Universal Journal of Agricultural Research 3(1): 15-22, 2015 http://www.hrpub.org
DOI: 10.13189/ujar.2015.030104

A Study on the Accumulation of Proline- An Osmoprotectant Amino Acid under Salt Stress in Some Native Rice Cultivars of North Kerala, India

EA Joseph[1], VV Radhakrishnan[2], KV Mohanan[1,2,*]

[1]Interuniversity Centre for Plant Biotechnology, Department of Botany, University of Calicut, India
[2]Genetics and Plant Breeding Division, Department of Botany, University of Calicut, India

Abstract Salinity is a major yield reducing factor in coastal as well as arid irrigated rice production systems. Salt induced abiotic stress is an acute problem, interrupting the metabolic processes of plants, resulting in reduced growth and productivity. Proline is an amino acid which increases in plants under abiotic stress. Salinity stress affects the metabolism of plants leading to severe crop damage and loss of productivity. Oxidative stress is one consequence of salinity that may be responsible for much of the damage. We inspected the immediate accumulation of proline in salinity induced stress in seven native rice (*Oryza sativa* L.) cultivars. Among the seven, five namely *Orthadian, Chovvarian*, *Kuttusan, Kuthiru* and *Orkazhama* were collected from a saline rice tract of the region and two namely *Kunhutty* and *Veliyan* from a non-saline rice tract of the region. The plants were subjected to different levels of salt stress ranging from 0 to 200 mM of NaCl and the concentration of proline in the leaf samples was inspected. Results showed that proline concentration increased in all the cultivars studied in relation to increase in salt stress and it was progressive along with increase in stress. This property was shown by all the cultivars irrespective of the fact whether they evolved in a saline habitat or a non-saline habitat. However, proline accumulation was higher in the cultivars collected from the saline tract when compared to the cultivars collected from the non-saline tract.

Keywords *Oryza Sativa*, Proline, Salinity Stress, Salt Tolerance

1. Introduction

Rice is one of the world's most important cereal crops with exceptional agricultural and economic importance and is the staple food of more than 50% population worldwide. Asian farmers produce more than 90% of the total rice, with two countries, India and China, growing more than half of the total crop [1]. Various abiotic stresses including high or low temperatures, water scarcity, high salinity and heavy metals exert drastic antagonistic effects on plant metabolism and thereby plant growth, development and productivity. Salinity and drought are the two major abiotic constraints in rice production, affecting plant growth and productivity globally [2,3]. In Asia alone, 21.5 million ha of land area is thought to be salt affected, with India having 8.6 million ha of such area which constitutes a major part of problem soils in India [4]. Yield of rice, especially Asian rice (*Oryza sativa* L.), is highly susceptible to salinity [5]. In India and especially in coastal rice fields, soil salinity is a major stress that reduces rice productivity [6]. Salinity is detrimental to the various processes of plants such as seed germination, seedling growth and vigour, vegetative growth, flowering and fruit set and ultimately it results in diminished yield and quality [7]. Salinity is known to affect plant growth and metabolism through its osmotic effects, through specific toxic effects of ions, by disturbing membrane integrity and function thereby interfering with internal solute balance and uptake of essential nutrients [8,9]. However, improvement in salt tolerance of crop plants remains elusive, since salinity affects almost every aspect of the physiology and biochemistry of plants at both whole plant and cellular levels [10,11].

Osmotic adjustment is a mechanism to maintain water relations under osmotic stress. In order to survive and cope up with the conditions of salt stress, plants show a number of physiological and biochemical adaptations. It involves the accumulation of a range of osmotically active molecules/ions including soluble sugars, sugar alcohols, proline, proline betaine, glycine betaine, glycerol, mannitol, sorbitol, organic acids, calcium, potassium, chloride ions, abscisic acid and osmotin [12-14]. These compounds are small uncharged molecules highly soluble in water at physiological pH, which can therefore accumulate at high concentration in the cytosol of plant cells without causing any damage to cellular

structures, because they are generally excluded from the hydration sphere of macromolecules [15]. Compatible osmolytes, once accumulated, are thought to reduce the cellular water potential below external values, driving or preserving water in the cell, thus maintaining turgor pressure high enough to sustain growth. In rice, accumulation of proline is reported to be more in salt tolerant than in salt sensitive cultivars and has been implicated to confer tolerance to salinity stress.

Salt stress is caused by excessive accumulation of salt in the soil, either directly because of salinization, or indirectly because of water loss. As a consequence, the soil water potential progressively decreases, hampering and eventually halting the gradient of water flow from roots to apical shoot. The resulting osmotic stress may cause stomatal closure, reduced photosynthesis rate and growth inhibition. Another consequence of osmotic stress is the production of ROS and the accumulation of toxic ions such as Na^+ or Cl^- within the cell, causing severe damage to membrane structures, proteins, nucleic acids and lipids [16]. Rapid accumulation of free proline is a typical response to salt stress. When exposed to drought or a high salt content in the soil (both leading to water stress), many plants accumulate high amounts of proline, in some cases several times the sum of all the other amino acids [17]. Proline has been found to protect cell membranes against salt injury [18]. Sultana et al. [19] have suggested that proline accumulation in both salinized leaves and grains of rice plants is implicated in osmotic adjustment to salinity. In contrast, Lutts et al. [20] have argued that proline accumulated in salt-stressed calluses had a negligible effect on osmotic adjustment and did not play a role in salt resistance in rice callus cultures.

For many years, proline has been known to be involved in the response to a number of environmental stresses, particularly salt and drought stress. The accumulation of proline upon osmotic stress is well documented in a large number of different plant species [21-26]. However, a general agreement on the precise role of proline in the response of plants to stress is still lacking and several hypotheses have been proposed on the significance of the accumulation of proline caused by stress [27]. Among the plant compatible osmolytes (or plant protectants), proline is considered of major importance, as it has been reported to accumulate in a large number of species in response to stresses such as excess salinity, drought, cold, nutrient deficiency, heavy metals, pathogen infections and high acidity [9,13]. In addition, high concentrations of proline have been observed in halophytic plants grown in saline environments [28,29,27] in the root apical region of plants growing at low water potentials [30] in suspension cultures of plant cells adapted to water or NaCl stress [31,32] and in flowers and fruits of a large number of species under normal physiological conditions [33,34].

Salt stress responses of plants may depend upon salt type, concentration and genotype. Therefore, screening for salt-stress tolerant genotypes in important crops such as rice will help in ensuring future crop production. In addition, studying differential responses of genotypes with contrasting stress tolerances will help reveal the underlying salt stress tolerance mechanisms [2]. The present investigation was aimed to study the effects of NaCl stress towards proline accumulation in seven rice cultivars, five of them collected from a traditional saline rice tract and two from a traditional non-saline rice tract of Kerala State of India.

2. Materials and Methods

2.1. Germination of Seeds and Transfer to the Experiment Site

The experiment was conducted in the experimental rainout poly house of Department of Botany, University of Calicut, Kerala, India located at 11°35′N latitude and 75°48′E longitude in the first crop season of 2013. Seven native cultivars of rice including five cultivars namely *Orthadian, Orkazhama, Kuthiru, Kuttusan* and *Chovvarian* collected from one of the saline rice tracts of Kerala and two cultivars namely *Kunhutty* and *Veliyan* collected from one of the non-saline rice habitats of Kerala were used for the study. Enough number of good caryopses were taken from single plant in the case of each cultivar and washed in running tap water to remove infected and unfilled grains and dust particles. The seeds were soaked in distilled water and allowed to germinate in 10cm diameter Petri dishes covered with lid under room temperature. The water was changed every day. The seeds started to germinate from the third day onwards.

2.2. Plant Materials and Treatments

Table 1. Details of salinity treatment

Sl. No.	Treatment
T1	Control
T2	10mM (0.91dSm^{-1}) on 45th day
T3	10mM (0.91dSm^{-1}) on 45th day & 30mM (2.74dSm^{-1}) on 53rd day
T4	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dSm^{-1}) on 53rd day & 50mM (4.57dSm^{-1}) on 61st day
T5	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dSm^{-1}) on 53rd day, 50mM (4.57dSm^{-1}) on 61st day & 70mM (6.39dSm^{-1}) on 69th day
T6	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dSm^{-1}) on 53rd day, 50mM (4.57dSm^{-1}) on 61st day, 70mM (6.39dSm^{-1}) on 69th day & 100mM (9.13dSm^{-1}) on 77th day
T7	10mM (0.91dSm^{-1}) on 45th day, 30mM (2.74dSm^{-1}) on 53rd day, 50mM (4.57dSm^{-1}) on 61st day, 70mM (6.39dSm^{-1}) on 69th day, 100mM (9.13dSm^{-1}) on 77th day & 200mM (18.26dSm-1) on 85th day

On the 10th day, required numbers of the germinated seedlings were transferred to coloured plastic pots of 25cm

diameter filled with paddy soil mixed with enriched compost in 3:1 ratio. Two seedlings were initially planted per pot and after establishment of the seedlings the smaller among the two was removed. The plants were maintained in the experimental poly house under wetland conditions, always maintaining 3cm of water above the soil level. The soil was fertilized with 1g N: P: K =18: 18: 18 per pot at fortnightly intervals starting from the 30th day. Weeding was done manually whenever required. Plants were grown in plastic pots of 25cm diameter in Randomized Block Design with three replications.

The experimental treatment was started from the 45th day onwards starting from 10mM (0.91dSm^{-1}) to 200mM (18.26dSm^{-1}) aqueous solution of sodium chloride as detailed in Table 1.

2.3. Estimation of Proline

Estimation of proline was done according to the procedure adopted by Bates et al. [35]. Fully expanded rice leaves from the experimental plants were sampled. Purified proline (Himedia, India) was used to standardize the procedure for quantifying sample values. Acid-ninhydrin was prepared by warming 1.25g ninhydrin (Himedia, India) in 30ml glacial acetic acid (Merck) and 20ml 6M phosphoric acid (Merck, India), with agitation, until getting dissolved. Kept cool (stored at 4°C), the reagent remains stable for 24 hours. Approximately 0.5g of plant material was ground in a mortar with liquid nitrogen; homogenized in 10ml of 3% aqueous sulfosalicylic acid (w/v) (Himedia, India) and the homogenate filtered using Whatman #2 filter paper. 2ml of the filtrate was reacted with acid-ninhydrin and 2ml of glacial acetic acid (Merck, India) in a test tube, incubated for 1 hour at 100°C in a boiling water bath and the reaction terminated in an ice bath. The reaction mixture was extracted with 4ml toluene (Merck, India) mixed vigorously with a test tube stirrer for 15-20 seconds. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read spectrophotometrically (Thermo Scientific, USA) at 520nm using toluene for a blank. The proline concentration was determined from a standard graph and calculated in µg/g on the basis of fresh weight of the leaf samples [36].

2.4. Statistical Analysis

Each experiment was done in replicates of three and data expressed as mean ± standard error (SE) and significance of variation calculated at $P \geq 0.05$ using analysis of variance.

3. Results

Free proline content in the experimental plants was determined on the 90th day, and the results are presented in Table 2 and Figs. 1 and 2. Free proline content was significantly elevated in the salt stressed plants over control plants in all the rice cultivars under study. Proline content increased in proportion to increase in salt stress applied. Rapid accumulation of free proline is a typical response to salt stress and similar responses have been observed by earlier workers in rice [37].

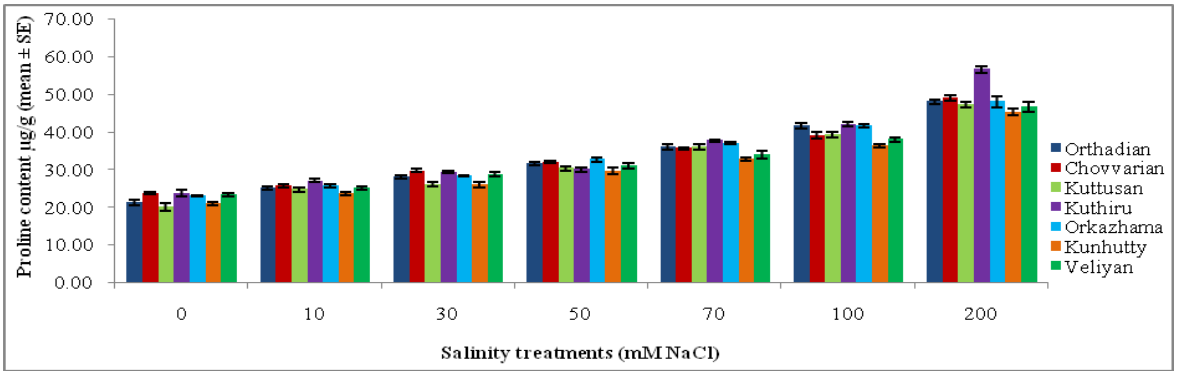

Figure 1. Levels of proline content in different rice cultivars studied under salt stress

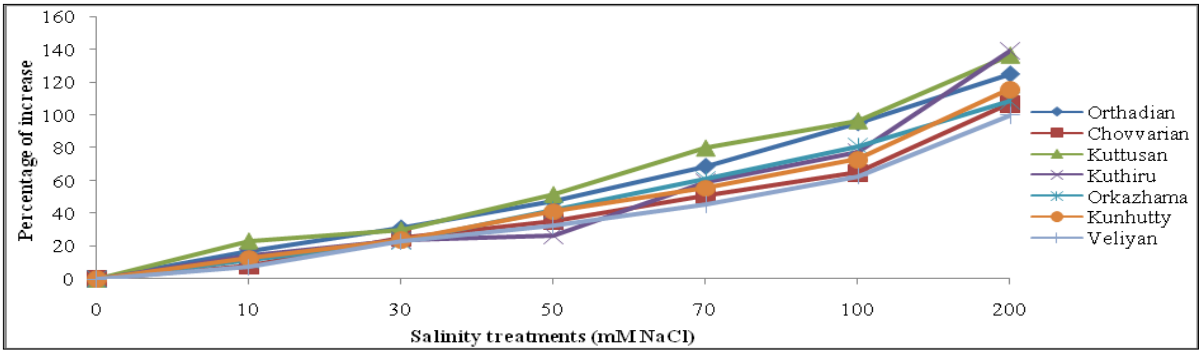

Figure 2. Percentage of increase in the concentration of proline in different rice cultivars under salt stress

Table 2. Concentration of proline in the rice cultivars studied under different levels of salinity stress and their percentages of increase over control

Cultivars/ Treatments	Mean±SE (µg/g)	CD @ 5%	% of increase over control
Orthadian			
Control	21.33±0.77	4.73	0.00
10mM	25.00±0.44		17.21
30mM	28.00±0.38*		31.27
50mM	31.67±0.45*		47.48
70mM	36.00±0.65*		68.78
100mM	41.67±0.67*		95.36
200mM	48.00±0.65*		125.04
Chovvarian			
Control	23.67±0.33	4.20	0.00
10mM	25.67±0.55		8.45
30mM	29.67±0.45*		25.35
50mM	32.00±0.22*		35.19
70mM	35.67±0.25*		50.70
100mM	39.00±0.87*		64.77
200mM	49.00±0.65*		107.01
Kuttusan			
Control	20.00±0.95	5.63	0.00
10mM	24.67±0.55		23.35
30mM	26.00±0.58*		30.00
50mM	30.33±0.55*		51.65
70mM	36.00±0.65*		80.00
100mM	39.33±0.77*		96.65
200mM	47.33±0.77*		136.65
Kuthiru			
Control	23.67±0.91	5.06	0.00
10mM	27.00±0.44		14.07
30mM	29.33±0.33*		23.91
50mM	30.00±0.58*		26.74
70mM	37.67±0.33*		59.15
100mM	42.00±0.65*		77.44
200mM	56.67±0.88*		139.42
Orkazhama			
Control	23.00±0.22	5.18	0.00
10mM	25.67±0.33		11.61
30mM	28.33±0.25*		23.17
50mM	32.67±0.67*		42.04
70mM	37.00±0.38*		60.87
100mM	41.67±0.50*		81.17
200mM	48.00±1.36*		108.70
Kunhutty			
Control	21.00±0.44	5.16	0.00
10mM	23.67±0.45		12.71
30mM	26.00±0.79		23.81
50mM	29.67±0.88*		41.29
70mM	32.67±0.45*		55.57
100mM	36.33±0.55*		73.00
200mM	45.33±0.77*		115.88
Veliyan			
Control	23.33±0.33	5.99	0.00
10mM	25.00±0.44		7.16
30mM	28.67±0.55		22.89
50mM	31.00±0.79*		32.88
70mM	34.00±0.95*		45.74
100mM	38.00±0.58*		62.88
200mM	46.67±1.20*		100.04

*shows significant variation at 5% level

Both the rice cultivars collected from the non-saline tract showed significant and gradual increase in proline content when salt stress was progressively applied. Earlier workers have also reported high accumulation of proline in salt sensitive rice under salt stress [38]. In consensus with this, our results show that the salt sensitive cultivars accumulate high amounts of proline in relation to increase of salinity stress. Among the two cultivars collected from the non-saline rice tract, increase in proline content was 115.88% in *Kunhutty* and 100.04% in *Veliyan* when exposed to 200mM NaCl.

In the case of the cultivars collected from the saline rice tract, increase in proline content varied from 107.01% to 139.42% under a salt stress of 200mM NaCl. The cultivar *Kuthiru* showed the highest quantum of accumulation of proline followed by *Kuttusan*, *Orthadiyan*, *Orkazhama* and *Chovvarian*. Capability of these cultivars to maintain comparatively good levels of growth and yield performance under salt stress has been demonstrated by earlier studies [39]. It is interesting to note that while the cultivars from the saline rice tract showed significantly higher levels of accumulation of proline from the salt treatment level of 30mM onwards, the cultivars from the non-saline rice tract showed significantly higher levels of accumulation of proline from the treatment level of 50mM only.

When exposed to high salt content in soil, many plants accumulate high amounts of proline, in some cases several times the sum of all other amino acids [40]. Proline is a known osmoprotectant and it plays an important role in osmotic balancing, protection of sub-cellular structures and enzymes and in increasing cellular osmolarity (turgor pressure) that provides the turgor necessary for cell expansion under stress conditions [41,28].

4. Discussion

Soil salinity is one of the major factors limiting crop production. Salt stress results in a wide variety of physiological and biochemical changes in plants. Accumulation of low molecular weight solutes such as proline, commonly referred to as compatible solutes is one such phenomenon [42]. By lowering water potentials, the accumulation of compatible osmolytes involved in osmoregulation allows additional water to be taken up from the environment, thus buffering the immediate effect of water shortages within the organism. High levels of proline enable the plant to maintain low water potentials. In addition to acting as osmoprotectant [43], proline also serves as a sink for energy to regulate redox potentials [44, 45], as a hydroxy radical scavenger [46], as a solute that protects macromolecules against denaturation [47], as means of reducing acidity in the cell [48] and acts as a storage compound and nitrogen source for rapid growth after stress [49]. Proline alleviates NaCl stress induced enhancement in oxygenase as well as carboxylase activities of Rubisco [50]. Matysik et al. [41] noticed that proline protects plants against free-radical induced damage by quenching of singlet oxygen. Proline accumulation is one of the adaptations of plants to salinity and water deficit [51,52,53]. Some workers did not observe any appreciable increase in free proline content due to stress [54,55] and some others consider enhanced proline level merely a stress effect, rather than a cause of stress tolerance [56].

Proline accumulation in leaf tissues has been suggested to result from a decrease in proline degradation, an increase in proline biosynthesis, a decrease in protein synthesis or proline utilization and hydrolysis of protein [57]. The higher accumulation of proline could be due to enhanced activities of ornithine aminotransferase (OAT) and pyrroline-5-carboxylate reductase (P5CR), the enzymes involved in proline biosynthesis [58], as well as due to inhibition of proline oxidase and proline dehydrogenase (PDH), proline catabolizing enzymes [59]. It has been reported that proline dehydrogenase also functions as pyrroline-5-carboxylate reductase (proline synthesizing enzyme) and catalyses the reaction with the same reactants and co-enzymes, but operating in an opposite direction [60]. Studying the effects of salt stress on enzyme activities involved in proline metabolism could provide valuable information on the physiological significance of its accumulation. KaviKishor et al. [61] reported that there was a surge in proline content in plants under salt stress. In many plants proline accumulates in excess of protein synthesis. Proline catabolism is repressed under osmotic stress, but once the stress is withdrawn, proline is oxidised to Δ^1-pyrroline-5-carboxylate (P5C) by proline dehydrogenase (PDH), also known as proline oxidase, the first enzyme in the proline degradation pathway. P5C is then converted back to glutamate by the enzyme P5Cdehydrogenase (P5CDH). Thus, both PDH and P5CDH form two important enzymes in the degradation of proline to glutamate in higher plants.

The present study has shown that proline concentration increases in all the cultivars of rice studied in relation to increase in salt stress and the increase is progressive along with increase in stress. This property was shown by all the cultivars irrespective of the fact whether they evolved in a saline habitat or a non-saline habitat. However, proline accumulation was higher in salt tolerant rice cultivars when compared to sensitive cultivars. The accumulation of compatible solutes may help to maintain the relatively high water content necessary for plant growth and cellular functions. Plants respond to a variety of stresses by accumulating certain specific metabolites, the most conspicuous being amino acids in general and proline in particular. Less than 5% of the total pool of free amino acids in plants under stress free conditions is provided by proline. In many plants under various forms of stress, the concentration increases up to 80% of the amino acid pool [41]. Abiotic stresses like drought and salinity cause significant crop loss worldwide [62,63]. They affect plants through different mechanisms, causing osmotic stress and ionic imbalance, in addition to inducing oxidative stress [64]. The exact role of proline in inducing stress tolerance has to

be further investigated in terms of the mechanisms involved.

5. Conclusions

Salinity is a major yield reducing factor in rice production systems. Salinity induces abiotic stress interrupting the metabolic processes of plants, resulting in reduced growth and productivity. Production and accumulation of some compounds in higher concentration is a phenomenon usually associated with salt stress response in plants. Proline is an amino acid which increases in plants under abiotic stress. The accumulation of proline under salinity induced stress in seven native rice (*Oryza sativa* L.) cultivars including five namely *Orthadian, Chovvarian*, *Kuttusan, Kuthiru* and *Orkazhama* collected from a saline rice tract of the study region and two namely *Kunhutty* and *Veliyan* from a non-saline rice tract of the region were studied presently. The plants were subjected to different levels of salt stress ranging from 0 to 200 mM of NaCl and the concentration of proline in the leaf samples was inspected. Proline concentration increased in all the cultivars studied in relation to increase in salt stress and it was progressive along with increase in stress. This property was shown by both the groups of cultivars irrespective of the fact whether they were collected from the saline habitat or the non-saline habitat. However, proline accumulation was higher in the first group of cultivars when compared to the second group of cultivars.

REFERENCES

[1] IRRI, 2011. International Rice Research Institute, Philippines. http://www.irri.org.

[2] Kumar, V., V. Shriram, P.B. KaviKishor, N. Jawali and M.G. Shitole, 2010. Enhanced proline accumulation and salt stress tolerance of transgenic *indica* rice by over expressing P5CSF129A gene. Plant Biotech. Rep. 4(1): 37-48.

[3] Tavakkoli E., F. Fatehi, S. Coventry, P. Rengasamy and G.K. McDonald, 2011. Additive effects of Na^+ and Cl^- ions on barley growth under salinity stress. J. Exp. Bot., 62(6): 2189-2203.

[4] Sahi, C., A. Singh, E. Blumwald and A. Grover, 2006. Beyond osmolytes and transporters: novel plant salt-stress tolerances related genes from transcriptional profiling data. Physiol. Plant. 127: 1-9.

[5] Munns, R. and M. Tester, 2008. Mechanisms of salinity tolerance. Ann. Rev. Plant Biol. 59:651–681.

[6] Kumar, V., V. Shriram, T.D. Nikam, N. Jawali and M.G. Shitole, 2008. Sodium chloride induced changes in mineral elements in *indica* rice cultivars differing in salt tolerance. J. Plant Nutr. 31(11): 1999-2017.

[7] Sairam, R.K. and A. Tyagi, 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr. Sci. 86(3): 407-421.

[8] Poljakoff-Mayber, A., 1975. Morphological and anatomical changes in plants as a response to salinity stress. In: Poljakoff-Mayber, A. and J. Gale (eds.), Plants in Saline Environments. Springer-Verlag, Berlin, pp: 97–117.

[9] Pitman, M.G. and A. Lauchli, 2002. Global impact of salinity and agricultural ecosystems. In: Lauchli, A. and U. Luttge (eds.), Salinity: Environment-Plants-Molecules. Kluwer, Dordrecht, The Netherlands, pp: 3–20.

[10] Murphy, K.S.T. and M.J. Durako, 2003. Physiological effects of short-term salinity changes on *Ruppia maritima*. Aquat. Bot. 75: 293 – 309.

[11] Cuartero, J., M.C. Bolarin, M.J. Asins and V. Moreno, 2006. Increasing salt tolerance in the tomato. J. Exp. Bot. 57: 1045–1058.

[12] Hare, P.D. and W.A. Cress, 1997. Metabolic implications of stress induced proline accumulation in plants. Plant Growth Regul. 21:79–102.

[13] Sairam, R.K., G.C. Srivastava, S. Agarwal and R.C. Meena, 2005. Differences in response to salinity stress in tolerant and susceptible wheat genotypes. Biol. Plant. 49(1): 85-91.

[14] Farooq, M., A.Wahid, N. Kobayashi, D. Fujita and S.M.A. Basra, 2009. Plant drought stress: effects, mechanisms and management. Agron. Sustain. Dev. 29: 185–212.

[15] Low, P.S., 1985. Molecular basis of the biological compatibility of nature's osmolytes. In: Gilles, R. and M. Gilles-Baillien (eds.), Transport Processes, Iono- and Osmoregulation. Springer-Verlag, Berlin, pp. 469–477.

[16] Apel, K. and H. Hirt, 2004. Reactive oxygen species: metabolism, oxidative stress and signal transduction. Ann. Rev. Plant Biol. 55: 373–399.

[17] Mansour, M.M.F., 2000. Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant. 43(4): 491–500.

[18] Mansour, M.M.F., 1998. Protection of plasma membrane of onion epidermal cells by glycine betaine and proline against NaCl stress. Plant Physiol. Biochem. 36(10): 767–772.

[19] Sultana, N., T. Ikeda and R. Itoh, 1999. Effect of NaCl salinity on photosynthesis and dry matter accumulation in developing rice grains. Environ. Exp. Bot. 42: 211–220.

[20] Lutts, S., J. M. Kinet and J. Bouharmon, 1996. Effects of various salts and of mannitolon ion and proline accumulation in relation to osmotic adjustment in rice (*Oryza sativa* L.) callus cultures. J. Plant Physiol. 149: 186–195.

[21] Yoshiba, Y., T. Kiyosue, T. Katagiri, H. Ueda, K. Wada, Y. Harada and K. Shinozaki, 1995. Correlation between the induction of a gene for Δ^1-pyrroline-5-carboxylate synthetase and the accumulation of proline in *Arabidopsis* under osmotic stress. Plant J. 7: 751–760.

[22] Peng, Z., Q. Lu and D.P. Verma, 1996. Reciprocal regulation of Δ^1-pyrroline-5-carboxylate synthetase and proline dehydrogenase genes controls proline levels during and after osmotic stress in plants. Mol. Gen. Genet. 253: 334–341.

[23] Verbruggen, N., X.J. Hua, M. May and M. Van Montagu, 1996. Environmental and developmental signals modulate proline homeostasis: Evidence for a negative transcriptional regulator. Proc. Natl. Acad. Sci. USA. 93: 8787–8791.

[24] Nakashima, K., R. Satoh, T. Kiyosue, K. Yamaguchi-Shinozaki and K. Shinozaki, 1998. A gene encoding proline dehydrogenase is not only induced by proline and hypoosmolarity, but is also developmentally regulated in the reproductive organs of *Arabidopsis*. Plant Physiol. 118: 1233–1241.

[25] Mattioli, R., D. Marchese, S. D'Angeli, M. M. Altamura, P. Costantino and M. Trovato, 2008. Modulation of intracellular proline levels affects flowering time and inflorescence architecture in *Arabidopsis*. Plant Mol. Biol. 66: 277–288.

[26] Székely, G., E. Ábrahám, Á. Cséplo, G. Rigo, L. Zsigmond, J. Csiszár, F. Ayaydin, N. Strizhov, J. Jásik, E. Schmelzer, C. Koncz and L. Szabados, 2008. Duplicated P5CS genes of *Arabidopsis* play distinct roles in stress regulation and developmental control of proline biosynthesis. Plant J. 53: 11–28.

[27] KaviKishor, P.B., Z. Hong, G.H. Miao, C.A.A. Hu and D.P.S. Verma, 1995. Over expression of Δ^1-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol. 108: 1387–1394.

[28] Briens, M. and F. Larher, 1982. Osmoregulation in halophytic higher plants: a comparative study of soluble carbohydrates, polyols, betaines and free proline. Plant Cell Environ. 5: 287–292.

[29] Treichel, S., 1986. The influence of NaCl on delta1-pyrroline-5-carboxylate reductase in proline accumulating cell suspension cultures of *Mesembryanthemum nodiflorum* and other halophytes. Plant Physiol. 67: 173–181.

[30] Kant, S., P. Kant, E. Raveh and S. Barak, 2006. Evidence that differential gene expression between the halophyte *Thellungiella halophila* and *Arabidopsis thaliana* is responsible for higher levels of the compatible osmolyte proline and tight control of Na^+ uptake in T. *halophila*. Plant Cell Environ. 29: 1220–1234.

[31] Handa, S., A.K. Handa, P.M. Hasegawa and R.A. Bressan, 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. Plant Physiol. 80: 938–945.

[32] Voetberg, G.S. and R.E. Sharp, 1991. Growth of the maize primary root tip at low water potentials. III. Role of increased proline deposition in osmotic adjustment. Plant Physiol. 96: 1125–1130.

[33] Rhodes, D., S. Handa and R.A. Bressan, 1986. Metabolic changes associated with adaptation of plant cells to water stress. Plant Physiol. 82: 890–903.

[34] Chiang, H.H. and A.M. Dandekar, 1995. Regulation of proline accumulation in *Arabidopsis* during development and in response to desiccation. Plant Cell Environ. 18: 1280–1290.

[35] Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water stress studies. Plant Soil 39: 205–207.

[36] Bagdi, D.L. and B.P. Shaw, 2013. Analysis of proline metabolic enzymes in *Oryza sativa* under NaCl stress. J. Environ. Biol. 34: 677-681.

[37] Lin, C.C., T.Y. Hsu and H.C. Kao, 2002. The effect of NaCl on proline accumulation in rice leaves. Plant Growth Regul. 36: 275-285.

[38] Rama, K., 1997. Salt stress responses in rice: protein profile, proline levels and molecular characterization of an AsrcDNA. Ph.D. Thesis, University of Madras, Tamil Nadu, India.

[39] Abhilash, J.E. and K.V.Mohanan, 2013. A study on the effect of salinity stress on the growth and yield of some native rice cultivars of Kerala State of India. Agriculture, Forestry and Fisheries 2(3): 141-150.

[40] Kumar, V., V. Shriram, T.D. Nikam, N. Jawali and M.G. Shitole, 2009. Antioxidant enzyme activities and protein profiling under salt stress in *indica* rice genotypes differing in salt tolerance. Arch. Agron. Soil Sci. 55(4): 379-394.

[41] Matysik, J., A.B. Bhalu and P. Mohanty, 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. Curr. Sci. 82: 525–532.

[42] Yancy, P.H., M.E. Clark, S.C. Hand, R.D. Bowlus and G.N. Somero, 1982. Living with water stress: evolution of osmolyte systems. Science 217: 1214–1223.

[43] Christian, J.H., 1955. The influence of nutrition on the water relation of *Salmonella oranienburg*. Aust. J. Biol. Sci. 8: 75–82.

[44] Saradhi, A. and P.P. Saradhi, 1991. Proline accumulation under heavy metal stress. J. Plant Physiol. 138: 554–558.

[45] Blum, A. and A. Ebercon, 1976. Genotypic responses in Sorghum to drought stress. III. Free proline accumulation and drought resistance. Crop Sci. 16: 428–431.

[46] Smirnoff, N. and Q.J. Cumbes, 1989. Hydroxyl radical scavenging activity of compatible solutes. Phytochemistry 28: 1057–1060.

[47] Schobert, B. and H. Tschesche, 1978. Unusual solution properties of proline and its interaction with proteins. Biochem. Biophys. Acta. 541: 270–277.

[48] Venkamp, J.H., J.E.M. Lampe and J.T.M. Koot, 1989. Organic acids as sources of drought induced proline synthesis in field bean plants, *Vicia faba* L. J. Plant Physiol. 133: 654–659.

[49] Singh, T.N., D. Aspinall, L.G. Paleg and S.F. Boggess, 1973. Stress metabolism. II. Changes in proline concentration in excised plant tissues. Aust. J. Biol. Sci. 26: 57–63.

[50] Sivakumar, P., P. Sharmila and P. Parthasaradhi, 2000. Proline alleviates salt stress induced enhancement in ribulose-1, 5-bisphosphate oxygenase activity. Biochem. Biophys. Res. Commun. 279: 512–515.

[51] Giridara Kumar, S., K.V. Madhusudhan, N. Sreenivasulu and C. Sudhakar, 2000. Stress responses in two genotypes of mulberry (*Morus alba* L.) under NaCl salinity. Indian J. Exp. Biol. 38: 192–195.

[52] Ramanjulu, S. and C. Sudhakar, 2000. Proline metabolism during dehydration in two mulberry genotypes with contrasting drought tolerance. J. Plant Physiol. 157: 81–85.

[53] Ramanjulu, S. and C. Sudhakar, 2001. Alleviation of NaCl salinity stress by calcium is partly related to the increased proline accumulation in mulberry (*Morus alba* L.) callus. J. Plant Biol. 28: 203–206.

[54] Dix, P.J. and R.S. Pearce, 1981. Proline accumulation in NaCl

resistant and sensitive cell lines of *Nicotiana sylvestris*. Zeitschrift fur Pflanzenschuts Physiologie 102: 243–248.

[55] Jain, R.K., R.S. Dhawan, D.R. Sharma and J.B. Chowdhury, 1987. Salt tolerance and proline accumulation: a comparative study in salt tolerant and wild type cultured cells of egg plant. Plant Cell Rep. 6: 382– 384.

[56] Moftah, A.H. and B.E. Michel, 1987. The effect of sodium chloride on solute potential and proline accumulation in soybean leaves. Plant Physiol. 83: 238–240.

[57] Charest, C. and C.T. Phan, 1999. Cold acclimation of wheat (*Triticum aestivum* L.): Properties of enzymes involved in proline metabolism. Physiol. Plant. 80: 159–168.

[58] Kohl, D.H., J.J. Lin, G. Shearer and K.R. Schubert, 1990. Activities of the pentose phosphate pathway and enzymes of proline metabolism in legume root nodules. Plant Physiol. 94: 1258–1264.

[59] Kandpal, R.P., C.S. Vaidyanathan, M. Udaykumar, K.S. Krishnasastry and N. Appaji-Rao, 1981. Alternation in the activities of the enzyme of proline metabolism in ragi (*Eleusine coracana*) leaves during water stress. J. Biosci.3: 361–369.

[60] Huang, A.H.C. and A.J. Cavalieri, 1979. Proline oxidase and water stress induced proline accumulation in spinach leaves. Plant Physiol. 63: 531–535.

[61] KaviKishor, P.B., S. Sangam, R.N. Amrutha, P. Sri Laxmi, K R. Naidu, K.R.S.S. Rao, S. Rao, K.J. Reddy, P. Theriappan and N. Sreenivasulu, 2005. Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr. Sci. 88: 424-438.

[62] Boyer, J.S., 1982. Plant productivity and environment. Science 218: 443–448.

[63] Owens, S., 2001. Salt of the earth. Genetic engineering may help to reclaim agricultural land lost due to salinization. EMBO Rep. 2: 877–879.

[64] Hasegawa, P M., R A. Bressan, J.K. Zhu and H.J. Bohnert, 2000. Plant cellular and molecular responses to high salinity. Ann. Rev. Plant Physiol. Plant Mol. Biol. 51: 463–499.