



Review

Approaches in modulating proline metabolism in plants for salt and drought stress tolerance: Phytohormones, mineral nutrients and transgenics



Tasir S. Per^{a,1}, Nafees A. Khan^{a,**,1}, Palakolanu Sudhakar Reddy^b, Asim Masood^a,
Mirza Hasanuzzaman^c, M. Iqbal R. Khan^{a,d,*}, Naser A. Anjum^e

^a Plant Physiology and Biochemistry Laboratory, Department of Botany, Aligarh Muslim University, Aligarh 202002, India

^b Cell, Molecular Biology and Genetic Engineering Group, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502 324, Telangana, India

^c Department of Agronomy, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka 1207, Bangladesh

^d Crop and Environmental Sciences Division, International Rice Research Institute, 4030 Los Banos, Philippines

^e CESAM-Centre for Environmental & Marine Studies and Department of Chemistry, University of Aveiro, 3810-19 Aveiro, Portugal

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ABSTRACT

Major abiotic stress factors such as salt and drought adversely affect important physiological processes and biochemical mechanisms and cause severe loss in crop productivity worldwide. Plants develop various strategies to stand healthy against these stress factors. The accumulation of proline (Pro) is one of the striking metabolic responses of plants to salt and drought stress. Pro biosynthesis and signalling contribute to the redox balance of cell under normal and stressful conditions. However, literature is meager on the sustainable strategies potentially fit for modulating Pro biosynthesis and production in stressed plants. Considering the recent literature, this paper in its first part overviews Pro biosynthesis and transport in plants and also briefly highlights the significance of Pro in plant responses to salt and drought stress. Secondly, this paper discusses mechanisms underlying the regulation of Pro metabolism in salt and drought-exposed plant *via* phytohormones, mineral nutrients and transgenic approaches. The outcome of the studies may give new opportunities in modulating Pro metabolism for improving plant tolerance to salt and drought stress and benefit sustainable agriculture.

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* Corresponding author. Crop and Environmental Sciences Division, International Rice Research Institute, Los Banos 4030, Philippines.

** Corresponding author. Plant Physiology and Biochemistry Laboratory, Department of Botany, Aligarh Muslim University, Aligarh 202002, India.

E-mail addresses: naf9@lycos.com (N.A. Khan), amu.iqbal@gmail.com (M.I.R. Khan).

¹ Equally contributed authors.

1. Introduction

The increasing severity of salt and drought stresses in the changing environmental scenario has resulted in considerable loss in crop productivity worldwide, mainly due to reduced land area fit for agricultural purposes (Golladack et al., 2014). Approximately 6% of the land area is affected by salinity (Flowers et al., 1977), whereas about 45% of the agricultural land area worldwide are subjected to continuous or frequent drought conditions (Bot et al., 2000). It has been estimated that increasing drought stress will cause up to 30% loss in global crop production by 2025 (Zhang, 2011). Plants under salt and drought stress exhibit osmotic and oxidative stresses as a common response in order to develop phenotypical and physiological adaptations to these stress factors (Golladack et al., 2014). One of the common responses shown by plants to mitigate salt/drought stress is the synthesis and accumulation of organic solutes known as osmoprotectants or compatible solutes, such as proline (Pro), glycine betaine, choline, O-sulphate, sugars and polyols (Iqbal et al., 2014; Khan et al., 2014; Klepek et al., 2005; Szabados and Savouré, 2010). Among the major compatible solutes, Pro is a low molecular weight cyclic imino acid and is known to provide osmotic adjustments in plants under stressful environments (Szabados and Savouré, 2010; Kaur and Asthir, 2015). It diminishes the effects of reactive oxygen species (ROS) by stabilizing antioxidant system through osmotic adjustments and protecting the integrity of cell membranes (Banu et al., 2009; Reddy et al., 2015; Szabados and Savouré, 2010). The significance of elevated Pro level in several plants exposed to varied stresses including salt, drought, heat, chilling, metal/metalloid and UV-B radiations has been reported (Szabados and Savouré, 2010; Iqbal et al., 2014). There is a general agreement that Pro also up-regulates some salt and drought tolerant genes (Khedr et al., 2003; Moustakas et al., 2011; Naliwajski and Skiodowska, 2014; Patade et al., 2014; Teh et al., 2015).

Phytohormones are chemical messengers that interact with Pro metabolism to increase stress tolerance in plants. The available scanty reports have shown that phytohormones interact synergistically with Pro metabolism and stress tolerance (Khan et al., 2013; Iqbal et al., 2014). However, the role of phytohormones in regulation of Pro biosynthesis is yet to be completely explored. Studies have also been focused to identify the regulators that can potentially interact synergistically or antagonistically with Pro metabolism and influence salt and drought tolerance. Macronutrients are important metabolic constituents and components of various signalling pathways, and have been shown to improve plant tolerance against various abiotic stresses (Anjum and Lopez-Lauri, 2011). Reports are available on the regulation of Pro metabolism by some mineral elements, such as nitrogen (N), phosphorus (P), sulfur (S) and calcium (Ca) under stress conditions including salt and drought stresses (Albert et al., 2012; Jaleel et al., 2007; Misra and Gupta, 2006; Moat et al., 2003; Shabbir et al., 2016; Singh et al., 1997; Verdoy et al., 2006), but the informations are less critically analyzed. To fill the highlighted knowledge-gap, this paper overviews the significance of Pro metabolism in salt and drought stress resistance and explores the role of phytohormones, mineral nutrients and transgenics in the modulation of its biosynthesis, accumulation and functions in plants.

2. An overview of proline biosynthesis and its regulation

In plants, Pro biosynthesis takes place via two pathways, namely, glutamate (Glu) and ornithine (Orn) pathways (Fig. 1). Under normal conditions, the biosynthesis of Pro takes place in the

cytosol; whereas under stress condition, Pro production can also be possible in chloroplasts (Székely et al., 2008; Rejeb et al., 2014). It has been shown that Pro synthesis occurs mainly from Glu under osmotic stress (Rejeb et al., 2014). In Glu pathway, Pro biosynthesis begins with the phosphorylation of Glu to form γ -glutamyl phosphate, which is reduced to an intermediate glutamic-5-semialdehyde (GSA) by the action of bifunctional enzyme Δ^1 -pyrroline-5-carboxylate synthetase (P5CS; EC 2.7.2.11/1.2.1.41), which is spontaneously cyclized into pyrroline-5-carboxylate (P5C). This intermediate P5C is finally reduced to Pro by the enzymatic catalysis of Δ^1 -pyrroline-5-carboxylate reductase (P5CR; EC 1.5.1.2). In Orn pathway, Orn can be transaminated to GSA by the activity of ornithine δ -aminotransferase (δ -OAT; EC 2.6.1.13) and subsequently gets converted to Pro via P5C (Szabados and Savouré, 2010). It has, however, been shown in *Arabidopsis thaliana* that δ -OAT generates P5C exclusively for catabolic branch which ultimately gives Glu as an end product (Funck et al., 2008). It has also been found that biosynthesis of Pro in *Arabidopsis* occurs predominantly or exclusively via the Glu pathway and does not depend on Glu produced by Arg and Orn catabolism. Therefore, it can be said that in plants, at least in *Arabidopsis*, biosynthesis of Pro occurs exclusively through Glu pathway (Funck et al., 2008). Catabolism of Pro occurs in mitochondria and this process is catalyzed by two enzymes such as Pro dehydrogenase (PDH; EC 1.5.99.8) and P5C dehydrogenase (P5CDH; EC 1.5.1.12) leading to the production of initial biosynthetic product Glu. The information of enzymes and genes involved in Pro biosynthesis and regulation of expression of Pro biosynthetic genes in animals, bacteria and plants has been thoroughly reviewed (Fichman et al., 2015). In plants, under environmental stress conditions, metabolism of Pro is regulated through a balance between its anabolism and catabolism. The production of Pro is promoted and its degradation suppressed during salt or drought stress. In some plant species including *A. thaliana*, two closely related P5CS genes have been identified (Savouré et al., 1995; Yoshiba et al., 1995; Strizhov et al., 1997; Zhang et al., 2014; Wang et al., 2015). In *Arabidopsis*, P5CS1 gene was found to show ubiquitous expression in almost all organs and is induced by drought and salt stress (Savouré et al., 1995; Yoshiba et al., 1995; Strizhov et al., 1997). Similarly, PDH genes were identified from various plant species (Funck et al., 2010; for review see Servet et al., 2012; Zhang et al., 2014; Wang et al., 2015). Among two genes PDH1 and PDH2 identified in *A. thaliana* (Funck et al., 2010), PDH1 is considered the main isoform involved in degradation of Pro. The expression of PDH1 has been shown to be down-regulated under water stress (Kiyosue et al., 1996). Wang et al. (2015) found that Pro level peaked at 300 mM NaCl in seashore mallow (*Kosteletzkya virginica*) and the expression of KvP5CS1 was up-regulated under salt stress and the expression of KvOAT showed a slight increase, while the expression of KvPDH was not changed much and slightly decreased before 12 h, and then returned to the original level. The up-regulated expression of KvP5CS1 supported the idea that it played a more important role than KvOAT for Pro accumulation under salt stress. Parida et al. (2008) imposed drought on plants of drought-tolerant (Ca/H 680) and drought-sensitive (Ca/H 148) genotypes of cotton (*Gossypium hirsutum*) after 45 days of sowing by withholding irrigation for 14 days. After 14 days of drought induction, the drought stressed plants were re-irrigated for 7 days for recovery, and a significant increase in Pro levels and the activity of P5CS in leaves of both drought-tolerant and drought-sensitive genotypes was noted, whereas the activity of P5CR increased minimally and the activity of δ -OAT remained unchanged. The activity of PDH, however, decreased under drought stress in both the genotypes. Contrarily, during recovery, the levels of Pro and the activity of P5CS and P5CR

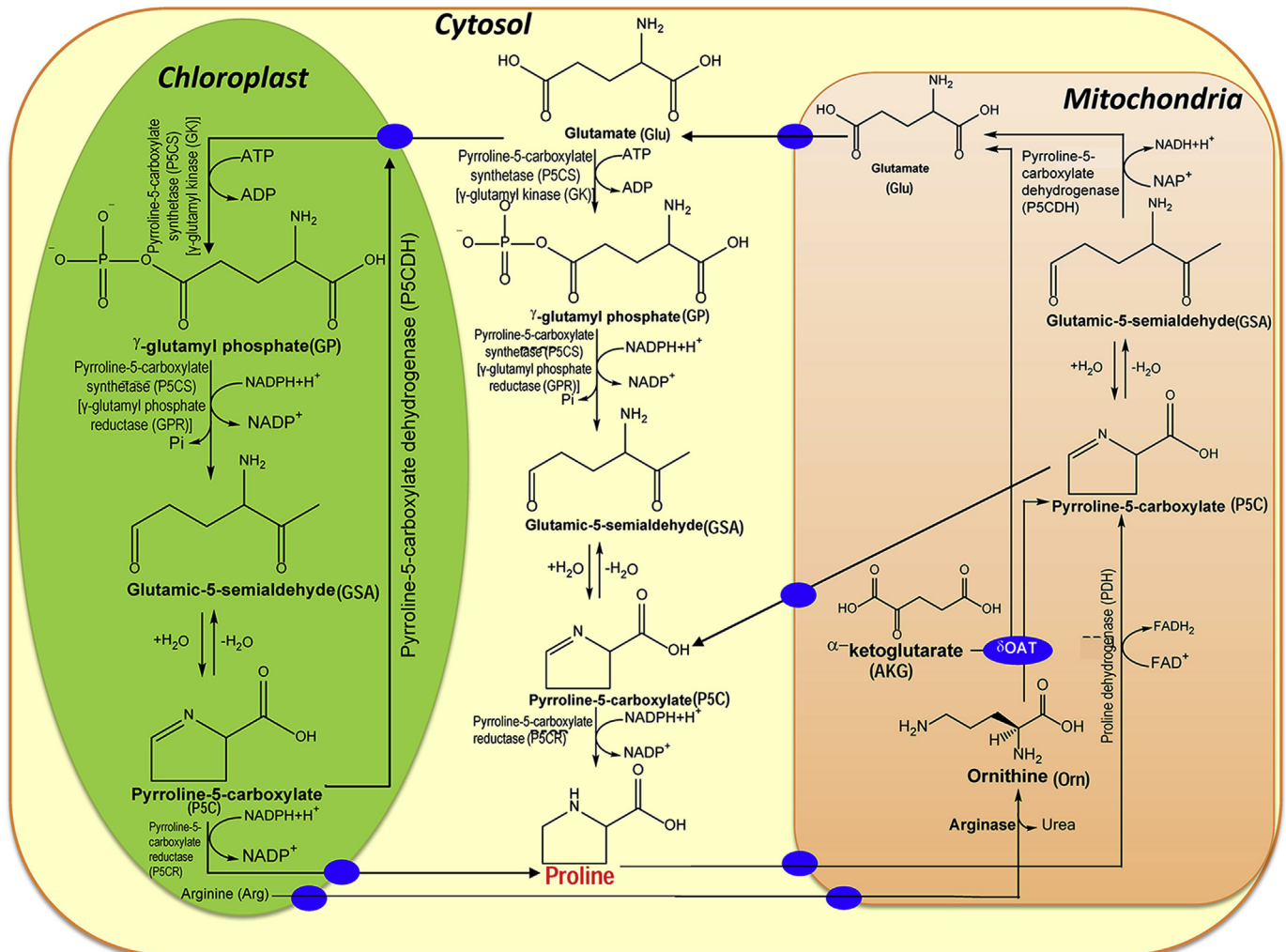


Fig. 1. Schematic representation of proline biosynthesis and metabolism in plant cell. In plants, proline biosynthesis takes place through two chemical pathways (i) glutamate and (ii) ornithine pathways. In glutamate pathway, glutamate is converted to an intermediate glutamic-5-semialdehyde through γ -glutamyl phosphate and then to pyrroline-5-carboxylate, which is finally reduced to proline by the enzymatic catalysis of P5CR. In ornithine pathway, ornithine is transaminated to glutamic-5-semialdehyde in mitochondria through pyrroline-5-carboxylate. Pyrroline-5-carboxylate can be transported to cytosol and produce proline or is converted to glutamic-5-semialdehyde, which is converted to glutamate and transported to cytosol to produce proline through glutamate pathway. Glutamate in cytosol can be transported to chloroplast and is converted to pyrroline-5-carboxylate through γ -glutamyl phosphate and glutamic-5-semialdehyde. Pyrroline-5-carboxylate is reduced to proline. Szabados and Savouré (2010) have presented the synthesis of proline in different compartments. GK— γ -glutamyl kinase; GPR— γ -glutamyl phosphate reductase; P5CDH—pyrroline-5-carboxylate dehydrogenase; P5CR—pyrroline-5-carboxylate reductase; P5CS—pyrroline-5-carboxylate synthetase; δ -OAT— ornithine δ -aminotransferase.

decreased almost equal to their respective controls, whereas the activity of PDH increased. In *A. thaliana*, it was found that during stress only *P5CS2* gene was required for Pro accumulation among the two closely related *P5CS* genes, *P5CS1* and *P5CS2*. Production of knockout mutant of *P5CS1* resulted in the reduction of stress-induced Pro synthesis and accumulation of ROS (Székely et al., 2008). It is generally believed that the level of Pro is mainly regulated by P5CS and PDH enzymes, but the regulation by P5CDH also seems to be important. In alfalfa (*Medicago sativa*) plants exposed to 170 or 100 mM NaCl for 7 or 20 days or to the increasing NaCl concentrations from 50 mM to 200 mM for 3 h and then recovered in a fresh medium, two PDH genes, *MsPDH* and a partial sequence of *MsP5CDH* genes were identified and cloned. *P5CDH* transcript levels were found to decrease in a salt concentration-dependent manner, while steady-state transcript levels remained unchanged for *MsP5CS* and *MsP5CDH*. Contrary to the salt-dependent repression, a rapid induction of *MsPDH* transcription occurred at a very early stage of the recovery process, independent of earlier salt treatments. Thus, Pro accumulation under salt stress resulted from

the decreased expression of two PDHs rather than the increased expression of *P5CS* in *M. sativa* (Miller et al., 2005). They also showed up-regulation of *PDH* gene during recovery from stress condition. This increase in expression of *PDH* during recovery from stress could be the result of accumulated Pro and hypoosmolarity. This phenomenon was explained by Satoh et al. (2002, 2004) who showed that a core cis-acting element, ACTCAT, was found in the *PDH* promoter necessary for the efficient expression of *PDH* in response to Pro and hypoosmolarity. It was named as Pro- or hypoosmolarity-responsive element (PRE). Later, several basic leucine zipper protein (bZIP) transcription factors were identified as candidates for binding to this element. Regulation of genes encoding *P5CS* has also been found to differ in response to osmotic stress. In *M. truncatula*, two *P5CS* genes showed different regulation at transcript level in response to osmotic stress. Under osmotic stress *MtP5CS1* transcript levels remained unaffected, while *MtP5CS2* transcript levels were strongly accumulated in shoots of salt stressed plants (Armengaud et al., 2004). Metabolism of Pro is tightly regulated by environmental and endogenous signals

(phytohormones) which are discussed separately in the following section. To cope with the environmental stresses, plants have been shown to adopt a memory response upon primary stress exposure to facilitate a quicker and stronger reaction to recurring stresses. Feng et al. (2016) reported that light signal was positively involved in salt-induced transcriptional memory of *P5CS1* and subsequent Pro accumulation. Furthermore, HY5-dependent light signalling was required for the maintenance of salt-induced *H3K4me3* in *P5CS1* during the recovery stage. This mechanism is likely operating during other stress as well, and could shed light on future research into the concerted effects of different environmental factors on plant response to stresses.

The current knowledge has highlighted the nature of relationships between Pro biosynthesis and salt-drought abiotic stress tolerance in plants. In many plants, Pro accumulation is a common response to abiotic stress, including salt and drought stresses which depends on expression of *P5CS* gene. *P5CS*, the rate-limiting enzyme involved in the regulation of Pro accumulation in higher plants is encoded by *P5CS1* (root and shoot tissue) and *P5CS2* (dividing cells) (Székely et al., 2008). In earlier studies, expression

of *P5CS1* in different tissues of the plant development is inducible by various abiotic stresses including drought, salinity or low temperature stress (Savouré et al., 1995; Strizhov et al., 1997; Yoshida et al., 1999; Abrahám et al., 2003). The present review discusses how Pro biosynthesis gene regulates the salt and drought stress tolerance. We have analyzed the expression of *P5CS* gene under both salt and drought stress treatments in two model plants i.e., *Arabidopsis* and rice (*Oryza sativa*) using the publicly available microarray data from Affymetrix microarray platform using Genevestigator (Fig. 2A and B; Supplementary Tables 1 and 2) (Hruz et al., 2008; Zimmermann et al., 2008). Expression in shoot and root samples under salt and drought stress has been represented in the percent expression potential values (0–100%). Analysis of *ArabidopsisAtP5CS* gene, a key enzyme in Pro biosynthetic pathway showed that drought and salinity stresses trigger Pro biosynthesis. The gene was temporally, spatially and differentially expressed in response to different stress stimuli. High transcript abundance was found in the shoots of plants subjected to salinity stress after 3 h, which gradually decreased up to 12 h. Similarly, shoots of plants subjected to drought stress exhibited high expression levels in the

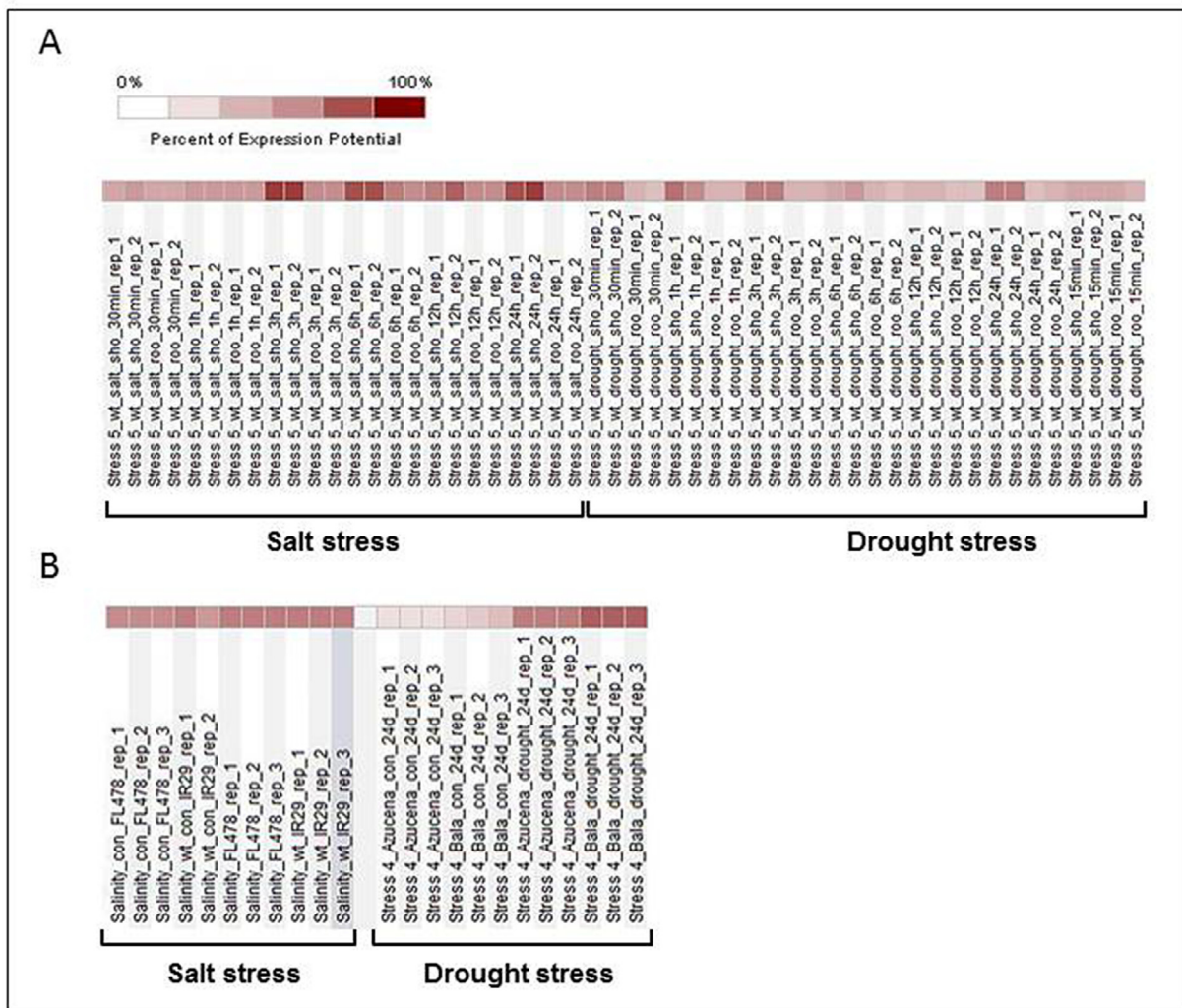


Fig. 2. Meta-analysis approach for proline biosynthetic gene *P5CS* in response to salt and drought stress conditions. A. Expression potential (%) of *P5CS* gene in shoot and root parts in response to salt and drought stress conditions in *Arabidopsis*. B. Expression potential (%) of *P5CS* gene in leaves of rice in response to salt and drought stress conditions. Affymetrix microarray data obtained from the Genevestigator toolbox. White, light brown and dark brown colors respectively indicate no perceptible, average level and high transcript abundance. The values indicate the percent expression potential in expression in shoot and root samples under salt and drought stress conditions. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

initial hours of stress induction (1 h) and then subsided by 12 h. Interestingly, the expression of *AtP5CS* in shoots peaked again at 24 h in response to both salt and drought stress (Fig. 2A). Although the gene expression in root tissue was at a basal level at all -time points, it was higher in response to salt stress as compared to drought stress. A similar pattern was observed in rice, *OsP5CS* gene expression was higher in salt stress compared to drought stress (Fig. 2B). Overexpression of *P5CS1* increased Pro accumulation, while mutants restricted Pro accumulation (Kishor et al., 1995; Székely et al., 2008; Zhang et al., 1995). These observations suggest that Pro biosynthesis was significantly affected by *P5CS* under salt and drought stress conditions. Differential expression profiles of *AtP5CS* and *OsP5CS* under salt and drought stress in different tissues strongly suggest that genes play distinct roles based on stress response and development (Fig. 2). This might be attributed to the ionic imbalance generated by imposition of salt stress, which required biosynthesis of osmoprotectants such as Pro.

3. Transport of proline

As Pro is a vital osmoregulator in plants, its transport is supposed to influence plant tolerance against stress conditions (Girousse et al., 1996; Lehmann et al., 2010; Sharma et al., 2011). The amino acid transporters in plants are generally classified into two superfamilies that include the amino acid, polyamine, and choline transport superfamily, and the amino acid transporter family (ATF) superfamily (Waditee et al., 2002). Moreover, the latter includes five sub-classes of the transporters, the amino acid permeases, the lysine, histidine, the Pro (ProT) and the putative auxin transporters. Eight different amino acid transporter clones have been isolated and characterized in *Arabidopsis* using yeast mutants (Rentsch et al., 1996). Among these transporters, two encoding specific Pro transporters (ProT1 and ProT2) were distantly related to the amino acid/auxin permease (AAP) family. So at present, ProT, which belongs to the ATF or AAAP family, has been shown to be localised at the plasma membrane and is likely involved in the intercellular and long-distance Pro transport (Rentsch et al., 2007). However, the molecular mechanism of ProT-mediated transport of Pro is poorly understood (Lehmann et al., 2011). Genes encoding transporters of Pro have been isolated from various plant species such as *A. thaliana* (Grallath et al., 2005; Rentsch et al., 1996), tomato (*Solanum lycopersicum*; Schwacke et al., 1999), rice (*O. sativa*; Igarashi et al., 2000) and barley (*Hordeum vulgare*; Fujiwara et al., 2010; Ueda et al., 2001). The expression of genes encoding two specific Pro transporters (*ProT1* and *ProT2*) was found to be expressed during stressful conditions in *A. thaliana*. The higher expression level of *ProT1* was found under salt stress, while the *ProT2* was strongly expressed in water deficit conditions. The increase in *ProT2* transcript expression reveals that *ProT2* plays an important role in transport of N, suggesting increased Pro export during water stress (Rentsch et al., 1996). The southern blot analysis revealed that *OsProT* has a gene family in *O. sativa* genome, like *AtProT* and *LeProT* and *OsProT* that specifically transported L-Pro (Igarashi et al., 2000). Ueda et al. (2001) analyzed that the *HvProT* mRNA strongly expressed in root cap cells under salt stress in *H. vulgare*. Later, tissue-specific over-expression of this transporter in *Arabidopsis* root tip led to increased Pro content of the root apex and increased growth under control conditions (Ueda et al., 2008). Besides the *ProT* genes, encoding amino acid/auxin permease (AAAP) and the amino acid/polyamine/choline families also mediate Pro uptake across the plasma membrane (Rentsch et al., 2007). Along with Glu, the AAP family of proteins is also involved in the proton-coupled uptake of Pro (Kishor et al., 2014). Recent studies on the function of ProT have helped to gain new insights of Pro metabolism. Zhang et al. (2014) isolated four ProT-homologous genes and after

combining their results of RNA sequencing and NCBI blast, they confirmed the presence of four homologous genes encoding ProT in chrysanthemum (*Chrysanthemum lavandulifolium*), but only three homologous *ProT* genes were found in other plants like *A. thaliana* (Grallath et al., 2005; Rentsch et al., 1996) and *S. lycopersicum* (Schwacke et al., 1999). The TMHMM programme predicted that there were likely 9 to 11 transmembrane domains in ClProT proteins, which was in agreement with the prediction for this protein in *A. thaliana* (Grallath et al., 2005) and *H. vulgare* (Fujiwara et al., 2010). This property indicated that ClProT might be localised at the plasma membrane and could facilitate Pro transport. However, phylogenetic analysis showed that ClProT1 and ClProT4 were not clustered within the ProT subfamily, which might result from a lack of information on *ProT* genes and the specificity of *ProT* genes in different species. Therefore, the affiliation and role in Pro metabolism or the translocation of other amino acids can be determined through further research on the functional analysis of ClProT. Zhang et al. (2014) also found that the expression patterns of four ClProT genes varied among different organs and under stress conditions, but the expression patterns of each ClProT under various stresses were the same. Among the four ClProT genes, ClProT2 was induced under various stress conditions. The cis-acting element involved in circadian control was found in the ClProT promoters. The cis-acting regulatory elements in the ClProT promoters indicated the crucial role of Pro metabolism in plant resistance to environmental stresses (Zhang et al., 2014).

Both inter- and intracellular transport of Pro is critical for maintaining cellular homeostasis, but the intracellular movement of Pro plays an important role in augmenting stress resistance (Verslues and Sharma, 2010). The transport of amino acids is regulated not only by endogenous but also by environmental signals in plants. Pro transport can be induced by salt stress in plants such as *M. sativa* and maize (*Zea mays*), suggesting that it plays a role in osmotic adjustment. In *M. sativa*, Pro transport played an important role in adaptation of this plant to osmotic stress by changing Pro concentration (Girousse et al., 1996). Under low water potential, Pro deposition in the elongation zone of *Z. mays* roots was not due to the increase in biosynthesis of Pro, but due to its enhanced transport (Verslues and Sharp, 1999). It was also evident from the findings of Raymond and Smirnov (2002) that Pro requirement was more than its biosynthesis under low water potential in *Z. mays* seedlings. This shortage was, however, fulfilled by the degradation of Pro-rich storage proteins of the endosperm, suggesting Pro accumulation in root tip was controlled by its transport and utilization rates. Stines et al. (1999) suggested that accumulation of Pro in grapevine (*Vitis vinifera*) was due to the increase in transport of Pro as no increase in the expression of *P5CS* gene was observed. Thus, Pro transport needs to be studied in more detail to gain better insight into its role in tolerance of plants to stress.

4. Phytohormones in regulation of proline metabolism under salt and drought stress

Intensive research has revealed the involvement of phytohormones in plant growth and development and also in plant responses to varied stress factors (Khan et al., 2012). Nevertheless, phytohormones were reported to play essential roles in regulation of Pro metabolism in plants under environmental stress conditions including salt and drought stress (Iqbal et al., 2014; Khan et al., 2012; Verslues and Bray, 2006). However, the detailed information on the role of auxin, gibberellins (GA), cytokinin, ethylene, ABA and salicylic acid (SA) in regulation of Pro metabolism is meager. However, certain reports are available which suggest the importance of various phytohormones in enhancing accumulation of Pro

and lowering of stressful environment in plants. Most of the studies are related to the exogenous application of different phytohormones on different plant species. Overall results of these findings reveal the additive effect of phytohormones on Pro accumulation for alleviation of stressful environment within the plants. Exogenous application of 0.3 mM indole-3-acetic acid (IAA) has been found to reduce Pro accumulation in wheat (*Triticum aestivum*) under salt (150 mM NaCl) and drought stress (6% polyethylene glycol) (Sadiqov et al., 2002). In several instances GA has been found to exhibit positive relationship with Pro accumulation. Foliar application of GA₃ on *Z. mays* plants was found to counteract the adverse effects of salt stress through increased Pro and macro (Ca, K, Mg, N, Na, P) and micronutrients (Fe, Mn and Zn) accumulation, and also in maintaining the cell membrane permeability (Tuna et al., 2008). The interaction of GA₃ and Ca²⁺ in linseed (*Linum usitatissimum*) (Khan et al., 2010), and GA₃ and N in *O. sativa* (Jang et al., 2008) resulted in increased Pro production. Thus, it is suggested that GA interaction with mineral elements promotes Pro accumulation and enhances plant stress tolerance. Following drought stress, bioactive cytokinin levels decreased in the whole plants of tobacco (*Nicotiana tabacum*), remaining relatively higher in preferentially protected upper leaves and in roots. Cytokinin suppression was less pronounced in Pro transformants over-expressing a gene for the Pro biosynthetic enzyme *P5CSF129A* and indicated a mild elevation of drought stress tolerance. This could be associated with enhanced sink-source polarization, achieved by a gradient of bioactive cytokinin in favour of upper leaves (Dobra et al., 2010). Ethylene has been reported to interact with other signalling molecules and regulates Pro accumulation and increases heat stress tolerance in *T. aestivum* (Khan et al., 2013) and salt stress tolerance in mustard (*Brassica juncea*) (Iqbal et al., 2015). Recently, involvement of ethylene signalling in increasing Pro accumulation, and eventual osmotic adjustment has been also reported (Cui et al., 2015).

ABA is a key component in integrating various signals and controlling downstream stress responses, and can also influence the accumulation of Pro by regulating the expression of *P5CS* and *P5CR* in plants during abiotic stress (Verslues and Bray, 2006). Accumulation of *AtP5C1* mRNA, one of the two genes responsible for Pro biosynthesis in *Arabidopsis* has been shown to be regulated by ABA signalling under osmotic and salt stress through the cis-acting ABA responsive (ABRE) element (Strizhov et al., 1997). The stimulation of Pro synthesis by ABA and salt stress has correlation with the activation of *P5CS1* expression in *Arabidopsis* (Abrahám et al., 2003). In *B. rapa* leaf discs, ABA directly induced the accumulation of Pro under osmotic stress and also influenced the mobilization of Pro when the stress was over (Trotel-Aziz et al., 2003). Both salt and drought stresses were reported to induce *Arabidopsis P5CS1* that was also activated by an ABA-dependent and ABA-insensitive controlled regulatory pathways and H₂O₂-derived signals (Verslues et al., 2007). Savouré et al. (1997) showed that stress induction of *P5CS1* occurred independently of ABA upon cold and osmotic stress. This was supported by Sharma and Verslues (2010) indicating that there was significant ABA-independent expression of *P5CS1* at lower water potential. This was demonstrated by comparing ABA-deficient (*aba2-1*), Pro accumulation deficient (*p5cs1-4*) and double (*p5cs1-4/aba2-1*) mutants. From these data, Pro metabolism appears to be tightly regulated by environmental conditions (Rejeb et al., 2014). The greater ABA stimulation of Pro accumulation in these mutants was argued to be caused by altered H₂O₂ content as opposed to other metabolic changes (Verslues et al., 2007). The interaction of ABA with Ca²⁺ signalling pathway can also modulate Pro accumulation in plants under stress condition. To this end, exogenous ABA-application up-regulated salt stress-responsive (*OsCam1-1*) gene expression and

also enhanced Pro accumulation by calmodulin signal transduction cascade in salt stressed *O. sativa* (Sripinyowanich et al., 2013). Transgenic lines of *O. sativa* over-expressing salt-inducible calmodulin gene *OsCam1-1* played an important role in biosynthesis of ABA by up-regulating two genes, *ABA aldehyde oxidase* and *9-cis-epoxycarotenoid dioxygenase 3* involved in ABA biosynthesis. In addition, the salinity-exposed transgenic *O. sativa* lines were tolerant to salt stress and maintained their shoot and root dry weight (Saeng-ngam et al., 2012). These studies showed the mutual interlinking of ABA and Ca²⁺ signalling pathways in stressed plants to accumulate Pro as an instrument of stress tolerance. A link between Pro accumulation and ABA may also involve sugars. Sucrose has been found to show inhibitory effect on ABA-induced accumulation of Pro and transcription factor ABI4 in *Arabidopsis* (Verslues and Bray, 2006). Moreover, the expression of ABA-dependent *P5CS1* was inhibited with brassinolide pre-treatment (Abrahám et al., 2003).

SA has been shown to induce Pro metabolism and confers tolerance to plants against both salt and drought stresses (Khan et al., 2014, 2015; Misra and Saxena, 2009; Nazar et al., 2015). Application of SA (0.5 M) increased Pro accumulation in shoots and roots of lentil (*Lens culinaris*) under salt stress through increased activity of *P5CR* and γ -GK and decreased activity of PDH (Misra and Saxena, 2009). Pre-soaking of *H. vulgare* seeds with SA resulted in the accumulation of Pro and increased photosynthetic pigments (Chl *a*, *b* and carotenoids) and membrane integrity under salt stress (El-Tayeb, 2005). SA-treatment increased the activity of nitrate reductase (NR; EC 1.7.1.1) and glutamine synthase (GS; EC 6.3.1.2) in cucumber (*Cucumis sativus*) (Jing-hong et al., 2012), and NR activity in amaranth (*Amaranthus hybridus*) (Umebese et al., 2009) under drought stress. Recently, Nazar et al. (2015) have shown that supplementation of 0.5 mM SA increased Pro accumulation through its influence on the activity of γ -GK and Pro oxidase under drought, and improved photosynthetic characteristics (net photosynthesis, stomatal conductance, and intercellular CO₂ concentration and Rubisco activity) and growth (leaf area and plant dry mass) of *B. juncea*. The authors also found SA-application mediated decrease in oxidative stress as a result of increased Pro content. SA also showed interaction with Ca²⁺ signalling (Du et al., 2009). This link between SA and Ca²⁺ signalling was further supported by the study of Al-Whaibi et al. (2012), where the authors reported higher increase in Pro concentration with the combined treatment of SA and Ca²⁺. SA has also been found to interact with N assimilation to influence Pro metabolism and protection of photosynthesis in *T. aestivum* subjected to water deficit (Bhupinder and Usha, 2003) and in salinity exposed *B. juncea* (Nazar et al., 2011).

Nitric oxide (NO) is another cell signalling molecule reported to play an important role in Pro metabolism. The treatment of *O. sativa* seedlings with less than 10 μ M NO increased expression of *P5CS* and enhanced quantum yield of PS II under salt stress (Uchida et al., 2002). In *T. aestivum* seedlings, NO application reduced oxidative damage by the enhanced activity of SOD and CAT and accumulation of Pro (Ruan et al., 2002). Pro accumulation in *T. aestivum* seedlings was in a phase dependent manner, mainly by enhancing the activity of *P5CS* within the first 4 days after treatment and inhibiting the activity of PDH 4 days later up to 8 days. Pro accumulation and oxidative stress were lower after application of low concentrations of sodium nitroprusside (SNP; donor of NO) in *B. rapa* under salt stress, and the reduction in Pro content was related to the increased activity of PDH (Lopez-Carrion et al., 2008). Fan et al. (2012) reported that exogenous SNP increased free Pro content and activity of *P5CS* and PDH in *C. sativus* seedling leaves and roots under salt stress. Although reports are available regarding the role of NO in increased Pro accumulation under abiotic stresses, but the study by Xiong et al. (2012) showed that NO-induced drought tolerance in

O. sativa was not related to Pro accumulation. Additionally, foliar spray of 100 μM SNP resulted in increased endogenous NO content and stomatal closure, decreased transpiration rate that cumulatively enhanced the adaptive responses against drought stress in *O. sativa*. The involvement of N in NO-mediated regulation of Pro metabolism has also been shown. For instance, an increased activity of NR, GS and N content was found with NO application under salinity stress (Manai et al., 2012). The modulation of Pro metabolism can be independent of NO production in plants under stress (such as water deficit). To this end, though a relationship between NO production, ABA and Pro accumulation was found in *M. truncatula* under water deficit stress, the content of Pro and the expression of genes encoding enzymes of Pro biosynthesis and degradation Pro were not affected by NO scavenger (cPTIO) under water deficit stress (Planchet et al., 2014). The interrelationships among NO, Ca^{2+} and ABA (Sokolovski et al., 2005), however, need further studies to establish their participation in Pro metabolism and plant stress tolerance. Nevertheless, more exhaustive studies on the interplay of phytohormones with other signalling molecules are required for getting insights into the role and underlying mechanisms of phytohormones mediated control of Pro production and plant stress resistance.

5. Nutrients in modulation of proline metabolism under salt and drought stress

Mineral nutrients minimize the negative effects of stress conditions through their involvement in various metabolic activities and play role in the modulation of the components of various signalling pathways (Anjum and Lopez-Lauri, 2011). There are reports on the role of macronutrients in stress tolerance, but information lacks with regard to micronutrients. Notably, there exists a close relation of N metabolism with Pro level and metabolism in plants (Sánchez et al., 2001). Pro metabolism significantly affected in green bean (*Phaseolus vulgaris*) plants supplied with different N doses (Sánchez et al., 2001). The applied N as NH_4NO_3 at 5.4, 11.6, 17.4 and 23.2 mM to the nutrient solution indicated that high N dose was characterized by the accumulation of NO_3^- , NH_4^+ and Pro in root and foliar organs. The enzymes, δ -OAT and P5CS, involved in Pro biosynthesis varied in behavior depending on the N status, but Pro appeared to be synthesized mainly by the enzyme δ -OAT (Sánchez et al., 2001). In another study, a positive correlation between Pro accumulation and N supply was reported in drought stressed sugar beet (*Beta vulgaris*) plants; wherein excess N supply was found to induce Pro levels (Monreal et al., 2007). In root nodules, elevated Pro accumulation was argued as a consequence of increased activity of PDH which was found to increase under salt stress in nitrogen-fixing *Rhizobia* or bacteroids (Kohl et al., 1994). Pro oxidation has been suggested to provide high energy for fixing atmospheric N in root nodules. The significance of Pro accumulation in legume nodule formation, N_2 -fixation and plant tolerance to osmotic stress has been reported (Verdoy et al., 2006). Accumulation of Pro in environmentally stressed plant cells including those of legume roots and nodules has been shown, but the regulation of Pro accumulation under stress is poorly understood. Transformation of the leguminous *M. truncatula* plant with the P5CS gene derived from moth bean (*Vigna aconitifolia*) displayed high Pro accumulation and N_2 fixation compared to wild-type plants and was also less affected by salt treatment (Armengaud et al., 2004). The production of Pro has been shown to be related to N assimilation and salt tolerance, and N differentially regulated Pro production to alleviate the adverse effect of salinity on photosynthesis in mustard (Iqbal et al., 2014, 2015). In salt and drought exposed *M. truncatula*, a significant increase in the expression of *MtP5CS3* was reported in shoots and nodulating roots, whereas the

expression of *MtP5CS1* was constitutive and *MtP5CS2* induced only under 100 μM ABA sprayed plants (Kim and Nam, 2013). Moreover, RNA silencing of *MtP5CS1* or *MtP5CS2* genes induced the expression of *MtP5CS3* gene, while that of *MtP5CS3* resulted in decreased free Pro content and also affected root nodule number. However, under salt stress (100 mM NaCl), the loss-of function mutant (*Mtp5cs3*) resulted in lesser Pro accumulation, fewer nodules formation, and comparatively lower N fixation than the wild type. Thus, *MtP5CS3* was suggested to play a crucial role in regulating Pro accumulation during symbiotic N_2 fixation under salt stress (Kim and Nam, 2013). N as a supplement has also been shown to regulate Pro accumulation in *Lotus corniculatus* under osmotically stressed conditions. An increased Pro accumulation was found both in nitrate-fed plants and ammonium-fed plants, however, a higher Pro concentration was observed in ammonium-fed plants than in nitrate-fed plant after 24 h of drought stress (Díaz et al., 2005). Glu and Orn pools are closely linked to N metabolism and were shown to be affected under drought stress (Pinheiro et al., 2001). Deficiency of water may differentially affect the concentration of Glu and Orn depending on plant N status. Thus, it can be concluded that accumulation of Pro is dependent on the availability of its precursors as well as on N availability under drought stress (Pinheiro et al., 2001). Albert et al. (2012) also reported the role of Pro in remobilization of N and in enhancing its use efficiency in *B. napus*. It was shown that application of low-N stress to *Jatropha curcas* resulted in induced expression of *JcP5CS* mRNA while the activity of P5CS enzyme and the content of free Pro in leaf reduced. However, the seedlings grown in low-N conditions were suddenly exposed to drought stress, the expression of *JcP5CS* gene was greatly induced. Both the activity of P5CS and the content of free Pro were also increased conferring drought tolerance to plants (Wang et al., 2011). N-dependent accumulation of Pro was suggested to offer an important advantage, where it could be metabolized to allow reallocation of energy, C and N from the older leaves to the younger tissues (Carillo et al., 2008).

Phosphorus is an essential element and is not readily available to plants. Also, S stands fourth after N, P and K in terms of its role in plants (Anjum and Lopez-Lauri, 2011). However, few reports are available on the relation of P and S with Pro in plants. Studies confirmed the role of P in inducing salt and drought stress tolerance in different plant species. White clover (*Trifolium repens*) grown in dry soil and supplied with P showed increase in drought tolerance. However, plants grown with low-P showed severe wilting symptoms indicated by desiccation and drying of leaves and stolons, and some with eventual mortality (Singh et al., 1997). The exact mechanism about how supply of P to these plants enhances drought tolerance is still not clear. It seems that P supplementation induces osmotic adjustment thereby improving drought tolerance relative to low-P plants. The induced osmotic adjustment helps in maintaining turgor pressure, protoplast volume and results in expansive growth, such as leaf expansion under water stress. Moreover, application of P led to greater osmotic adjustment, Pro concentration and leaf expansion rate. P-mediated osmotic adjustment and plant stress tolerance via the modulation of solutes such as Pro, betaine, and choline have been reported (Salinas et al., 2013; Singh and Sale, 2000). Recently, Shabbir et al. (2016) have shown that the supplementation of combined N, P and K in *T. aestivum* increased Pro accumulation and the activity of both the enzymes of N assimilation and of antioxidant system maximally in drought tolerant genotype. Plants also adapt to extreme conditions of abiotic stresses either by synthesizing S-rich compounds, such as reduced glutathione (GSH) or by osmotic adjustment through accumulation of Pro (Anjum et al., 2014). Both these compounds share L-Glu as a common biosynthesis precursor (Moat et al., 2003). However, detailed efforts are lacking to explore interrelationship of

GSH and Pro to obstruct abiotic stresses. A coordinated role of S and N supplementation in increasing Pro accumulation has been reported in *B. juncea* plants under salt stress (Rais et al., 2013). The authors observed significantly increased Pro accumulation with combined application of S and N that alleviated salt stress effects on photosynthetic efficiency and growth of *B. juncea*.

Calcium is known to induce halo-tolerance in many crop plants and play an important role in osmoprotection (Srivastava et al., 2013). Generally, Ca^{2+} ions ameliorate the effects of salt stress on growth of plants by altering various metabolic processes. Pro metabolism has been widely reported to be modulated on Ca^{2+} application to salt treated plants (Jaleel et al., 2007; Misra and Gupta, 2006; Murugan and Sathish, 2005; Sharma and Dhanda, 2015). Calcium chloride application (5.0 mM) to NaCl (200 mM)-stressed plants increased the Pro concentration by decreasing the level of Pro oxidase and increasing the activity of γ -GK and P5CR (Misra and Gupta, 2006). A significant increases in activity of P5CS and Pro concentration were reported in NaCl-exposed calli supplemented with CaCl_2 (Murugan and Sathish, 2005). Ca can play a positive role in ABA-mediated gene induction of P5CS in plants under drought and salinity (Knight et al., 1997). The authors suggested the involvement of Ca^{2+} and some other signalling factor in the induction of P5CS transcript and expression of *AtP5CS* gene during stress. The supplementation of CaCl_2 mediated increase in Pro peroxidase level and decrease in γ -GK activity in NaCl-stressed plants was argued as a cause of low Pro concentration in NaCl-exposed plants (Jaleel et al., 2007). In another study, they showed that NaCl-stressed plants when treated with CaCl_2 resulted in lower free amino acid and Pro content than plants treated with NaCl alone. Contradictory results have also been reported in literature where Ca^{2+} either prevented or did not affect stress-induced Pro accumulation (Sadiqov et al., 2002; Sharma and Dhanda, 2015). In salt stressed plants, the accumulation of Pro was found independent to Ca^{2+} treatment (Sadiqov et al., 2002). Pro content was found to increase with NaCl stress, however, the combined treatment of NaCl and CaCl_2 resulted in significant decrease in Pro content (Sharma and Dhanda, 2015). Reports also indicate the association of a loss of cellular Ca^{2+} with NaCl induced accumulation of Pro via inhibition of PDH activity in marine green macroalga *Ulva fasciata* (Lee and Liu, 1999).

6. Proline and stress tolerance using transgenic approach

The contribution of Pro in stress tolerance using transgenic plants has been the focus of researchers for many years (Hmida-Sayari et al., 2005; Karthikeyan et al., 2011; Kishor et al., 1995; Kumar et al., 2010; Surekha et al., 2014). The transgenic plants with a gene introduced for P5CS from *V. aconitifolia* in *N. tabacum* under the control of CaMV 35 S promoter showed 10–18 fold increase in Pro production (Kishor et al., 1995). Elevated levels of Pro caused by over-expression of *V. aconitifolia* P5CS in transgenic *O. sativa* conferred enhanced tolerance to salt stress (Zhu et al., 1998). An increase in Pro level decreased free radicals under osmotic stress in *N. tabacum* plants engineered for hyper-accumulation of Pro by over-expression of P5CS and enabled them to grow in salt containing medium (Siripornadulsil et al., 2002). Recently, Zhang et al. (2015) reported that over-expression of *StP5CS* gene from *S. torvum* increased salt tolerance in transgenic vegetable soybeans. Transgenic *S. tuberosum* containing P5CS gene from *Arabidopsis* showed improved tolerance to 100 mM NaCl stress (Hmida-Sayari et al., 2005). The higher Pro accumulation facilitates maintenance of osmotic potential under water stress and enhances biomass production and flower development under salt stress. Similarly, insertion of P5CS gene into *T. aestivum* using *Agrobacterium*-mediated gene transfer resulted in increased

production of Pro and tolerance to salt stress by up to 200 mM NaCl (Sawahel and Hassan, 2002). Elevated accumulation of Pro in transgenic *T. aestivum* plants was reported to reduce membrane lipid peroxidation and controlled ROS-accrued damages during drought condition (Vendruscolo et al., 2007). Pro content also increased in leaves and roots of transgenic *N. tabacum* over-expressing P5CS under drought stress (Ehsanpour et al., 2012). A higher Pro accumulation, osmotic adjustment, higher photosynthetic rate, and improved water stress tolerance were achieved with the introduction of P5CS gene of *V. aconitifolia* in *Citrus sinensis* (Molinari et al., 2004). Increase in Pro content, growth, chlorophyll, and relative water content with reduced lipid peroxidation were reported in transgenic plants (Kumar et al., 2010) in Indica rice carrying mutagenized *V. aconitifolia* P5CS cDNA (*P5CSF129A*), and in pigeonpea (*Cajanus cajan*) carrying mutagenized version of *P5CSF129A* (Surekha et al., 2014). In addition to P5CS, δ -OAT enzyme has also been reported to play a role in increasing Pro biosynthesis and takes part in tolerance to salt and drought stress. It has been found that over-expression of δ -OAT gene can increase Pro biosynthesis and subsequent higher biomass and germination rate under osmotic stress conditions (Roosens et al., 2002). *O. sativa* plants over-expressing *Arabidopsis* δ -OAT gene exhibited higher tolerance to drought and salt stress due to 5 to 15- fold increase in Pro content. Furthermore, the over-expression of δ -OAT also improved the yield of transgenic plants under stress conditions (Wu et al., 2003). Apart from the synthesis and accumulation of Pro, its degradation has also been found to participate in the resistance of plants to salt stress. To this end, suppression of Pro degradation by generating antisense transgenic *Arabidopsis* plants with an *AtProDH* cDNA was reported to improve freezing and high salinity tolerance (600 mM NaCl) in transgenic plants (vs. wild-type plants) (Nanjo et al., 1999). The transgenic plants developed by heterologous expression of Pro genes to confer salt and drought stress tolerance are listed in Table 2.

7. Significance of exogenously applied proline in responses of plants to salt and drought stresses

The exposure of plants to salt and drought stress impairs physiological and biochemical functions such as turgor, growth, photosynthetic rate, stomatal conductance and also damages cellular components. Under salt and drought stresses, the over-production of ROS increased oxidative stress which ultimately caused cell death, and as a result affected growth and development of plants (Khan et al., 2012). Pro plays an important role in the protection of plants grown under adverse environmental stress conditions by regulating osmotic adjustment, working as a molecular chaperone and protecting the integrity of proteins and enzymes (Szabados and Saviouré, 2010). It induces dehydrins in *Pantratum maritimum* to levels comparable to those under salt stress conditions (Khedr et al., 2003). Pro upregulates genes responsible for stress tolerance and mitigates the damages caused by salt (Patade et al., 2014; Teh et al., 2015) and drought stress (Moustakas et al., 2011). Pro can also reduce the effects of ROS, and overcome or repair stress injuries by stabilizing antioxidants system through osmotic adjustments and protecting the integrity of cell membranes (Banu et al., 2009; Szabados and Saviouré, 2010). Pro-mediated stabilization of proteins, DNA and membranes through ROS-scavenging has also been reported (reviewed in Matysik et al., 2002). In fact, the low ionization potential of Pro can enable this compound to readily form a reversible charge-transfer complex with $^1\text{O}_2$ and efficiently quenches ROS (Alia et al., 2001).

Several studies have shown that exogenously applied Pro can benefit plants exposed to salt and drought stress. To this end, exogenously applied Pro has also been reported to alleviate salt-

induced oxidative stress in many plants (Hasanuzzaman et al., 2014; Hoque et al., 2007; Nounjan et al., 2012) by reducing the Na^+/K^+ ratio, increasing endogenous Pro and transcript levels of *P5CS* and *P5CR*. Nounjan et al. (2012) have shown a decrease in the activity of superoxide dismutase (SOD; EC 1.15.1.1), ascorbate peroxidase (APX; EC 1.11.1.11), peroxidase (POX; EC 1.11.1.7) and catalase (CAT; EC 1.11.1.6) by exogenously applied Pro. However, it seems to be contradictory with other studies showing increase in antioxidant metabolism by exogenously applied Pro under stress conditions. In melon (*Cucumis melo*) exogenously applied 0.2 mM

Pro maintained membrane integrity by lowering the content of H_2O_2 and malondialdehyde (MDA), increased fresh and dry weight, chlorophyll content, net photosynthetic rate, efficiency of photosystem II (PS II) and activity of SOD, POD, CAT, APX, dihydroascorbate reductase (DHAR) and glutathione reductase (GR; EC 1.8.1.7) in roots of two cultivars under 100 mM NaCl stress condition (Yan et al., 2013). Addition of Pro to the culture medium of peanut (*Arachis hypogea*) proved beneficial to plants and protected membrane lipids from peroxidative damage caused by salt stress (Jain et al., 2001). Kamran et al. (2009) reported that pre-soaking of

Table 1
Representative studies investigating the role of exogenous application of proline in the modulation of major metabolic processes and their consequences in plants under salt and drought stress.

Stress	Duration of stress	Plant	Proline applied	Metabolism influenced	Consequence	Reference	
Salt	100 mM NaCl, 3 days and 5 days after starting treatment	<i>Cucumis melo</i>	0.2 mM	Antioxidant metabolism	Increased plant growth, chlorophyll content, net photosynthetic rate and actual efficiency of photosystem II	Yan et al. (2013)	
	100 mmol L ⁻¹ NaCl, 8 days after treatment	<i>C. sativus</i>	10 mM	Antioxidant metabolism (SOD, POD, CAT and APX activities)	Alleviated plant growth inhibition	Huang et al. (2009)	
	0.23, 3.13, 6.25 dS/m, 45 days after sowing	<i>Helianthus annuus</i>	2.5, 5.0 and 7.5 mM	Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids and growth (plant height, number of leaves, shoot fresh and dry weights/plant)	Improved plant growth	Sadak (2015)	
	200 mM NaCl, 11 days	<i>Nicotiana tabacum</i>	20 mM	Antioxidant metabolism (CAT, POX)	Provided protection against NaCl-induced cell death	Banu et al. (2009)	
	100 and 200 mM NaCl, young leaves (fully expanded leaves that developed soon after the onset of the different treatments)	<i>Olea europaea</i>	25 mM	Ion homeostasis (Na^+ , K^+ and Ca^{2+})	Improved plant growth	Ahmed et al. (2011)	
	150 and 300 mM NaCl for 48 h	<i>Oryza sativa</i>	5.0 mM	Antioxidant metabolism AsA, GSH and GSH/GSSG, APX, MDHAR, DHAR, GR, GPX, CAT, and Gly I activities	Increased tolerance to oxidative damage by up-regulating the antioxidant defense system	Hasanuzzaman et al. (2014)	
	25 mL 100 mmol L ⁻¹ NaCl, 3rd day and the 7th day after seed soaking	<i>O. sativa</i>	15 and 30 mM	Carbohydrate metabolism alpha-amylase, beta-amylase isoenzyme	Alleviated inhibitory effects on seed germination	Hua-long et al. (2014)	
	150 mM NaCl, 30 days after culture	<i>O. sativa</i>	5.0 mM	Plant height, root length	Increased plant growth	Teh et al. (2015)	
	200 mmol/L NaCl, 6 days	<i>O. sativa</i>	10 mM	Antioxidant metabolism (SOD, CAT, APX, POX)	Alleviated plant growth inhibition	Nounjan and Theerakulpisut (2012)	
	100 mM NaCl, 6 days	<i>O. sativa</i>	10 mM	Proline (<i>P5CS</i> and <i>P5CR</i> genes) and antioxidant metabolism (SOD, CAT, APX, POX)	Plants exhibited a pronounced beneficial effect during recovery period showing higher percentage of growth recovery in phosphorus plus sulfur treatment compared with sulfur	Nounjan et al. (2012)	
	100 mM NaCl, 7 days	<i>O. sativa</i>	1.0 mM	Ion homeostasis K^+/Na^+ ratio	Induced salt tolerance	Sobahan et al. (2012)	
			<i>O. sativa</i>	1.0 mM	Osmotic balance	Alleviated detrimental effect on growth and photosynthesis	Deivanai et al. (2011)
			<i>O. sativa</i>	1.0 mM	Osmotic balance	Improved plant growth	Xavier (2011)
	Drought	100, 200, 300 and 400 mM NaCl 75, 150, 225 or 300 mM NaCl, 10 days	<i>Pancratium maritimum</i>	5.0 mM	Osmoregulation (Ubiquitin, dehydrins)	Improved salt tolerance by increasing stress protective proteins	Khedr et al. (2003)
6.57 dS m ⁻¹ , almost 4 months		<i>Solanum lycopersicum</i>	10 and 20 mg L ⁻¹	Photosynthesis (chlorophyll a fluorescence parameters)	Increased plant growth and chlorophyll a fluorescence	Kahlaoui et al. (2014)	
150 mM NaCl, 15 days		<i>S. melongena</i>	10 and 20 mM	Water use efficiency	Counteracted the adverse effects on plant growth	Shahbaz et al. (2013)	
100 mM NaCl, 1 month		<i>Saccharum officinarum</i>	20 mM	Antioxidant metabolism (SOD, GR, APX, GPX, CAT)	Increased plant growth rate	Patade et al. (2014)	
300 mM NaCl, 24 and 48 h		<i>Vigna radiate</i>	15 mM	Antioxidant metabolism (GSH content, GPX, GR)	Reduced oxidative stress	Hossain and Fujita (2010)	
4 week		<i>Arabidopsis thaliana</i>	10 mM	Proline accumulation and chlorophyll fluorescence	Induced tolerance to photoinhibition	Moustakas et al. (2011)	
63 days		<i>Triticum aestivum</i>	20 mM	Growth (shoot and root fresh and dry weights, shoot length, total leaf area)	Improved growth and yield	Kamran et al. (2009)	
21 days interval		<i>Zea mays</i>	30 mM	Secondary metabolites and fatty acids	Ameliorated the adverse effects on seed oil composition	Ali et al. (2013)	
15 days		<i>Z. mays</i>	30 mM	Ion homeostasis (K^+ , Ca^{2+} , N and P)	Increased plant growth	Ali et al. (2008)	
15 days		<i>Z. mays</i>	30 mM	Growth and photosynthetic rate	Counteracted the adverse effects of on plant growth	Ali et al. (2007)	

T. aestivum seeds with Pro alleviated the adverse effects of drought stress on growth and grain yield. Moustakas et al. (2011) have shown that Pro interacts with the signalling pathway of total soluble sugars and quenching of ROS, and promotes drought tolerance in *A. thaliana*. The positive effect of Pro in plants under salt and drought stress is not only limited to physiological parameters but also improves yield and quality of seed oil (Ali et al., 2013). Thus, Pro alleviates stress by serving as a significant osmolyte and also by its ROS-scavenging ability. Table 1 summarizes studies on the role of exogenous Pro in protection of plants under salt and drought stress conditions.

The accumulation of Pro in plants can positively affect light and dark components of photosynthesis under stress conditions (Anjum et al., 2014). Endogenous level of Pro was evidenced to protect complex II of the mitochondrial electron transport chain under salt stress by providing a reducing potential through its oxidation by PDH and P5CDH in mitochondria (Hamilton and Heckathorn, 2001; Kishor et al., 2005). Pro has also been reported to increase activity of methylglyoxal detoxification enzymes, glutathione-S-transferase (GST; EC 2.5.1.18) and GSH redox state in salt stressed *N. tabacum* cells (Hoque et al., 2008; Islam et al., 2009).

Elevated accumulation of Pro led to the protection of photochemical efficiency of PS II and prevented lipid peroxidation during drought stress (Molinari et al., 2007). The inhibition of Pro biosynthesis and NADPH-NADP⁺ conversion by antisense *P5CR* in transgenic soybean (*Glycine max.*) led to drought-hypersensitivity; whereas, over-expression of *P5CR* prevented damage to PS II by maintaining NADPH level and improved drought tolerance (De Ronde et al., 2004). Studies showing influence of Pro on antioxidant metabolism have also been well documented. High Pro accumulation due to the expression of a feedback-insensitive *P5CS* from *V. aconitifolia* differentially regulated APX, CAT and SOD in transgenic *Citrus paradisi* under drought conditions (de Carvalho et al., 2013). In contrast, few reports are available on the negative influence of Pro accumulation in plants. Exogenous application of Pro in petunia (*Petunia hybrida*) resulted in increased Pro accumulation in transgenic and wild type plants (Yamada et al., 2005). However, the wild-type plants showed 18-times higher Pro accumulation than non-treated plants that exhibited suppressed growth (Yamada et al., 2005). In another study, all *HspP5CS* lines and control line *HspGUS4* showed similar basal thermo-tolerance without Pro induction (Lv et al., 2011). It was argued that Pro

Table 2
Transgenics developed from proline biosynthetic pathway genes that conferred drought and salt stress tolerance.

Stress	Gene	Source	Host	Reference	
Salt	<i>P5CS</i>	<i>Vigna aconitifolia</i>	<i>Triticum aestivum</i>	Sawahel and Hassan (2002)	
	<i>P5CS</i>	<i>Vigna aconitifolia</i>	<i>Daucus carota</i>	Han and Hwang (2003)	
	<i>P5CS</i>	Not known	<i>Olea europaea</i> (Olive)	Behelgardy et al. (2012)	
	<i>P5CS</i>	<i>Arabidopsis</i>	<i>Nicotiana tabacum</i>	Rastgar et al. (2011)	
	<i>P5CS1</i>	<i>Phaseolus vulgaris</i>	<i>Arabidopsis</i>	Ji-Bao et al. (2010)	
	<i>P5CR</i>	<i>Triticum aestivum</i>	<i>Arabidopsis</i>	Ma et al. (2008)	
	<i>ProDH</i>	Not known	<i>N. tabacum</i>	Kolodyazhnaya et al. (2006)	
	<i>ProBA</i>	<i>Bacillus subtilis</i>	<i>Arabidopsis</i>	Zeng et al. (2005)	
	<i>ProBA</i>	<i>Escherichia coli</i>	<i>N. tabacum</i>	Sokhansandzh et al. (1997)	
	<i>Anti-ProDH</i>	Antisense suppression	<i>Arabidopsis</i>	Nanjo et al. (1999)	
	<i>OsP5CS2</i>	<i>Vigna aconitifolia</i>	<i>Oryza sativa</i>	Hur et al. (2004)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>Cicer arietinum</i>	Ghanti et al. (2011)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>Oryza sativa</i>	Karthikeyan et al. (2011)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>O. sativa</i>	Kumar et al. (2010)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>Larix leptoeuropaea</i>	Gleeson et al. (2005)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>Medicago truncatula</i>	Verdoy et al. (2006)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>N. tabacum</i>	Kishor et al. (1995)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>Oryza sativa</i>	Su and Wu (2004)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>O. sativa</i>	Anoop and Gupta (2003)	
	<i>P5CS</i>	<i>V. aconitifolia</i>	<i>O. sativa</i>	Zhu et al. (1998)	
	<i>P5CS</i>	<i>Arabidopsis</i>	<i>Solanum tuberosum</i>	Hmida-Sayari et al. (2005)	
	<i>P5CSF129A</i>	<i>V. aconitifolia</i>	<i>Cajanus cajan</i>	Surekha et al. (2014)	
	<i>P5CSF129A</i>	<i>V. aconitifolia</i>	<i>N. tabacum</i>	Hong et al. (2000)	
	δ -OAT	<i>Arabidopsis</i>	<i>N. plumbaginifolia</i>	Roosens et al. (2002)	
	δ -OAT	<i>Arabidopsis</i>	<i>O. sativa</i>	Wu et al. (2003)	
	<i>P5CSF129A</i>	<i>Vigna aconitifolia</i>	<i>Sorghum bicolor</i>	Reddy et al. (2015)	
	Drought	<i>P5CS</i>	<i>Arabidopsis</i> , <i>Oryza sativa</i>	<i>Petunia</i>	Yamada et al. (2005)
		<i>P5CS1</i>	<i>Phaseolus vulgaris</i>	<i>Arabidopsis</i>	Ji-Bao et al. (2010)
		<i>P5CS1</i>	<i>Arabidopsis</i>	<i>Arabidopsis</i>	Yoshiba et al. (1999)
		<i>P5CS2</i>	<i>Phaseolus vulgaris</i>	<i>N. tabacum</i>	Chen et al. (2008)
		<i>P5CSF129A</i>	<i>Vigna aconitifolia</i>	<i>Citrus paradisi</i> × <i>C. trifoliata</i> (Swingle citrumelo)	de Campos et al. (2011)
		<i>P5CSF129A</i>	<i>V. aconitifolia</i>	<i>N. tabacum</i>	Pospisilova et al. (2011)
<i>P5CSF129A</i>		<i>V. aconitifolia</i>	<i>Cicer arietinum</i>	Bhatnagar-Mathur et al. (2009)	
<i>P5CSF129A</i>		<i>V. aconitifolia</i>	<i>Festuca arundinaceae</i>	Li et al. (2005)	
<i>P5CR</i>		<i>Arabidopsis</i>	<i>Glycine max</i>	Kocsy et al. (2005)	
<i>OAT</i>		<i>Oryza sativa</i>	<i>Oryza sativa</i>	You et al. (2012)	
<i>P5CS</i>		<i>V. aconitifolia</i>	<i>Citrus</i>	Molinari et al. (2004)	
<i>P5CS</i>		<i>V. aconitifolia</i>	<i>N. tabacum</i>	Kishor et al. (1995)	
<i>P5CS</i>		<i>V. aconitifolia</i>	<i>Oryza sativa</i>	Su and Wu (2004)	
<i>P5CS</i>		<i>V. aconitifolia</i>	<i>O. sativa</i>	Zhu et al. (1998)	
<i>P5CS</i>		<i>V. aconitifolia</i>	<i>Triticum aestivum</i>	Vendruscolo et al. (2007)	
<i>P5CR</i>		<i>Arabidopsis</i>	<i>Glycine max</i>	De Ronde et al. (2004)	
δ -OAT		<i>Arabidopsis</i>	<i>O. sativa</i>	Wu et al. (2003)	
<i>P5CSF129A</i>		<i>Vigna aconitifolia</i>	<i>Sorghum bicolor</i>	Reddy et al. (2015)	

accumulation under heat stress decreased the thermo-tolerance by increasing ROS production via the Pro/P5C cycle and ABA-inhibition and ethylene biosynthesis (Lv et al., 2011). Earlier, exogenous Pro-mediated exaggeration and the inhibitory effects of heat stress were reported in *Arabidopsis* seedlings (Rizhsky et al., 2004). A schematic presentation of Pro-mediated control of major metabolic processes, and salt and drought stress resistance is shown in Fig. 3.

8. Conclusions and future prospects

Plants adaptation to salt and drought stress is a complex phenomenon and involves a range of osmolytes production. Pro is one of the osmolytes and signalling molecules widely reported to accumulate in plants under salt and drought stress conditions. Pro protects plants against drought and salinity stress mainly by maintaining osmotic adjustment, ROS-scavenging, and modulating antioxidant metabolites and major enzymatic components of antioxidant defense system. Phytohormones regulate Pro production and also stress tolerance. It is, thus, expected that there is a close relationship between phytohormones and Pro metabolism. The unraveling of these relationships could enhance the understanding of regulatory points involved in phytohormone-mediated Pro metabolism. The unavailability of nutrients to plants under salt

and drought stress brings many unavoidable consequences in plants. Exploration of physiological/biochemical and molecular insights into the occurrence of antagonism and synergism among major mineral nutrients, and their cumulative role in Pro metabolism, and plant drought/salt tolerance can be an important aspect of future research. Up-regulation of Pro biosynthesis enzymes or the over-expression of the Pro biosynthesis enzyme-genes has been resulted into enhanced tolerance of plants to drought and salinity stress. Therefore, identifying the genes reported to regulate Pro biosynthesis, and their modulation can benefit sustainable agriculture by improving drought and salt stress tolerance in plants and. Further, identification of signal transduction pathways, promoter elements and other transcription factors involved in Pro metabolism, and their relationship with phytohormones can enlighten the molecular mechanisms involved in Pro-accumulation and its role in salt and drought stress resistance in plants. The outcome of these studies may be useful in translating information to other salinity-sensitive crops.

Authors contribution

All authors are contributed equally.

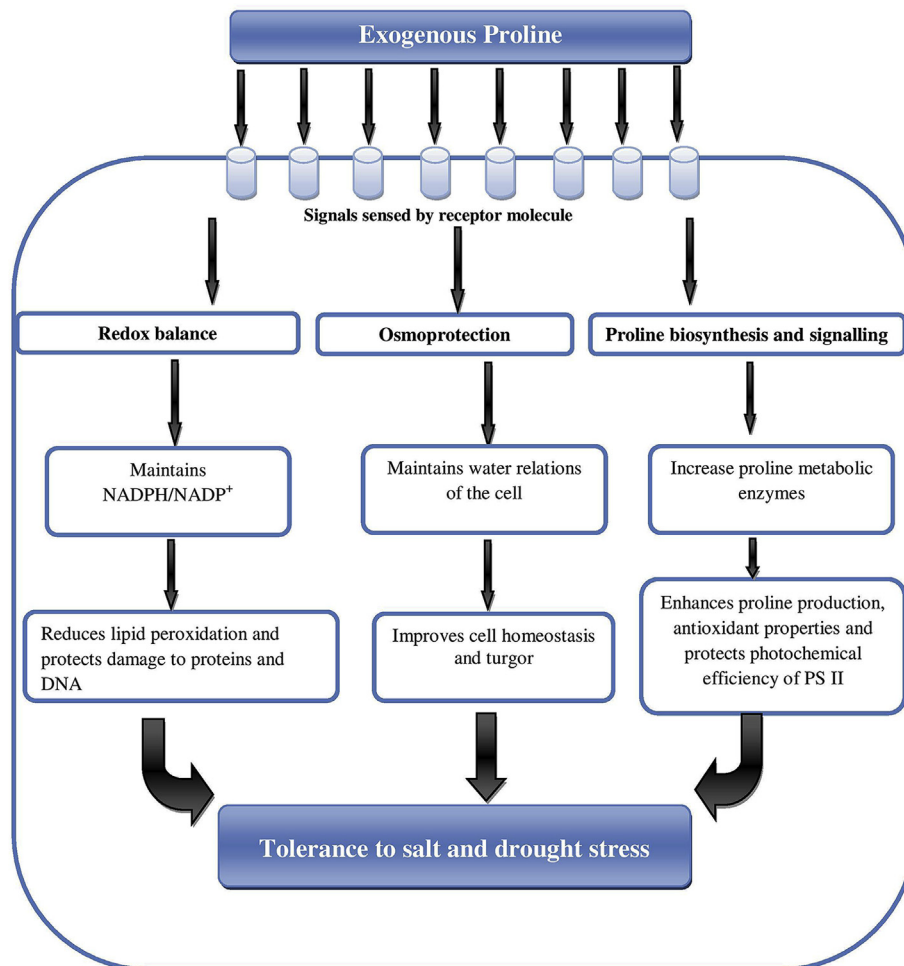


Fig. 3. Schematic representation of potential mechanism involved in salt and drought tolerance after exogenous application of proline. APX; ascorbate peroxidase, AsA; ascorbic acid, CAT; catalase, GSH; reduced glutathione, GST; glutathione S-transferase, NADPH/NADP⁺; Nicotinamide dinucleotide phosphate (reduced/oxidized), POX; peroxidase, PS II; photosystem II, ROS; reactive oxygen species, SOD; superoxide dismutase.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.plaphy.2017.03.018>.

References

- Abrahám, E., Rigó, G., Székely, G., Nagy, R., Koncz, C., Szabados, L., 2003. Light-dependent induction of proline biosynthesis by abscisic acid and salt stress is inhibited by brassinosteroid in *Arabidopsis*. *Plant Mol. Biol.* 51, 363–372.
- Ahmed, C.B., Magdich, S., Rouina, B.B., Sensoy, S., Boukhris, M., Abdullah, F.B., 2011. Exogenous proline effects on water relations and ions contents in leaves and roots of young olive. *Amino Acids* 40, 565–573.
- Albert, B., Le Cahérec, F., Niogret, M.F., Faes, P., Avice, J.C., Leprot, L., Bouchereau, A., 2012. Nitrogen availability impacts oilseed rape (*Brassica napus* L.) plant water status and proline production efficiency under water-limited conditions. *Planta* 236, 659–676.
- Ali, Q., Anwar, F., Ashraf, M., Saari, N., Perveen, R., 2013. Ameliorating effects of exogenously applied proline on seed composition, seed oil quality and oil antioxidant activity of maize (*Zea mays* L.) under drought stress. *Intl. J. Mol. Sci.* 14, 818–835.
- Ali, Q., Ashraf, M., Athar, H.U.R., 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot.* 39, 1133–1144.
- Ali, Q., Ashraf, M., Shahbaz, M., Humera, H., 2008. Ameliorating effect of foliar applied proline on nutrient uptake in water stressed maize (*Zea mays* L.) plants. *Pak. J. Bot.* 40, 211–219.
- Alia, Mohanty, P., Matysik, J., 2001. Effect of proline on the production of singlet oxygen. *Amino Acids* 21, 195–200.
- Al-Whaibi, M.H., Siddiqui, M.H., Basalah, M.O., 2012. Salicylic acid and calcium induced protection of wheat against salinity. *Protoplasma* 249, 769–778.
- Anjum, N.A., Aref, I.M., Duarte, A.C., Pereira, E., Ahmad, I., Iqbal, M., 2014. Glutathione and proline can coordinately make plants withstand the joint attack of metal (loid) and salinity stresses. *Front. Plant Sci.* 5, 662.
- Anjum, N.A., Lopez-Lauri, F., 2011. Plant nutrition and abiotic stress tolerance III. In: *Plant Stress*, vol. 5 (Special Issue 1, ISBN 978-4-903313-59-7).
- Anoop, N., Gupta, A.K., 2003. Transgenic *Indica* rice cv IR-50 over-expressing *Vigna aconitifolia* Δ^1 -Pyrroline-5-carboxylate synthetase cDNA shows tolerance to high salt. *J. Plant Biochem. Biotechnol.* 12, 109–116.
- Armengaud, P., Thiery, L., Buhot, N., Grenier-de March, G., Savouré, A., 2004. Transcriptional regulation of proline biosynthesis in *Medicago truncatula* reveals developmental and environmental specific features. *Physiol. Plant* 120, 442–450.
- Banu, M.N.A., Hoque, M.A., Watanabe-Sugimoto, M., Matsuoka, K., Nakamura, Y., Shimoishi, Y., Murata, Y., 2009. Proline and glycinebetaine induce antioxidant defense gene expression and suppress cell death in cultured tobacco cells under salt stress. *J. Plant Physiol.* 166, 146–156.
- Behelgardy, M.F., Motamed, N., Jazii, F.R., 2012. Expression of the *P5CS* gene in transgenic versus nontransgenic olive (*Olea europaea*) under salinity stress. *World Appl. Sci. J.* 18, 580–583.
- Bhatnagar-Mathur, P., Vadez, V., Devi, M.J., Lavanya, M., Vani, G., Sharma, K.K., 2009. Genetic engineering of chickpea (*Cicer arietinum* L.) with the *P5CSF129A* gene for osmoregulation with implications on drought tolerance. *Mol. Breed.* 23, 591–606.
- Bhupinder, S., Usha, K., 2003. Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regul.* 39, 137–141.
- Bot, A., Nachtergaele, F., Young, A., 2000. Land Resource Potential and Constraints at Regional and Country Levels (No. 90). Food & Agriculture Org.
- Carillo, P., Mastrodonato, G., Nacca, F., Parisi, D., Verlotta, A., Fuggi, A., 2008. Nitrogen metabolism in durum wheat under salinity: accumulation of proline and glycinebetaine. *Funct. Plant Biol.* 35, 412–426.
- Chen, J.B., Jing, R.L., Mao, X.G., Wang, S.M., 2008. A response of transgenic tobacco with common bean *PvP5CS2* gene to drought stress [J]. *J. Plant Gen. Res.* 9, 186–189.
- Cui, M., Lin, Y., Zu, Y., Efferth, T., Li, D., Tang, Z., 2015. Ethylene increases accumulation of compatible solutes and decreases oxidative stress to improve plant tolerance to water stress in *Arabidopsis*. *J. Plant Biol.* 58, 193–201.
- de Campos, M.K.F., de Carvalho, K., de Souza, F.S., Marur, C.J., Pereira, L.F.P., Besspalhok Filho, J.C., Vieira, L.G.E., 2011. Drought tolerance and antioxidant enzymatic activity in transgenic 'Swingle'citrumelo plants over-accumulating proline. *Environ. Exp. Bot.* 72, 242–250.
- de Carvalho, K., de Campos, M.K.F., Domingues, D.S., Pereira, L.F.P., Vieira, L.G.E., 2013. The accumulation of endogenous proline induces changes in gene expression of several antioxidant enzymes in leaves of transgenic Swingle citrumelo. *Mol. Biol. Rep.* 40, 3269–3279.
- De Ronde, J.A., Cress, W.A., Krüger, G.H.J., Strasser, R.J., Van Staden, J., 2004. Photosynthetic response of transgenic soybean plants, containing an *Arabidopsis* *P5CR* gene, during heat and drought stress. *J. Plant Physiol.* 161, 1211–1224.
- Deivanai, S., Xavier, R., Vinod, V., Timalata, K., Lim, O.F., 2011. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *J. Stress Physio. Biochem.* 7, 157–174.
- Díaz, P., Borsani, O., Márquez, A., Monza, J., 2005. Osmotically induced proline accumulation in *Lotus corniculatus* leaves is affected by light and nitrogen source. *Plant Growth Regul.* 46, 223–232.
- Dobra, J., Motyka, V., Dobrev, P., Malbeck, J., Prasil, I.T., Haisel, D., Gaudinova, A., Havlova, M., Gubis, J., Vankova, R., 2010. Comparison of hormonal responses to heat, drought and combined stress in tobacco plants with elevated proline content. *J. Plant Physiol.* 167, 1360–1370.
- Du, L., Ali, G.S., Simons, K.A., Hou, J., Yang, T., Reddy, A.S.N., Poovaiah, B.W., 2009. Ca^{2+} /calmodulin regulates salicylic-acid-mediated plant immunity. *Nature* 457, 1154–1158.
- Ehsanpour, A.A., Zarei, S., Abbaspour, J., 2012. The role of over expression of *P5CS* gene on proline, catalase, ascorbate peroxidase activity and lipid peroxidation of transgenic tobacco (*Nicotiana tabacum* L.) plant under in vitro drought stress. *J. Cell Mol. Res.* 4, 43–49.
- El-Tayeb, M.A., 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.* 45, 215–224.
- Fan, H.F., Du, C.X., Guo, S.R., 2012. Effect of nitric oxide on proline metabolism in cucumber seedlings under salinity stress. *J. Am. Soc. Hortic. Sci.* 137, 127–133.
- Feng, X.J., Li, J.R., Qi, S.L., Lin, Q.F., Jin, J.B., Hua, X.J., 2016. Light affects salt stress-induced transcriptional memory of *P5CS1* in *Arabidopsis*. *Proceed. Natl. Acad. Sci.* 113, E8335–E8343.
- Fichman, Y., Gerdes, S.Y., Kovács, H., Szabados, L., Zilberstein, A., Csonka, L.N., 2015. Evolution of proline biosynthesis: enabozymology, bioinformatics, genetics, and transcriptional regulation. *Biol. Rev.* 90, 1065–1099.
- Flowers, T.J., Troke, P.F., Yeo, A.R., 1977. The mechanism of salt tolerance in halophytes. *Annu. Rev. Plant Physiol.* 28, 89–121.
- Fujiwara, T., Mitsuya, S., Miyake, H., Hattori, T., Takabe, T., 2010. Characterization of a novel glycinebetaine/proline transporter gene expressed in the mestome sheath and lateral root cap cells in barley. *Planta* 232, 133–143.
- Funck, D., Eckard, S., Müller, G., 2010. Non-redundant functions of two proline dehydrogenase isoforms in *Arabidopsis*. *BMC Plant Biol.* 10 (70).
- Funck, D., Stadelhofer, B., Koch, W., 2008. Ornithine- δ -aminotransferase is essential for arginine catabolism but not for proline biosynthesis. *BMC Plant Biol.* 81, 40.
- Ghanti, S.K.K., Sujata, K.G., Kumar, B.V., Karba, N.N., Reddy, K.J., Rao, M.S., Kishor, P.K., 2011. Heterologous expression of *P5CS* gene in chickpea enhances salt tolerance without affecting yield. *Biol. Plant.* 55, 634–640.
- Girousse, C., Bournoville, R., Bonnemain, J.L., 1996. Water deficit-induced changes in concentrations in proline and some other amino acids in the phloem sap of alfalfa. *Plant Physiol.* 111, 109–113.
- Gleeson, D., Lelu-Walter, M.A., Parkinson, M., 2005. Overproduction of proline in transgenic hybrid larch (*Larix x leptoeuropaea* (Dengler)) cultures renders them tolerant to cold, salt and frost. *Mol. Breed.* 15, 21–29.
- Golldack, D., Li, C., Mohan, H., Probst, N., 2014. Tolerance to drought and salt stress in plants: unraveling the signaling networks. *Front. Plant Sci.* 5, 10.3389/fpls.2014.00151.
- Grallath, S., Weimar, T., Meyer, A., Gumy, C., Suter-Grottemeyer, M., Neuhaus, J.M., Rentsch, D., 2005. The AtProT family. Compatible solute transporters with similar substrate specificity but differential expression patterns. *Plant Physiol.* 137, 117–126.
- Hamilton, E.W., Heckathorn, S.A., 2001. Mitochondrial adaptations to NaCl. Complex I is Prot. by anti-oxidants small heat shock proteins, whereas complex II is Prot. by proline betaine. *Plant Physiol.* 126, 1266–1274.
- Han, K.H., Hwang, C.H., 2003. Salt tolerance enhanced by transformation of a *P5CS* gene in carrot. *J. Plant Biotech.* 5, 149–153.
- Hasanuzzaman, M., Alam, M.M., Rahman, A., Hasanuzzaman, M., Nahar, K., Fujita, M., 2014. Exogenous proline and glycinebetaine mediated upregulation of antioxidant defense and glyoxalase systems provides better protection against salt-induced oxidative stress in two rice (*Oryza sativa* L.) varieties. *BioMed. Res. Intl.* <http://dx.doi.org/10.1155/2014/757219>.
- Hmida-Sayari, A., Gargouri-Bouzd, R., Bidani, A., Jaoua, L., Savouré, A., Jaoua, S., 2005. Overexpression of Δ^1 -pyrroline-5-carboxylate synthetase increases proline production and confers salt tolerance in transgenic potato plants. *Plant Sci.* 169, 746–752.
- Hong, Z., Lakkinen, K., Zhang, Z., Verma, D.P.S., 2000. Removal of feedback inhibition of Δ^1 -pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant Physiol.* 122, 1129–1136.
- Hoque, M.A., Banu, M.N.A., Nakamura, Y., Shimoishi, Y., Murata, Y., 2008. Proline and glycinebetaine enhance antioxidant defense and methylglyoxal detoxification systems and reduce NaCl-induced damage in cultured tobacco cells. *J. Plant Physiol.* 165, 813–824.
- Hoque, M.A., Banu, M.N.A., Okuma, E., Amako, K., Nakamura, Y., Shimoishi, Y., Murata, Y., 2007. Exogenous proline and glycinebetaine increase NaCl-induced ascorbate–glutathione cycle enzyme activities, and proline improves salt tolerance more than glycinebetaine in tobacco Bright Yellow-2 suspension-cultured cells. *J. Plant Physiol.* 164, 1457–1468.
- Hossain, M.A., Fujita, M., 2010. Evidence for a role of exogenous glycinebetaine and

- proline in antioxidant defense and methylglyoxal detoxification systems in mung bean seedlings under salt stress. *Physiol. Mol. Biol. Plants* 16, 19–29.
- Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem, W., Zimmermann, P., 2008. Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinforma.* <http://dx.doi.org/10.1155/2008/420747>.
- Hua-long, L., Han-jing, S., Jing-guo, W., Yang, L., De-tang, Z., Hong-wei, Z., 2014. Effect of seed soaking with exogenous proline on seed germination of rice under salt stress. *J. Northeast Agric. Univ.* 21, 1–6 (English edition).
- Huang, Y., Bie, Z., Liu, Z., Zhen, A., Wang, W., 2009. Protective role of proline against salt stress is partially related to the improvement of water status and peroxidase enzyme activity in cucumber. *Soil Sci. Plant Nutr.* 55, 698–704.
- Hur, J., Jung, K.H., Lee, C.H., An, G., 2004. Stress-inducible OsP5CS2 gene is essential for salt and cold tolerance in rice. *Plant Sci.* 167, 417–426.
- Igarashi, Y., Yoshida, Y., Takeshita, T., Nomura, S., Otomo, J., Yamaguchi-Shinozaki, K., Shinozaki, K., 2000. Molecular cloning and characterization of a cDNA encoding proline transporter in rice. *Plant Cell Physiol.* 41, 750–756.
- Iqbal, N., Umar, S., Khan, N.A., 2015. Nitrogen availability regulates proline and ethylene production and alleviates salinity stress in mustard (*Brassica juncea*). *J. Plant Physiol.* 178, 84–91.
- Iqbal, N., Umar, S., Khan, N.A., Khan, M.I.R., 2014. A new perspective of phytohormones in salinity tolerance: regulation of proline metabolism. *Environ. Exp. Bot.* 100, 34–42.
- Islam, M.M., Hoque, M.A., Okuma, E., Banu, M.N.A., Shimoishi, Y., Nakamura, Y., Murata, Y., 2009. Exogenous proline and glycinebetaine increase antioxidant enzyme activities and confer tolerance to cadmium stress in cultured tobacco cells. *J. Plant Physiol.* 166, 1587–1597.
- Jain, M., Mathur, G., Koul, S., Sarin, N., 2001. Ameliorative effects of proline on salt stress-induced lipid peroxidation in cell lines of groundnut (*Arachis hypogaea* L.). *Plant Cell Rep.* 20, 463–468.
- Jaleel, C.A., Gopi, R., Sankar, B., Manivannan, P., Kishorekumar, A., Sridharan, R., Panneerselvam, R., 2007. Studies on germination, seedling vigour, lipid peroxidation and proline metabolism in *Catharanthus roseus* seedlings under salt stress. *South Afr. J. Bot.* 73, 190–195.
- Jang, S.W., Hamayun, M., Sohn, E.Y., Shin, D.H., Kim, K.U., Lee, B.H., Lee, I.J., 2008. Effect of elevated nitrogen levels on endogenous gibberellin and jasmonic acid contents of three rice (*Oryza sativa* L.) cultivars. *J. Plant Nutr.* 31, 171, 181–186.
- Ji-Bao, C., LiYing, Z., XinGuo, M., ShuMin, W., RuiLian, J., 2010. Response of PvP5CS1 transgenic Arabidopsis plants to drought-and salt-stress. *Acta Agron. Sin.* 36, 147–153.
- Jing-hong, H., Yang, Y., Qing-mao, S., Chun-juan, D., Zhi-gang, Z., 2012. Effect of exogenous salicylic acid on nitrogen assimilation of cucumber seedling under drought stress. *Acta Hort.* 39, 81–90.
- Kahlaoui, B., Hachicha, M., Rejeb, S., Rejeb, M.N., Hanchi, B., Mislé, E., 2014. Response of two tomato cultivars to field-applied proline under irrigation with saline water: growth, chlorophyll fluorescence and nutritional aspects. *Photosynthetica* 52, 421–429.
- Kamran, M., Shahbaz, M., Ashraf, M., Akram, N.A., 2009. Alleviation of drought-induced adverse effects in spring wheat (*Triticum aestivum* L.) using proline as a pre-sowing seed treatment. *Pak. J. Bot.* 41, 621–632.
- Karthikeyan, A., Pandian, S.K., Ramesh, M., 2011. Transgenic indica rice cv. ADT 43 expressing a Δ^1 -pyrroline-5-carboxylate synthetase (P5CS) gene from *Vigna aconitifolia* demonstrates salt tolerance. *Plant Cell Tissue Organ Cult.* 107, 383–395.
- Kaur, G., Asthir, B., 2015. Proline: a key player in plant abiotic stress tolerance. *Biol. Plant* 59, 609–619.
- Khan, M.I.R., Asgher, M., Khan, N.A., 2014. Alleviation of salt-induced photosynthesis and growth inhibition by salicylic acid involves glycinebetaine and ethylene in mungbean (*Vigna radiata* L.). *Plant Physiol. biochem.* 80, 67–74.
- Khan, M.I.R., Fatma, M., Per, T.S., Anjum, N.A., Khan, N.A., 2015. Salicylic acid-induced abiotic stress tolerance and underlying mechanisms in plants. *Front. Plant Sci.* 6, 462.
- Khan, M.I.R., Iqbal, N., Masood, A., Per, T.S., Khan, N.A., 2013. Salicylic acid alleviates adverse effects of heat stress on photosynthesis through changes in proline production and ethylene formation. *Plant Signal.Behav.* 8 <http://dx.doi.org/10.4161/psb.26374>.
- Khan, M.N., Siddiqui, M.H., Mohammad, F., Naeem, M., Khan, M.M.A., 2010. Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defence system and osmoprotectant accumulation. *Acta Physiol. Plant* 32, 121–132.
- Khan, N.A., Nazar, R., Iqbal, N., Anjum, N.A. (Eds.), 2012. *Phytohormones and Abiotic Stress Tolerance in Plants*. Springer Science & Business Media.
- Khedr, A.H.A., Abbas, M.A., Wahid, A.A.A., Quick, W.P., Abogadallah, G.M., 2003. Proline induces the expression of salt-stress-responsive proteins and may improve the adaptation of *Pancreaticum maritimum* L. to salt-stress. *J. Exp. Bot.* 54, 2553–2562.
- Kim, G.B., Nam, Y.W., 2013. A novel Δ^1 -pyrroline-5-carboxylate synthetase gene of *Medicago truncatula* plays a predominant role in stress-induced proline accumulation during symbiotic nitrogen fixation. *J. Plant Physiol.* 170, 291–302.
- Kishor, K., Polavarapu, B., Sreenivasulu, N., 2014. Is proline accumulation per se correlated with stress tolerance or is proline homeostasis a more critical issue? *Plant Cell Environ.* 37, 300–311.
- Kishor, K.P.B., Sangam, S., Amrutha, R.N., Laxmi, P.S., Naidu, K.R., Rao, K.R., Rao, S., Reddy, K.J., Theriappan, P., Sreenivasulu, N., 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. *Curr. Sci.* 88, 424–438.
- Kishor, P.K., Hong, Z., Miao, G.H., Hu, C.A.A., Verma, D.P.S., 1995. Overexpression of [Δ^1]-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiol.* 108, 1387–1394.
- Kiyosue, T., Yoshida, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., 1996. A nuclear gene encoding mitochondrial proline dehydrogenase, an enzyme involved in proline metabolism, is upregulated by proline but downregulated by dehydration in *Arabidopsis*. *Plant Cell* 8, 1323–1335.
- Klepek, Y., Geiger, D., Stadler, R., Kleibl, F., Landouar-Arsivaud, L., Lemoine, R., Hedrich, R., Sauer, N., 2005. Arabidopsis polyol transporter 5, a new member of the monosaccharide transporter-like superfamily, mediates H⁺-symport of numerous substrates, including myo-inositol, glycerol, and ribose. *Plant Cell* 17, 204–218.
- Knight, H., Anthony, J., Knight, M.R., 1997. Calcium signalling in *Arabidopsis thaliana* responding to drought and salinity. *Plant J.* 12, 1067–1078.
- Kocsy, G., Laurie, R., Szalai, G., Szilágyi, V., Simon-Sarkadi, L., Galiba, G., De Ronde, J.A., 2005. Genetic manipulation of proline levels affects antioxidants in soybean subjected to simultaneous drought and heat stresses. *Physiol. Plant* 124, 227–235.
- Kohl, D.H., Straub, P.F., Shearer, G., 1994. Does proline play a special role in bacteroid metabolism? *Plant Cell Environ.* 17, 1257–1262.
- Kolodyazhnaya, I., Titov, S.E., Kochetov, A.V., Komarova, M.L., Romanova, A.V., Koval, V.S., Shumny, V.K., 2006. Evaluation of salt tolerance in *Nicotiana glauca* plants bearing an antisense suppressor of the proline dehydrogenase gene. *Genetika* 42, 278–281.
- Kumar, V., Shriram, V., Kishor, P.K., Jawali, N., Shitole, M.G., 2010. Enhanced proline accumulation and salt stress tolerance of transgenic indica rice by over-expressing P5CSF129A gene. *Plant Biotechnol. Rep.* 4, 37–48.
- Lee, T.M., Liu, C.H., 1999. Correlation of decreased calcium contents with proline accumulation in the marine green macroalga *Ulva fasciata* exposed to elevated NaCl contents in seawater. *J. Exp. Bot.* 50, 1855–1862.
- Lehmann, S., Funck, D., Szabados, L., Rentsch, D., 2010. Proline metabolism and transport in plant development. *Amino Acid.* 39, 949–962.
- Lehmann, S., Gummy, C., Blatter, E., Boeffel, S., Fricke, W., Rentsch, D., 2011. In planta function of compatible solute transporters of the AtProT family. *J. Exp. Bot.* 62, 787–796.
- Li, Z., Congli, H., Xiuhai, Z., Mingqing, C., Zheng, L., Gang, W., Zhongyi, W., 2005. Transferring P5CS gene into tall fescue (*Festuca arundinacea* Schreb) via microprojectile bombardment. *Acta Agron. Sin.* 32, 653–657.
- Lopez-Carrion, A.I., Castellano, R., Rosales, M.A., Ruiz, J.M., Romero, L., 2008. Role of nitric oxide under saline stress: implications on proline metabolism. *Biol. Plant* 52, 587–591.
- Lv, W.T., Lin, B., Zhang, M., Hua, X.J., 2011. Proline accumulation is inhibitory to *Arabidopsis* seedlings during heat stress. *Plant Physiol.* 156, 1921–1933.
- Ma, L., Zhou, E., Gao, L., Mao, X., Zhou, R., Jia, J., 2008. Isolation, expression analysis and chromosomal location of P5CR gene in common wheat (*Triticum aestivum* L.). *South Afr. J. Bot.* 74, 705–712.
- Manai, J., Zaghdoud, C., Debouba, M., Donia, H.G., Bouthour, 2012. Role of nitric oxide in saline stress: implications on nitrogen metabolism. *Afr. J. Plant Sci.* 6, 376–382.
- Matysik, J., Bhalu, B., Mohanty, P., 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* 82, 525–532.
- Miller, G., Stein, H., Honig, A., Kapulnik, Y., Zilberstein, A., 2005. Responsive modes of *Medicago sativa* proline dehydrogenase genes during salt stress and recovery dictate free proline accumulation. *Planta* 222, 70–79.
- Misra, N., Gupta, A.K., 2006. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in *Catharanthus roseus* seedlings. *J. Plant Physiol.* 163, 11–18.
- Misra, N., Saxena, P., 2009. Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. *Plant Sci.* 177, 181–189.
- Moat, A.G., Foster, J.W., Spector, M.P., 2003. *Biosynthesis and metabolism of amino acids*. In: Moat, A.G., Foster, J.W., Spector, M.P. (Eds.), *Microbial Physiology*. John Wiley and Sons, New York, pp. 503–544.
- Molinari, H.B.C., Marur, C.J., Daros, E., De Campos, M.K.F., De Carvalho, J.F.R.P., Pereira, L.F.P., Vieira, L.G.E., 2007. Evaluation of the stress-inducible production of proline in transgenic sugarcane (*Saccharum spp.*): osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiol. Plant* 130, 218–229.
- Molinari, H.B.C., Marur, C.J., Filho, J.C.B., Kobayashi, A.K., Pileggi, M., Júnior, R.P.L., Pereira, L.F.P., Vieira, L.G.E., 2004. Osmotic adjustment in transgenic citrus rootstock Carrizo citrange (*Citrus sinensis* Osb. x *Poncirus trifoliata* L. Raf.) overproducing proline. *Plant Sci.* 167, 1375–1381.
- Monreal, J.A., Jiménez, E.T., Remesal, E., Morillo-Velarde, R., García-Mauriño, S., Echevarría, C., 2007. Proline content of sugar beet storage roots: response to water deficit and nitrogen fertilization at field conditions. *Environ. Exp. Bot.* 60, 257–267.
- Moustakas, M., Sperdouli, I., Kouna, T., Antonopoulou, C.I., Therios, I., 2011. Exogenous proline induces soluble sugar accumulation and alleviates drought stress effects on photosystem II functioning of *Arabidopsis thaliana* leaves. *Plant Growth Regul.* 65, 315–325.
- Murugan, K., Sathish, D.K., 2005. Ameliorative Effect by calcium on NaCl salinity stress related to proline metabolism in the callus of *Centella asiatica* L. *J. Plant Biochem. Biotechnol.* 14, 205–207.
- Naliwajski, M.R., Skłodowska, M., 2014. Proline and its metabolism enzymes in cucumber cell cultures during acclimation to salinity. *Protoplasma* 251,

- 201–209.
- Nanjo, T., Kobayashi, M., Yoshida, Y., Kakubari, Y., Yamaguchi-Shinozaki, K., Shinozaki, K., 1999. Antisense suppression of proline degradation improves tolerance to freezing and salinity in *Arabidopsis thaliana*. *FEBS Lett.* 461, 205–210.
- Nazar, R., Iqbal, N., Syeed, S., Khan, N.A., 2011. Salicylic acid alleviates decreases in photosynthesis under salt stress by enhancing nitrogen and sulfur assimilation and antioxidant metabolism differentially in two mungbean cultivars. *J. Plant Physiol.* 168, 807–815.
- Nazar, R., Umar, S., Khan, N.A., Sareer, O., 2015. Salicylic acid supplementation improves photosynthesis and growth in mustard through changes in proline accumulation and ethylene formation under drought stress. *South Afr. J. Bot.* 98, 84–94.
- Nounjan, N., Nghia, P.T., Theerakulpisut, P., 2012. Exogenous proline and trehalose promote recovery of rice seedlings from salt-stress and differentially modulate antioxidant enzymes and expression of related genes. *J. Plant Physiol.* 169, 596–604.
- Nounjan, N., 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, 309–315.
- Parida, A.K., Dagaonkar, V.S., Phalak, M.S., Aurangabadkar, L.P., 2008. Differential responses of the enzymes involved in proline biosynthesis and degradation in drought tolerant and sensitive cotton genotypes during drought stress and recovery. *Acta Physiol. Plant* 30, 619–627.
- Patade, V.Y., Lokhande, V.H., Suprasanna, P., 2014. Exogenous application of proline alleviates salt induced oxidative stress more efficiently than glycinebetaine in sugarcane cultured cells. *Sugar Tech.* 16, 22–29.
- Pinheiro, C., Chaves, M.M., Ricardo, C.P., 2001. Alterations in carbon and nitrogen metabolism induced by water deficit in the stems and leaves of *Lupinus albus* L. *J. Exp. Bot.* 52, 1063–1070.
- Planchet, E., Verdu, I., Delahaie, J., Cukier, C., Girard, C., Morère-Le Paven, M.C., Limami, A.M., 2014. Abscisic acid-induced nitric oxide and proline accumulation in independent pathways under water-deficit stress during seedling establishment in *Medicago truncatula*. *J. Exp. Bot.* <http://dx.doi.org/10.1093/jxb/eru088>.
- Pospisilova, J., Haisel, D., Vankova, R., 2011. Responses of transgenic tobacco plants with increased proline content to drought and/or heat stress. *Amer. J. Plant Sci.* 2, 318–324.
- Rais, L., Masood, A., Inam, A., Khan, N., 2013. Sulfur and nitrogen co-ordinately improve photosynthetic efficiency, growth and proline accumulation in two cultivars of mustard under salt stress. *J. Plant Biochem. Physiol.* 1, 101.
- Rastgar, J.F., Yamchi, A., Hajirezaei, M., Abbasi, A.R., Karkhane, A.A., 2011. Growth assessments of *Nicotiana tabacum* cv. Xanthitrans formed with *Arabidopsis thaliana* P5CS under salt stress. *Afr. J. Biotechnol.* 10, 8539–8552.
- Raymond, M.J., Smirnov, N., 2002. Proline metabolism and transport in maize seedlings at low water potential. *Ann. Bot.* 89, 813–823.
- Reddy, P.S., Jogeswar, G., Rasineni, G.K., Maheswari, M., Reddy, A.R., Varshney, R.K., Kishor, P.K., 2015. Proline over-accumulation alleviates salt stress and protects photosynthetic and antioxidant enzyme activities in transgenic sorghum [*Sorghum bicolor* (L.) Moench]. *Plant Physiol. Biochem.* 94, 104–113.
- Rejeb, K.B., Abdelly, C., Savouré, A., 2014. How reactive oxygen species and proline face stress together. *Plant Physiol. Biochem.* 80, 278–284.
- Rentsch, D., Hirner, B., Schmelzer, E., Frommer, W.B., 1996. Salt stress-induced proline transporters and salt stress-repressed broad specificity amino acid permeases identified by suppression of a yeast amino acid permease-targeting mutant. *Plant Cell* 8, 1437–1446.
- Rentsch, D., Schmidt, S., Tegeder, M., 2007. Transporters for uptake and allocation of organic nitrogen compounds in plants. *FEBS Lett.* 581, 2281–2289.
- Rizhsky, L., Liang, H., Shuman, J., Shulaev, V., Davletova, S., Mittler, R., 2004. When defense pathways collide. The response of *Arabidopsis* to a combination of drought and heat stress. *Plant Physiol.* 134, 1683–1696.
- Roosens, N.H., Al Bitar, F., Loenders, K., Angenon, G., Jacobs, M., 2002. Over-expression of ornithine- δ -aminotransferase increases proline biosynthesis and confers osmotolerance in transgenic plants. *Mol. Breed.* 9, 73–80.
- Ruan, H., Shen, W., Ye, M., Xu, L., 2002. Protective effects of nitric oxide on salt stress-induced oxidative damage to wheat (*Triticum aestivum* L.) leaves. *Chin. Sci. Bull.* 47, 677–681.
- Sadak, M.S., 2015. Pre-sowing Seed Treatment with Proline improves some growth, biochemical aspects, yield quantity and quantity of two sunflower cultivars grown under seawater salinity stress. *Scientia* 9, 60–69.
- Sadiqov, S.T., Akbulut, M., Emedov, V., 2002. Role of Ca^{2+} in drought stress signaling in wheat seedlings. *Biochem. Mosc.* 67, 491–497.
- Saeng-ngam, S., Takpirom, W., Buaboocha, T., Chadchawan, S., 2012. The role of the OsCam1-1 salt stress sensor in ABA accumulation and salt tolerance in rice. *J. Plant Biol.* 55, 198–208.
- Salinas, R., Sánchez, E., Ruiz, J.M., Lao, M.T., Romero, L., 2013. Phosphorus levels influence plasma membrane H^+ -ATPase activity and K^+ , Ca^{2+} , and Mg^{2+} assimilation in Green Bean. *Commun. Soil Sci. Plant Anal.* 44, 456–464.
- Sánchez, E., López-Lefebvre, L.R., García, P.C., Rivero, R.M., Ruiz, J.M., Romero, L., 2001. Proline metabolism in response to highest nitrogen dosages in green bean plants (*Phaseolus vulgaris* L. cv. Strike). *J. Plant Physiol.* 158, 593–598.
- Satoh, R., Fujita, Y., Nakashima, K., Shinozaki, K., Yamaguchi-Shinozaki, K., 2004. A novel subgroup of bZIP proteins functions as transcriptional activators in hypoosmolarity-responsive expression of the ProDH gene in *Arabidopsis*. *Plant Cell Physiol.* 45, 309–317.
- Satoh, R., Nakashima, K., Seki, M., Shinozaki, K., Yamaguchi-Shinozaki, K., 2002. ACTCAT, a novel cis-acting element for proline- and hypoosmolarity-responsive expression of the ProDH gene encoding proline dehydrogenase in *Arabidopsis*. *Plant Physiol.* 130, 709–719.
- Savouré, A., Hua, X.J., Bertauche, N., Montagu, M.V., Verbruggen, N., 1997. Abscisic acid-independent and abscisic acid-dependent regulation of proline biosynthesis following cold and osmotic stresses in *Arabidopsis thaliana*. *Mol. Gen. Genet.* 254, 104–109.
- Savouré, A., Jaoua, S., Hua, X.J., Ardiles, W., Van Montagu, M., Verbruggen, N., 1995. Isolation, characterization, and chromosomal location of a gene encoding the Δ^1 -pyrroline-5-carboxylate synthetase in *Arabidopsis thaliana*. *FEBS Lett.* 372, 13–19.
- Sawahel, W.A., Hassan, A.H., 2002. Generation of transgenic wheat plants producing high levels of the osmoprotectant proline. *Biotechnol. Lett.* 24, 721–725.
- Schwacke, R., Grallath, S., Breikreuz, K.E., Stransky, E., Stransky, H., Frommer, W.B., Rentsch, D., 1999. LeProT1, a transporter for proline, glycine betaine, and γ -amino butyric acid in tomato pollen. *Plant Cell* 11, 377–391.
- Servet, C., Ghelis, T., Richard, L., Zilberstein, A., Savoure, A., 2012. Proline dehydrogenase: a key enzyme in controlling cellular homeostasis. *Front. Biosci.* 17, 607–620.
- Shabbir, R.N., Waraich, E.A., Ali, H., Nawaz, F., Ashraf, M.Y., Ahmad, R., Ahmad, S., Irfan, M., Hussain, S., Ahmad, Z., 2016. Supplemental exogenous NPK application alters biochemical processes to improve yield and drought tolerance in wheat (*Triticum aestivum* L.). *Environ. Sci. Poll. Res.* 1–12.
- Shahbaz, M., Mushtaq, Z., Andaz, F., Masood, A., 2013. Does proline application ameliorate adverse effects of salt stress on growth, ions and photosynthetic ability of eggplant (*Solanum melongena* L.)? *Sci. Hortic.* 164, 507–511.
- Sharma, A., Dhanda, S., 2015. Application of calcium chloride to mitigate salt stress in *Vigna radiata* L. *Cultiv. Int. J. Curr. Microbiol. App. Sci.* 4, 764–769.
- Sharma, S., Verslues, P.E., 2010. Mechanisms independent of abscisic acid (ABA) or proline feedback have a predominant role in transcriptional regulation of proline metabolism during low water potential and stress recovery. *Plant Cell Environ.* 33, 1838–1851.
- Sharma, S., Villamor, J.G., Verslues, P.E., 2011. Essential role of tissue-specific proline synthesis and catabolism in growth and redox balance at low water potential. *Plant Physiol.* 157, 292–304.
- Singh, D.K., Sale, P.W., 2000. Growth and potential conductivity of white clover roots in dry soil with increasing phosphorus supply and defoliation frequency. *Agron. J.* 92, 868–874.
- Singh, D.K., Sale, P.W., McKenzie, B.M., 1997. Water relations of white clover (*Trifolium repens* L.) in a drying soil, as a function of phosphorus supply and defoliation frequency. *Aust. J. Agric. Res.* 48, 675–681.
- Siripornadulsil, S., Traina, S., Verma, D.P.S., Sayre, R.T., 2002. Molecular mechanisms of proline-mediated tolerance to toxic heavy metals in transgenic microalgae. *Plant Cell* 14, 2837–2847.
- Sobahan, M.A., Akter, N., Ohno, M., Okuma, E., Hirai, Y., Mori, I.C., Murata, Y., 2012. Effects of exogenous proline and glycinebetaine on the salt tolerance of rice cultivars. *Biosci. Biotechnol. Biochem.* 76, 1568–1570.
- Sokhansandh, A., Neumyvakin, L.V., Moseiko, N.A., Piruzian, E.S., 1997. Transfer of bacterial genes for proline synthesis in plants and their expression by various plant promoters. *Genetika* 33, 906–913.
- Sokolovski, S., Hills, A., Gay, R., Garcia-Mata, C., Lamattina, L., Blatt, M.R., 2005. Protein phosphorylation is a prerequisite for intracellular Ca^{2+} release and ion channel control by nitric oxide and abscisic acid in guard cells. *Plant J.* 43, 520–529.
- Sripinyowanich, S., Klomsakul, P., Boonburapong, B., Bangyeekhun, T., Asami, T., Gu, H., et al., 2013. Exogenous ABA induces salt tolerance in indica rice (*Oryza sativa* L.): the role of *OsP5CS1* and *OsP5CR* gene expression during salt stress. *Environ. Exp. Bot.* 86, 94–105.
- Srivastava, A.K., Rai, A.N., Patade, V.Y., Suprasanna, P., 2013. Calcium Signaling and its Significance in Alleviating Salt Stress in Plant. in: *Salt Stress in Plants*. Springer, New York, pp. 197–218.
- Stines, A.P., Naylor, D.J., Høj, P.B., Van Heeswijck, R., 1999. Proline accumulation in developing grapevine fruit occurs independently of changes in the levels of Δ^1 -pyrroline-5-carboxylate synthetase mRNA or protein. *Plant Physiol.* 120, 923.
- Strizhov, N., Abraham, E., Okresz, L., Blickling, S., Zilberstein, A., Schell, J., Koncz, C., Szabados, L., 1997. Differential expression of two *P5CS* genes controlling proline accumulation during salt-stress requires ABA and is regulated by ABA1, ABI1 and AXR2 in *Arabidopsis*. *Plant J.* 12, 557–569.
- Su, J., Wu, R., 2004. Stress-inducible synthesis of proline in transgenic rice confers faster growth under stress conditions than that with constitutive synthesis. *Plant Sci.* 166, 941–948.
- Surekha, C., Kumari, K.N., Aruna, L.V., Suneetha, G., Arundhati, A., Kishor, P.K., 2014. Expression of the *Vigna aconitifolia* *P5CSF129A* gene in transgenic pigeonpea enhances proline accumulation and salt tolerance. *Plant Cell Tissue Organ Cult.* 116, 27–36.
- Szabados, L., Savouré, A., 2010. Proline: a multifunctional amino acid. *Trends Plant Sci.* 15, 89–97.
- Székely, G., Ábrahám, E., Cséplő, Á., Rigó, G., Zsigmond, L., Csizsár, J., Ayaydin, F., Strizhov, N., Jásik, J., Schmelzer, E., Koncz, C., 2008. Duplicated *P5CS* genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. *Plant J.* 53, 11–28.
- Teh, C.Y., Mahmood, M., Shaharuddin, N.A., Ho, C.L., 2015. In vitro rice shoot apices as simple model to study the effect of NaCl and the potential of exogenous proline and glutathione in mitigating salinity stress. *Plant Growth Regul.* 1–11.

- Trotel-Aziz, P., Niogret, M.F., Deleu, C., Bouchereau, A., Aziz, A., Larher, F.R., 2003. The control of proline consumption by abscisic acid during osmotic stress recovery of canola leaf discs. *Physiol. Plant* 117, 213–221.
- Tuna, A.L., Kaya, C., Dikilitas, M., Higgs, D.E.B., 2008. The combined effects of gibberellic acid and salinity on some antioxidant enzyme activities, plant growth parameters and nutritional status in maize plants. *Environ. Exp. Bot.* 62, 1–9.
- Uchida, A., Andre, T.J., Takashi, H., Temhiro, T., Tetsuko, T., 2002. Effects of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. *Plant Sci.* 163, 515–523.
- Ueda, A., Shi, W., Sanmiya, K., Shono, M., Takabe, T., 2001. Functional analysis of salt-inducible proline transporter of barley roots. *Plant Cell Physiol.* 42, 1282–1289.
- Ueda, A., Shi, W., Shimada, T., Miyake, H., Takabe, T., 2008. Altered expression of barley proline transporter causes different growth responses in *Arabidopsis*. *Planta* 227, 277–286.
- Umebese, C.E., Olatimilehin, T.O., Ogunlusi, T.A., 2009. Salicylic acid protects nitrate reductase activity, growth and proline in Amaranth and tomato plants during water deficit. *Am. J. Agric. Biol. Sci.* 4, 224–229.
- Vendruscolo, E.C.G., Schuster, I., Pileggi, M., Scapim, C.A., Molinari, H.B.C., Marur, C.J., Vieira, L.G.E., 2007. Stress-induced synthesis of proline confers tolerance to water deficit in transgenic wheat. *J. Plant Physiol.* 164, 1367–1376.
- Verdoy, D., Caba de la Peña, T., Redondo, F.J., Lucas, M.M., Pueyo, J.J., 2006. Transgenic *Medicago truncatula* plants that accumulate proline display nitrogen-fixing activity with enhanced tolerance to osmotic stress. *Plant Cell Environ.* 29, 1913–1923.
- Verslues, P.E., Bray, E.A., 2006. Role of abscisic acid (ABA) and *Arabidopsis thaliana* ABA-insensitive loci in low water potential-induced ABA and proline accumulation. *J. Exp. Bot.* 57, 201–212.
- Verslues, P.E., Kim, Y.S., Zhu, J.K., 2007. Altered ABA, proline and hydrogen peroxide in an *Arabidopsis* glutamate: glyoxylate aminotransferase mutant. *Plant Mol. Biol.* 64, 205–217.
- Verslues, P.E., Sharma, S., 2010. Proline Metabolism and its Implications for Plant-environment Interaction. *The Arabidopsis Book/American Society of Plant Biologists*, p. 8.
- Verslues, P.E., Sharp, R.E., 1999. Proline accumulation in maize (*Zea mays* L.) primary roots at low water potentials. II. Metabolic source of increased proline deposition in the elongation zone. *Plant Physiol.* 119, 1349–1360.
- Waditee, R., Hibino, T., Tanaka, Y., Nakamura, T., Incharoensakdi, A., Hayakawa, S., Suzuki, S., Futsuhara, Y., Kawamitsu, Y., Takabe, T., Takabe, T., 2002. Functional characterization of betaine/proline transporters in betaine-accumulating mangrove. *J. Biol. Chem.* 277, 18373–18382.
- Wang, H., Tang, X., Wang, H., Shao, H.B., 2015. Proline accumulation and metabolism-related genes expression profiles in *Kosteletzkya virginica* seedlings under salt stress. *Front. Plant Sci.* 6, 792.
- Wang, W.G., Li, R., Liu, B., Li, L., Wang, S.H., Chen, F., 2011. Effects of low nitrogen and drought stresses on proline synthesis of *Jatropha curcas* seedling. *Acta Physiol. Plant* 33, 1591–1595.
- Wu, L., Fan, Z., Guo, L., Li, Y., Zhang, W., Qu, L.J., Chen, Z., 2003. Over-expression of an *Arabidopsis* δ -OAT gene enhances salt and drought tolerance in transgenic rice. *Chin. Sci. Bull.* 48, 2594–2600.
- Xavier, R., 2011. Role of exogenous proline in ameliorating salt stress at early stage in two rice cultivars. *J. Stress Physiol. Biochem.* 7.
- Xiong, J., Zhang, L., Fu, G., Yang, Y., Zhu, C., Tao, L., 2012. Drought-induced proline accumulation is uninvolved with increased nitric oxide, which alleviates drought stress by decreasing transpiration in rice. *J. Plant Res.* 125, 155–164.
- Yamada, M., Morishita, H., Urano, K., Shiozaki, N., Yamaguchi-Shinozaki, K., Shinozaki, K., Yoshida, Y., 2005. Effects of free proline accumulation in petunias under drought stress. *J. Exp. Bot.* 56, 1975–1981.
- Yan, Z., Guo, S., Shu, S., Sun, J., Tezuka, T., 2013. Effects of proline on photosynthesis, root reactive oxygen species (ROS) metabolism in two melon cultivars (*Cucumis melo* L.) under NaCl stress. *Afr. J. Biotechnol.* 10, 18381–18390.
- Yoshida, Y., Kiyosue, T., Katagiri, T., Ueda, H., Mizoguchi, T., Yamaguchi-Shinozaki, K., Wada, K., Harada, Y., Shinozaki, K., 1995. Correlation between the induction of a gene for δ^1 -pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis thaliana* under osmotic stress. *Plant J.* 7, 751–760.
- Yoshida, Y., Nanjo, T., Miura, S., Yamaguchi-Shinozaki, K., Shinozaki, K., 1999. Stress-responsive and developmental regulation of Δ^1 -pyrroline-5-carboxylate synthetase 1 (P5CS1) gene expression in *Arabidopsis thaliana*. *Biochem. Biophys. Res. Comm.* 261, 766–772.
- You, J., Hu, H., Xiong, L., 2012. An ornithine δ -aminotransferase gene OsOAT confers drought and oxidative stress tolerance in rice. *Plant Sci.* 197, 59–69.
- Zeng, Y., Chen, X., Miao, L., Cao, J., 2005. Construction of plant expression vector of the proBA gene from *Bacillus subtilis* mutant and analysis of the salt tolerance of transgenic *Arabidopsis thaliana*. *J. Anhui Agric. Univ.* 32, 316–322.
- Zhang, C.S., Lu, Q., Verma, D.P.S., 1995. Removal of feedback inhibition of Δ^1 -pyrroline-5-carboxylate synthetase, a bifunctional enzyme catalyzing the first two steps of proline biosynthesis in plants. *J. Biol. Chem.* 270, 20491–20496.
- Zhang, G.C., Zhu, W.L., Gai, J.Y., Zhu, Y.L., Yang, L.F., 2015. Enhanced salt tolerance of transgenic vegetable soybeans resulting from overexpression of a novel Δ^1 -pyrroline-5-carboxylate synthetase gene from *Solanum torvum* Swartz. *Hortic. Env. Biotechnol.* 56, 94–104.
- Zhang, J., 2011. China's success in increasing per capita food production. *J. Exp. Bot.* 62, 3707–3711.
- Zhang, M., Huang, H., Dai, S., 2014. Isolation and expression analysis of proline metabolism-related genes in *Chrysanthemum lavandulifolium*. *Gene* 537, 203–213.
- Zhu, B., Su, J., Chang, M., Verma, D.P.S., Fan, Y.L., Wu, R., 1998. Overexpression of a Δ^1 -pyrroline-5-carboxylate synthetase gene and analysis of tolerance to water- and salt-stress in transgenic rice. *Plant Sci.* 139, 41–48.
- Zimmermann, P., Laule, O., Schmitz, J., Hruz, T., Bleuler, S., Gruissem, W., 2008. Genevestigator transcriptome meta-analysis and biomarker search using rice and barley gene expression databases. *Mol. Plant* 1, 851–857.