

Review Proline Biosynthesis and its Role in Abiotic Stress

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Abstract – Crop productivity is severely affected by various abiotic stresses such as high and low temperature, draught and salinity. When exposed to stressful conditions plant adopts various strategies to overcome stress, one of these is accumulation of compatible osmolytes to maintain water potential under stress conditions. Glycine betaine (GB) and proline are two major organic osmolytes. Proline, besides acting as an excellent osmolyte, plays three major roles during stress, i.e., as a metal chelator, an antioxidative defense molecule by bringing concentrations of reactive oxygen species (ROS) within normal ranges and a signaling molecule. It also stabilizes membranes, thereby preventing electrolyte leakage. A large body of data suggests a positive correlation between proline accumulation and plant stress. Here we review the pathway of synthesis of proline and important genes involved in its biosynthesis, role of proline under stress condition.

Keywords – Proline, Abiotic Stress, P5CS, P5CS, PDH, ROS.

I. INTRODUCTION

Various abiotic stresses affect plant growth, development and final yield of food grains. Drought, salinity, ionic imbalance and extreme temperature being the major abiotic stress (Figure 1). Among these drought and salinity are most widely spread (Ashraf, 1994). In India about 7 M ha of land is salt- or sodicity - affected (Hollington, 1998) and this is becoming worse due to unreasonable use of water, increasing scarcity of fresh water, improper land irrigation and fertilization practices. Much land is becoming secondary salinized. Up to 45% of the world agricultural lands are under continuous or frequent drought, (Bot *et al.*, 2000). Generally, plants experience drought stress either when the water supply to roots becomes hard or when the transpiration rate becomes very high (Manivannan *et al.*,

2007). In comparison to salinity, drought and ionic imbalance, changes in ambient temperature occur more rapidly. These changes in ambient temperature further aggravate the adverse effects of other stresses, including drought and salinity, on crop production and quality. For instance, heat stress adversely affects grain quality and final crop yield in 40% of the irrigated wheat growing area of the world (Fischer and Byerlee, 1991). With the tremendously increasing population the demand for food grains have increased in recent years. So to meet the food grain requirement with increasing abiotic stresses there is need for development of varieties tolerant to these hostile stress conditions. For development of crop plants tolerant to abiotic stress require thorough knowledge of the mechanism adopted by plants to abiotic stress tolerance. A complex mechanism is present in plants at cellular and molecular level to combat abiotic stress. Environmental stresses provoke numerous plant responses, varying from altered gene expression to metabolic processes. Plants imply basic five mechanisms to overcome salt stress i.e. ion selectivity, ion accumulation, osmotic adjustment, organic solutes and water use efficiency (Shannon, 1997). Osmotic adjustment in plants is made by synthesis of a number of low-molecular-weight compounds which accumulate in plants in response to stress. These compounds help in stabilizing cellular structures, specific enzymes, photosynthetic complexes and other macromolecules. They are also involved in scavenging of reactive oxygen species. Proline and glycine betaine are the most commonly occurring osmolytes under stress conditions. This review describes the biosynthesis of proline its accumulation, degradation and its role in abiotic stress tolerance.



Fig. 1. The various abiotic stress affecting plant growth

II. PROLINE METABOLISM IN PLANTS

Its Synthesis

Proline is a proteinogenic amino acid without imino group. It has an exceptional conformational rigidity and is essential for primary metabolism. In plants, there are two pathways for synthesis of proline one is from glutamate and other is from ornithine. However glutamate pathway is most preferred pathway for proline biosynthesis than from ornithine as the key enzyme i.e. ornithine – delta-aminotrasferase involved in proline synthesis from ornithine is downregulated (Szekely *et al.*, 2008). In synthesis of proline from glutamate pathway, glutamate is reduced to glutamate-semialdehyde (GSA) by the pyrroline-5-carboxylate synthetase (P5CS) enzyme and spontaneously converted to pyrroline-5-carboxylate (P5C) (Savoure, *et al.*, (1995) (Figure 2). P5C reductase (P5CR) is reduced to the P5C intermediate to proline (Szoke, *et al.*, 1992). Under stress conditions, P5CS1 accumulates in the chloroplasts, leading to enhanced proline biosynthesis in the plastids. In most plant species, P5CS is encoded by two genes and P5CR is encoded by one (Szoke, *et al.*, 1992).

The alternative pathway for proline synthesis from ornithine. Ornithine is converted to glutamic semialdehyde (GSA) by ornithine – delta-aminotrasferase. GSA is spontaneously converted to P5C, which is converted to proline by P5CR (Delauney, *et al.*, 1993, Roosens, *et al.*, 1998). The ornithine pathway uses arginine and produces P5C and glutamate in mitochondria. Mitochondrial P5C can be recycled to proline in the cytosol by P5CR.

Its Degradation

Proline is oxidized to P5C by sequential action of proline dehydrogenase (PDH) and P5C dehydrogenase (P5CDH) in mitochondria. PDH is often been referred as proline oxidase (POX). P5CDH is encoded by single gene while PDH is encoded by two genes. P5CDH gene has been identified in Arabidopsis and tobacco (*Nicotiana tabacum*) (Kishor, *et al.*, 2005). Both PDH and P5CDH are present in the matrix side of inner mitochondrial membrane (Elthon and Stewart 1981, 1982). PDH is an oxygen dependent flavoprotein in plants (Elthon and Stewart 1982). The location of proline catabolic pathway in mitochondria suggests that its role in contributing carbon to TCA cycle.

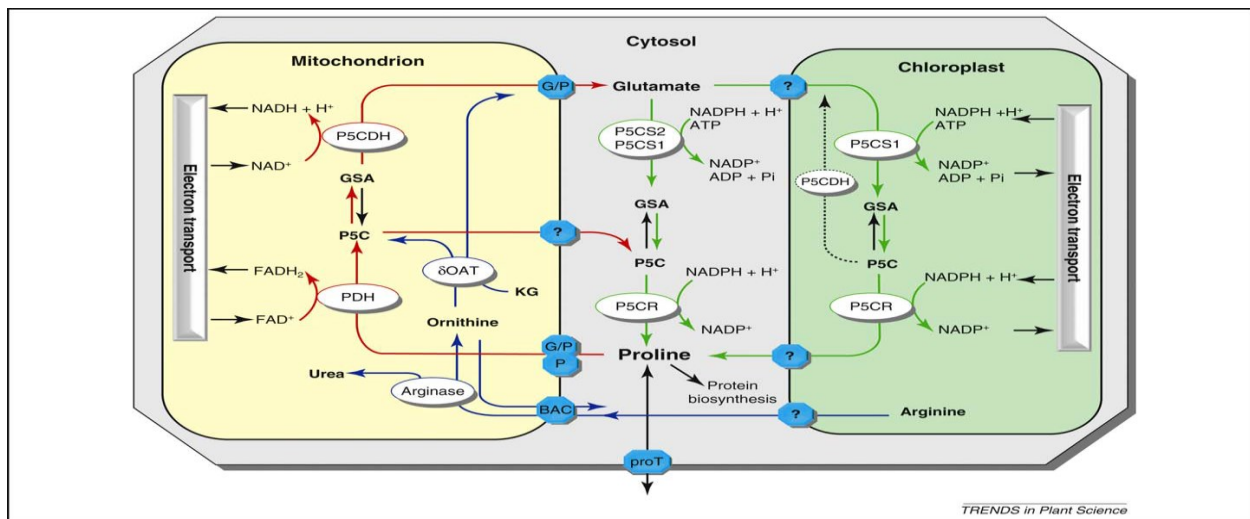


Fig. 2. Proline metabolism pathway in higher plants. The biosynthetic pathway, catabolic pathway and the ornithine pathway is indicated by green, red and blue line respectively. The proline biosynthesis occur in the cytosol whereas the catabolic pathway occurs inside mitochondria. Enzymes are depicted as ellipses and transporter proteins as blue octagons (Szabodos, and A. Savoure *et al.*, 2009)

III. GENES INVOLVED IN PROLINE BIOSYNTHESIS

Pyrroline-5-Carboxylate Synthetase (P5CS)

P5CS (EC 2.7.2.11/1.2.1.41) is a bifunctional enzyme that converts glutamate into the intermediate glutamic semialdehyde, which spontaneously cyclizes into D¹-pyrroline-5-carboxylate (P5C) (Verslues and Sharma, 2010). P5CS gene was first cloned from *Vigna aconitifolia* (moth bean) by complementation (Hu *et al.*, 1992). *V. aconitifolia* P5CS have leucine zipper sequences in each of the enzymatic domains (Hu *et al.*, 1992), which function intramolecularly to maintain the tertiary structure of the enzyme or intermolecularly in protein-protein interaction. This hexameric enzyme has a molecular weight of 450 kDa,

and is up-regulated by transcriptional mechanisms under stress conditions.

Proline act as a competitive inhibitor and decreases the affinity of *Vigna* P5CS for glutamate whereas ADP act a competitive inhibitor of glutamate kinase activity of P5CS. P5CS has two enzymatic domains correspond to the ProB and ProA proteins in *Escherichia coli* and as *tomPRO1* and *tomPRO2* in tomato (Fujita *et al.*, 1998). *tomPRO*, encodes two polypeptides resembling the bacterial operon, whereas *tom-PRO2* encodes a protein similar to eukaryotic P5CS. Most of the *tomPRO1* mutants had lower specific activities and increased inhibition constants (20-fold to 3500-fold). In plants also two different forms of P5CS are found, P5CS1 and P5CS2. The two different forms results from independent evolutionary duplication events (Turchetto-

Zolet *et al.*, 2009). These two duplicated enzymes perform non-reductant functions and their genes shows different expression pattern (Ginzberg *et al.*, 1998). P5CS1 is localized in chloroplast and is induced by dehydration and high stress whereas isoform1 *i.e.* P5CS2 has a cytoplasmic distribution and is involved in embryo and seedling development. In the absence of stress, P5CS1 is more diffuse, raising the possibility of stress-induced relocalization of the enzyme. P5CS1 is subjected to feedback inhibition by proline (Hu *et al.*, 1992). Both P5CS1 and P5CS2 of *A. thaliana* have a leucine zipper region in each domain. These motifs are not present in an α helix and do not match the normal consensus of four heptad repeats (Savoure *et al.*, 1997). These leucine zipper regions participate in protein-protein interaction (perhaps with reduced affinity) needed to maintain P5CS1 structure (Savoure *et al.*, 1997). In *A. thaliana* a knockout of P5CS1 causes a reduction of stress-induced proline synthesis, hypersensitivity to salt stress and an accumulation of reactive oxygen species (ROS) (Szekely *et al.*, 2008). Decreased activity of glutathione-S-transferase (GST) and glutathione reductase (GR) was observed in the transgenic species, indicating that the glutathione detoxifying pathway was subsequently affected by the absence of P5CS isoform1. These observations suggest a link between glutathione and proline metabolism in plants. In plants under water stress the transcript level of P5CS is increased, activity of P5CS is increased and proline degradation is inhibited by inhibition of proline dehydrogenase (PRODH) activity (Ginzberg *et al.*, 1998). Mutants of P5CS1 are hypersensitive to salt, osmotic stress and have low water potential (Szekely *et al.*, 2008).

Proline accumulation under stress is regulated by Abscisic (ABA) but when ABA was applied in absence of stress it was insufficient to induce high level of proline (Savoure *et al.*, 1997; Sharma and Verslues, 2010). An ABA-independent regulation of P5CS1 was found on quantitative comparison of *P5CS1* expression to known

ABA-induced genes, implying that the underlying mechanisms controlling P5CS1 expression are distinct from that of many commonly studied stress marker genes (Sharma and Verslues, 2010). P5CS1 expression has also been found to be stimulated by light and nitric oxide (Verslues and Sharma, 2010). In contrast, P5CS1 expression was decreased by brassinosteroid application. Other than effects of plant hormones, the signal transduction controlling *P5CS1* is not well known. Calcium signaling is likely to be involved: by using a *Glycine max* (soybean) salt-inducible calmodulin, Yoo *et al.*, (2005) described a mechanism whereby calmodulin activated a MYB transcription factor which then activated a number of downstream genes including P5CS1 (Verslues and Sharma, 2010). Another calcium signaling component, phospholipase C, upregulated expression of P5CS1 in *Arabidopsis* under salt but not osmotic stress. In contrast to the P5CS1, gene expression of P5CS2 has little or no transcriptional up-regulation under stress (Strizhov *et al.*, 1997; Szekely *et al.*, 2008) and all indications are that it has only a minor role in proline accumulation induced by abiotic stress (Strizhov *et al.*, 1997; Szekely *et al.*, 2008). P5CS2 can be induced by pathogen response and may be more actively involved in plant-pathogen interaction. Also, P5CS2 is expressed more abundantly in actively dividing callus and cell suspension culture (Strizhov *et al.*, 1997). P5CS2 mutants are embryo lethal despite the presence of functional P5CS1 (Szekely *et al.*, 2008), suggesting a specialized developmental role of P5CS2. While exogenous proline can rescue some P5CS2 mutants, the mutant plants were sterile (Szekely *et al.*, 2008). How P5CS expression affects development and whether the different functions of P5CS1 and P5CS2 may also involve different post-translational regulation and/or compartmentation of the two remains to be established. The enzymatic properties of *Arabidopsis* P5CS2 have been relatively little studied. Post translational regulation is also unknown for either P5CS1 or P5CS2.

Table 1. Genes that are involved in the network of proline biosynthesis (Kishore *et al.*, 2005).

Name of Gene	E.C. number	Prosrite signature	Localization	Organism
Pyroline-5-carboxylate synthetase (P5CS)	2.7.2.11	Glutamate-5-kinase	Unknown (may be chloroplastic)	Plants
Pyroline-5 carboxylate reductase (P5CR)	1.5.1.2	Delta-1-pyrroline-5-carboxylate	Cytosolic; chloroplastic	Plants
Proline dehydrogenase (PDH)	1.5.99.8	Aldehyde dehydrogenase-g and c	Mitochondrial inner active site membrane	Plants
Glutamic-g-semialdehyde dehydrogenase (GSDH)	1.2.1.41	Gamma-glutamyl phosphate reductase	Mitochondrial inner membrane	Plants
Pyroline-5-carboxylate dehydrogenase (P5CDH)	1.5.1.12	No hit in EXPASY	Mitochondrial; cytosolic	Plant
Prolyl hydroxylase	1.14.11.2	Thioredoxin family site	Unknown	Plant
Acetyl-CoA: glutamate N-acetyl transferase (Ac GACT)	2.3.1.35	Hydrolytic activity on acetyl-L-ornithine	Chloroplastic	Plants
N-Acetylglutamate kinase (Ac GK)	2.7.2.8	N-Acetylglutamate 5 phospho-transferase active site	Mitochondrial	Plants
Acetyl glutamic-g-semialdehyde dehydrogenase - (Ac GSD)	1.2.1.38	N-acetyl-gamma-glutamyl- phosphate reductase active site	Chloroplastic	Prokaryotes
Ornithine carbamoyl transferase (OCT)	2.1.3.3	Aspartate and ornithine carbamoyl-transferases signature	Mitochondrial; Cytosolic	Plants
Arginino succinate synthetase (ASS)	6.3.4.5	Argininosuccinate synthase signature 1	Cytosolic	Plant
Arginino succinate lyase (ASL)	4.3.2.1	Fumarate lyases signature	Cytosolic	Plants
Arginase (ARG)	3.5.3.1	Arginase family signatures	Mitochondrial	Plants
Pyroline-2-carboxylate reductase (P2CR)	1.5.1.1	Pyroline-2-carboxylate reductase signature	Unknown	Plants

IV. ROLE OF PROLINE UNDER STRESS CONDITIONS

Plants when exposed to any stressful conditions accumulates proline in large quantity (Hsu *et al.*, 2003; Kishore *et al.*, 2005). Besides acting as an osmolyte for osmotic adjustment, proline contributes to stabilizing sub-cellular structures (e.g. membranes and proteins), scavenging free radicals and buffering cellular redox potential under stress conditions, alleviating cytoplasmic acidosis and maintaining appropriate NADP + /NADPH ratios compatible with metabolism (Hare and Cress, 1997). In addition, on removal of stressful conditions results in breakdown of proline which provide sufficient reducing agents that support mitochondrial oxidative phosphorylation and generation of ATP for recovery from stress and repairing of stress-induced damages (Hare and Cress, 1997). Proline is also known to induce expression of salt stress responsive genes, which possess proline responsive elements (e.g. *PRE*, *ACTCAT*) in their promoters (Satoh *et al.*, 2002; Chinnusamy *et al.*, 2005). Reactive oxygen species severely damage the plant by their peroxidation activity of membrane lipid components. Plants synthesize various metabolite to scavenge the ROS such as glutathione, ascorbic acid, and many antioxidant enzymes. But during severe stress conditions the amount of ROS exceeds the limit which can be scavenged by the plant system. So during severe stress proline prevent excess formation of ROS. Proline also act as a chaperon and stabilizes the structure of proteins in cytosol. Its accumulation also buffers the cytosolic pH. (Hare and Cress 1997).

Proline as an Osmolyte

The cellular response of salt tolerant plant to both long term and short term salinity stress includes the synthesis and accumulation of compatible solutes which acts as osmoprotectants. These are relatively small, polar, uncharged, non-toxic compounds that can stabilize proteins and cellular structures and increase the osmotic pressure of the cell (Yancey *et al.*, 1982). They mainly include proline, glycine betaine, sugar and polyols. The concentration of these compatible solutes within the cell is maintained either by irreversible synthesis of the compounds or by a combination of synthesis and degradation. Osmolytes maintains water status inside cell and sub-cellular structures and play a protective role from the denaturing effects of osmotic stress (Ashraf and Foolad, 2007). Proline is an important osmolyte among all the osmolytes. Various studies have indicated an important role of proline in maintain osmotic balance during abiotic stress conditions (Ashraf and Foolad, 2005; Munns, 2005; Khan *et al.*, 2009).

Proline as Scavenger of ROS

Abiotic stress induces production of reactive oxygen species (ROS) including superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH^\cdot). All these compounds being strong oxidizing agents are detrimental to cell integrity and cause oxidative damage to different cellular components by deteriorating protein, fragmenting DNA, lipid peroxidation of membrane lipids and eventually cell death (Groß *et al.*, 2013, Farooq *et al.*,

2008). During normal condition the concentration of these ROS is maintained low by the activity of low molecular mass antioxidants such as ascorbic acid and reduced glutathione and a diverse array of enzymes such as superoxide dismutases (SOD), catalases (CAT), ascorbate peroxidases (APX), glutathione *S*-transferases (GST) and glutathione peroxidases (GPX) are produced by plants during stress (Haliwell *et al.*, 1986). During stress conditions beside increased activity of the antioxidants, accumulation of compatible solutes such as proline also increases. Accumulation of proline has been shown to protect plants against damage by reactive oxygen species. Several studies have reported proline as a scavenger of ROS (Matysik and Bhalu, 2002., Smirnov, 1989). Experiments conducted by Alia *et al.*, 1999, have shown proline as an excellent quencher for singlet oxygen. Hoque *et al.*, 2006 reported that the activities of antioxidative enzymes viz. catalase (CAT), peroxidase (POX) and superoxide dismutase (SOD) were significantly enhanced when proline was applied exogenously in tobacco suspension cultures exposed to salinity stress.

Proline in Maintaining Redox Balance

Under stress condition there occurs a reduction in NADP + /NADPH ratio due to suppression of Calvin cycle resulting in higher accumulation of NADPH. This further decreases the electron supply in the electron transport chain due to suppression of electron acceptor NADP + (Chaves, *et al.*, 2009). During stress condition proline accumulation increases in chloroplast which help in restoring the normal NADP + /NADPH ratio as proline biosynthesis being a reductive pathway involves the use of NADPH for conversion of glutamate to P5C and then finally to proline and in turns generates NADP +. NADP + can be used as an electron acceptor thus decreasing the formation of ROS (Figure 3). Thus accumulation of proline contributes in sustaining the electron flow between photosynthetic excitation centers, stabilize the redox balance, and reduce photoinhibition and damage of the photosynthetic apparatus (Hare, and Cress, 1997). On restoration of normal conditions catabolism of proline occurs which generates reducing equivalents that support mitochondrial oxidative phosphorylation. This process does not involve NADPH oxidation as electrons enter the electron transport system directly at the level of a flavoprotein. The shift in proline metabolism in relation to stress conditions play an important regulatory function in plants cellular metabolism (Bogges, *et al.*, 1978).

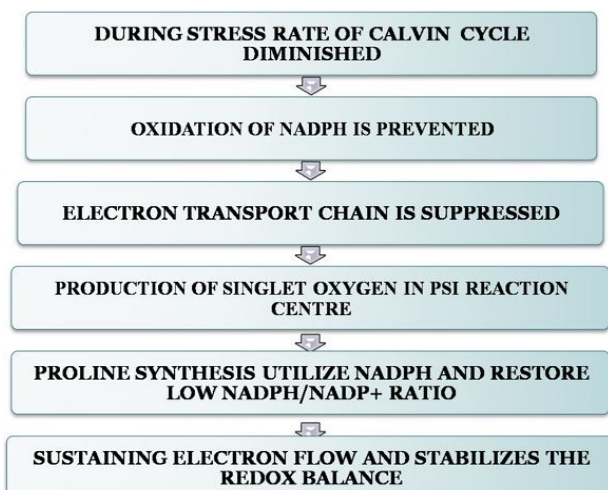


Fig. 3. Proline role in maintaining appropriate NADP + /NADPH ratios compatible with metabolism.

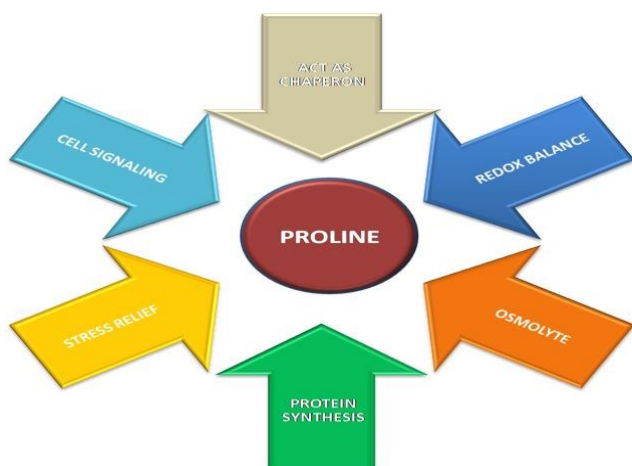


Fig. 4. Functions of proline in plants. Proline is used for protein synthesis, functions as an osmolyte, contributes to the maintenance of the redox balance, stress relief, signaling and development.

V. CONCLUSION

Understanding the mechanism involved in proline biosynthesis, degradation and transport of proline during stress is essential for developing stress tolerance in plants. By studying the various genes involved in proline metabolism and identifying the promoter related to these genes, the regulatory mechanism of these genes could be known. So engineering strategies targeting the promoter and their related genes involved in proline biosynthesis can be aimed at enhancing proline concentration in chloroplast during stress.

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Research Publications Published

- **Rana, V.**, Ram, S., Nehra, K., and Sharma, I. 2015. Differential Expression analysis of salt stress related genes *TaSRG* and *TaRUB1* in contrasting wheat genotypes. *J. Wheat Res.* 7(1): 71-73.
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Book Chapter

- **Rana, V.**, Ram, S., and Nehra, K. 2014. R.K. Salar *et al.*, Molecular basis of salt tolerance in wheat and other crop plants (eds.), *Biotechnology: Prospects and Applications*, DOI 10.1007/978-81-322-1683-4_2, © Springer India 2014.

Abstract Published

- **Rana, V.**, Ram, S., and Nehra, K. 2013. Proline biosynthesis and its role in abiotic stress. BIOSYM- 2013 (pp 15) March 15-16, 2013, DCRUST, Murthal (Sonapat).
- **Rana, V.**, Ram, S., and Nehra, K. 2013. Understanding proteomics technology under salinity stress. “National Symposium on “Emerging Trends in Agri Bioinformatics” (ETAB), December 16-17, 2013.



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