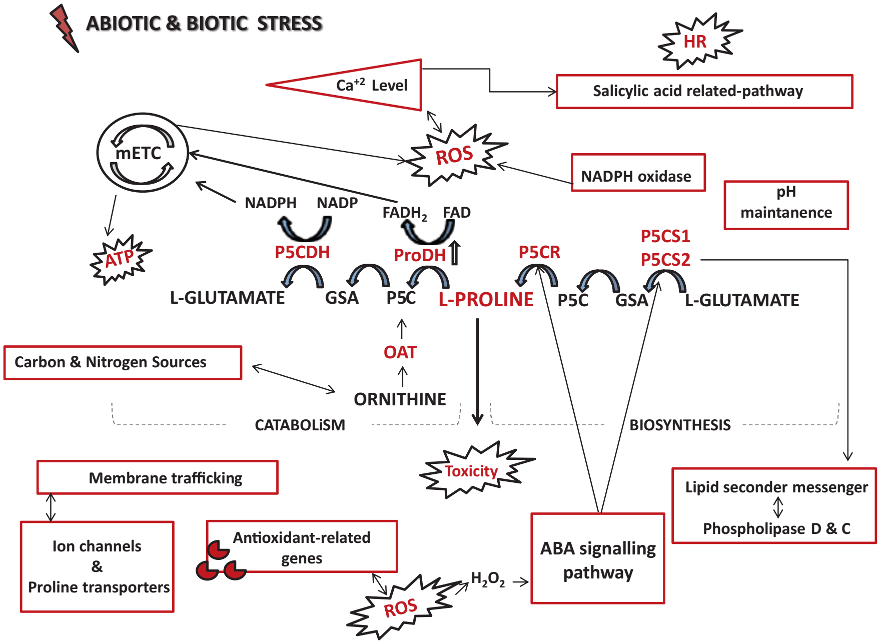

Fig. 1 Model of proline-related and proline-regulated processes in the plant metabolism. Proline biosynthesis and catabolism-related enzymes contribute to different physiological processes in development and stress responses. P5CS pyrroline-5-carboxylate synthase, P5CDH pyrroline-5-carboxylate dehydrogenase, P5CR pyrroline-5-carboxylate reductase, ProDH proline dehydrogenase, HR hypersensitive response

transcription factor (Yoo et al. 2005). P5CS2 affected development of reproductive organs, and this was proposed to be related to flowering regulator CONSTANS genes (Samach et al. 2000).

Expression level of P5CS, which encodes the enzyme that catalyzes the rate-limiting step in proline biosynthesis, was increased in response to salinity and drought. In addition, transcript level of P5CR encoding gene was also found out to be upregulated in the leaves of *A. thaliana* and in the roots of soybean and pea under osmotic stress (Delauney and Verma 1990; Williamson and Slocum 1992; Verbruggen et al. 1993; Liang et al. 2013). Transcription of P5CS is tightly regulated by proline levels by feedback inhibition (Zhang et al. 1995; Liang et al. 2013). On the other hand, proline levels are determined by the activities of proline dehydrogenase (proDH), P5CR, and pyrroline-5-carboxylate dehydrogenase (P5CDH) which are transcriptionally regulated and alter ROS-mediated signaling processes (Liang et al. 2013).

The analyses of the promoter regions of several stress marker genes by bioinformatics tools have revealed that many of them had at least one proline-responsive element (PRE) in their promoter regions although their expressions were not affected by proline (Sharma and Verslues 2010). For example, a bZIP transcription factor which has a proline binding element was related to the induction of proDH by exogenous proline treatment. However, at stress conditions, the presence of neither proline nor ABA did not alter proDH expression (Sharma and Verslues 2010). Accordingly, bHLH-related two G-BOX motifs were found at *Oryza sativa* P5CS promoters. Overexpression of bHLH leads to enhanced osmotic and cold stress tolerance with increased proline levels (Liu et al. 2014, 2015; Jin et al. 2016). Similarly, OsP5CS2 and OsP5CR promoters had CACG NAC-core motif in their promoter regions, and overexpression of NAC genes increased drought and salt tolerance in relation to proline accumulation (Liu et al. 2013; Hong et al. 2016). Moreover, P5CS expression can be negatively regulated by different proteins such as annexins. These proteins are light-dependent Ca^{+2} and phospholipid binding proteins, and annexin mutants have increased P5CS expression which leads to drought and salt tolerance (Huh et al. 2010).

Exogenous treatment of plants with proline or proline precursors affected the expression of different stress-related genes which resulted in tolerance against not only to abiotic stresses but to biotic stress. A recent confirmation was reported by Wang et al. (2017). Amino acid permease 1 (AAP1)-mediated proline uptake has improved salt stress tolerance in *A. thaliana* (Wang et al. 2017). When treated with the precursor of proline, P5C increased HR-like responses against pathogens by the activation of AvrB and AvrRpt2 genes (Funck et al. 2008). Chen et al. (2011) reported that proline affected calcium-mediated production of H_2O_2 and increased NDR1 expression-activated SA signaling pathway which lead to pathogenesis-related (PR) gene expression. In abiotic stress responses, exogenous proline was reported to be also responsible for protection of plants; however, there are controversial results which indicated the negative impact of proline on growth and metabolic processes. *A. thaliana* plants treated with proline at salt stress conditions had growth inhibition and accelerated senescence (Yamada et al. 2005). Antioxidant enzymes Cu/ZnSOD and MnSOD encoding genes were upregulated in rice plants when treated with proline under salinity; however, in the absence of NaCl, the expression of these genes was suppressed (Nounjan et al. 2012). In the light of these findings, regulation of biosynthesis and catabolism of proline within the plant cells seemed to be more effective than exogenous proline treatment.

3 Glycinebetaine

Glycinebetaine (GB) is the most common and best-known compatible solute that is found in several organisms including bacteria and plants (Castiglioni et al. 2018). GB is biosynthesized by two pathways; the most common route is via the oxidation of choline, while the other one is a bacteria-specific glycine methylation pathway (Fig. 2). In plants, choline is oxidized to betaine aldehyde by a ferredoxin-dependent choline monooxygenase (CMO) which is then converted to GB by the activity of betaine aldehyde dehydrogenase (BADH) (Nuccio et al. 1998; Ahmad et al. 2013). Plants are divided into two classes: GB accumulators and non-accumulators

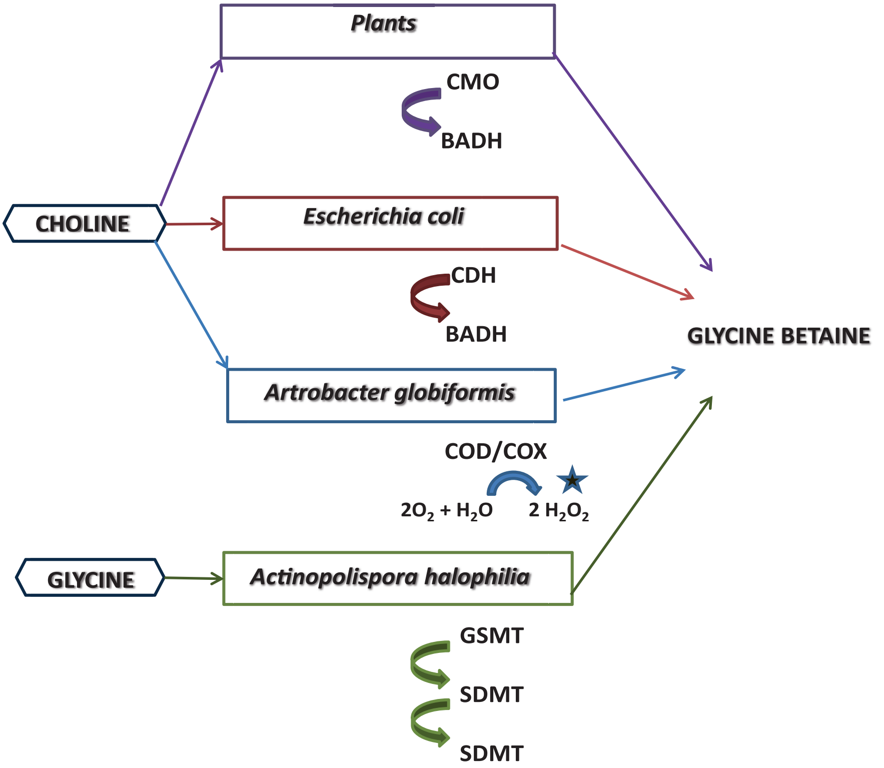

Fig. 2 Biosynthesis of glycinebetaine from different precursor molecules in different organisms. CMO choline monooxygenase, COD choline oxidase, BADH betaine aldehyde dehydrogenase, CDH choline dehydrogenase, GSMT glycine sarcosine methyltransferase, SDMT sarcosine dimethylglycine methyltransferase

according to their ability for GB biosynthesis. Accumulator plants such as sugar beet, spinach, and mangrove have the ability to well-adapt to drought and salinity conditions (Bor et al. 2003; Ahmad et al. 2013). Under osmotic stress-imposing conditions, even exogenous GB treatment was found out to have a protective role in plants; therefore, engineering non-accumulator plants for genes related to GB biosynthesis was proposed to be important for increasing yield of crop plants (Castiglioni et al. 2018; Bor and Ozdemir 2018).

Crop plants such as rice, carrot, tomato, and potato are non-accumulators of GB, and in the recent years, transgenic studies for GB were accelerated for increasing crop biomass and yield (Ahmad et al. 2013). In GB-synthesizing transgenic rice plants, more than 165 genes were upregulated and 76 genes were downregulated (Kathuria et al. 2009; Ahmad et al. 2013). Within the upregulated genes, 50 of them were related to the alleviation of various stress effects, and 115 of them were involved in regulation of gene expression, membrane transport, growth and development, signal transduction, and metabolism (Kathuria et al. 2009). GB functions at important processes such as osmoprotection, destabilization of DNA, refolding and thermal stabilization of proteins, maintenance of membrane integrity, and protection of enzymes (rubisco, rubisco activase, malate dehydrogenase, etc.) which are all remarkable components of plant tolerance to abiotic stresses (Chen and Murata 2011; Ahmad et al. 2013). Wei et al. (2017) reported that the activity of ion channels and transporters was regulated by GB which provided high potassium and low sodium levels conferring to salt tolerance in transgenic tomato plants. On the other hand, codA-and BADH-transgenic tomato plants had differential regulation of cell wall invertase, protein kinase, sucrose transporter, cyclin-dependent kinase, auxin transcription factor, and miniature zinc-finger protein (IMA) encoding genes which might be responsible for flower and fruit development (Wei et al. 2017). A generalized scheme for the processes and contribution of these genes to overall plant metabolism and stress responses was given in Fig. 3.

In the case of stress-coping mechanisms, the possibility of different interactions between GB and stress-related metabolites was proposed (Fig. 3). For instance, maize plants treated with a nitric oxide (NO) inhibitor (*N*ω-nitro-L-arginine methyl ester; L-NAME) had reduced BADH gene expression which leads to low GB levels (Phillips et al. 2018). NO is known to contribute to ROS detoxification, regulation of antioxidant enzymes, and compatible solutes during abiotic and biotic stresses

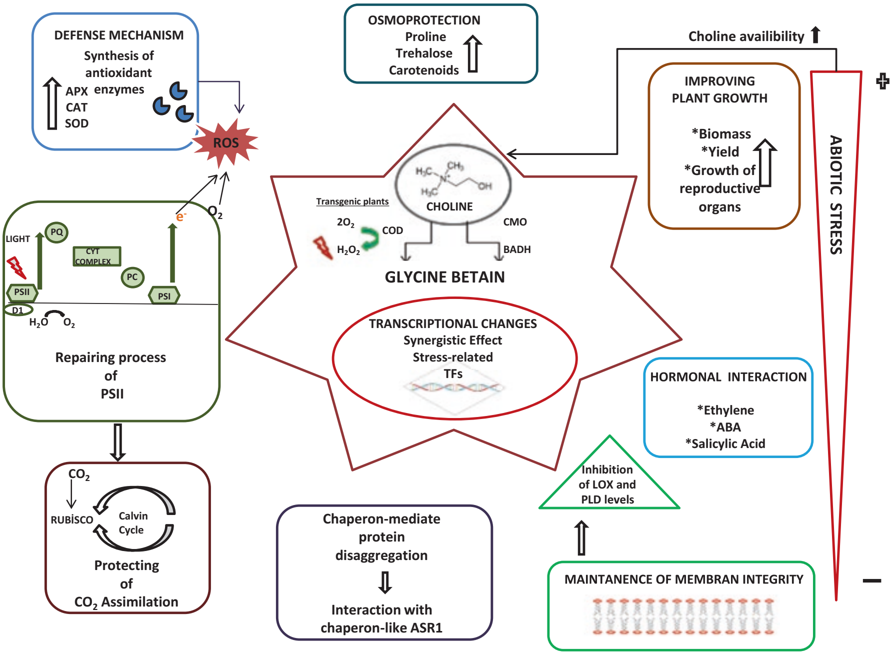

Fig. 3 The direct and indirect contribution of glycinebetaine metabolism to stress-coping processes in plants. SOD superoxide dismutase, CAT catalase, APX ascorbate peroxidase, CMO choline monooxygenase, COD choline oxidase, BADH betaine aldehyde dehydrogenase, ABA abscisic acid, PLD phospholipase D, LOX lipoxygenase

(Uchida et al. 2002; Zhang et al. 2006; Guo et al. 2009; Phillips et al. 2018). Several metabolic routes are affected by GB accumulation and/or exogenous GB treatment. As indicated before, GB served not only by protecting proteins and enzymes but also by triggering transcription of stress-responsive genes or their transcription factors. Antioxidant enzymes, fatty acid metabolism-related enzymes such as lipoxygenase (LOX) and phospholipase-D (PLD) are among the most important enzymes which are regulated by GB levels.

4 Trehalose

Trehalose is synthesized from uridine diphosphate glucose (UDP-Glc) and glucose-6-phosphate (G6P) via trehalose-6-phosphate synthase (TPS) enzyme which dephosphorylated to a more effective form, trehalose-6-phosphate (T6P) by the activity of trehalose-6-phosphate phosphatase (Figueroa et al. 2016). In *A. thaliana*, T6P proposed to act as a signaling molecule in the regulation of sucrose level in order to provide optimal level of sucrose within the cell (Fig. 4). *Oryza sativa* TPS overexpressing lines, trehalose, and proline levels were highly induced with or without stress treatment. Expression of stress-related genes such as ELIP, HSP70, CRP, DHN6, LEA14A, and WS118 were increased up to twofold in these plants as compared to wild-type plants (Li et al. 2011). Increased level of T6P was related to the activation of nitrate reductase (NR) and phosphoenolpyruvate carboxylase (PEPC) through posttranslational modifications (Figueroa et al. 2016). Protein kinases, protein phosphatases, and other enzymes involved in these modifications were proposed to be the potential targets of T6P (Fig. 4).

Trehalose was reported to serve as a compatible solute for the stabilization of membranes and biomolecules (Fernandez et al. 2010). In plant cells, trehalose is synthesized at low levels as compared to other compatible solutes such as proline, glycinebetaine, mannitol, etc. Hence, its being a common compatible solute is still under debate. High levels of trehalose were detected only in resurrection plants and in specific organs upon stress exposure (Avonce et al. 2004; Schluepmann et al. 2003; Grennan 2007; El-Bashiti et al. 2005; Garg et al. 2002; Fernandez et al. 2010). Since trehalose and T6P levels are usually very low in plants, they were proposed to have regulatory or sensing roles for source-sink relationship. Trehalose pathway might be a facilitator between the cellular compartments via regulation of different transcription factors under different environmental stresses (Table 1 and Fig. 5). T6P was thought to be a negative-feedback regulator for the adjustment of sucrose levels by interaction with SnRK1 (Bledsoe et al. 2017). T6P-sucrose interaction is adjusted according to developmental stage, tissue and cell type, and various environmental factors such as low temperature stress (Figueroa et al. 2016).

Various studies indicated the importance of trehalose metabolism at transcriptional, translational, and posttranslational levels for controlling and regulating stress responses in plants (Table 1). In plant cells, sucrose:T6P ratio affects important metabolic processes in multiple ways via induction or repression of several

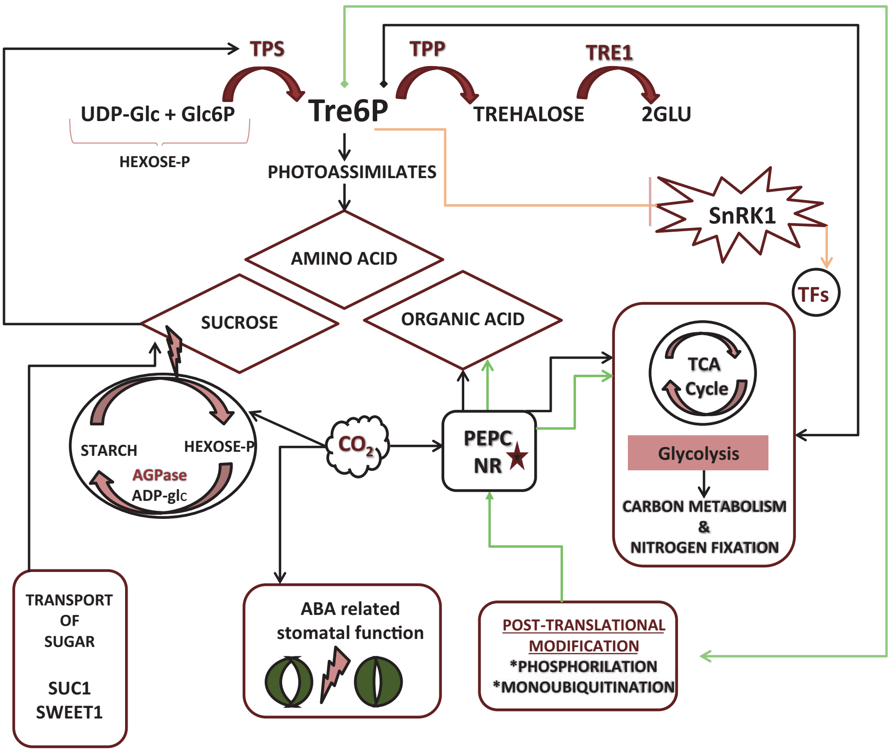

Fig. 4 The interaction of trehalose pathway with different metabolic processes. TPS trehalose phosphate synthase, TPP trehalose phosphate phosphatase, TRE trehalose, T6P trehalose-6-phosphate, ABI4 ABA insensitive 4; Glc-6P glucose-6-phosphate, AGPase ADP-glucose-pyrophosphorylase, PEPC phosphoenolpyruvate carboxylase, ABA abscisic acid, SnRK sucrose non-fermenting receptor kinase, FUS3 mitogen-activated kinase, bZIP11 basic leucine zipper transcription factor 11

stress-responsive transcription factors (Fig. 4). For instance, increased T6P levels resulted in the repression of SnRK1 which is a key transcriptional regulator that responds to carbon and energy supply (Nuccio et al. 2015). Therefore, T6P influences SnRK1-upregulated genes negatively and SnRK1-downregulated genes positively. Another transcription factor bZIP11 which affects the regulation of carbohydrate metabolism is also regulated by T6P. The developmental phase transitions, carbohydrate, and amino acid metabolisms are regulated by bZIPs (Tsai and Gazzarini 2014). Accordingly, it has been suggested that OsTPP7 contributes to anaerobic germination tolerance by modulating local T6P:sucrose ratios in germinating tissues which lead to upregulation of MYBS1 and CIPK15 genes for regulating amylase activation for increased starch mobilization (Kretzshmer et al. 2015).

Trehalase catalyzes the hydrolysis of trehalose into two glucose monomers which was reported to be important for osmotic regulation and stress responses (Lunn 2007; Avonce et al. 2010; Houtte et al. 2013). *A. thaliana* had one trehalase

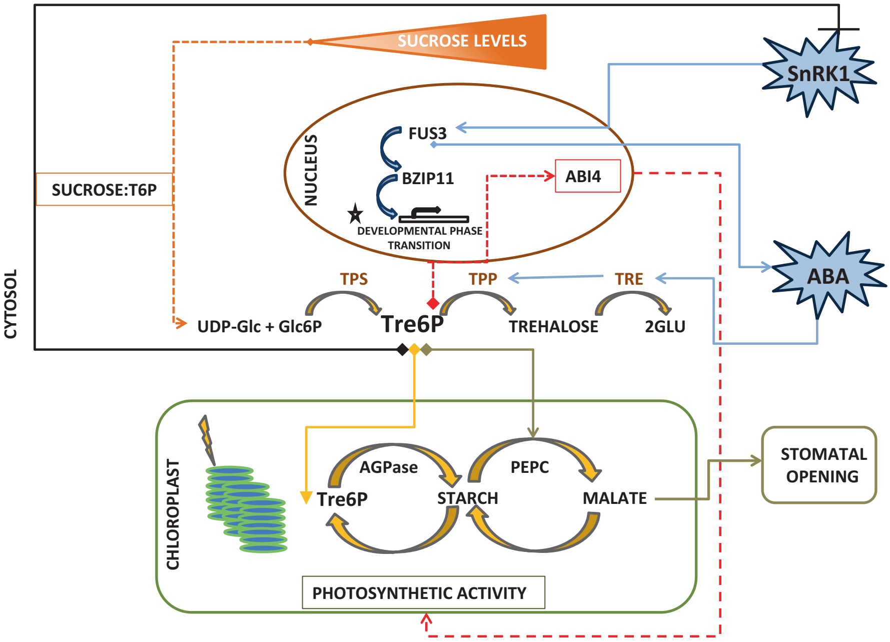

Fig. 5 Impact of T6P-mediated photoassimilates partitioning on the plant metabolism. TPS trehalose phosphate synthase, TPP trehalose phosphate phosphatase, TRE trehalose, T6P trehalose-6-phosphate, SnRK sucrose non-fermenting receptor kinase, ABA abscisic acid, PEPC phosphoenolpyruvate carboxylase, NR nitrate reductase, SUC1 sucrose transporter 1

encoding gene, TRE1, that has a MYB4 binding site in its promoter region (Lunn 2007; Avonce et al. 2010; Houtte et al. 2013). Besides this, a W-box promoter motif was identified in the AtTRE1 promoter for MYB102 and WRKY transcription factors which are known to be involved in ABA signaling at dehydration and osmotic stress conditions (Houtte et al. 2013). Since both MYB4 and MYB102 are members of the R2R3-type MYB family, these transcription factors can induce AtTRE1 expression during developmental processes such as guard cell differentiation (Houtte et al. 2013). Genetic control of trehalase would be a good tool for adjusting endogenous trehalose levels; therefore, drought tolerance might be manipulated by regulation of AtTRE1 (Houtte et al. 2013). Increased trehalase activity affected the sensitivity of guard cells to exogenous ABA treatments; thus, AtTRE1 may be essential for the ABA-induced stoma closure. One confirmation was reported from a study with Attre1-1 and Attre1-2 mutants which were unable to close their stomata in response to the ABA treatments (Houtte et al. 2013). On the other hand, hydrolysis of trehalose would be essential for different developmental processes. AtTRE1 was strongly upregulated during senescence in *A. thaliana* which indicated the contribution of trehalose degradation during programmed cell death (Yamada et al. 2005).

Although upregulation of trehalose biosynthesis and exogenous trehalose treatments both have protective and regulatory functions in various plants such as tomato, tobacco, and rice under drought, salt, and cold stresses, we are still far from explaining the exact mode of action of trehalose in plants. Despite increasing stress tolerance in plants, overexpression of trehalose pathway-related genes has frequently resulted in dwarfism, delay in flowering, and abnormalities in leaf and root morphologies (Li et al. 2011). Exogenous trehalose treatment in rice resulted in reduced damage under salinity which was proposed to be related to preservation of root integrity, decreased Na^+ accumulation, and regulation of the genes responsible for osmotic adjustment (Garcia et al. 1997; Bae et al. 2005; Fernandez et al. 2010).

5 Conclusion

Understanding stress-coping mechanisms is among the hot topics of plant science not only for basic scientific curiosity but also for improving agricultural yield and performance. Plants have evolved sophisticated stress tolerance mechanisms against abiotic and biotic stresses of which can be introduced to crop plants by transgenic approaches. Stress tolerance is a complex network of gene activation and signaling transduction routes; therefore, manipulation of one metabolic process may lead to undesired or unsufficient effects. Among these mechanisms, accumulation of osmoprotectant solutes was found out to be the most effective and compatible one since most of the crop plants have at least one type of these molecules or their precursors. Studies presented in this chapter might give an idea for how the biosynthetic and catabolic routes of these three molecules might be manipulated by genetic approach for improvement of stress responses in plants. Different characteristics and features of these molecules and how they affected transcription of stress-responsive and stress-related genes were discussed in detail. All of these molecules have an impact and ameliorative effect on stress tolerance in plants, and one might consider carefully for choosing the best candidate. Proline, for being a component of free amino acid; glycinebetaine, for being the most compatible solute among these molecules; and trehalose, for being an unusual sugar molecule with ability to preserve water, are all promising for regulating and controlling stress tolerance processes in plants.

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