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REVIEW

Proline: a key player in plant abiotic stress tolerance

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Abstract

Dramatic accumulation of proline is a common physiological response in plants exposed to various abiotic stresses. Accumulation of proline could be due to *de novo* synthesis, decreased degradation, lower utilization, or hydrolysis of proteins. Extensive intercellular proline transport occurs between the cytosol, chloroplasts, and mitochondria due to its compartmentalized metabolism. Although all functions of proline in stress tolerance are still a matter of debate, it is suggested that proline contributes to stabilization of sub-cellular structures, scavenging free radicals, and buffering cellular redox potential. It also chelates heavy metals, modulates cellular functions, and even triggers gene expression. Apparently, proline acts as stress-related signal exhibiting cross tolerance to a range of different stresses. Besides these significant roles, its metabolism is found to be coupled to several key pathways such as pentose phosphate, tricarboxylic acid, or urea cycles and contributes to, *i.e.*, purine synthesis and the phenylpropanoid pathway. Although the molecular basis of regulation of proline metabolism is still largely obscure, the genetic engineering of proline content could lead to new opportunities to achieve plant stress tolerance.

Additional key words: cross tolerance, free radicals, glutamate, ornithine, pentose phosphate pathway, proline metabolism.

Introduction

Being sessile, plants are subjected to various types of environmental stresses. Salinity, water deficit, temperature extremes, heavy metal toxicity, nutrient deficiency, and UV-radiation are among the major environmental constraints to crop productivity worldwide. There are many cellular mechanisms by which organisms ameliorate the effects of environmental stresses; for instance, plants accumulate an array of metabolites including compatible osmolytes, particularly amino acids such as proline. Although a clear-cut relationship between proline accumulation and stress adaptation has been under question, it is generally believed that the increase in proline content following stress is beneficial for the plant (Verbruggen and Hermans 2008). A very high content of cellular proline (up to 80 % of the amino acid pool under stress in comparison with 5 % under normal conditions) has been documented due to its increased synthesis and decreased degradation under a variety of stress conditions in many plant species (Szabados and Savoure 2009).

As multifunctional amino acid, proline seems to have diverse roles under stress conditions, such as stabilization of proteins, membranes, and subcellular structures, and protecting cellular functions by scavenging reactive oxygen species (ROS). Compartmentalization of proline biosynthesis and degradation in the cytosol, chloroplast, and mitochondria adds to the complexity of functional diversification of proline metabolism. The enhanced rate of proline biosynthesis in the chloroplast can contribute to the stabilization of redox balance and maintenance of cellular homeostasis by dissipating the excess of reducing potential when electron transport chain is saturated during adverse conditions (Taiz and Zeiger 2010). Proline catabolism in the mitochondria is connected to oxidative respiration and administers energy to resume growth after stress. Although proline pretreatment can ameliorate the phytotoxicity of heavy metals by reducing the concentration of ROS (Viehweger 2014), it may also function as protein compatible hydrotrope (Srinivas and

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Abbreviations: ABA - abscisic acid; APX - ascorbate peroxidase; CAT - catalase; GR - glutathione reductase; GSA - glutamate semialdehyde; H_2O_2 - hydrogen peroxide; O_2^- - superoxide; $OH^\bullet$ - hydroxyl radical; OAT - ornithine aminotransferase; PPP - pentose phosphate pathway; P5C - pyrroline-5-carboxylate; P5CDH - P5C dehydrogenase; P5CS - pyrroline-5-carboxylate synthetase; P5CR - P5C reductase; PDH - proline dehydrogenase; POX - proline oxidase; PP - pentose pathway; ROS - reactive oxygen species; SA - salicylic acid; SOD - superoxide dismutase; TCA - tricarboxylic acid.

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Balasubramanian 1995, Verslues and Sharma 2010) alleviating cytoplasmic acidosis and maintaining appropriate $NADP^+$/NADPH ratios compatible with cell metabolism (Sharma and Verslues 2010, Filippou *et al.* 2014). Proline appears to function as metabolic signal that regulates metabolite pools and influences plant growth and development (Verbruggen and Hermans 2008). Alternatively, proline might confer a protective effect by inducing stress-protective proteins (Khedr *et al.* 2003, Chinnusamy *et al.* 2005) and expression of salt stress responsive genes possessing proline responsive elements (*e.g. PRE*, ACTCAT) in their promoters (Satoh *et al.* 2002, Oono *et al.* 2003, Chinnusamy *et al.* 2005). In fact, proline synthesis could be one of the earliest metabolic responses triggered in the signal transduction pathway that links the perception of many environmental stresses to the elucidation of physiological response at the cellular level (Song *et al.* 2005). Therefore, to clarify whether a change in proline content is actually a signaling component, and how it regulates gene expression and adjusts metabolic process will undoubtedly be a challenge question and needs more exploration. Keeping in view the diverse roles of proline in plants, the present review focuses on metabolism, transport, regulation, and key linking pathways of proline to stress tolerance.

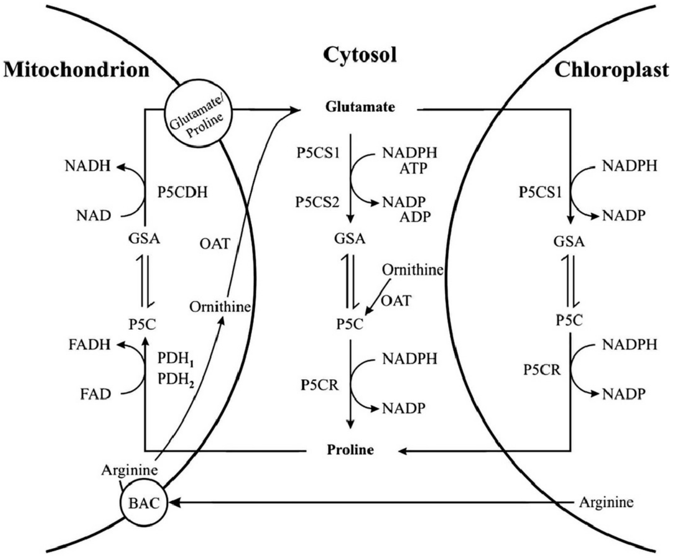

Fig. 1. A schemetic representation of proline metabolism in different cell organelles. Biosynthesis of proline occurs in the chloroplast or cytosol from glutamate *via* pyrroline-5-carboxylate synthetase (P5CS) and δ-pyrroline-5-carboxylate (P5C) reductase (P5CR) whereas catabolism occurs in the mitochondrion where proline is oxidized to P5C and glutamate through a sequential action of proline dehydrogenase (PDH) and pyrroline-5-carboxylate dehydrogenase (P5CDH). BAC - basic amino acid transporter, GSA - glutamate-γ-semialdehyde, OAT - ornithine aminotransferase. Adapted from Szadabos and Savoure (2010).

Metabolism of proline in plants

A pathway for the biosynthesis of proline in plants was elucidated first in *E. coli* in the year 1952 (Vogel and Davis 1952). Tissues undergoing rapid cell divisions and elongation, such as shoot apical meristems, accumulate a high proline content which appears to be involved in floral transition and embryo development (Lehmann *et al.* 2011). Housekeeping genes for proline biosynthesis were reported first in the cytosol of plants and thereafter in the chloroplast (Székely *et al.* 2008). Proline is synthesized by either the glutamate pathway or the ornithine pathway. Glutamate acts as key route involving two successive reductions catalyzed by pyrroline-5-carboxylate synthetase (P5CS; EC 2.7.2.11) and P5C reductase (P5CR). The P5CS is a bifunctional enzyme encoded by two homologous *P5CS* genes. It catalyzes the activation of glutamate by phosphorylation and also the reduction of the labile intermediate γ-glutamyl phosphate into glutamate semialdehyde (GSA) which spontaneously cyclizes to δ-pyrroline-5-carboxylate (P5C) and then it is finally reduced to proline by δ1-pyrroline-5-carboxylate reductase (P5CR; EC 1.5.1.2) (Verbruggen and Hermans 2008, Miller *et al.* 2009; Fig. 1). Under many stresses, proline accumulation is correlated with the activity of P5CS which is key regulatory and rate limiting enzyme in the biosynthetic pathway (Strizhov *et al.* 1997). The *P5CS* genes were cloned and characterized from several plants such as *Arabidopsis thaliana* (Savoure *et al.* 1995), *Solanum lycopersicum* (Fujita *et al.* 1998), *Oryza sativa* (Hur *et al.* 2004), and *Sorghum bicolor* (Su *et al.* 2011). In some species, these genes show different expression patterns. For instance, *P5CS* genes have different functions in plant development and during stress response (Verbruggen and Hermans 2008). In *Arabidopsis*, P5CR was found to be encoded by a single gene though alternative splicing might generate two different proteins (Hare and Cress 1997). Two isozymes of P5CR, comprising P5CR-1 and P5CR-2, were found to be differentially expressed in chloroplasts of *Spinacea oleracea* (Murahama *et al.* 2001).

The second route of proline biosynthesis encompasses ornithine as precursor which can be transaminated to P5C by ornithine-δ-aminotransferase (OAT; EC 2.6.1.13) *via* an intermediate product, pyrroline-2-carboxylate (Delauney and Verma 1993; Fig. 1). The ornithine pathway seems to predominate in mitochondria under a high nitrogen supply whereas the glutamate pathway acts during osmotic stress and nitrogen limitation (Sánchez *et al.* 2001). The significance of ornithine and OAT in proline biosynthesis has been questioned, as proline content was unaffected in *Arabidopsis oat* knockout mutants. Instead, it was suggested that OAT facilitates nitrogen recycling from arginine through P5C which is converted to glutamate by P5CDH (Funck *et al.* 2008).

On the contrary, proline catabolism occurs predominantly in mitochondria involving proline dehydrogenase (PDH) or proline oxidase (POX) producing P5C from proline, and P5C dehydrogenase (P5CDH) which converts P5C to glutamate (Fig. 1). The PDH is encoded by two genes whereas a single *P5CDH* gene has been identified in *Arabidopsis* and tobacco (Ribarits *et al.* 2007). The PDH and P5CDH uses FAD and NAD as electron acceptors and generate FADH and NADH, respectively, and deliver electrons for mitochondrial respiration (Deuschle *et al.* 2004). Thus, the biosynthetic enzymes (P5CS1, P5CS2, and P5CR) were predicted to be localized in the cytosol and chloroplasts whereas mitochondrial localization was predicted for the enzymes, such as PDH1, PDH2, P5CDH, and OAT, involved in proline catabolism (Szabados and Savouré 2009). Proline accumulation increases in plants by transferring the genes associated with the biosynthetic pathway. In cactus pear, it was found that salt stress stimulates the expression of *P5CS* that lead to an enhanced proline accumulation (Silva-Ortega *et al.* 2008). The activities of P5CS and P5CR were found to be significantly increased in leaves of *Morus alba* (Chaitanya *et al.* 2009) and tobacco (Stein *et al.* 2011) with decreasing leaf water potential. On the contrary, it was seen that the proline content decreases when *P5CS* in *Arabidopsis* is knocked-out reducing proline synthesis (Szekely *et al.* 2008). Also, the transcription of antisensing *ProDH* improves production of proline in tobacco (Stein *et al.* 2011). In mulberry, activity of proline dehydrogenase is reduced with a progressive increase in water stress. Similarly, there are studies about the proline alternative pathway involving ornithine transaminase enzymes where its activity was found to be elevated in *Morus alba* and cashew under low leaf water potentials (Chaitanya *et al.* 2009, Rocha *et al.* 2012).

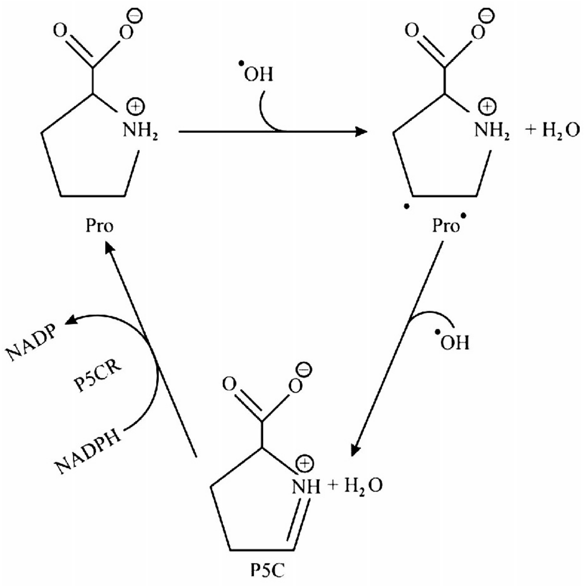

Fig. 2. The proline-proline cycle. Proline captures the first hydroxyl radical (OH•) by H-abstraction on C5, followed by a second H-abstraction which captures another OH• yielding δ-pyrroline-5-carboxylate (P5C). The P5C is then recycled back to proline by the action of the pyrroline-5-carboxylate reductase (P5CR)/NADPH enzymatic system. Pro• - proline radical. Adapted from Signorelli *et al.* (2014).

Proline transport in various compartments of cell

An extensive intracellular proline transport occurs between the cytosol, chloroplasts, and mitochondria as implied by compartmentalization of proline metabolism. Yu *et al.* (1983) suggested that uptake of proline in mitochondria is active process employing various transporters. Plant amino acid transporters are classified into two superfamilies; the amino acid proline transporter family (ATF) and amino acid polyamine choline (APC) transporters (Lopez *et al.* 2000). Different amino acid transporters have been isolated and characterized from *Arabidopsis* and tomato. At least three transporters of proline (ProT1, ProT2, and AAP6) belonging to the ATF superfamily have been identified in *Arabidopsis thaliana* (Grallath *et al.* 2005). The *ProT1* expresses ubiquitously in all organs of plants, but the highest content was found in the phloem of the floral stalk that enters the carpel where it is down-regulated after fertilization (Mattioli *et al.* 2008). Under water or salinity stress, a strong expression of *ProT2* is recorded whereas AAP6 transcripts are detected mainly in sink tissues (Waditee *et al.* 2002). In general, an increase in proline export or transport is evident by accumulation of ProT2 transcripts under salt-stress (Lehmann *et al.* 2011). Two proline carriers have been identified in mitochondria of *Triticum durum*: a proline uniporter that facilitates proline transport into the mitochondrial matrix; and a proline/glutamate antiporter which appears to have an important role in the proline/glutamate shuttle between the mitochondrial matrix and the cytosol (Martino *et al.* 2006). Basic amino acid transporters have been reported to deliver arginine and ornithine through the mitochondrial membranes (Palmieri *et al.* 2006).

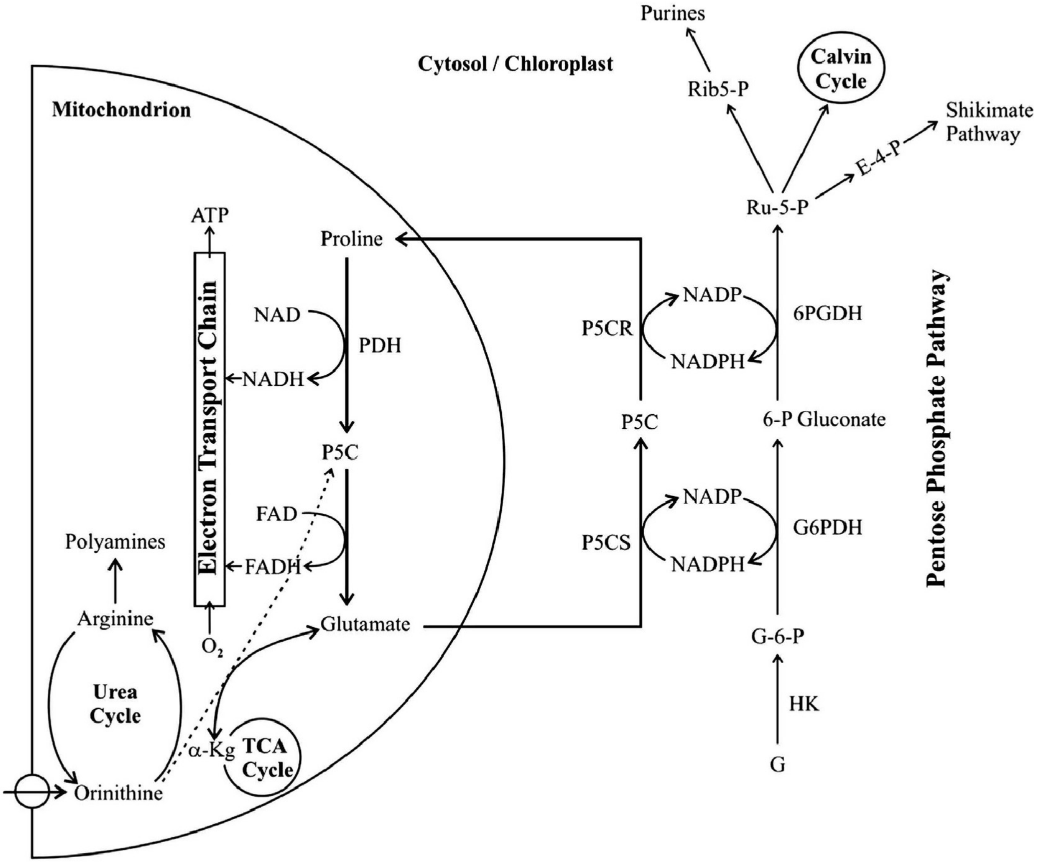

Fig. 3. Proline linked key pathways. A model for proline metabolism and a link with the tricarboxylic acid cycle (TCA), pentose phosphate pathway, urea cycle, and shikimate and phenylpropanoid pathways. α-Kg - α-ketoglutarate, E-4-P - erythrose-4-phosphate, G - glucose, G-6-P - glucose-6-phosphate, G6PDH - glucose-6-phosphate dehydrogenase, 6-P-gluconate - 6-phosphogluconate, 6PGDH - 6-phosphogluconate dehydrogenase, P5C - δ-pyrroline-5-carboxylate, P5CR - pyrroline-5-carboxylate reductase, P5CS - pyrroline-5-carboxylate synthase, Ru-5-P - ribulose-5-phosphate, Rib-5-P - ribose-5-phosphate. Adapted from Shetty (1997).

Regulation of proline metabolism

Proline biosynthesis is found to be upregulated by a high radiation and osmotic stress whereas proline catabolism is activated in the dark and during stress relief (Hayashi *et al.* 2000, Arora and Saradhi 2002). It was found that high external concentrations of sucrose and glucose cause a strong accumulation of proline in *Brassica napus* leaf discs (Larher *et al.* 1993). Verslues and Bray (2006) demonstrated an inhibitory effect of sucrose on abscisic acid (ABA) induced proline accumulation and suggested the transcription factor ABI4 connects ABA and sugar-signaling in regulation of proline accumulation. The availability of nitrogen has also been demonstrated to affect proline accumulation in several plant species. It was observed that proline content in tobacco leaves increases when plants are grown on a medium containing high concentrations of NH_4NO_3 (Kishor *et al.* 1995). Promoter analysis of *PDH1* identified the proline and hypo-osmolarity-responsive element motif ACTCAT necessary for activation of the *PDH* gene (Usha and Malik 2012). Basic leucine zipper protein transcription factors (AtbZIP-2,-11,-44,-53) have been identified as candidates for binding to this motif (Kiran and Abdin 2012). Expression analysis revealed that *PDH1* transcription is repressed during daylight and induced in darkness suggesting that irradiance possesses opposite effects on *P5CS1* and *PDH1* transcription (Masoud *et al.* 2013). This regulation may be probably due to transcriptional control of proline metabolism enzymes since the *AtP5CS* mRNA content increases whereas sucrose mediate a negative regulation of *AtPDH1* and *AtPDH2* by involving bZIP transcription factors (Weltmeier *et al.* 2006, Hanson *et al.* 2008).

Secondary messengers, such as nitric oxide or hydrogen peroxide, are known to mediate ABA signals that affect proline metabolism under stress conditions (Desikan *et al.* 2000, Neill *et al.* 2002, Yang *et al.* 2010). An important plant regulator that appears to be involved in regulation of proline metabolism is salicylic acid (SA). Several reports indicated a promoting effect of SA on proline accumulation even in the absence of stress (Kanade 2008, Yusuf *et al.* 2008, Misra and Saxena 2009). The P5CS2 has been found to be activated by avirulent bacteria, SA, and ROS signals which trigger a hypersensitive response (Fabro *et al.* 2004). Phospholipase D also functions as negative regulator of proline accumulation (Thiery *et al.* 2004) whereas calcium signaling and phospholipase C triggers *P5CS* transcription and proline accumulation during salt stress (Parre *et al.* 2007). In a halophyte *Thellungiella halophila*, phospholipase D functions as positive regulator whereas phospholipase C exerts a negative control on proline accumulation (Mohamed *et al.* 2012).

Roles of proline in abiotic stress tolerance

Proline is one of the most widely distributed compatible solutes that accumulates in plants during adverse environmental constraints and plays an important role in plant stress tolerance. Besides acting as excellent osmolyte, proline plays major roles as metal chelator, antioxidative defense molecule, and signaling molecule during stress. It imparts stress tolerance by modulating mitochondrial functions, influences cell proliferation, triggers specific gene expression, and stabilizes membranes thereby preventing electrolyte leakage and bringing concentrations of ROS within normal ranges leading to stress recovery. Foliar application of proline under heavy metal stress has shown to be effective method to reduce the toxicity of metals by activating protective mechanisms in plants (Emamverdian *et al.* 2015). Likewise, connection between an increased proline content and a higher biomass was found under osmotic stress in *Arabidopsis* (Roosens *et al.* 2002) and rice (Wu *et al.* 2003). In fact, proline plays multifarious roles including adaptation, recovery, and signaling when it comes to combating stress in plants.

Osmotic adjustment and protection: Proline possesses a rigid structure, carries no charge at the neutral pH and maintains a high solubility in water. Sivakumar *et al.* (2000) elucidated that proline alleviates ribulose-1,5-bisphosphate activity even at a concentration of 50 mM NaCl and thus potentiating its role in protecting photosynthetic activity under stress. Wu and Bolen (2006) suggested that proline stabilizes protein structures by driving burial of the peptide backbone and facilitates protein folding. Proline is known to prevent the aggregation of P39A cellular retinoic acid-binding protein (an aggregation prone protein) under salt stress (Ignatova and Gierasch 2006). Accumulation of proline results in an increase in cellular osmolarity that drives influx of water or reduces its efflux thus providing pressure potential necessary for cell expansion (Joseph *et al.* 2015).

Reactive oxygen species scavenging: The sequential reduction of oxygen through the addition of electrons leads to the formation of a number of ROS including singlet oxygen ($^1O_2$), superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^\bullet$). Oxidative stress occurs when formation of ROS exceeds their scavenging potential. Plants have developed various protective mechanisms to eliminate or reduce the deleterious effects of ROS through antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), and non-enzymatic antioxidants (ascorbate, glutathione, and tocopherol), *etc.* (Beak and Skinner 2003, Woo *et al.* 2007, Rejeb *et al.* 2014). It was proposed that proline is effective hydroxyl radical scavenger (Smirnoff and Cumbes 1989). Proline could also deactivate $^1O_2$ by physical quenching (Alia *et al.* 2001, Matysik *et al.* 2002). Further investigations reported that free and polypeptide-bound proline reacts with H_2O_2 and $OH^\bullet$ and forms stable free radical adducts of proline and hydroxyproline derivatives (4-hydroxyproline and 3-hydroxyproline) (Kaul *et al.* 2008). Recent findings also depicted that proline cannot quench $^1O_2$ in an aqueous buffer, which leads to reconsider an assumption about a likely role of proline in $^1O_2$ scavenging in plants under stress (Signorelli *et al.* 2013). Nevertheless, a mechanistic evidence was collected by using density functional theory coupled with polarizable continuum model (DFT/PCM) to sustain the role for proline as protective $OH^\bullet$ scavenger under stress in plants. It was proposed proline-proline cycle operation (Fig. 2) in which proline captures the first $OH^\bullet$ by H-abstraction followed by the second H-abstraction which also captures another $OH^\bullet$ yielding P5C which is then recycled back to proline by the action of the P5CR/NADPH enzymatic system (Signorelli *et al.* 2014).

Several studies have attributed that exogenous proline application enhances activity of antioxidative enzymes in chickpea (Kaushal *et al.* 2011), sea daffodil (Khedr *et al.* 2003), lentil (Molla *et al.* 2014), sugarcane (Patade *et al.* 2014), olive (Ahmed *et al.* 2010), and melon (Yan *et al.* 2011) under various stress conditions (Table 1). Proline as pre-sowing seed treatment has also been found to ameliorate the adverse effects of water deficit in *Triticum aestivum* (Kamran *et al.* 2009). Likewise, Xu *et al.* (2009) suggested that proline could contribute to detoxification of O_2^1 radicals by increasing activity of SOD in *Solanum nigrum* under Cd stress. The activities of APX, monodehydroascorbate reductase, and dehydroascorbate reductase, which are components of the ascorbate-glutathione cycle, are significantly enhanced by exogenous proline in tobacco cultures exposed to salinity stress (Hoque *et al.* 2007a). Aggarwal *et al.* (2011) showed an increase in content of ascorbate and glutathione, reduced glutathione/disulfide ratio, and higher activities of APX, GR, and CAT together with decrease in H_2O_2 and malondialdehyde content in salt-treated mung bean that had been pre-treated with proline (Table 1). Reports also revealed that plants may over-accumulate proline and mitigate the deleterious effects of drought-induced oxidative stress by lowering lipid peroxidation (De Campos *et al.* 2011). In transgenic citrus plants carrying the heterologous gene *P5CS112A*, a high endogenous proline causes an increase in transcription of cytosolic APX and chloroplast GR and Cu/Zn SOD isoforms (De Carvalho *et al.* 2013).

Redox buffer for reductants: An enhanced proline synthesis under abiotic stress may be mechanism to maintain redox potential at values suitable for metabolism (Hare *et al.* 1998). It is well known that stomatal closure during osmotic stress limits carbon uptake and subsequently decreases NADPH consumption by the Calvin cycle. Recently, it was discovered (Signorelli *et al.* 2014) that the P5C-proline cycle involves cycling proline and P5C *via* ProDH and P5CR and delivers electrons to mitochondrial electron transport without producing glutamate. During stress conditions, an enhanced rate of proline biosynthesis in chloroplasts maintains a low NADPH:NADP ratio and contributes to sustain electron flow between photosynthetic excitation centers and thus stabilizes the redox balance and reduces photoinhibition and damage to the photosynthetic apparatus (Székely 2008). Thus, oxidation of NADPH accompanying proline synthesis restores the terminal electron acceptor of the photosynthetic electron transport chain and possibly increases the cellular NADP/NADPH ratio (Shetty 2004). In the study on transgenic soybean plants, it was observed that inhibition of proline biosynthesis and NADPH-NADP conversion by antisense *P5CR* lead to drought hypersensitivity whereas overexpression of *P5CR* results in a moderate drought tolerance indicating thereby that proline biosynthesis is important for maintaining $NADP^+$ pools during stress (De Ronde *et al.* 2004). Hebbelmann *et al.* (2012) reported that *Arabidopsis* knockout mutants lacking chloroplast NADP dependent malate dehydrogenase do not use excess reducing equivalents in the Calvin cycle for carbon assimilation in response to a high irradiance. These mutants rather use a combination of compensatory strategies to maintain redox homeostasis, particularly adjusting photorespiration and proline biosynthesis, which dissipate excess reducing equivalents, sustain photosynthesis, and prevent photoinhibition.

Source of energy: The proline biosynthetic pathway from glutamate, although short, involves an extremely high rate of consumption of reductants (NADH and NADPH). Rapid oxidation of proline appears to be excellent means of storing energy since the oxidation of one molecule of proline can yield 30 ATP equivalents (Hu *et al.* 1992, Szabados and Savouré 2009). In *Arabidopsis*, expression of *ProDH* has been detected in lateral and main roots especially in the central stele and meristem suggesting that proline catabolism may be necessary to provide energy when roots of rehydrated plants elongate (Nakashima *et al.* 1998, Kishor *et al.* 2005). Upregulation of the proline catabolic genes typically observed in flowers, siliques, and seeds has been found to be consistent with the need to provide a plant with energy throughout the whole reproductive phase (Mattioli *et al.* 2008). It is thus believed that this feature contributes substantially to a role for proline in plants as resource of value either in acclimation to stress or in recovery upon relief from stress.

Link with key pathways: Proline occupies a central place in metabolism and is connected to other pathways through both ornithine and glutamate. Proline biosynthesis consumes a reductant in the form of NADPH (Verbruggen and Hermans 2008) and was found to be linked with the pentose phosphate pathway (PPP) as way of moving the reductants and buffering the redox status of the chloroplast (Sarkar *et al.* 2011). In the PPP, glucose-6-phosphate is oxidized to glucono-δ-lactone and 6-phosphogluconate by glucose-6-phosphate dehydrogenase and 6-phosphogluconolactonase. Oxidation of 6-phosphogluconate to ribulose-5-phosphate is mediated by 6-phosphogluconate dehydrogenase (Fig. 3). Ribulose-5-phosphate formed can enter the Calvin cycle through phosphoribulokinase or further metabolize to glyceraldehyde-3-phosphate through a series of reactions in the non-oxidative phase of the PPP. The production of ribulose-5-phosphate also generates two molecules of NADPH and one molecule of CO_2. This NADPH is utilized for synthesis of proline and assimilation of CO_2 by carbon fixation. This may prove beneficial under stress conditions where limited CO_2 occurs due to stomatal closure. The resulting proline acts as osmoticum for coping with water loss and can be transported into mitochondria to pass the reductant directly into the electron transport chain or can be transported to non-photosynthetic tissue (such as roots) as substrate for continued growth or osmotic adjustment (Spielbauer *et al.* 2013). The pentose phosphate pathway and photosynthetic carbon reduction cycle also provides carbon in the form of erythrose-4-phosphate which together with phospho*enol*pyruvate acts as precursor for phenylalanine biosynthesis *via* the shikimic acid pathway (Shetty 2004) and supports purine nucleotide biosynthesis, too (Hayat *et al.* 2012; Fig. 3).

On the contrary, the mitochondrial location of proline degradation and the presence of glutamate dehydrogenase in the matrix suggest that this process may contribute carbon for the TCA cycle (Sarkar *et al.* 2011). Besides contributing carbon for the TCA cycle, mitochondrial degradation of proline to 2-oxoglutarate may also provide reducing equivalents needed to support the mitochondrial electron transport and generation of ATP for recovery from stress and repair of stress-induced damage (Araújo *et al.* 2013). It was observed that consumption of proline in barley leaves recovering from drought stress could theoretically contribute to the TCA cycle at a rate sufficient to account for 20 % of total respiratory activity (Guo *et al.* 2009). A tight link between ornithine and arginine has also been found in a study where arginine was hydrolysed to ornithine by the action of arginase involving the urea cycle (Fig. 3). In addition, arginine and ornithine are also the precursors of polyamines (putrescine, spermidine and spermine) which play important roles in developmental processes and stress tolerance (Alcazar *et al.* 2010).

Table1. Exogenous application of proline and its protective role in plants under abiotic stresses. P_N - net photosynthesis rate; F_v/F_m - maximum quantum yield of photosystem II photochemistry; Chl - chlorophyll.

Name of the crop	Proline	Effects	References
Chickpea (*Cicer arietinum)*	10 µM	reduced oxidative injury by elevating enzymatic and non-enzymatic antioxidants	Kaushal *et al.* 2011
Mung bean (*Phaseolus vulgaris*)	50 µM	stimulated components of the ascorbate-glutathione cycle, increased activities of antioxidative enzymes, decreased lipid peroxidation and H_2O_2 content	Aggarwal *et al.* 2011
Melon (*Cucumis melo*)	200 µM	increased fresh and dry masses, increased P_N, F_v/F_m, and Chl content, reduced O_2^- and the H_2O_2 content, enhanced activities of antioxidative enzymes	Yan *et al.* 2011
Sea daffodil (*Pancratium maritimum*)	5 mM	protected protein turnover machinery against stress-damage and up-regulated stress protective proteins	Khedr *et al.* 2003
Lentil (*Lens culinaris*)	15 mM	induced upregulation of glutathione transferase and glyoxalase	Molla *et al.* 2014
Wheat (*Triticum aestivum*)	20 mM	improved shoot and root fresh and dry masses, shoot length, and grain yield	Kamran *et al.* 2009
Tobacco (*Nicotiana tabacum*)	20 mM	increased fresh mass, enhanced activities of antioxidative enzymes	Hoque *et al.* 2007b
Sugarcane (*Saccharum officinarum*)	20 mM	alleviated salt induced oxidative stress by improving guaicol peroxidase activity	Patade *et al.* 2014
Olive (*Olea europaea*)	50 mM	modulated the antioxidative defense system, increased photosynthetic activity and plant growth, maintained a suitable plant water status	Ahmed *et al.* 2010

Manipulating proline metabolism for abiotic stress tolerance

Proline engineering is mediated *via* accumulation or overexpression of *P5CS*, *P5CR*, and *OAT*, or by repression of *ProDH*. Kishor *et al.* (1995) produced transgenic tobacco ectopically expressing *Vigna aconitifolia P5CS*and accumulating more proline than the wild type. Soybean plants expressing *P5CR* under the control of an inducible heat shock promoter were found to accumulate higher amounts of proline than the antisense counterparts, thereby conferring drought stress tolerance (De Ronde *et al.* 2004). Karthikeyan *et al.* (2011) observed five times more proline content in transgenic chickpea and rice plants transformed with the *P5CS* gene from . *aconitifolia* than their non-transformed counterparts.

Increased or decreased *ProDH* expression in sense and anti-sense transgenic plants led to 50 % decreased or 25 % increased proline content, respectively (Stein *et al.* 2011). Reports in the literature indicate that decreasing proline catabolism by *PDH* antisense strategy does not alter plant development (Mani *et al.* 2002). A slight decrease in seed germination was observed in PDH RNAi tobacco plants (Ribarits *et al.* 2007). A change in proline content does not show any correlation with osmotolerance (Mani *et al.* 2002). However, Nanjo *et al.* (1999) generated anti-sense transgenic *Arabidopsis* plants using *AtProDH* cDNA and proposed a positive correlation between an increased proline accumulation and freezing or salt tolerance. Efficient expression of P5CSF129A transgene enhances proline accumulation in transgenic pigeonpea as compared with non-transgenic plants under stress conditions (Prasad *et al.* 2004, Kumar *et al.* 2010, Krishna *et al.* 2011).

Conclusion and future perspectives

In short, this review discusses the accumulation of proline, the intrinsic and extrinsic factors that influence its accumulation, and its diverse functioning in plant abiotic stress. Since the engineering of proline metabolism can lead to opportunities to improve plant stress tolerance, the examples of transgenic plants engineered to have enhanced proline content as well as the effects of exogenous application of some osmolytes are also described. Besides this much understanding, numerous questions related to proline genetics and a molecular basis remain unanswered till date. An exciting area of future direction must be dissecting the regulatory signaling cascades in the stress responses linked with proline accumulation.

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