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## Osmolytes: Proline metabolism in plants as sensors of abiotic stress

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**Abstract:** Proline accumulation occurs in a large range of plant species in retaliation to the numerous abiotic stresses. An exclusive research pattern suggests there is a pragmatic relation between proline accumulation and plant stress tolerance. In this review, we will discuss the metabolism of proline accumulation and its role in stress tolerance in plants. Pertaining to the literature cited clearly indicates that not only does it acts as an osmolyte, it also plays important roles during stress as a metal chelator and an antioxidative defence molecule. Moreover, when applied exogenously at low concentrations, proline enhanced stress tolerance in plants. However, some reports point out adverse effects of proline when applied at higher doses. Role of proline gene in seed germination, flowering and other developmental programmes; thus creation of transgene overexpressing this gene would provide better and robust plants. In this context this review gives a detailed account of different proline gene over-expressed in all the transgenic crops so far.

**Keywords:** Abiotic stress, Osmoprotectant, Proline, ROS, Transgenic

### INTRODUCTION

Plants being exposed to different environmental stresses learn and or adopt to these stresses in a number of ways. Thus among these stresses, Osmolytes production stress, in particular that is caused due to abiotic stress such as drought and salinity is the most critical problem that limits plant growth and crop productivity in agriculture (Boyer, 1982). Plant physiological development and productivity is largely affected by many environmental stresses such as drought, high salinity, and low temperature. These stresses trigger expression of an array of gene in response. Thus the expressed products of these genes function not only in stress response but also in stress tolerance. In the signal transduction network from the first discrimination of stress signals to stress-responsive gene expression, many concerned transcription factors and *cis*-acting elements in the stress-induced promoters function for plant mitigation to environmental stresses. Recent a lot of advances have been made in studying the complex cascades of gene regulation in drought and cold stress responses, especially in identifying specificity and cross talk in stress signaling. In this review article, we highlight transcriptional regulation of gene expression in response to abiotic stresses, with particular emphasis on the role of transcription factors and *cis*-acting elements in stress-inducible promoters. Genes expressed during stress conditions function not only in protecting cells from stress by producing important

metabolic products/bio-molecules, but also in regulating genes for signal transduction in the stress response. Thus, these gene products are classified into two groups (Fowler and Thomashow, 2002; Kreps *et al.*, 2002; Seki *et al.*, 2002). The primary group includes proteins that mainly function in stress subjection, such as osmotin, antifreeze proteins, chaperones, LEA (late embryogenesis abundant) proteins, RNA-binding proteins, and it also includes important catalyzing bio-molecules i.e enzymes for osmolyte biosynthesis such as water channel proteins, proline, sugar and proline transporters, The second group contains protein factors which play important role in regulation of signal transduction and gene expression machinery that probably works in response to stress reflex. This included an array of different transcription factors, suggesting that various transcriptional regulatory mechanisms function in the drought-, cold-, or high-salinity-stress signal transduction pathways (Seki *et al.*, 2003). Many works indicate that there are more than 300 genes that have been identified as being stress-inducible (Kazuo and Kazuko, 2006). Among these different physiologically activated genes, more than half of the drought induced genes are also induced by high salinity, indicating the existence of significant cross talk between the drought and high-salinity responses. The section of small molecules known as "compatible osmolytes" includes certain amino acids (notably proline), quaternary ammonium compounds (e.g. glycinebetaine, proline betaine,  $\beta$ -alanine betaine,

and choline-*O*-sulfate), and the tertiary sulfonium compound 3-dimethylsulfoniopropionate (DMSP). Throughout their life cycle, plants are subjected to various types of environmental stresses, water deficit, temperature extremes, toxic metal ion concentration and UV radiations depending upon the severity of stress. The environmental factors retard the growth and productivity of plants to different degrees. In reflex response to different stresses plants accumulate large quantities of different types of compatible solutes (Serraj and Sinclair, 2002). Thus accumulation of osmolyte compounds, usually called 'osmotic adjustment' or 'osmoregulation', is certainly a remedial measure to overcome the negative consequence of water deficit condition in plant's growth and survival. It has been proposed since long before as a remedial mechanism for drought and salt tolerance (Martin, 1930; Bernstein, 1961), but it has gained fame during the last 20 years. Compatible solutes are low molecular weight, highly soluble organic compounds that are usually non-toxic at high cellular concentrations. These solutes provide immunity, to plants from environmental-induced stress by regulating cellular osmotic adjustment, ROS detoxification, protection of membrane integrity and enzymes/protein stabilization (Ashraf and Foolad, 2005; Bohnert and Jensen, 1996; Yancey, 1994). These include proline, sucrose, polyols, trehalose and quaternary ammonium compounds (QACs) such as glycine betaine, alanine betaine, proline betaine and pipercolate betaine. Expedition of the action of these solutes would provide a clear cut evidence to combat environmental stresses, which is important as it gives direct hope to genetically manipulate plants to withstand this condition. There are many cellular mechanisms by which organisms mitigate the effects of abiotic stresses; for instance, accumulation of compatible osmolytes such as proline is one such phenomenon. The phenomenon of proline accumulation is known to occur under water deficit (Naidu *et al.*, 1991), Salinity (Munns, 2005; Rhodes and Hanson 1993), low temperature (Hare *et al.*, 1998), heavy metal exposure (Bassi and Sharma, 1993a; Bassi and Sharma, 1993b; Schat *et al.*, 1997; Sharma and Dietz, 2006) and UV radiations, etc. Apart from acting as 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 (Ashraf *et al.*, 2007). In many plant species, proline accumulation under salt stress has been correlated with stress tolerance, and its concentration has been shown to be generally higher in salt tolerant than in salt sensitive plants (Fougère *et al.*, 1991; Gangopadhyay *et al.*, 1997; Madan *et al.*, 1995; Petrusa and Winicov, 1997). It may also act as protein compatible hydrotrope (Strizhov *et al.*, 1997), alleviating cytoplasmic acidosis and maintaining appropriate NADP<sup>+</sup>/NADPH ratios

compatible with metabolism. Work relating to this i.e. proline osmoprotectant is reviewed here. Some generalizations can be made: Firstly, the availability of the precursor to synthesize the osmoprotectants could limit the amount of osmoprotectant made in a transgenic host. Secondly, negative physiological consequences of diverting the precursor to the osmoprotectants away from primary metabolism should be considered (Su and Wu 2004). Thirdly, despite the availability of physiological data and techniques for assessing stress tolerance in plants, but still transgenic plants are rarely had been put to test their functioning which calls for their examination. Proline accumulation normally occurs in cytoplasm where it plays the role of molecular chaperons stabilizing/conditioning the structure of proteins and its accumulation by buffering cytosolic pH and maintaining cell redox status. It has also been proposed that its accumulation may be part of stress signal cascade influencing adaptive responses thus taking in account this feature would be beneficial to incur stress tolerance via engineering transgenic over expressing proline gene (Hoque *et al.*, 2008).

#### **SENSORS OF ABIOTIC STRESS- OSMOLYTES**

There are many mechanisms at cellular level through which organisms ameliorate the effects of environmental stresses; for instance, accumulation of compatible osmolytes such as proline is one such phenomenon. Many plants, including halophytes, accumulate compatible osmolytes, such as proline (Pro), glycine betaine and sugar alcohols, when they are exposed to drought or salinity stress (Hellebust, 1976; Csonka, 1989; McCue KF, Hanson, 1990; Delauney and Verma, 1993). The accumulation of Pro has been observed not only in plants but also in eubacteria, marine invertebrates, protozoa, and algae (Delauney and Verma, 1993; Roosens *et al.*, 2002). It has been suggested that compatible osmolytes do not interfere with normal biochemical reactions and act as osmoprotectants during osmotic stress. Among known compatible solutes, Proline is probably the most widely distributed osmolytes. Results of investigations of the relationship between the expression of these genes and the accumulation of Proline under water stress indicate that the level of Pro in plants is mainly regulated at transcriptional level during water stress. Moreover, the overproduction of Pro results in the increased tolerance of transgenic tobacco plants to osmotic stress. Thus tolerance to abiotic stress, especially to salt and improved plant growth, was observed in a variety of transgenics that were engineered for overproduction of proline (Kavi Kishor *et al.*, 1995; Bohnert and Shen, 1999; Kavi Kishor *et al.*, 2005). Proline seems to have diverse roles under osmotic stress conditions, such as stabilization of proteins, membranes and sub-cellular structures and protecting cellular functions by scavenging reactive

oxygen species (Sasaki *et al.*, 2005).

Salinity is detrimental to the various processes of crops such as seed germination, seedling growth and vigor, vegetative growth, flowering and fruit set and ultimately it causes diminished economic yield and also quality of produce (Stewart and Larher, 1980). Rice crop is important not only as food crop but also due to its medicinal value (Bajaj and Mohanty, 2005), due to virtue of it, it acts as a model monocot system for various biotechnological, metabolic, genetic engineering and functional genomics development studies worldwide (Munns and Tester, 2008). However, the yield of rice, especially Asian rice, is severely susceptible to salinity (Sairam *et al.*, 2005). In India and especially the rainfed rice is hindered by three major abiotic stresses namely drought, submergence and Salinity (Rice Knowledge Management Portal, 2011).

### **PROLINE AND ITS FUNCTION IN OSMOREGULATION**

Proline plays versatile functions in plants. As amino acid it is a one of the building blocks of protein structure, but it also plays a major role in of stress osmolytes solute under environmental stress conditions. Proline synthesis has been associated with tissues undergoing rapid cell divisions, such as shoot apical meristems, and appears to be involved in floral transition and embryo development. Lofty levels of proline can be found in pollen and seeds, where it serves as compatible solute, where it acts as dehydration protector of cellular structures during plant development. The agglomeration of proline at various terrain such as cells, tissues and other vital organs such as vascular bundles are controlled by reciprocity of biosynthesis, degradation, and cellular transport arcade. Thus, both the unique properties of proline and its variegated action through two most widely studied transporter, both general amino acid permeases and selective compatible solute transporters indicates its prime position to be use in production of abiotic resilient plants engineered through manipulating it genes (Armengaud *et al.*, 2004).

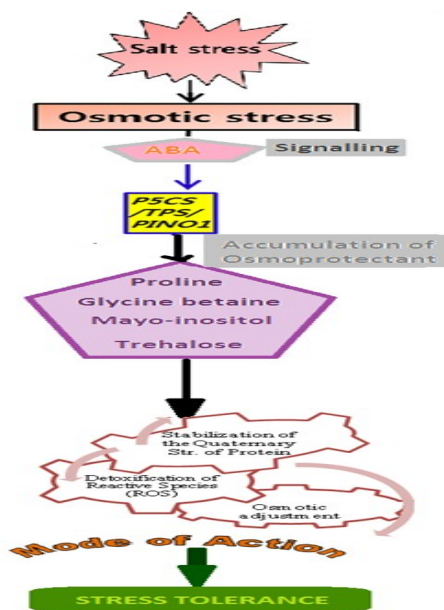
All the mechanisms encompassing the proline action be it accumulation or degradation shows that mechanisms regulating proline differ substantially from other amino acids (Yu *et al.*, 1983). Proline accumulation is a common metabolic riposte, of higher plants to water deficits, and salinity stress, and has been the subject of numerous reviews over the last 20 years (Stewart and Larher, 1980; Thompson, 1980; Stewart, 1981; Hanson and Hitz, 1982; Samaras *et al.*, 1995; Taylor, 1996; Rhodes *et al.*, 1999). This versatile amino acid has highest water solubility and is accumulated by leaves of many halophytic higher plant species grown in saline environments (Stewart and Lee, 1974; Briens and Larher, 1982; Treichel, 1986), in leaf tissues and

shoot apical meristems of plants undergoing water stress (Barnett and Naylor, 1966; Boggess *et al.*, 1978; Jones *et al.*, 1980) in desiccating pollen (Hongqi *et al.*, 1982), in root apical regions growing at low water potentials (Voetberg and Sharp, 1991), and in suspension cultured plant cells reorganized to water stress (Tal and Katz, 1980; Rhodes, 1987), or NaCl stress (Tal and Katz, 1980; Rhodes and Handa, 1989; Thomas *et al.*, 1992). Proline shields membranes and proteins against the adverse effects of elevated concentrations of inorganic ions and extreme temperature (Pollard *et al.*, 1979; Paleg *et al.*, 1981; Nash *et al.*, 1982). Proline may also function as a protein-compatible hydrotrope (Srinivas and Balasubramaniam, 1995) and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989). The proline gathered in response to water stress or salinity stress in plants is primarily confined in the cytosol (Leigh *et al.*, 1981; Pahlich *et al.*, 1981; Ketchum *et al.*, 1991). In addition, the transient accumulation of proline, might serve as a safety valve to calibrate cellular redox state during stress (Shen *et al.*, 1999; Kuznetsov and Shevyakova, 1999).

### **PROLINE METABOLISM AND ITS IMPLICATIONS FOR PLANT-ENVIRONMENT INTERACTION**

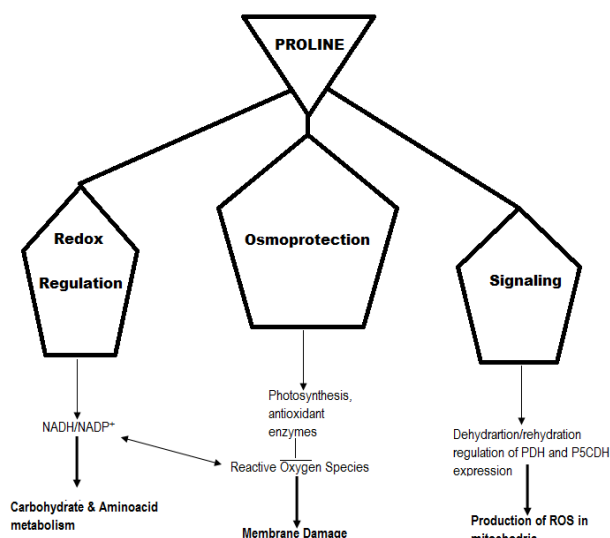
Very high agglomeration of cellular proline (upto 80% of the amino acid pool under stress and 5% under normal condition) has been documented in many plant species (Choudhary *et al.*, 2005; Widodo *et al.*, 2009). The increase in osmoprotectants is achieved either by amendment of metabolism (increasing biosynthesis and/or decreasing degradation) or by transport (increase uptake and/or decrease export) which also depends upon the type of stress and the type of species under consideration. Unlike other amino acids, proline has cyclized amino nitrogen that has significant influence on the conformation as well as uniqueness of polypeptides. Proline is also a prime component of structural proteins in animals and plants besides being a known osmoprotectants capable of mitigating the footprint of drought, salt, and temperature stress in plants (Rodriguez and Redman, 2005).

Proline, and its metabolism, is eminent from other amino acids in several ways. In plants proline is synthesized from glutamate as well from arginine/ornithine. The most fundamental is that proline is the only one of the proteogenic amino acids where the  $\alpha$ -amino group is present as a secondary amine. While this may seem like a distinction more important to chemists than plant biologists, the unique properties of proline are highly relevant to understanding its role in plants. Second important feature of proline has been studied a lot and recorded that its accumulation is caused by most of stresses relating to environmental stresses (Hare *et al.*, 1998). The role of proline and



**Fig. 1.** An overview of salinity stress.

sulphur metabolism during osmotic stress tolerance in plants has been emphasized recently (Verma, 1999). Gene involved in biosynthesis of enzymes for biosynthesis and degradation of Proline is very well documented. Results of exploration of the relationship between the expression of these genes and the agglomeration of proline under deficit water stress indicate that the level of proline in plants is mainly regulated at micro-cellular transcriptional level during water stress. Moreover in this context, the overproduction of proline results in the increased tolerance of transgenic tobacco plants to osmotic stress was reported by Dobra *et al.* (2011).



**Fig. 2.** Potential roles of proline during abiotic stress (Made by the author).

The basic of proline metabolism involves two enzymes catalyzing proline synthesis from glutamate in the cytoplasm or chloroplast, two enzymes catalyzing proline catabolism back to glutamate in the mitochondria, as well as an alternative pathway of proline synthesis via ornithine (Fig. 3). The inter conversion of proline and glutamate is sometimes referred to as the “proline cycle”. The transcriptional up regulation of proline synthesis from glutamate and down regulation of proline catabolism during strain condition provides a conception to control proline levels, although inconsistency to this pattern has been observed (Stines *et al.*, 1999). This is not the only side of the story however, as posttranslational modulation, of these enzymes has been little inspected and the role of ornithine as a proline precursor remains obscure (Phang, 1985). Likewise, the proline cycle may at first seem to be an ineffectual cycle; however, apprehending the intrinsic corporative modulation of this cycle and metabolic flux is the clue to understanding the proline metabolism biochemistry.

Proline not only being an important constituent of protein it is also a very versatile molecule playing an important part in osmoprotectant, cellular signal molecule during stress condition. After plant exposed to salt stress in *Arabidopsis* it accounted for 20% of the amino acid pool (Verbruggen *et al.*, 1996). There are two different pathways in proline biosynthesis in higher plants: the ornithine and the glutamate pathways. The plant glutamate pathway is quite different from those in microbes and human. In bacteria and human, the conversion of glutamate to glutamate-5-semialdehyde (GSA) is catalyzed by two successive reactions, whereas, in higher angiosperms the conversion is catalyzed by a bi-functional enzyme in a single step reaction (Hu *et al.*, 1992). This brings us to the conclusion that many research activities are being drawn specifically studying salinity and drought tolerance induced by proline (Williamson and Slocum, 1992).

The pathway for the biosynthesis of proline in plants was elucidated by reference to the pathway in *Escherichia coli* (Leisinger, 1987). Fig. 3 shows the proline biosynthesis and metabolism pathway in plants. The pathway in bacteria begins with the ATP-dependent phosphorylation of the  $\gamma$ -carboxy group of L-glutamic acid (L-GIU) by  $\gamma$ -glutamyl kinase ( $\gamma$ -GK). The product of  $\gamma$ -GK is reduced to glutamic- $\gamma$ -semialdehyde (GSA) by GSA dehydrogenase (GSADH), with which  $\gamma$ -glutamyl kinase forms an obligatory enzyme complex. GSA cyclizes spontaneously to form  $\Delta^1$ -pyrroline-5-carboxylate (P5C), which is finally reduced to proline by P5C reductase (P5CR). It has been suggested that, in plants, proline is synthesized either from Glu or from ornithine and that the pathway from Glu is the primary route for the synthesis of Pro under conditions of osmotic stress and

**Table 1.** Transgenic plants developed for abiotic stress tolerance through the manipulation of proline metabolism related genes.

S. No.	Gene	Targeted Trait	Species	References	
1	Pyrroline-5-carboxylate synthetase (P5CS)	Salinity stress tolerance	<i>Nicotiana spp.</i>	Kavi Kishor <i>et al.</i> , 1995	
		Increased proline accumulation		Zhang <i>et al.</i> , 1995	
		Salinity stress tolerance		Sokhansanj, <i>et al.</i> , 2006	
		Increased proline accumulation and osmotic stress tolerance		Yamchi <i>et al.</i> , 2007	
		Increased proline accumulation and salinity stress tolerance		Razavizadeh and Ehsanpour, 2009	
		Abiotic stress tolerance		Dobra <i>et al.</i> , 2010	
		Increased proline accumulation and salinity stress tolerance		Jazii <i>et al.</i> , 2011	
		Salinity stress tolerance		Mahboobeh and Akbar, 2013	
		Salinity stress tolerance		Ibragimova <i>et al.</i> , 2015	
		Drought and salinity tolerance		Zhu <i>et al.</i> , 1998	
		Increased proline accumulation and osmotic stress tolerance		Hong <i>et al.</i> , 2000	
		Salinity stress tolerance		<i>Oryza sativa</i>	Anoop and Gupta, 2003
		Drought and salt stress conditions			Su and Wu, 2004
		Enhanced salt and cold stress tolerance	Hur <i>et al.</i> , 2004		
		Salinity stress tolerance	<i>Arabidopsis thaliana</i>	Kumar <i>et al.</i> , 2010	
		Salinity stress tolerance		Karthikeyan <i>et al.</i> , 2011	
		Drought and salinity stress tolerance		Priya <i>et al.</i> , 2015	
		Hypersensitivity to osmotic stress	<i>Triticum aestivum</i>	Nanjo <i>et al.</i> , 1999	
		Drought and salinity stress tolerance		Chen <i>et al.</i> , 2010	
		Salinity stress tolerance		Chen <i>et al.</i> , 2013	
		Salinity stress tolerance	<i>Triticum aestivum</i>	Sawahel and Hassan, 2002	
		Proline accumulation and water stress tolerance	<i>Triticum aestivum</i>	Vendruscoloa <i>et al.</i> , 2007	
		Tolerance to toxic heavy metals	<i>Chlamydomonas reinhardii</i>	Siripornadulsil <i>et al.</i> , 2002	
		Salinity stress tolerance	<i>Daucus carota</i>	Han and Hwang, 2003	
		Drought stress tolerance	<i>Citrus spp.</i>	Molinari <i>et al.</i> , 2004	
		Drought stress tolerance		De Campos <i>et al.</i> , 2011	
Proline accumulation and salt tolerance	<i>Solanum tuberosum</i>	Hmida-Sayari <i>et al.</i> , 2005			
Cold, salt, and freezing stress tolerance	<i>Larix leptoeuropaea</i>	Gleeson <i>et al.</i> , 2005			
Osmotic stress tolerance	<i>Medicago truncatula</i>	Verdoy <i>et al.</i> , 2006			
Water deficit stress tolerance	<i>Saccharum officinarum</i>	Molinari <i>et al.</i> , 2007			
Proline accumulation and salt tolerance	<i>Cicer arietinum</i>	Ghanti <i>et al.</i> , 2011			
Salinity stress tolerance	<i>Olea europaea</i>	Behelgardy <i>et al.</i> , 2012			
Salinity stress tolerance	<i>Lilium spp.</i>	Li <i>et al.</i> , 2013			
Drought stress tolerance	<i>Hordeum vulgare</i> var. <i>nudum</i>	Deng <i>et al.</i> , 2013			
Proline accumulation and salt tolerance	<i>Cajanus cajan</i>	Surekha <i>et al.</i> , 2014			
Proline accumulation and salt tolerance	<i>Saccharum officinarum</i>	Guerzoni <i>et al.</i> , 2014			
Proline accumulation and salt tolerance	<i>Sorghum bicolor</i>	Surender Reddy <i>et al.</i> , 2015			
Salinity stress tolerance	<i>Brassica napus</i>	Szymon <i>et al.</i> , 2015			
Salinity stress tolerance	<i>Nicotiana tabacum</i>	LaRosa <i>et al.</i> , 1991			
2	Pyrroline-5-carboxylate reductase (P5CR)	Enhanced heat and drought stress tolerance	<i>Glycine max</i>	De Ronde <i>et al.</i> , 2000	
		Osmotic and drought stress tolerance	<i>Arabidopsis thaliana</i>	De Ronde <i>et al.</i> , 2004	
		Salinity, drought and ABA stress tolerance		Ma <i>et al.</i> , 2008	
Salinity stress tolerance	<i>Ipomea batata</i>	Liu <i>et al.</i> , 2014			

Contd.

**Table 1. Contd.**

3	Proline dehydrogenase ( <i>ProDH</i> )	Salt and freezing stress tolerance	<i>Arabidopsis thaliana</i>	Nanjo <i>et al.</i> , 1999
		Hypersensitivity to proline		Mani <i>et al.</i> , 2002
		Elevated salt tolerance	<i>Nicotiana tabacum</i>	Kolodyazhnaya <i>et al.</i> , 2006
		Increased proline content and drought stress tolerance	<i>Nicotiana tabacum</i>	Kochetov <i>et al.</i> , 2004
		Enhanced water stress tolerance	<i>Arabidopsis thaliana</i>	Ueda <i>et al.</i> , 2008
		Over-expression for Osmotic stress tolerance	<i>Arabidopsis thaliana</i> and <i>Nicotiana tabacum</i>	Miller <i>et al.</i> , 2009
4	<i>ProBA</i>	Salinity and water stress tolerance through elevated proline content	<i>Nicotiana tabacum</i>	Ibragimova <i>et al.</i> , 2012
		Enhanced oxidative stress tolerance	<i>Arabidopsis thaliana</i>	Monteoliva <i>et al.</i> , 2014
		Increased proline content and drought stress tolerance	<i>Arabidopsis thaliana</i>	Chen <i>et al.</i> , 2007
5	Ornithine- $\delta$ -aminotransferase ( $\delta$ -OAT)	Increased proline biosynthesis and osmotic tolerance	<i>Nicotiana tabacum</i>	Roosens <i>et al.</i> , 2002
		Drought and salinity tolerance	<i>Oryza sativa</i>	Wu <i>et al.</i> , 2003
		Multiple abiotic stress tolerance		You <i>et al.</i> , 2012

nitrogen limitation, while the pathway from ornithine predominates at high levels of available nitrogen (Delauney *et al.*, 1993).

The other degree that controls production of Pro in plants is the degradation or metabolism of Pro. L-Proline is oxidized to P5C in plant mitochondria by proline dehydrogenase (oxidase) (*ProDH*; EC 1.5.99.8), and P5C is converted to L-GIU by P5C dehydrogenase (*P5CDH*) (Boggess *et al.*, 1975; Elthon and Stewart, 1981). Such oxidation of Pro is repressed during the buildup of Pro under water stress and is triggered in rehydrated plants (Stewart *et al.*, 1977; Rayapati and Stewart, 1991). *ProDH* and *P5CDH* catalyze reactions that are the opposite of those catalyzed by *P5CS* and *P5CR*, respectively, in the biosynthesis of Proline. Although stress-induced proline accumulation is evolutionarily conserved in a wide range of plants, its regulatory mechanism is subject to considerable variation. In most plant species studied, proline accumulation during stress is the result of reciprocal action of increased biosynthesis and inhibited degradation (Kavi Kishor *et al.*, 2005).

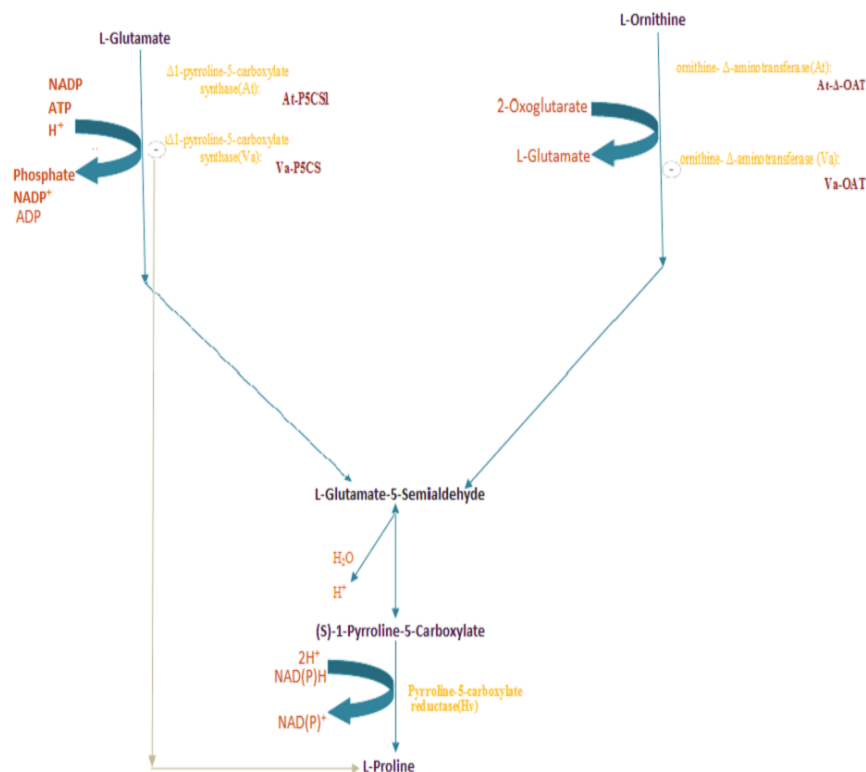
#### DEGRADATION OF PROLINE

Proline degradation in eukaryotes takes place in mitochondria and thus in plants are compartmentalized spatially from the biosynthetic pathway. The catabolism of Proline commences with the oxidation of proline to P5C by proline dehydrogenase (*PDH*), using FAD as cofactor. P5C is sequentially converted to glutamate by pyrroline-5-carboxylate dehydrogenase (*P5CDH*) using  $\text{NAD}^+$  (Fig. 3). Whereas in eukaryotes two enzymes catalyze these sequential steps in proline degradation, in bacteria both mono- and bifunctional enzymes exist (Tanner, 2008). Two homologous genes have been diagnosed to encode proline dehydrogenase in *Arabidopsis* and tobacco (Mani *et al.*, 2002; Ribarits *et al.*, 2007; Verbruggen and Hermans, 2008; Funck *et al.*, 2008), while the available literature is scarce. In

contrast, the enzyme catalyzing the second step of proline degradation (*P5CDH*) is ciphered by a single copy gene in all monocot and dicot species analyzed so far (Ayliffe *et al.*, 2005; Deuschle *et al.*, 2006; Mitchell *et al.*, 2006). Biochemical investigation revealed the presence of two *P5CDH* activities with slightly divergent characteristics in *Nicotiana plumbaginifolia* and *Zea mays* (Elthon and Stewart, 1982; Forlani *et al.*, 1997). But the present scenario does not clearly reveal that whether both activities arise from a single gene or if a second *P5CDH* gene is exist in these species.

#### Role of proline metabolism in plant for interaction and development of stress tolerance:

It is or it is not the agglomeration proline responsible for adaptive retaliation; to abiotic stress has been argued since Kemble and McPherson (Kemble and Macpherson, 1954) first documented proline accumulation in wilted ryegrass. Evident correlations between high proline and greater stress caused injury led to conclude that proline was the first manifestation of damage which results in decreased growth and metabolism rather than an adaptive retaliation (Stewart and Hanson, 1980). Indeed, it seems contradictory that plants synthesize large amounts of proline and at the same time restricting carbohydrate biosynthesis by restricting photosynthesis which severely retards growth and reproductive performance during drought (Boyer, 2010). However, controlled experiments demonstrated that proline agglomeration occurs even in plant tissues/cell where growth prevails and injury is nominal (Voetberg and Sharp, 1991; Ober and Sharp, 1994) and also occurs to remarkable extent under mild and moderate stress treatments (Sharma and Verslues, 2010). Proline also accumulates to elevated high levels in pollen and this is likely related to pollen desiccation tolerance (Schwacke *et al.*, 1999). Moreover, a number of groups have pointed out a higher proline in more drought adapted varieties of wild or cultivated plants (Ben Hassine *et al.*, 2008; Parida *et al.*, 2008; Evers *et*



**Fig. 3.** Pathways for the biosynthesis and metabolism of proline in higher plants ([http://themedicalbiochemistry page.org/amino-acid-metabolism.php](http://themedicalbiochemistry.page.org/amino-acid-metabolism.php)).

*al.*, 2010). Also, the salt-tolerant *Arabidopsis* relative *Thellungiella halophila* agglomerates more proline at salinity stress than the standard Columbia-0 ecotype of *Arabidopsis* (Kant *et al.*, 2006). Proline play a role as a precursor for proline- or hydroxyproline-betaines which is even more effective osmoprotectants for plants in acute dry environments (Hanson *et al.*, 1994). With advancement of molecular techniques data has clearly indicated that proline metabolism is actively controlled by stress induced signals and the usage of reverse genetics (such as p5cs1 mutants; Szekely *et al.*, 2008) has made possible a more direct tests of the requirement of proline accumulation for stress resistance. The main challenging question is how proline contributes to plant stress resistance, the metabolic modulation that allows elevated levels of proline to accumulate, and whether fine-tuning of proline metabolism is useful for biotechnological improvement of plants which is yet to be answered.

#### Genetic engineering for abiotic stress tolerance

**By overexpression of proline genes:** Metabolic engineering is the directed improvement of cellular properties through the modification of species biochemical reactions or the introduction of new ones, with the use of recombinant DNA technology (Stephanopoulos, 1999). Osmoprotectant accumulation is only one facet of a myriad of stress-tolerant traits found in nature.

Since oxidative stress is a component of drought and salinity, manipulations aimed at improving oxidative stress tolerance have also resulted in salinity tolerance (Roxas *et al.*, 1997). This could be done either via repeatedly engineering the gene or by crossing and selecting transgenic plants engineered for different traits. For example, manipulation of genes involved in ion transport together with osmoprotectant synthesis can be expected to increase a cell's ability to withstand salinity stress. The gene products involved in ion homeostasis have been identified by the use of yeast model systems (Serrano *et al.*, 1999) and by analyzing mutants altered for salt sensitivity (Wu *et al.*, 1996; Liu *et al.*, 2000). Osmoprotectant synthesis in naturally stress-tolerant species is highly regulated by stress. In addition to the use of stress inducible promoters for engineering osmoprotectant synthesis pathways, genes involved in stress signal sensing are additionally useful for engineering stress tolerant plants.

**Stress tolerance via proline:** Kavi Kishor *et al.* (2005) reported over expression of proline in transgenic tobacco and the transgenic plants produced increased root biomass under moisture deficient stress. While in one other recent study, the *OsP5CS1* and *OsP5CS2* genes were co-expressed in tobacco that converted transgenic plants with high

levels of proline accumulation as well as reduced oxidative harm to cells under abiotic stress conditions (Zhang *et al.*, 2005). Similar proline production was also reported in transgenic rice, wheat and carrot plants overexpressing P5CS gene that showed resistance to salinity stress (Zhu *et al.*, 1998; Sawahel *et al.*, 2002; Han *et al.*, 2003).

Transgenic *Arabidopsis* plants that expressed P5CS antisense gene were constitute with morphological aberrations, and the plants were hypersensitive to water-influx stress which was seen by Nanjo *et al.* (1999). In addition to playing a role in safeguarding vital proteins, it was also projected that proline would play a viable, role in ROS scavenging; this was conceptualized by Smirnoff and Cumbes (1989). In transgenic *Arabidopsis P5CS* mutant lines, it was proposed that the ROS scavenging enzymes demonstrated significantly lower activities. This evidence clearly points out the possibilities that either proline protects the enzymes of the glutathione–ascorbate cycle or increases their activities during osmotic stress. Hmida-Sayari *et al.* (2005) concluded a study of transfer the *Arabidopsis P5CS* gene into potato in which the stress induced promoter got activated as a result of stress condition and thus its subsequent effect was observed on the plant's growth, tuber morphology and yield and the data compiled accordingly. Transgenic potato plants accumulated high proline content compared to those at control under high salt stress (100 mM NaCl) and, in turn, exhibited improved salinity tolerance by diminished tuber yield and weight when compared to that of non-transgenic control. In addition, some other studies were conducted on transgenic petunias and pigeon pea (*Cajanus cajan*) with the *P5CS* gene that rendered these plants abiotic stress tolerant (Surekha *et al.*, 2013). Petunia was modified with pyrroline-5-carboxylate Synthetase genes (*AtP5CS* from *A. thaliana* L. or *OsP5CS* from *Oryza sativa* L.) the results showed more proline production which resulted in water stress tolerance for a period of 14 days. Modified pigeon pea was also mutagenized with the alternate version (*P5CSF129A*) of wild *P5CS* gene from *Vigna aconitifolia*. The resultant transgenic plants showed enhanced level of proline content than their non-transgenic counterparts. About four times higher proline content was observed in the T<sub>1</sub> transgenic plants compared to that of nontransgenic under 200 mM NaCl stress, this study was done by Surekha *et al.* (2013). As a result of correspondingly levels high proline accumulation, under salt stress the transgenic plants displayed more chlorophyll, better growth, and relative water content and reduced levels of lipid peroxidation. These findings indicated the importance of proline biosynthesis in transgenic plants when compared osmotic stress induced by salt and drought stresses.

Huang *et al.* (2013) have scrutinized HtP5CS, HtOAT and HtPDH enzyme activities and gene expression mosaic of putative *HtP5CS1*, *HtP5CS2*, *HtOAT*, *HtPDH1*, and *HtPDH2* genes. Ashfaque *et al.* (2014) applied H<sub>2</sub>O<sub>2</sub> on plants to demonstrated the different parameters related to water and salinity stress. The application of both 50 and 100nM H<sub>2</sub>O<sub>2</sub> minimized the extremity of salt stress by reducing the level of Na<sup>+</sup> and Cl<sup>-</sup> content; and thereby elevating the levels of proline and N assimilation. The outcome was increased water relations, photosynthetic pigments and growth under salinity stress. This was evident even under non saline condition where application of H<sub>2</sub>O<sub>2</sub> increased all the parameters under study. Szymon *et al.* (2015) showed the role of priming-induced modulation of activities of concerned genes and proline turnover enzymes, and its interplay with higher levels of hydrogen peroxide, in enhancing the seed germination capability under various stress. During the events such as priming and post-priming germination the buildup of proline was analogous with strong up-regulation of the *P5CSA* gene, down-regulation of the *PDH* gene along with production of hydrogen peroxide. *P5CSA* transcript was up-regulated which was found in consistent with the expanded activity of P5CS gene and the other genes intricate such as ornithine-δ-aminotransferase (OAT), pyrroline-5-carboxylate synthetase (P5CS), and proline dehydrogenase (PDH) was examined in detail which clearly indicated the role of proline biosynthesis as well as proline metabolism genes in rendering plant tolerant to various abiotic stresses.

## Conclusion

Fabricating transgenic plants utilizing biotechnological approaches has become an eminent tool in plant-stress biology. The abiotic stresses effects the plant at all possible levels of organization i.e. morphological, physiological, biochemical and molecular which demands attention. Thus comprehending the apparatuses that regulate gene expression and the possibility to transfer genes from other organisms into plants will definitely expand our horizon to master the plant genetically. The use of new and useful approaches combining molecular genetics, physiological, biochemical and other related techniques will definitely provide us the better understanding to exploit it to the prime extent to get mastered transgenic crops. The abiotic stresses such as salinity and drought are more complex traits, controlled by many genes. Transgenic plant development for these stresses has utilized many single genes. However, much emphasis has been placed on Proline, its function and metabolism. This review focuses on the current status of research on osmoprotectant proline genetic engineering and their overexpression to abiotic stress tolerance in transgenic plants.

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