Int J Pharm Bio Sci 2013 Apr; 4(2): (B) 933 - 941

Research Article **BioChemistry**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

COMPATIBLE SOLUTE ACCUMULATION, OSMOTICUM MAINTANANCE AND GROWTH IN *PANICUM MILIACEUM* L. EXPOSED TO SALINITY

JITHA BHASKARAN, PAUL AJITHKUMAR I AND R. PANEERSELVAM*

Department of Botany, Annamalai University, Annamalai Nagar- 608002, Tamilnadu, India

ABSTRACT

Salinity is one of the most important constrain affecting crop growth and productivity. Hence studying salinity at morphological and biochemical point of view has become the basis for understanding salinity and plant interactions. Under stress, plants survive by enhancing the osmolyte accumulation by complex metabolic activities. Present investigation deals with the effect of salinity on growth, photosynthetic pigments and compatible solute accumulation in *Panicum miliacium*. Five NaCl regimes were used, 0 mM, 50 mM, 100 mM, 150 mM and 200 mM. Plants were treated with above mentioned NaCl on 30, 40, 50 and 60 days after sowing. From the data obtained we understand that in accord with the increase in salinity, shoot and root growth and photosynthetic pigments content reduced considerably, whereas organic solutes like proline, glycinebetaine and sugar increased considerably. Based on the data recorded it was concluded that reduced shoot and root growth, decreased photosynthetic pigments and remarkable accumulation of compatible solute make the plant survive salinity stress.

KEYWORDS*:* Compatible solutes, Osmotic stress, Salinity, Glycinebetaine, Proline, Salt stress.

R. PANEERSELVAM
Department of Botany, Annamalai University,
Annamalai Nagar- 608002, Tamilnadu, India
rpselvam9@gmail.com.

INTRODUCTION

Today, of various abiotic stresses, soil salinity is known to cause considerable crop losses. Approximately 33% of irrigated land world-wide has been affected by salinity [1]. In plantsalinity is one of the major abiotic factors contributing to the reduced growth physiological activity, developmental process and productivity and limiting plant geographical distribution in semi-arid and arid region [2,3]. Most crop plants are glycophytes, plants whose growth is reduced even in very low levels of salinity. In these plants, excess salt (NaCl) in the soil solution interferes with seed germination, inhibiting plant growth and metabolism [4]. In extreme cases salinity even causes partial or even complete mortality. Limited success in improving crop salt tolerance has been mainly achieved due to the lack of knowledge on the plant-NaCl interaction affecting fundamental physiological, biochemical and cellular processes which inturn affect plant growth and development [5,6]. For the better understanding on NaCl- plant interaction, in this investigation we have dealt with the changes in morphology, chlorophyll pigments and osmolyte accumulation under salinity.Salinity results in the accumulation of low-molecular mass compounds, termed as compatible solutes. Compatible solutes do not interfere with normal metabolic pathways [7]. They balance the regulation and co-ordination of cytoplasm and vacuolar volumes. These solutes mainly include proline and glycine betaine [8]. When plants are exposed to salt stress, they accumulate nitrogenous compounds (NCC) like amino acids which play a role in salt tolerance. Other compatible solutes are carbohydrate such as sugars and starch [9], which contributes in osmotic adjustment, carbon storage, and radical scavenging. Under salt stress accumulation of proline and betaine and their relative role in osmotic adjustment have been studied in terrestrial plants [10,11]. Due to an increase in the intercellular solutes, osmotic potential is lowered and this can be regarded as an adaptive mechanism of plants to external stress [12]. These solutes have a key role in maintaining the osmoticum, protect sub-cellular structures, and rectify the oxidative damage caused by free radicals formed due to abiotic stresses [13].Proso millet (*Panicum miliaceum L.)* is an annual grass and its origin goes back in history at least as far as 2000 B.C. when it was reported to have been grown in the central regions of Europe. This plant is especially well suited to dry climates such as central Russia, the Middle East, northern India, Africa, Manchuria, and the Great Plains area of North America. Proso millet was first introduced to Canada in the 17th century, and was used in a limited way as a forage crop in the early 1900's. In recent years a demand for birdseed renewed interest in proso millet. Some farmers are growing proso millet for this use in the market. The primary objective of the present study was to determine the extent to which NaCl induces changes in growth and important compatible solutes in *Panicum mileaceum (P. miliacium).*

MATERIALS AND METHODS

Seeds of *P. miliaceum* belonging to the family Poeceae were collected from Kollimalai, Tamil Nadu and were identified by Tamil Nadu Agriculture University, Coimbatore, Tamil Nadu. The experiment was laid out in a Completely Randomized Block Design (CRBD). Pot cultures and the treatment procedures were carried out in the month of June-August (2012) in the Botanical Garden and the biochemical analysis was conducted in Stress – Physiology Laboratory, Department of Botany, Annamalai University, Tamil Nadu, India. The pots were filled with soil containing mixture of red soil, sand and farm yard manure at 1:1:1 ratio. Four concentrations of NaCl used for the treatment were 50 mM, 100 mM, 150 mM and 200 mM and 0 mM served as control. For each treatment five replicates were maintained. Treatments were imposed on the plant on 30, 40, 50 and 60 DAS (days after sowing). The length between

shoot tip and point of the root shoot transition region was taken as shoot length. Root length was recorded by measuring below the point of root-shoot transition to the fibrous root and the length of lateral roots was taken as total root length. The shoot and root length are expressed in centimeters per plant. Chlorophyll contents were measured from the *P. miliacium* leaves according to Arnon method [14]. Fresh leaves (1 g) were extracted with 80 % acetone (v/v) and chlorophyll contents were estimated spectrophotometrically at 645 and 663 nm using Hitachi U-2000 spectrophotometer and were expressed in terms of mg chlorophyll present g^{-1} fresh mass. Glycinebetaine was estimated by the method of Grieve and Grattan [15]. Briefly, finely ground dried plant tissue (0.5 g) was stirred with 20 cm3 distilled water for 24 h and filtered. The filtrate was diluted with equal volume of 1 M H2SO4, made into aliquots of 0.5 cm3 in microcentrifuge tubes, cooled over ice for 1 h and to each of these were added 0.2 cm3 cold KI-I2 reagent. The reactants were gently stirred, stored at 4 °C overnight and centrifuged at 12 000 *g* for 15 min at 4 °C to get the precipitated periodide crystals. The crystals were dissolved in 1,2-dichloroethane, and absorbance was measured at 365 nm after 2 h. Glycinebetaine dissolved in 1 M H2SO4 served as standard. Free proline was assayed spectrophotometrically by the ninhydrin method [16]. The plant material was homogenized in 3% aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of acid ninhydrin and glacial acetic acid, which was boiled at 100°C for 1 h. After termination of reaction in ice bath, the reaction mixture was extracted with toluene, and absorbance was read at 520 nm using L-proline as standard. Soluble sugars were quantified following the phenolsulfuric acid method [17]. 100 mg dry weight of shoots was extracted in 80% (v/v) methanol heated to 70°C in a water bath. The extract was then centrifuged at 5,000 × g for 10 min. The supernatant was used for the estimation of soluble sugarconcentrations. The reaction mixture consisted of 5% phenol and 98% sulphuric acid. Once the extract had cooled, its absorbance was determined at 490 nm using D-glucose as standard. Total free amino acids were extracted and estimated by following the method of Moore and Stein [18]. Five hundred milligrams of fresh plant material was homogenized in a mortar and pestle with 80% boiled ethanol. The extract was centrifuged at 800 g for 15 minutes and the supernatant was made up to 10 ml with 80% ethanol. In 25 ml test tube, ethanol extract was taken and neutralized with 0.1 N NaOH using the methyl red indicator to which ninhydrin reagent was added. The contents were boiled in a boiling water bath for 20 minutes, and then 5ml of diluting solution was added, cooled and made up to 25 ml with distilled water. The absorbance was read at 570 nm.

RESULT AND DISCUSSION

Salt stress factors may induce osmotic stress, oxidative stress and protein denaturation in plants, which lead to adaptive responses such as stunted growth, reduced chlorophyll content and accumulation of compatible solutes.

(i) Effect of NaCl on growth

From the data of the present investigation it is clear that salt stress decreases shoot and root length (Table 1), and this is because of reduced water content due to decreased water adsorption caused as a result of osmotic stress and drought stress induced due to salinity [19, 20]. Under NaCl stress shoot and root length decreased considerably but the decrease was higher in shoot when compared with that of the root. This can be blamed on salinity which causes root damage leading to decrease in the number of lateral roots and increase in girth. Our finding was in accordance with the observations made by Misra et al. [21]. At morphological point of view, the most typical symptom of salinity stress is decreased growth which in turn is caused due to the inhibition in cell elongation and cell division controlled by different auxins and its synthesis is controlled by salinity [22]. NaCl stress induced a significant

decrease in root length. This may be due to salinity which causes root damage leading to decrease in the number of lateral roots and increase in girth. Our finding goes hand in hand with the observations made by Misra et al. [23]. Decrease in shoot length may prevent excess water loss by reducing the number of active stomata and transpiration rate.

(ii) NaCl effects on chlorophyll pigments

As the plant matured in the treated ones the chlorophyll content gradually decreased. At 200mM minimum chlorophyll content was observed (Table 2). According to Turan et al. [24] and Najafi et al. [25] the decrease in chlorophyll content during salt stress may be due to the increased activity of chlorophyll degrading enzyme namely chlorophyllase. Chl *a*/*b* ratio increased under salinity stress suggesting more damage to Chl *b* than Chl *an* under salt stress. In all treated plants, leaf Chl a/Chl b ratio increased as compared to the control, indicating that Chl b was degraded at a higher rate than Chl a, likely in relation with the fact that the first step in Chl b degradation involves its conversion to Chl a [26]. The reduction in chlorophyll contents is a common phenomenon happening under salinity stress, being membranous bound; its stability is dependent on membrane stability, which under for salinity tolerance condition seldom remains intact [27]. According to Molazem et al. [28] all the photosynthetic pigments decreased when exposed to salinity.

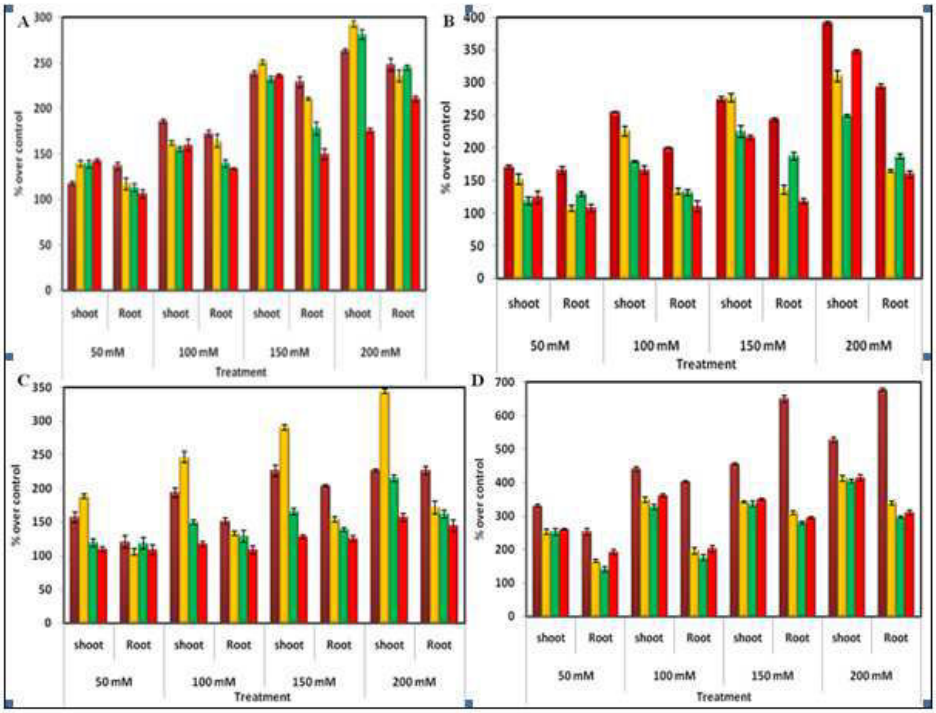
(iii) NaCl effects on compatible solute accumulation

We have recorded an increased in the content of osmolyte in all the treatments. The accumulation of compatible solutes such as sugars, proline or glycine betaine in plants benefit stressed cells by protecting and stabilizing macromolecules and structures from damage caused by abiotic stress [29]. When the level of NaCl in vacuoles increases, osmotic pressure is created in the cytoplasm and in order to balance this osmoticum, compatible solute accumulation is also enhanced. An aliphatic quaternary ammonium compound (QAS), glycine betaine has been found to accumulate in a large number of plants exposed to salt stress and is considered to be one of the most predominant and effective osmoprotectant. Salt stress caused an increase in the glycine betaine content in leaves and roots of millet studied when compared to control. At 200 mM of the NaCl treatment maximum value of GB was recorded (Fig 1.A) and it was found to be 79.43 µg g^{-1} dry weight in shoot and 67.46 µg g^{-1} dry weight in root. Glycine-betaine is synthesized from choline in two steps, and it is catalyzed by monooxygenase (CMO) and betaine aldehyde dehydrogenase (BADH) respectively. Both these enzyme activities are induced in plants under salinity [30] in crops including rice [31] and sorghum [32]. Proline, which is regarded as a non-toxic solute, increased under NaCl stress and was found to be concentration dependent. A significant increase proline content was observed in all the treatments when compared all the four treatments and it showed the highest value at 200 mM of NaCl stress (Fig. 1.B) and the highest values being 0.554 µg g^{-1} dry weight in shoot and 0.433 µg g^{-1} dry weight in root. Increased proline in the stressed plants may be an adaptation to overcome the stress conditions. Proline accumulation is due to the stimulation of proline biosynthesis and this happens due to an increase in pyrroline-5-carboxylase activity, and a decrease of proline dehydrogenase activity [33]. Increase in proline content and its accumulation impart tolerance to salinity [34]. Our results were in agreement with the results of other reports which includes the work carried out on sorghum [35], *Brassica juncea* [36]. Proline accumulation was recorded high in the Nostoc species under NaCl stress[37]. Yet another compatible solute, sugar accumulation was also prominent in all those plants under NaCl treatment. Total sugar content increased in all the treatments when compared to control. Among all treatments, 200 mM showed the higher total sugar level when in comparison with the others. Sugar content was found to be 1.56 mg g^{-1} dry weight and 0.851 mg g^{-1} dry weight in shoot and root respectively (Fig. 1.C). A significant increase in the percentage of control

was observed in leaf when compared to root. The accumulation of sugar may be due to the decomposition of starch under salt stress. The accumulation of soluble sugars in response to salinity and water stress is of common occurrence and was well documented by Murakeozy *et al*. [38, 39]. It is believed with the increasing salinity, sucrose accumulation also increases which contribute to osmotic adjustment [40]. Our report is in agreement with many previous studies [41,42]. Under salinity, sugar accumulation has a major role in the osmotic adjustment in comparison with other organic solutes [43]. The nitrogen containing compound (NCC) was found to increase in all the treatments. Amino acid content in leaves and roots of *P. miliaceum* increased with the increase in concentration of NaCl and it showed its maximum expression in 200 mM NaCl treatment (Fig. 1.D) with the values reaching 17.41 mg g^{-1} dry weight and 13.47 mg g^{-1} dry weight in shoot and root respectively. Accumulated amino acid may be occurring in response to the change in osmotic adjustment of their cellular contents [44]. Under salt stress, these compounds play a key role in osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells, and scavenging of free radicals. NCC accumulation is usually correlated with plant salt tolerance [45]. Amino acid content increased under drought condition in *astragali* [46,47]. A significant increase in the accumulation of compatible solutes especially proline, glycine betaine and sugars with the increase in the level of salinity may have contributed to the osmotic adjustment in the cytoplasm and in minimizing the oxidative damage. Reduced shoot and root growth and decrease in chlorophyll content can also be regarded as an adaptive mechanism which a plant adapts to minimize the water loss by reduced stomatal count and thus lowering the transpiration rate and photosynthetic rate respectively.

Figure 1

NaCl stress induced changes in compatible solute accumulation.



A.Glycinebetaine content; B. Proline content; C. Soluble sugar content; D. Aminoacid content. The symbol coding scheme is as follows: 30 days after sowing (Brown), 40 days after sowing (Yellow), 50 days after sowing (Green) and 60 days after sowing (Red).

Table 1
NaCl stress induced changes in shoot and root length of P. miliacium at different stages of growth.

Growth Stages	Control	50 mM	100 mM	150 mM	200 mM
Shoot (cm/plant)					
30 DAP	54.23 ± 0.004	49.38 ± 0.001	43.48 ± 0.006	37.08 ± 0.020	33.42 ± 0.010
40 DAP	78.00 ± 0.021	72.04 ± 0.030	68.47 ± 0.031	50.04 ± 0.018	48.00 ± 0.020
50 DAP	93.47 ± 0.002	83.00 ± 0.041	78.13 ± 0.006	71.38 ± 0.049	54.49 ± 0.019
60 DAP	106.05 ± 0.071	92.20 ± 0.018	86.90 ± 0.30	78.43 ± 0.031	58.48 ± 0.199
Root (cm/plant)					
30 DAP	24.34 ± 0.043	19.33 ± 0.177	13.08 ± 0.004	10.56 ± 0.054	6.84 ± 0.050
40 DAP	42.92 ± 0.170	24.38 ± 0.201	22.84 ± 0.104	16.59 ± 0.065	12.58 ± 0.720
50 DAP	51.28 ± 0.032	43.93 ± 0.071	37.93 ± 0.008	22.92 ± 0.025	17.92 ± 0.039
60 DAP	57.84 ± 0.049	54.93 ± 0.018	46.93 ± 0.080	29.93 ± 0.044	21.75 ± 0.008

(values are the mean ± S.D of 6 replicates expressed in cm/plant)

Table 2
NaCl stress induced changes in photosynthetic pigments of P. miliacium at different stages of growth.

Growth Stages	Control	50 mM	100 mM	150 mM	200 mM
Chlorophyll a					
30 DAP	0.504 ± 0.092	0.483 ± 0.023	0.475 ± 0.015	0.421 ± 0.034	0.403 ± 0.010
40 DAP	0.562 ± 0.043	0.549 ± 0.117	0.532 ± 0.023	0.481 ± 0.006	0.430 ± 0.004
50 DAP	0.571 ± 0.043	0.563 ± 0.044	0.534 ± 0.302	0.499 ± 0.043	0.442 ± 0.007
60 DAP	0.577 ± 0.014	0.570 ± 0.044	0.536 ± 0.002	0.500 ± 0.111	0.458 ± 0.203
Chlorophyll b					
30 DAP	0.276 ± 0.054	0.231 ± 0.003	0.173 ± 0.010	0.121 ± 0.042	0.101 ± 0.011
40 DAP	0.296 ± 0.081	0.253 ± 0.014	0.221 ± 0.043	0.154 ± 0.001	0.104 ± 0.041
50 DAP	0.307 ± 0.003	0.263 ± 0.008	0.224 ± 0.001	0.175 ± 0.039	0.116 ± 0.018
60 DAP	0.322 ± 0.061	0.283 ± 0.080	0.227 ± 0.007	0.179 ± 0.017	0.120 ± 0.030
***Chlorophyll a/b* ratio**					
30 DAP	1.826 ± 0.004	1.558 ± 0.003	2.746 ± 0.032	3.479 ± 0.108	3.990 ± 0.017
40 DAP	1.899 ± 0.156	2.170 ± 0.291	2.407 ± 0.073	3.123 ± 0.091	4.135 ± 0.072
50 DAP	1.848 ± 0.001	2.141 ± 0.603	2.384 ± 0.003	2.851 ± 0.102	3.810 ± 0.301
60 DAP	1.792 ± 0.200	2.014 ± 0.821	2.361 ± 0.710	2.793 ± 0.004	3.650 ± 0.025

(Values are mean ± S.D. of 6 samples, n-6 and expressed in mg/g fresh weight)

ACKNOWLEDGMENTS

Authors want acknowledge UGC-BCR for providing the financial support for carrying out this project.

REFERENCES

1. Athar HR, M. Ozturk and Ashraf M, Ed. Strategies for crop improvement against salt and water stress: An overview. In: Salinity and water stress: Improving crop efficiency. Springer-Verlag, The Netherlands, 44–256, (2009).
2. Bray EA, Bailey-Serres J, Weretilnyk E, Ed. Responses to abiotic stresses. Biochemistry and Molecular Biology of Plants, American Society of Plant Biologists, Rockville. 158–1249, (2000).
3. Anitha T and Usha R. Effect of salinity stress on physiological, biochemical and antioxidant defense systems of high yielding cultivars of soyabean. International journal of Pharama and Biosciences, 851 – 864, (2012).

4. Kingsbury RW, Epstein E. Salt sensitivity in wheat-A case for specific ion toxicity. Plant Physiol. 80,651-654, (1986).
5. Chinnusamy V, Jagendorf A, Zhu JK. Understanding and improving salt tolerance in plants. Crop Sci, 45:437–448, (2005).
6. Munns R, Tester M. Mechanisms of Salinity Tolerance. Annual Review of Plant Biology, 59:651-681, (2008).
7. Zhifang G, Loescher WH. Expression of a celery mannose-6-phosphate reductase in Arabidopsos thaliana enhances salt tolerance and induces biosynthesis of both mannitol and a glucosyl-mannitol dimmer. Plant Cell Environ, 26:275-283, (2003).
8. Ghoulam C, Foursy A, Fares K. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ. Exp. Bot, 47:39-50, (2002).
9. Parida AK, Das AB, Das P. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, Bruguiera parviflora, in hydroponic cultures. J. Plant Biol, 45:28-36, (2002).
10. Niknam V, Bagherzadeh M, Ebrahimzadeh H, Sokhansanj A. Effect of NaCl on biomass and contents of sugars, proline and proteins in seedlings and leaf explants of Nicotiana tabacum grown in vitro, Biol. Plant, 48:613-615, (2004).
11. Cherian S, Reddy MP, Ferreira RB. Transgenic plants with improved dehydration- stress tolerance: progress and future prospects. Biol. Plant, 50:481-495, (2006).
12. Rahman MS, Miyake H, Takeoka Y. Effects of exogenous glycinebetaine on growth and ultrastructure of salt-stressed rice seedlings (Oryza sativa L.). Plant Prod. Sci, 5:33-44, (2002)
13. Attipali RR, Kolluru VC, Munusamy V. Drought induced responses of photosynthesis and antioxidant metabolism in higher plants. J Plant Physiol, 161:1189–1202, (2004).
14. Arnon DI. Copper enzymes in isolated chloroplasts. polyphenoloxidase in beta vulgaris. Plant physiol, 24:1-15, (1849).
15. Grieve CM, Grattan SR. Rapid assay for determination of water soluble quaternary ammonium compounds. Plant Soil, 70:303– 307, (1983).
16. Bates S, Waldren RP, Teare ID. Rapid determination of the free proline in water stress studies. Plant Soil, 39:205–208, (1973).
17. Robyt JF, White BJ. Biochemical Techniques- Theory and Practice, 267–275, (1987).
18. Moore S, Stein WH. Photometric method for use in the chromatography of amino acids. J. Biol. Chem, 176,367-388, (1948).
19. Cavalcanti F, Lima JP, Silva S, Viegas R, Silveria J. Roots and leaves display contrasting oxidative response during salt stress and recovery in cowpea, Journal of Plant Physiology., 164: 591-600, (2007)
20. Munn R. Comparative physiology of salt and water stress, Plant cell and environment, 25:239-250, (2002)
21. Misra N, Murumu B, Singh P, Misra M. Growth and proline accumulation in mungbean seedlings as effected by sodium chloride, Biol Plant, 38,531, (1996)
22. Ndayiragije A, Lutts SD. Do exogeneous polyamines have an impact on the response of a salt sensitive rice culivar to NaCl?, J. Plant Physiol, 163, 506-16, (2006).
23. Misra N, Gupta AK, Dwivedi UN. Changes in free amino acids and stress protein synthesis in two genotypes of green gram under salt stress. J Plant Sci, 1:56-66, (2006).
24. Turan M.A, Hassan A, Elkarim A, Taban N, Taban S. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentration on maize plant', African Journal of Agricultural Research, 4:893-897, (2009).
25. Najafi F, Khavari RA, Rastgar F, Sticklen M. 'Physiological changes in Pea (Pisum sativum L. cv. Green Arrow) under NaCl

salinity', Pakistan Journal of Biological Sciences., 9(5):974-978, (2006).
26. Fang Z, Boukamp JC, Solomos T. Chlorophyllase activities and chlorophyll degradation during leaf senescence in nonyellowing mutant and wild type of Phaseolus vulgaris L. J Exp Bot, 49,503–510, (1998).
27. Ashraf M, Foolad MR. Pre-sowing seed treatment-a shotgun approach to improve germination, plant growth and crop yield under saline non-saline conditions. Advances in Agronomy, 88:223-271, (2005).
28. Molazem D, Qurbanov EM, Dunyamaliyev SA. Role of Proline, Na and Chlorophyll Content in Salt Tolerance of Corn (Zea mays L.). American-Eurasian J. Agric. & Environ. Sci, 9(3): 319-324, (2010).
29. Bohnert HJ, Jensen RG. Strategies for engineering water stress tolerance in plants, Trends Biotech, 14,89–97, (1996).
30. Arakawa K, Katayama M, Takabe T. Levels of betaine and betaine aldehyde dehydrogenase activity in the green leaves and etiolated leaves and roots of barley. Plant Cell Physiol, 31:797–803, (1990)
31. Mohanty A, Kathuria H, Ferjani A, Sakamoto A, Mohanty P, Murata, Tyagi AK. Transgenics of an elite indica rice variety Pusa Basmati 1 harbouring the codA gene are highly tolerant to salt stress. Theor. Appl. Genet, 106,51–57, (2002).
32. Yang WJ, Rich PJ, Axtell JD, Wood KV, Bonham CC, Ejeta G, Mickelbart MV, Rhodes D. Genotypic variation for glycinebetaine in sorghum, Crop Sci, 43:162–169, (2003).
33. Delauney AJ, Verma DPS. Proline biosynthesi and osmoregulation in plants. Plant Journal, 4:215-223, (1993).
34. Misra N, Gupta AK. Effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content in Catharanthus roseus seedlings. J. Plant Physiol, 163(1):11-8, (2006).
35. Zhu X, Gong H, Chen G, Wang S, Zhang C. Different solute levels in two spring wheat cultivars induced by progressive field water stress at different developmental stages. J Arid Environ, 62,1–14, (2005).
36. Yusuf M, Hasan SA, Ali B, Hayat S, Fariduddin Q and Ahmad A. Effect of salicylic acid on salinity-induced changes in Brassica juncea. J. Integ. Plant Biol, 50:1-7, (2008).
37. Devirami GVNS and Gyana prasuna R. Effect of fungicides on proline content of Nostac species. Int J Pharm Bio Sci, 3(4),152–157, (2012).
38. Murakeozy EP, Nagy Z, Duhaze C, Bouchereau A, Tuba Z. Seasonal changes in the levels of compatible osmolytes in three halophytic species of inland saline vegetation in Hungary. J. Plant Physiol, 160:395-401, (2003).
39. Garg AK, Ownes JK, Wu RJ. Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stress. Proc Natl Acad Sci U S A, 99:15898-15903, (2002).
40. Martino CD, Delfine S, Pizzuto R, Loreto F, Fuggi A. Free amino acids and glycinebetaine in leaf osmoregulation of spinach responding to increasing salt stress. New Phytol, 158:455-463, (2003).
41. Fougere F, Le-Rudulier D, Streeter JG. Effects of salt stress on amino acid, organic acid, and carbohydrate composition of roots, bacteroids and cytosol of alfalfa (Medicago sativa L.). Plant Physiol, 96:1228-1236, (1991).
42. Hsu SY, Hsu YT, Kao CH. The effect of polyethylene glycol on proline accumulation in rice leaves. Biol. Plant. 46:73-78, (2003).
43. Atienza SG, Faccioli P, Perrotta G, Dalfino G, Zschiesche W, Humbeck K, Stanca AM., Cattivelli L. Large-scale analysis of transcripts abundance in barley subjected to several single and combined abiotic stress conditions, Plant Sci, 167:1359-1365, (2004).
44. Shao H.B, Chuc LY, Wu G, Zhang JH, Lua ZH, Hug Y. Changes of some anti-oxidative physiological indices under soil

water deficits among 10 wheat (Triticum aestivum L.) genotypes at tillering stage. Colloids and Surfaces B: Biointerfaces, 54:143–149, (2007).
45. Mansour, MMF Nitrogen containing compounds and adaptation of plants to salinity stress. Biol. Plant, 43:491-500, (2000).
46. Tan Y, Zongsuo L, Hongbo S, Feng D. Effect of water deficits on the activity of anti-oxidative enzymes and osmoregulation among three different genotypes of Radix astragali at seedling stage. Coll. Surf. B. Biointerfaces, 49:60-65, (2006).
47. Maricle BR., Cobos DR, Campbell CS. Biophysical and morphological leaf adaptations to drought and salinity in salt marsh grasses. J. Environ. Exp. Bot, 60:458-467, (2008).