Proline accumulation and osmotic stress: an overview of *P5CS* gene in plants

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ABSTRACT: Under osmotic stresses, proline accumulation is an important response of plants to these conditions. Proline is a compatible osmolyte which affects many cellular and molecular aspects of a plant in both normal and stressful situations. Proline is shown to be involved in plant development in normal conditions and in conferring resistance to a plant under biotic and abiotic stresses. Therefore, many surveys have already been conducted to unveil its mechanisms and signaling pathways, so that it might be considered as an insight into resolving growing challenges of agriculture, drought and soil salinity. Δ^1-pyrroline-5-carboxylate synthetase (P5CS), one of the two main enzymes in the proline biosynthesis pathway of the glutamate precursor, has been demonstrated to play a significant role in proline accumulation in plants under water stresses. Regarding the role of P5CS under the osmotic stress, there are controversial observations in various plants which casts doubts regarding whether P5CS is a rate-limiting enzyme in the pathway or not. Obviously, transgene *P5CS* is proved to give higher resistance to transgenic plants under drought and salinity, by elevating proline content. In this review of literature, proline and its identified various functions in plants, characteristics of P5CS enzyme, signals, inducers and inhibitors of *P5CS* gene and the expression pattern of *P5CS* under differential conditions in studied plant species are discussed. Finally, some of the important features of the transgenic plants overexpressing *P5CS* have been summarized.

KEYWORDS: Abiotic Stress, Overexpressing, Proline, P5CS, Transgenic Plants

INTRODUCTION

It is not impenetrable today that the most influential obstacles to achieve high-yielding crops are osmotic stresses, particularly drought and soil salinity whereby dehydration causes loss of millions of tons of crops every year and half of the arable lands has already become arid due to soil salinization (66) inasmuch as they are growing at the worrisome rate of 3 hectare per minute (39). These stresses affect the plant with lowering the amount of water available for it, as well as osmotic potential of cells. These conditions cause deficiency in normal development and growth of the plant, reduction in its fertility and even death of the plant in severe and prolonged stresses (8). Therefore, investigation on improving plants resistance to osmotic stresses has drawn much attention among researchers. Proline, the most accumulated osmolyte, which accumulates to high levels in many plants in various stressful conditions such as drought, salinity, high and low temperature. Photo-damage, heavy metals and even pathogens, have been proven to play a significant role in adapting the plants to water stresses (30, 66, 69). Proline which is conspicuously more than just an osmo-protectant, has many functions under normal and stressful conditions in plants. Proline, as a cyclic amino

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acid, is an important part of many proteins involved in osmotic regulation, the plant cell wall and membrane. So, it is essential for their stability (62). Proline deficiency in plants causes defect in growth and development of flowers and seeds of *Arabidopsis thaliana* (33). Also, proline metabolism and catabolism, which help maintaining redox balance of the cell is required for efficient flowering and seedling of plants (18). It probably affects flowers and seeds by transporting carbon, nitrogen and reducing agents to them (66). Moreover, Proline deficiency is reported to postpone the flowering time (62). Accordingly, on long days, it was shown that one of the main proline biosynthesis pathway genes, *P5CS*, is the target gene for CONSTANS transcription factor, which mediates flowering in long days (49). Inhibition of proline biosynthesis has resulted in morphological abnormalities in leaves, inflorescence, epidermal and mesophyll cells and the vascular system of *Arabidopsis thaliana* (41). Proline is also involved in cell division (18) and embryogenesis (62) in plants. Despite its numerous roles in plant normal development, proline is most renowned for its functions under stressed conditions. Highly soluble in water, proline is a compatible solute, which confers resistance to many plants from algae and aquatic plants to higher plants like *Arabidopsis*, halophytes and various crops, under osmotic tensions. In water-deficit conditions, it retains osmotic potential (11, 29, 37) and redox balance of cells (21, 68, 72), scavenges free radicals and ROS as an antioxidant (24, 50, 54), protects macromolecules from denaturation as a chemical chaperone (45) and regulates cytosolic pH (54). Finally, under stressful conditions, proline is a nitrogen and carbon provider after rehydration (3), source of energy (66), metal chelator (34) and signal molecule (70). In plants, proline is synthesized from two precursors, L-Glutamate (Glu) and Arginine/Ornithine (Orn) (25). Although Glu pathway is believed to be dominant in many stressful and normal conditions, except for the case of excessive nitrogen (9), Orn pathway has been reported to play a crucial role in adult *Arabidopsis* under osmotic stress, while in young plants both pathways cooperate to accumulate proline (48). Recently, it has been shown that after the removal of severe stress, Orn cooperates with the Glu in the biosynthesis of proline (3). However, Glu pathway seems to have a central role in synthesizing proline under water stresses. In Glu pathway, glutamic-γ-semialdehyde (GSA) is synthesized by L-Glutamic acid including both phosphorylation and reduction activities of Δ^1-pyrroline-5-carboxylate synthetase (P5CS). In *E. coli*, GSA is formed by two distinct enzymes, γ-glutamyl Kinase (GK) and GSA dehydrogenase or γ-glutamyl phosphate reductase (GPR). GSA, then turns to Δ^1-pyrroline-5-carboxylate (P5C) in spontaneous cyclization reaction. Finally, P5C Reductase (P5CR) reduces P5C to L-Proline (25).

Proline degradation pathway regulation is also important for its accumulation under abiotic stresses and plant life. Proline accumulation in plants is the result of both increased proline biosynthesis and decreased proline degradation (76). Furthermore, the activation of proline degradation after rehydration (31, 42) is crucial to provide reducing potential for mitochondria and so as to continue the respiratory cycle of cells and plant life (62). Besides, inhibition of proline degradation is reported to be highly toxic in *Arabidopsis* (35, 40). Proline is degraded by two oxidation reactions in which, it is first oxidized by proline dehydrogenase/oxidase (ProDH) to P5C and after converting to GSA, spontaneously, P5C dehydrogenase (P5CDH) oxidizes GSA to Glu (20).

Generally, proline biosynthesis and a degradation cycle are required for balanced redox potential under water deficit pressure, flowering and seedling in normal development of plant (18, 19, 66). Proline distribution, intracellular and intercellular, also plays a critical role in rendering osmotic-resistance to various tissues under stress (66). Proline biosynthesis of Glu is mostly occurring in cytosol of plant cells and in chloroplast, when faced with water deficit (33, 63). Under stressful conditions, it is accumulated in cytosol to induce water diffusion into cells (36), while in the absence of stress, proline is transported to organelles, particularly vacuole and plastid (33). Vacuole, distributes proline to cytosol, in lack of water (76). By rehydration, proline is transported to mitochondria, where it is degraded to Glu (33). Transportation of proline between tissues and its accumulation is not the same in reported surveys. It seems that they are greatly dependent on the condition and species, but what is similar in all related studies is that proline can travel long distances as far as the height of the plant from roots to flowers, through both xylem and/or phloem. Proline is largely synthesized and accumulated in roots and leaves of plants under water stress (3, 12) and then transported mainly to meristems, dividing cells of root apex and sexual organs (33, 67).

Under normal conditions, proline accumulates chiefly in pollen, seeds and fruits (51, 56, 66).

In metabolism and catabolism of proline under water stress, P5CS and ProDH enzymes are believed to be rate-limiting and play a significant role in the regulation of the proline level in plants (76). P5CS, having two genes encoding it in some plants including *Arabidopsis* (57), is the only rate-limiting enzyme in the Glu-based pathway. In spite of an anomalous report in which no relation between the level of P5CS and proline was observed (56), there are many reports that show when the expression of *P5CS* is increased, proline accumulation reaches higher levels (11, 25, 51, 75, 79), which does not occur with the other enzyme of the pathway, P5CR, at least in that amount. For instance, overexpression of *P5CR* gene in tobacco led to 200 times more expression of *P5CR*, but no noticeable increase in proline level in transgenic tobacco was observed (63). On the other hand, *p5cs* mutants of *Arabidopsis* gathered less amount of proline and demonstrated no resistance to the hypo-osmotic condition (41). We believe that reconsidering different aspects of P5CS in this review, will shed light on known and unknown territories of the proline biosynthesis pathway which can be considered as an insight regarding the challenge of osmotic stresses in agriculture. Therefore, we discuss characteristics of P5CS enzyme and gene in various plants, *P5CS* expression pattern and transgenic plants over-expressing *P5CS* gene, in this review of literature.

P5CS Enzyme

As mentioned above, P5CS in plants, localized in cytosol and plastid in cells (62), consists of two domains functioning as kinase and dehydrogenase enzymes. Each domain has leucine zipper sequence, which is involved in preservation of tertiary structure of the enzyme, protein-protein interaction and probably the contribution of two domains (25). GK domain of P5CS, responsible for phosphorylation of Glu, depends on ATP (11), while the GPR domain requires NADPH as reducing agent (19). Accordingly, the presence of both ATP-binding and NAD(P)H-binding motifs in P5CS has been proposed (51, 60). The Leucine-rich region has also been reported to exist in sorghum P5CS (60). The P5CS1 protein of *Arabidopsis*, the second isolated *P5CS* gene among plants after mothbean (*Vigna aconitifolia*), is a poly peptide with 717 amino acids, which is estimated to weigh about 77.7 kDa (51, 57). While the native P5CS enzyme was shown to be approximately a 450 kDa protein, it was deducible that the P5CS functions as a hexamer with six similar subunits (79). The same had also been reported with two distinct enzymes in *E. coli* (55). Leucine zipper may have a role in formation of this quaternary structure, as it mediates protein-protein dimerization (1). Later, the approximate length and molecular weight were estimated for P5CS in grapevine (56), common bean (7) and sorghum (60). The activation of P5CS enzyme is inhibited by specific amount of proline, depending on some variables especially plant species (25, 56, 62). The inactivation of P5CS enzyme in grapevine mature fruits was between 33% and 50%, depending on the proline concentration (56). After removing the proline-binding residue in *E. coli* GK enzyme, the residues responsible for this feedback inhibition were also found in *V. aconitifolia* and recently in sorghum (60, 79). Mutation of Asp 126 or Phe 129 in *Vigna*, removed this inhibition and consequently more proline accumulated in transgenic plants (79). P5CSF129A, in which Phe 129 is replaced by Ala, was later used broadly to generate plants with higher levels of proline (discussed later). These two amino acids are also conserved in *Arabidopsis* (57). However, the same mutations didn't work in sorghum, but instead, Phe 128 and 141 were recognized to be the target residues of proline in sorghum (60). It is proposed that the accumulation of proline to higher levels under stressful conditions, might be due to the inactivation of feedback inhibition in these situations (11), but this inactivation is not complete (24).

P5CS gene inducers and inhibitors

In addition to regulation in protein level, expression of *P5CS* is regulated in transcription and probably post-transcription levels as well. Nevertheless, induction and inhibition signaling of *P5CS* has not been completely discovered so far. *P5CS* expression is shown to be induced, as recognized till now, mainly by various environmental factors and plant hormones. In an early survey, it was reported that drought and salt stress amplify *AtP5CS* transcription (75). To investigate transcription of *P5CS*, GUS enzyme was expressed under *AtP5CS1* promoter in *Arabidopsis* and tobacco (77). Transgenic plants displayed increased expression of *GUS* in dehydration and to lower contents in salinity, while low temperature (4°C) had no effect on the amount of *GUS*. In rice, salinity, dehydration, low temperature

induced *OsP5CS* (26). NaCl, also mediated the expression of *P5CS* in tomato (11). These studies plus other similar reports (2, 4, 25, 43, 53, 60, 80) prove that *P5CS* gene transcription in plants although varies by species, is promoted by osmotic stresses including dehydration, salinity, high and low temperature. Recently, it has been indicated that osmotic stresses increase DNA methylation modification of *P5CS* gene in rice (78). DNA methylation in plants is involved in response to biotic and abiotic stresses (47). Signaling pathway for the induction of *P5CS* expression is however not clear yet, but different expression patterns in various stresses, suggest distinct pathways for different stresses such as cold and water stresses (21). As proline accumulates in light (15), some groups studied the effect of light on *P5CS* transcription in *Arabidopsis*. They showed that the level of *AtP5CS1* mRNA was much higher in light than in dark (2, 22). However, in the saline condition, it was high in both light and darkness (22). In (22), the authors proposed that light and darkness might play an indirect role to regulate *P5CS* expression, probably by affecting water potential of leaves, in the meanwhile, light has a negative effect on *ProDH* expression. In light, photosynthesis is activated and so is sugar synthesis. Consequently, leaves face decreased osmotic potential. This stress, might be the reason for *P5CS* transcription promotion in light and the reverse in dark. Accordingly, no light-responsive element was found in the upstream of *SbP5CS* gene in sorghum (60). In freezing-resistant *eskimo1* mutant *Arabidopsis*, the 8-fold higher expression of *AtP5CS1* was observed (71). Also, in these plants abscisic acid (ABA)-dependent *RAB18* gene showed constitutive expression, while *RD29A*, mainly regulated by ABA-independent pathway, had no considerable change in transcription level. Although freezing has not been reported to stimulate *P5CS* up-regulation, the cold-response signaling pathway is involved in regulating *P5CS* and proline amount in *Arabidopsis*. Furthermore, proline might have a role in giving resistance to freezing in plants.

Predicting cis-acting elements of *SbP5CS* promoter revealed a MeJA-responsive motif (TGACG-motif) upstream of the gene (60). MeJA is a plant hormone, produced in response to abiotic and biotic stresses. Expectedly, MeJA treatment of sorghum seedlings mediated *SbP5CS* expression (60). Phenolic plant hormone, salicylic acid(SA), which is mostly recognized by its role in plant growth, development, photosynthesis and defense against pathogens, was reported to affect *AtP5CS2* expression positively in pathogenic condition (10). Later, finding a SA-responsive element, TCA-element, in promoter region of *SbP5CS* (60), increased the impact of SA on *P5CS* up-regulation. Likewise, a gibberellin (GA)-responsive element, GARE, was predicted as the upstream of *SbP5CS* gene, but no survey has been done yet to clarify involvement of GA in *P5CS* stimulation. Another plant stress-responsive hormone, ABA, has also been shown to induce *P5CS1 and P5CS2* expression in *Arabidopsis* (57, 75) and rice (58). An inconsistent report which showed that exogenous ABA had no effect on expression of *GUS* under *AtP5CS1* promoter (77) led to the hypothesis that ABA might regulate *P5CS* in post-transcription level (77). But further investigations signify the positive role of ABA in *P5CS* transcription. For instance, exogenous ABA treatment induced *OsP5CS* in rice (26) and even stronger ABA-responsive element (ABRE) has been found to exist in promoter region of *AtP5CS2* and *SbP5CS* (60, 77). Besides, another ABA-responsive cis-acting element exists in the upstream of *AtP5CS1*, *AtP5CS2* and *SbP5CS* genes which is the binding element for MYB transcription factor (21, 60). Calcium signals are also believed to activate the MYBs, which promote *P5CS* transcription (74). The abiotic response of plants is assorted into two main pathways, ABA-dependent signaling pathway and ABA-independent one. While many genes are controlled by either pathways in some cases, like *RD29A*, both pathways contribute in the regulation of the gene. Interestingly, expression pattern of *AtP5CS* is more like that of *Rd29A*, rather than ABA-dependently controlled genes such as *RAB18* (64). Considering all the observations, scientists suggest that *P5CS* is among the genes which are controlled by both pathways (21, 76).

To unveil upstream signaling the pathway of *P5CS*, a research was done on it in *Arabidopsis*. It showed that phospholipase D, which is involved in water stress responses, mediates ABA signal transduction (17). However, it inhibited proline accumulation by under-regulating *P5CS1* under normal and stressful conditions (64). Calcium accumulation in cytosol is one of the first responses of plant cells to water stress. It was reported that calcium played a significant role in proline accumulation under the saline condition, but it was not sufficient for up-regulation of *P5CS* expression; while, simultaneous treatment of *Arabidopsis* with $CaCl_2$ and Phospholipase D inhibitor resulted in higher *P5CS1*

mRNA level (64). Calcium, was also suggested to regulate Phospholipase D, as a downstream signal messenger (64). Despite some efforts, little is yet known in this respect and further surveys are needed to understand the *P5CS* and proline signaling pathways.

P5CS Expression under normal and osmotic stress conditions in non-transgenic plants

The expression pattern of *P5CS* gene, as an indicator of the way it affects proline accumulation in plants, was studied in various studies. In *Arabidopsis*, under the normal condition, *AtP5CS1* and *AtP5CS2* showed different expressions in various tissues. While no *P5CS* mRNA was notably expressed in roots, *AtP5CS1* transcript level was very high in Leaves, stems and flowers, with the highest level in leaves and there was no detectable amount in callus and cell suspension cultures. Conversely, *AtP5CS2* was expressed highly in dividing tissues especially in callus of *Arabidopsis* (57). In another report, the highest *AtP5CS1* mRNA was detectable in flowers, even though it was transcribed in any tissue (51). 10-day-old seedlings under 170 mM NaCl salinity, demonstrated to accumulate *P5CS* transcript just 4 hours after the treatment and reached the highest level after 8 hours. Then, this level started to decrease during 24 hours (51). Dehydration led to severe and immediate increase in *AtP5CS1* expression, reaching the maximum level in 6 hours and much lower effect on *P5CS2*. 25 mM NaCl, as was observed in previous study, promoted *P5CS* expression, but slower and in lower amount in comparison with dehydration. While *AtP5CS1* mRNA accumulated after 6 hours and persisted for 24 hours in roots, *AtP5CS2* showed a little increase in transcription just for 24 hours (57). In another work, dehydration promoted *AtP5CS1* expression an hour after treatment and its level reached the summit in 5 hours. Cold had slight impact on *P5CS* expression, too (75). In these studies, the transcript level of *AtP5CS1* was about 7 to 8 times higher in water and salt stress conditions in comparison with normal conditions. Also, proline accumulation was consistent at *P5CS* mRNA level.

In rice, *OsP5CS*, having about 75% similarity with *P5CS* in *Arabidopsis* and *Vigna*, up-regulated for 10 hours after the treatment of 250 mM NaCl and kept the trend for 24 hours. It subsided gradually to the normal level after 72 hours. Dehydration caused promoted expression of *OsP5CS* in 5 hours, which reached the maximum level in 10 hours and returned to the control amount after 24 hours. 4°C treatment of 10-day-old rice plants promoted *OsP5CS* mRNA level after 1 or 2 hours. Proline accumulation level showed consistency with *P5CS* mRNA level (26). In a recent study, activity of OsP5CS enzyme was reported to increase by about 19%, subjected to 425mM NaCl and led to proline accumulation (4).

Tomato *tomP5CS1*, interestingly consists of two distinct ORFs, just like that of *E. coli*, while *tomP5CS2* has characteristics like other plant *P5CS* genes. Though the transcript level of *tomP5CS* under 100 and 200 mM NaCl stress was about two times in comparison to control plants, rather lower than *Arabidopsis* and rice, proline accumulated in tomato much higher, up to 80 folds higher than control plants (11).

In alfalfa, *MsP5CS1* and *MsP5CS2* cDNAs were isolated and their expression under 90 mM NaCl was studied in roots of 6-day-old alfalfa seedlings (13). In this condition, *MsP5CS1* transcript showed an increase after 48 hours and it was still growing after 72 hours, but *MsP5CS2* expression got promoted at early hours, in 6 hours and it kept ascending for 72 hours after NaCl treatment. Induction level of *MsP5CS2* was obviously higher than that of *MsP5CS1*. Surprisingly, the increase in proline content of roots was not considerable during stress, while *P5CS* mRNA was accumulated up to 4 times higher than control plants. Finding two P5CS coding regions also in bean (7), shows that duplication of *P5CS* gene is common in plants. Isolated *P5CS1* and *P5CS2* genes in mentioned plants, positioned on nuclear genomes, have shown 65 to 80% similarity between two isoforms.

In grapevine, one coding region for VvP5CS was identified and its cDNA was isolated. Surprisingly, the accumulation of proline in mature fruits, up to 80 folds higher than that in leaves and roots was independent of *P5CS* transcript and the enzyme level (56).

In cactus pear (*Opuntia streptacantha*), an aquatic macrophyte, the expression pattern of isolated *P5CS* cDNA and the activity of the relative enzyme was studied. *OsP5CS* showed an increased expression under 75 to 350 mM NaCl stress after 6 to 9 days (53). This study, consistent with two other investigations (48, 69), deduced that P5CS might not be the rate-limiting enzyme in the Glu-based pathway, in salinity condition. These studies reported that while *P5CS* expression was promoted by NaCl, no sign of elevated activity of P5CS

enzyme was observed, meanwhile, proline reached high amounts in cactus pear and wheat. In sorghum, *SbP5CS1* and *SbP5CS2* genes were isolated and their characteristics were evaluated in salinity and drought (60). Both mRNAs accumulated in leaves and roots, under dehydration and 250 mM NaCl stresses. However, *SbP5CS1* transcript level was obviously higher than *SbP5CS2*. Under drought conditions, the up-regulation of genes started in 3 days, while salinity provoked their expression much earlier, but in a different manner. *SbP5C1* became stimulated in 4 hours and it reached the highest level after 12 hours in leaves, but after 24 hours in roots. *SbP5CS2* reached its lower highest level in 8 hours. Generally, the transcript levels were a bit higher in roots. Proline accumulation under drought was 60 folds higher than that of the control plants in 6 days. These numbers for salinity were at most 8 folds after 48 hours. Proline accumulation was highly consistent with *P5CS1* mRNA accumulation pattern under both stresses. Observing much lower *SbP5CS2* mRNA than *SbP5CS1* under stress, the authors assumed the gene to be a house-keeping gene, which is only involved in the proline metabolism (60).

Recently, *P5CS* cDNA was isolated from a drought- and salinity-resistant halophyte, *Nitraria tangutorum*, and its expression was characterized under various osmotic conditions (80). *NtP5CS* mRNA was shown to be up-regulated under 200 mM NaCl, 10% polyethylene glycol, 50°C and 4°C stresses, with the highest amount under heat, followed by salinity. Also, the proline level was in accordance with *NtP5CS* expression. However, the lack of data about the relative enzyme activity, makes any deduction impossible. Transformation of both *NtP5CS* and *AtP5CS* in *E. coli*, demonstrated that although both transgenic strains had improved growth under drought, salinity, heat and cold, a halophyte P5CS worked more efficiently than *Arabidopsis* P5CS in conferring osmotic resistance to *E. coli* (80).

Generally speaking, while there is no explicit similarity between expression patterns of *P5CS* in studied plants, it is deducible that *P5CS* responds to dehydration more quickly than to salinity, but many variables such as plant species, stress severity and plant organs are seemingly influential which should be mentioned. Considering the role of P5CS in proline biosynthesis as a rate-limiting enzyme, it seems that some researches' doubtfulness and inconsistent reports cannot lead to an accurate conclusion on the matter and it needs to be investigated more. These studies should be comprehensive, meaning that both Glu and Orn pathways as well as both P5CS and P5CR along with their transcripts and enzyme activity levels should be taken into account. The effect of elements like plant species, genotypes, a plant organ and its developmental stage, type of stress, severity and its duration transcriptional and post-transcriptional regulation of P5CS, with an unknown signaling pathway for proline and P5CS accumulation have complicated the role of P5CS and it is still unidentified.

P5CS overexpression in plants

Despite lack of sufficient knowledge about P5CS in plants, it is proved that except for one report, all transgenic plants overexpressing *P5CS* gene, are resistant to osmotic stresses. These pieces of evidence are summarized in Table 1. This resistance is the result of accumulating proline in higher levels than control plants (Table 1). The overexpression of *P5CS* gene has resulted in higher survival rate, improved tolerance and higher yield under osmotic stresses in important crops such as wheat, rice and potato. For example, by ectopic expression of Mothbean *P5CS* in wheat, the transgenic lines could tolerate the salinity up to concentration of 200 mM, which is a great success. Also, the overexpression of Mothbean *P5CSF129A* in Tobacco, increased the proline level twice as did *P5CS* of Mothbean, and consequently, the transgenic lines with *P5CSF129A*, showed much higher germination and much lower free radicles. Except for three works, in which *P5CS* gene has been transformed under inducible AIPC promoter (58, 81), all groups have overexpressed P5CS under a constitutive promoter. AIPC is a stress-inducible heterologous promoter which responds to ABA (59). As ABA accumulation is a plant response to osmotic stresses (14), an ABA-responsive promoter is accordingly induced in stressful conditions. It is believed that overexpressing genes under inducible promoters reduces undesirable side effects in transgenic plants in normal conditions (16, 65, 81). However, some of these studies reported no or an insignificant defect on plant development and growth in spite of using a constitutive promoter for *P5CS* gene transformation (5, 12, 23, 72). Owing to the inconspicuous role of P5CS in some studies, discussed above, simultaneous overexpression of both P5CS and P5CR might result in more resistant plants, at least under the salt stress. Moreover, overexpressing these genes under osmotic-responsive

Table 1. Transgenic plants with over-expressed *P5CS* gene.

Gene	Transformed plant species	Promoter	Proline Normal [a]	Proline Drought [b]	Proline Salinity [c]	Effects	Reference (s)
Mothbean *P5CS*	Tobacco	*CaMV 35S*	8 to 14	2	-	Later welting under drought stress. Higher biomass level, longer roots and more number of seeds under salinity	(29)
Mothbean *P5CS*	Rice	*AIPC*	1.5 to 2.5	-	-	Increased shoot length, shoot weight and root weight under salinity. Later wilting and increased shoot weight under drought	(81)
Mothbean *P5CS*	Tobacco	*CaMV 35S*	2.5 to 3	-	1.5	Higher germination and lower free radicals under salinity	(24)
Mothbean *P5CSF129A*			5 to 6		3	Much higher germination and much lower free radicals under salinity	
Mothbean *P5CS*	Wheat	*CaMV 35S*	12	-	2.5	Resistance to salinity and normal growth up to 200 mM NaCl	(52)
Mothbean *P5CS*	Rice	rice *Actin 1*	3.2	2 to 3.2	2.2 to 3.2	Higher shoot and root weight under drought and salinity	(58)
		AIPC	1.2 to 1.4	1.2 to 1.9	1.2 to 2	Much higher shoot and root weight under drought and salinity	
Mothbean *P5CSF129A*	Orange	*CaMV 35S*	2	2.5	-	Higher photosynthetic activity under drought	(37)
Arabidopsis P5CS	*Petunia*	*CaMV 35S*	2 to 3	-	-	High survivor percentage after drought	(72)
Rice *P5CS*			1.5 to 3.5				
Arabidopsis P5CS1	Potato	*CaMV 35S*	-	-	2.5 to 7	Resistance to salinity and normal growth up to 100 mM NaCl, and lower yield reduction under salinity	(23)
Mothbean *P5CS*	Wheat	*AIPC*	Same as NT	2	-	Higher membrane stability, lower oxidative damages, and higher photosynthesis under drought	(9)
Arabidopsis P5CS	Tobacco	*CaMV 35S*	7.5 to 35		3.3 to 11	Control plants germinated in the presence of NaCl concentration up to 50 mM and tolerated 100 mM NaCl during growth phase while transgenic plants were able to germinate in 200 mM NaCl and tolerated up to 250 mM NaCl during growth phase	(46, 73)
Mothbean *P5CSF129A*	Chickpea	*CaMV 35S*	2 to 6	1.3 to 2.9		High resistance and decreased free radicals under drought	(5)
Mothbean *P5CSF129A*	Rice	*CaMV 35S*	2.5 to 4	-	4.5 to 5.5	Higher plant height and weight, lower oxidative damages under salinity	(32)
Mothbean *P5CS*	Chickpea	*CaMV 35S*	10	-	-	lower membrane damage, and higher survival percentage under salinity	(12)
Mothbean *P5CSF129A*	Tobacco	*CaMV 35S*	8	1.8		No significant effect was observed	(44)
Mothbean *P5CS*	Rice	*CaMV 35S*	2.5 to 5	-	3.5 to 5	higher plant height and weight under salinity	(27)
Bean *P5CS1*	*Arabidopsis*	*CaMV 35S*	2.8	-	3.2 to 3.7	higher resistance under salinity	(6)
Bean *P5CS2*			2.7		7	much higher resistance under salinity	
Mothbean *P5CSF129A*	Pigeonpea	*CaMV 35S*	3.5 to 5	-	4 to 4.5	Higher plant height, water and Chlorophyll content, lower oxidative damages under salinity	(61)

[a] Proline content(fold) in comparison to non-transgenic (NT) plant in normal condition
[b] Proline content(fold) in comparison to NT plant under drought stress
[c] Proline content(fold) in comparison to NT plant under salt stress

promoters such as *rd29A* and recently characterized *Oshox24*, which results in higher resistance under osmotic stresses, with lowest phenotypic abnormality in normal conditions (28, 38), is suggested to be studied in further investigations.

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تجمع پرولین و تنش اسمزی: مروری بر ژن *P5CS* در گیاهان

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چکیده

تجمع پرولین تحت تنش‌های اسمزی، یکی از پاسخ‌های مهم گیاهان به این شرایط است. پرولین یک اسمولایت سازگار است که بسیاری از جنبه‌های سلولی و مولکولی گیاه را در هر دو شرایط عادی و تنش‌زا، تحت تأثیر قرار می‌دهد. پرولین نشان داده شده است که در تکامل گیاه در شرایط عادی و در مقاوم ساختن آن تحت تنش‌های زنده و غیرزنده، دخیل است. بنابراین، تا کنون مطالعات متعددی به منظور آشکارسازی مکانیسم‌ها و مسیر سیگنال‌دهی پرولین انجام شده است، تا شاید بتواند به عنوان چشم‌اندازی برای حل چالش‌های رو به رشد کشاورزی، یعنی خشکی و شوری خاک، مد نظر قرار گیرد. آنزیم Δ^1-pyrroline-5-carboxylate synthetase (P5CS)، به عنوان یکی از دو آنزیم اصلی در مسیر بیوسنتز پرولین از پیش ماده گلوتامات، نشان داده شده است که نقش چشمگیری در تجمع پرولین در گیاهان در شرایط تنش‌های کم‌آبی ایفا می‌کند. در مورد نقش P5CS تحت تنش اسمزی، مشاهدات بحث‌برانگیزی در گیاهان مختلف وجود دارد که باعث ایجاد ابهام در "محدودکننده" بودن آنزیم P5CS در این مسیر شده است. آنچه که آشکار است، ژن ترانس *P5CS* با بالابردن میزان پرولین، باعث افزایش مقاومت گیاهان تراریخت تحت شرایط خشکی و شوری می‌شود. در این مقاله‌ی مروری، پرولین و کارکردهای شناخته شده‌ی آن در گیاهان، مشخصه‌های آنزیم P5CS، سیگنال‌ها، محرک‌ها و بازدارنده‌های ژن *P5CS* و الگوی بیان آن تحت شرایط متفاوت در گونه‌های گیاهی مطالعه شده بحث گردیده است. در نهایت، خصوصیات گیاهان تراریخت تولیدشده با تشدید بیان ژن *P5CS* و نتایج این انتقال بررسی گردیده است.

کلمات کلیدی: پرولین، تنش غیرزنده، تشدید بیان، گیاهان تراریخت، P5CS