Review

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

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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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Contents

1. Introduction ........................................ 127
2. An overview of proline biosynthesis and its regulation ........................................ 127
3. Transport of proline ........................................ 130
4. Phytohormones in regulation of proline metabolism under salt and drought stress ........................................ 130
5. Nutrients in modulation of proline metabolism under salt and drought stress ........................................ 132
6. Proline and stress tolerance using transgenic approach ........................................ 133
7. Significance of exogenously applied proline in responses of plants to salt and drought stresses ........................................ 133
8. Conclusions and future prospects ........................................ 136

Authors contribution ........................................ 136
Acknowledgements ........................................ 137
Supplementary data ........................................ 137
References ........................................ 137

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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 (Golldack 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 (Goldack 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), glycerol betaine, choline, O-sulfate, 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 antioxidanit 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 (Khedir et al., 2003; Moustakas et al., 2011; Naliwajski and Sklodowska, 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 potenially interact synergistically or antagonistically with Pro metabolism and influence stress 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 (Széko 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 Δ¹-pyrroline-5-carboxylate synthetase (P5CS; EC 2.7.11.12.1.41), which is spontaneously cyclized into pyrroline-5-carboxylate (PSC). This intermediate PSC is finally reduced to Pro by the enzymatic catalysis of Δ¹-pyrroline-5-carboxylate reductase (P5CR; EC 1.5.1.12). In Orn pathway, Orn can be transaminated to GSA by the activity of ornithine-δ-amino transferase (δ-OAT; EC 2.6.1.13) and subsequently gets converted to Pro via PSC (Szabados and Savouré, 2010). It has, however, been shown in Arabidopsis thaliana that δ-OAT generates PSC exclusively for catalobic 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 catalobic. Therefore, it can be said that in plants, at least in Arabidopsis, biosynthesis of Pro occurs exclusively through Glu pathway (Funck et al., 2008). Catalobism of Pro occurs in mitochondria and this process is catalyzed by two enzymes such as Pro dehydrogenase (PDH; EC 1.5.99.8) and PSC dehydrogenase (PSCDH; 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 (Savoure 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 (Savoure 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
decreased almost equal to their respective controls, whereas the activity of PDH increased. In A. thaliana, it was found that during stress only \( \text{P5CS2} \) gene was required for Pro accumulation among the two closely related \( \text{P5CS} \) genes, \( \text{P5CS1} \) and \( \text{P5CS2} \). Production of knockout mutant of \( \text{P5CS1} \) 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, \( \text{MsPDH} \) and a partial sequence of \( \text{MsP5CDH} \) 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 \( \text{MsP5CS} \) and \( \text{MsP5CDH} \). Contrary to the salt-dependent repression, a rapid induction of \( \text{MsPDH} \) 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 \( \text{P5CS} \) 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 \( \text{P5CS} \) genes showed different regulation at transcript level in response to osmotic stress. Under osmotic stress \( \text{MtP5CS1} \) transcript levels remained unaffected, while \( \text{MtP5CS2} \) 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.

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 \( \gamma \)-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 proline-5-carboxylate through \( \gamma \)-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—\( \gamma \)-glutamyl kinase; GPR—\( \gamma \)-glutamyl phosphate reductase; P5CDH—pyrroline-5-carboxylate dehydrogenase; P5CR—pyrroline-5-carboxylate reductase; P5CS—pyrroline-5-carboxylate synthetase; \( \delta \)-OAT—ornithine \( \delta \)-aminotransferase.
(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 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) (Szekely 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 Arabidopsis AtP5CS 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

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**Fig. 2.** Meta-analyais 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; Lehnmann et al., 2010; Soma 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 AAP 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 (Lehnmann 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 (AAP) 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 CIPPro 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 CIPPro might be localised at the plasma membrane and could facilitate Pro transport. However, phylogenetic analysis showed that CIPProT1 and CIPProT4 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 CIPPro. Zhang et al. (2014) also found that the expression patterns of four CIPPro genes varied among different organs and under stress conditions, but the expression patterns of each CIPPro under various stresses were the same. Among the four CIPPro genes, CIPProT2 was induced under various stress conditions. The cis-acting element involved in circadian control was found in the CIPProT promoters. The cis-acting regulatory elements in the CIPPro 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 Smirnoff (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 auxins, 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.
Arabidopsis P5CS1 (2003). Both salt and drought stresses were reported to induce stress in plants during abiotic stress (Verslues and Bray, 2006). The greater ABA accumulation, and eventual osmotic adjustment has been also reported (Cui et al., 2015). 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) (Umehese et al., 2009) under drought stress. Recently, Nazar et al. (2015) have shown that pre-application 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 μM NO increased expression of PCS 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 oxynitrate-γ-GK and decreased activity of PDH (Misra and Saxena, 2009) and 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) (Umehese et al., 2009) under drought stress. Recently, Nazar et al. (2015) have shown that pre-application 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).

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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 (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 NH₄NO₃ 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 NO₃, NH₄ 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 bacterialor (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, N₂-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 N₂ 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 N₂ 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 halotolerance in many crop plants and play an important role in osmoprotection (Srivastava et al., 2013). Generally, Ca2+ 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 Ca2+ application to salt treated plants (Jaleel et al., 2007; Misra and Gupta, 2006; Murugan and Sathish, 2005; Sharma and Dhandha, 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 PSKR (Misra and Gupta, 2006). A significant increase in activity of PSKS and Pro concentration were reported in NaCl-exposed calli supplemented with CaCl2 (Murugan and Sathish, 2005). Ca can play a positive role in ABA-mediated gene induction of PSKS in plants under drought and salinity (Knight et al., 1997). The authors suggested the involvement of Ca2+ and some other signalling factor in the stress-induced Pro accumulation in *Arabidopsis* (Szabados and Savouré, 2015). The expression of PSKS gene during stress. The supplementation of CaCl2 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 CaCl2 resulted in lower free amino acid and Pro content than plants treated with NaCl alone. Contradictory results have also been reported in literature where Ca2+ either prevented or did not affect stress-induced Pro accumulation (Sadiqov et al., 2002; Sharma and Dhandha, 2015). In salt stressed plants, the accumulation of Pro was found independent to Ca2+ treatment (Sadiqov et al., 2002). Pro content was found to increase with NaCl stress, however, the combined treatment of NaCl and CaCl2 resulted in significant decrease in Pro content (Sharma and Dhandha, 2015). Reports also indicate the association of a loss of cellular Ca2+ 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 *P. 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 *P. aconitifolia* PSKS 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 PSKS and enabled them to grow in salt containing medium (Siripornadulsil et al., 2002). Recently, Zhang et al. (2015) reported that over-expression of *StPSKS* gene from *S. torvum* increased salt tolerance in transgenic vegetable soybeans. Transgenic *S. tuberosum* containing PSKS 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 PSKS 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 PSKS 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 PSKS 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* PSKS cDNA (P5CSF129A), and in pigeonpea (*Cajanus cajan*) carrying mutagenized version of P5CSF129A (Surekha et al., 2014). In addition to PSKS, 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 10 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 Savouré, 2010). It induces dehydrins in *Pancreatium maritimum* to levels comparable to those under salt stress conditions (Khadr et al., 2003). Pro upregulates genes responsible for stress tolerance and mitigates the damages caused by salt (Pathade 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 Savouré, 2010). Pro-mediated stabilization of proteins, DNA and membranes through ROS-scavenging has also been reported (reviewed in Maatjak et al., 2002). In fact, the low ionization potential of Pro can enable this compound to readily form a reversible charge-transfer complex with 1O2, 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 1.15.1.11), 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\(_2\)O\(_2\) 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 hypogaea) 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

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### 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.

<table>
<thead>
<tr>
<th>Stress</th>
<th>Duration of stress</th>
<th>Plant</th>
<th>Proline applied</th>
<th>Metabolism influenced</th>
<th>Consequence</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt</td>
<td>100 mM NaCl, 3 days and 5 days after starting treatment</td>
<td>Cucumis melo</td>
<td>0.2 mM</td>
<td>Antioxidant metabolism</td>
<td>Increased plant growth, chlorophyll content, net photosynthetic rate and actual efficiency of photosystem II</td>
<td>Yan et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>100 mmol L(^{-1}) NaCl, 8 days after treatment</td>
<td>C. sativus</td>
<td>10 mM</td>
<td>Antioxidant metabolism (SOD, POD, CAT and APX activities)</td>
<td>Alleviated plant growth inhibition</td>
<td>Huang et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>200 mM NaCl, 11 days</td>
<td>Nicotiana tabacum</td>
<td>20 mM</td>
<td>Antioxidant metabolism (CAT, POX)</td>
<td>Improved plant growth</td>
<td>Banu et al. (2009)</td>
</tr>
<tr>
<td></td>
<td>100 and 200 mM NaCl, young leaves (fully expanded leaves that developed soon after the onset of the different treatments)</td>
<td>Olea europaea</td>
<td>25 mM</td>
<td>Ion homeostasis (Na(^+), K(^+) and Ca(^{2+}))</td>
<td>Increased tolerance to oxidative damage by up-regulating the antioxidant defense system</td>
<td>Hasanuzzaman et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>150 and 300 mM NaCl for 48 h</td>
<td>Oryza sativa</td>
<td>5.0 mM</td>
<td>Antioxidant metabolism AsA, GSH and GSSG, APX, MDHAR, DHAR, GR, GPX, CAT, and Gly I activities</td>
<td>Alleviated inhibitory effects on seed germination</td>
<td>Hua-long et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>25 mL 100 mmol L(^{-1}) NaCl, 3rd day and the 7th day after seed soaking</td>
<td>O. sativa</td>
<td>15 and 30 mM</td>
<td>Carbohydrate metabolism alpha-amylase, beta-amylase isoenzyme</td>
<td>Alleviated plant growth inhibition</td>
<td>Teh et al. (2015)</td>
</tr>
<tr>
<td></td>
<td>150 mM NaCl, 30 days after culture</td>
<td>O. sativa</td>
<td>5.0 mM</td>
<td>Plant height, root length</td>
<td>Increased plant growth</td>
<td>Nounjan and Theerakulpisut (2012)</td>
</tr>
<tr>
<td></td>
<td>200 mmol/L NaCl, 6 days</td>
<td>O. sativa</td>
<td>10 mM</td>
<td>Antioxidant metabolism (SOD, CAT, APX, POX)</td>
<td>Alleviated plant growth inhibition</td>
<td>Nounjan et al. (2012)</td>
</tr>
<tr>
<td></td>
<td>100 mM NaCl, 6 days</td>
<td>O. sativa</td>
<td>10 mM</td>
<td>Proline (P5CS and P5CR genes) and antioxidant metabolism (SOD, CAT, APX, POX)</td>
<td>Plants exhibited a pronounced beneficial effect during recovery period showing higher percentage of growth recovery in phosphorus plus sulfur treatment compared with sulfur induced salt tolerance</td>
<td>Shahbaz et al. (2014)</td>
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<td></td>
<td>100 mM NaCl, 7 days</td>
<td>O. sativa</td>
<td>1.0 mM</td>
<td>Ion homeostasis K(^+)/Na(^+) ratio</td>
<td>Alleviated detrimental effect on growth and photosynthesis</td>
<td>Hossain and Fujita (2010)</td>
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<td></td>
<td>100, 200, 300 and 400 mM NaCl</td>
<td>O. sativa</td>
<td>1.0 mM</td>
<td>Osmotic balance</td>
<td>Improved plant growth</td>
<td>Xavier (2011)</td>
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<tr>
<td></td>
<td>75, 150, 225 or 300 mM NaCl, 10 days</td>
<td>Pancratium maritimum</td>
<td>1.0 mM</td>
<td>Osmotic balance</td>
<td>Improved plant growth</td>
<td>Khedr et al. (2005)</td>
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<td></td>
<td>6.57 dS/m, almost 4 months</td>
<td>Solanum lycopersicum</td>
<td>5.0 mM</td>
<td>Osmoregulation (Ubiquitin, dehydrins)</td>
<td>Increased plant growth and chlorophyll a fluorescence</td>
<td>Kablanoui et al. (2014)</td>
</tr>
<tr>
<td></td>
<td>150 mM NaCl, 15 days</td>
<td>S. melongena</td>
<td>10 and 20 mg L(^{-1})</td>
<td>Water use efficiency</td>
<td>Counteracted the adverse effects on plant growth</td>
<td>Shahzad et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>100 mM NaCl, 1 month</td>
<td>Saccharum officinarum</td>
<td>20 mM</td>
<td>Antioxidant metabolism (SOD, GR, APX, GPX, CAT)</td>
<td>Increased plant growth rate</td>
<td>Patade et al. (2014)</td>
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<tr>
<td></td>
<td>300 mM NaCl, 24 and 48 h</td>
<td>Vigna radiate</td>
<td>15 mM</td>
<td>Antioxidant metabolism (GSH content, GPX, GR)</td>
<td>Reduced oxidative stress</td>
<td>Khorasani et al. (2014)</td>
</tr>
<tr>
<td>Drought</td>
<td>63 days</td>
<td>Arabidopsis thaliana</td>
<td>10 mM</td>
<td>Proline accumulation and chlorophyll fluorescence</td>
<td>Improved tolerance to photoinhibition</td>
<td>Moustakas et al. (2011)</td>
</tr>
<tr>
<td></td>
<td>21 days interval</td>
<td>Trigonella foenum-graecum</td>
<td>20 mM</td>
<td>Growth (shoot and root fresh and dry weights, shoot length, total leaf area)</td>
<td>Improved growth and yield</td>
<td>Kamran et al. (2009)</td>
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<tr>
<td></td>
<td>15 days</td>
<td>Zea mays</td>
<td>30 mM</td>
<td>Secondary metabolites and fatty acids</td>
<td>Ameliorated the adverse effects on seed oil composition</td>
<td>Ali et al. (2013)</td>
</tr>
<tr>
<td></td>
<td>15 days</td>
<td>Z. mays</td>
<td>30 mM</td>
<td>Ion homeostasis (K(^+), Ca(^{2+}), N and P)</td>
<td>Increased plant growth</td>
<td>Ali et al. (2008)</td>
</tr>
<tr>
<td></td>
<td>15 days</td>
<td>Z. mays</td>
<td>30 mM</td>
<td>Growth and photosynthetic rate</td>
<td>Countered the adverse effects of on plant growth</td>
<td>Ali et al. (2007)</td>
</tr>
</tbody>
</table>
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. italicum. 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 alleviate stress by providing a reducing potential through its protective complex II of the mitochondrial electron transport chain (Anjum et al., 2014). Endogenous level of Pro was evidenced to maintain NAPDH level and improved drought tolerance (De Ronde et al., 2004). Studies showing influence of Pro on antioxidan't 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. |

<table>
<thead>
<tr>
<th>Stress</th>
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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.
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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.

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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.


