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Research Article

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Antioxidant marker Response of *Solanum melongena* to salinity stress

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ABSTRACT

Salinity stress has been a major factor, limiting the growth and productivity of the Solanum melongena (Brinjal) plants in the temperate regions. A pot experiment was conducted on 60 days old plants. Four replicates of the plant were subjected to stress levels of 25 mM NaCl and 50 mM NaCl for duration of 10 days with 3 day intervals. The response of S. melongena to this salinity stress was attributed by decrease in total chlorophyll and elevated levels of other stress biomarkers such as Proline, Flavonoids, Ascorbic acid (ASC) and Glutathione (GSH). Salinity induces generation of reactive oxygen species (ROS) which occurs via electron transport reactions in the mitochondria and chloroplasts. ASC and GSH are key components of non-enzymatic antioxidant system in Brinjal contributing to scavenging of ROS. Parallel elevation of these two antioxidants under salinity suggests efficient operation of GSH-cycle.

Key words: Ascorbate, Flavonoids, Glutathione, Proline, *Solanum melongena*, salinity.

INTRODUCTION

Abiotic stresses trigger a wide range of plant responses, from altered gene expression and cellular metabolism to changes in growth rates and crop yields. The duration, severity, and the rate at which the stress is imposed influence how a plant responds. Several adverse conditions in combination may elicit a response different from that of a single type of stress. Features of the plant, including organ or tissue identity, development age, and genotype, too influence the plants response to stress.

Salinity affected area is increasing day by day and spreading all over the country [1]. 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 [2]. Salinity can reduce evapo-transpiration by making soil water less available for plant and reduces potential energy of soil water solution[3]. Brinjal (*S. melongena*) belonging to Solanaceae family is considered one of the most popular vegetable in India. Its demand is increasing day by day throughout the year while production is far from the requirement and or varies year to year.

Brinjal or eggplant (*S. melongena* Linn.), is a very important common vegetable in India. It is often described as a poor person's vegetable because it is popular amongst small-scale farmers and low income consumers. It is featured in the dishes of virtually every household in India, regardless of food preferences, income levels and social status[4]. Low in calories and high in nutrition, the vegetable has very high water content and is a very good source of fibre, calcium, phosphorus, foliate, and vitamins B and C [5]. It is also used in Ayurveda medicine for curing diabetes, hyper-tension and obesity. In addition, dried brinjal shoots are used as fuel in rural areas [4].

Salt stress causes inhibition of growth and development, reduction in photosynthesis, respiration, and protein synthesis [6] [7]. Chlorophyll loss and accumulation of Proline is widely accepted as a marker for salt/drought stress [8][9][10], which protects the proteins against denaturation and also act as osmotic balancing agents[11][12]. Therefore, identifying which responses promote or maintain plant growth and development during stress is

important for understanding the stress response process. The ability to withstand stresses frequently becomes the limiting factor for plant growth, survival and geographical distribution. The study of the behaviour of plants under stress is of practical importance from the point of view of agricultural yield. The current study aims at identifying the morphological changes in the plant growth and development caused due to salinity stress and also to check for the varied levels of enzyme biomarkers during stress.

EXPERIMENTAL SECTION

Growth and stress conditions: The experiment was conducted on 60 days old plants growing under natural greenhouse conditions; day/night temperature and relative humidity were: 30/25 °C and 75/70 % respectively. The average photoperiod was 12 h light/12 h dark. Four replicates were taken wherein two of the replicates were subjected to 25 mM NaCl and other two to 50 mM NaCl on every third day for duration of 10 days.

Total Chlorophyll**:** The procedure of chlorophyll determination was based on the work of Mackinney[13]on the absorption of light by aqueous acetone (80%) extracts of chlorophyll at 663 and 645 nm. The concentrations of total chlorophyll, chlorophyll-a, -b and total chlorophyll were calculated by the formula of Arnon[14]as given below:

$$\text{Chl a (mg g}^{-1}) = [(12.7 \times A663) - (2.6 \times A645)] \times (V / 1000 \times wt)$$
$$\text{Chl b (mg g}^{-1}) = [(22.9 \times A645) - (4.68 \times A663)] \times (V / 1000 \times wt)$$
$$\text{Total Chl} = [(20.2 \times A645) + (8.02 \times A663)] \times (V / 1000 \times wt)$$

The chlorophyll stability index (CSI) was calculated according to the method of Murty and Majumdar [15] as the ratio between Chlorophyll content in stressed leaves and Chlorophyll content in control leaves and expressed in % [14].

Proline: The estimation was carried out according to the method of Bates [16]. Free proline was extracted from 100 mg of fresh tissue in 5 ml sulphosalicylic acid (3%) using a chilled pestle and mortar. The extract was filtered through Whatman No. 1 filter paper. The filtrate was collected and to this equal amount of Acid Ninhydrin reagent and equal volumes of glacial acetic acid was added (i.e., 5 ml of filtrate was obtained and to this 5 ml of each of the mentioned reagents were added). The contents were boiled for 1 h in a boiling water bath and cooled rapidly on ice. The colour was extracted in 4 ml toluene by vigorous shaking and the organic phase recorded at 520 nm against toluene as blank. Standard curve was prepared for different concentrations of Proline [16].

Total flavonoids: The estimation was carried out according to the method of Chang et al., (2002). Flavonoids were extracted from 1g of fresh tissue in 8 ml methanol and 2 ml distilled water using a chilled pestle and mortar. The extract was filtered using Whatman No. 1 filter paper and the filtrate was left exposed to air. Once evaporated partially, 6 drops of 2 M sulphuric acid was added to it followed by the addition of chloroform to the mixture in a 3:1 ratio. The chloroform layer was separated out using a separating funnel and was then exposed to air for 12 h. Once completely evaporated, the residue was dissolved in minimal amount of methanol and used for the estimation of flavonoids. Absorbance was read at 670 nm and the concentration of flavonoids was calculated from the standard graph [17].

Ascorbic Acid: Ascorbic acid estimation was carried out according to the procedure of Sadasivam and Manickam[18]determined by using the principle of oxidation of L-Ascorbic acid to dehydroascorbic acid and 2,3-diketogulonic acid followed by reaction with 2,4-dinitrophenylhydrazine. The leaf tissue (200 mg) from both control and stress plant was homogenized in 6 mL of 4% oxalic acid using a chilled pestle and mortar, followed by centrifugation at 4000 rpm for 10 min. The assay mixture consisted of 0.1 ml of brominated sample extract made up to 3.0 mL with distilled water, 1.0 ml of 2% DNPH reagent and 1-2 drops of thiourea. After incubation at 37 ºC for 3 h, the orange-red osazone crystals formed were dissolved by the addition of 7.0 ml of 80% sulphuric acid and absorbance was read at 540 nm [18].

Reduced Glutathione: GSH was estimated according to Beutler [19]. The tissue was homogenized with 3% metaphosphoric acid. DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] was added to supernatants cleared by centrifugation. The formation of 5-thio-2-nitrobenzoic acid, which is proportional to total glutathione concentration, was monitored at 412 nm at 25 °C against reagent controls [19].

RESULTS AND DISCUSSION

Chlorophyll: In the present study, the leaf chlorophyll content declined (Table 1, Fig 1) suggesting that salinity injury may involve severe chlorophyll photo-oxidation mediated by oxy-radical [20]. In addition to oxidative

damage, increased chlorophyllase activity and salt-induced weakening of protein-pigment-lipid complexes [21] have been implicated in chlorophyll degradation during stress conditions. Thus, the observed reduction in chlorophyll content under NaCl stress could be a result of both decreased synthesis and increased degradation. Decreased or unchanged chlorophyll level during salt stress has been reported in many species, depending on the duration and severity of stress [22]. Supporting results also include studies on bean plant *Phaseolus vulgaris* [23],*Catharanthus roseus*[24], cowpea (*Vigna unguiculata L.*)[25], *Vigna subterranean* [26] and Field bean (*Lablab purpureus*)[27] demonstrate that NaCl stress caused a decrease in total chlorophyll content. The chlorophyll stability index (CSI), an indicator of the stress tolerance capacity of plants exhibited a significant reduction under NaCl stress.

Table 1: Levels of total Chlorophyll and CSI % in leaves of Brinjal plants subjected to 25 mM and 50 mM NaCl stress

Sample	Total Chlorophyll (mg/g FWt)	Chlorophyll Stability Index (CSI) (%)
Control	3.529 ±0.9	100
Sample A	3.482 ±0.9	98.66
Sample B	2.042 ±0.5	57.86
Sample C	2.256 ±0.8	63.92
Sample D	2.156 ±1.3	61.09

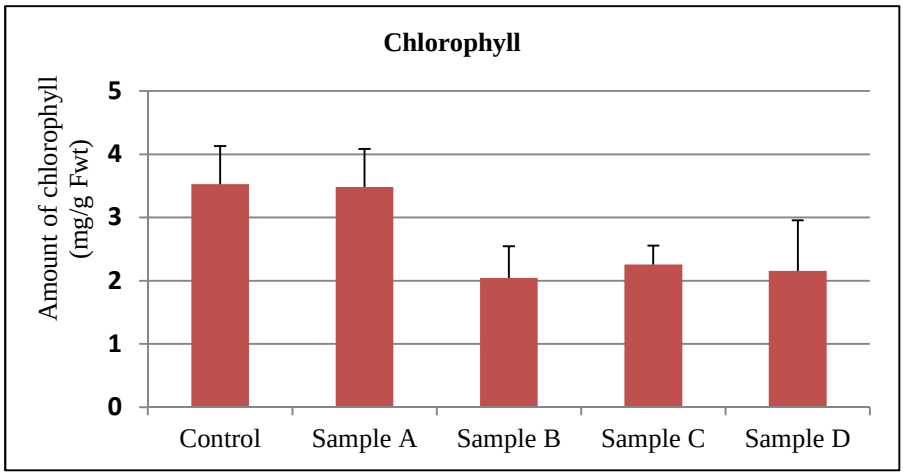


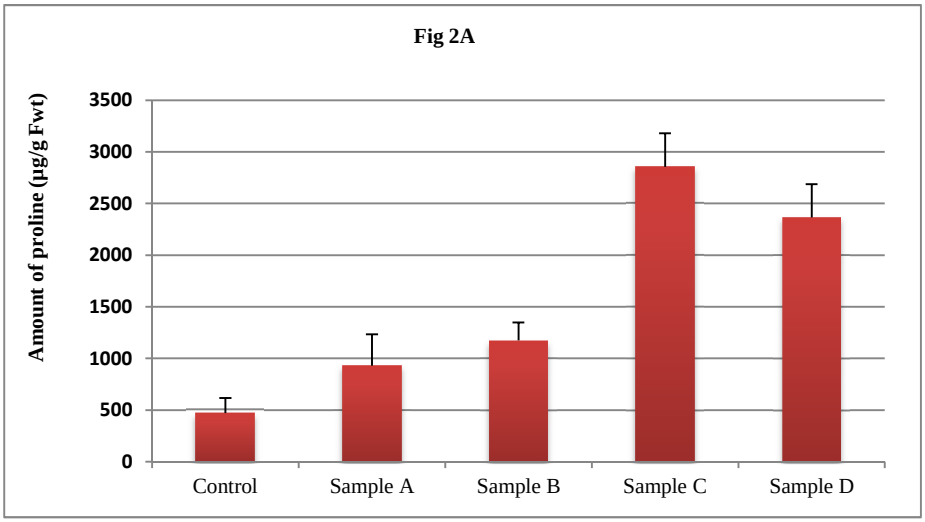
Fig 1: Levels of Total Chlorophyll of leaf tissue of Brinjal after treatment with 25 mM NaCl 3DAS (Sample A) and 5DAS (Sample B); 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D). Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$)

Proline: Osmoprotectants like proline are compounds that share the property of being uncharged at neutral pH and have high solubility in water. Moreover, at high concentrations they have little or no perturbing effect on macromolecule-solvent interactions. Therefore it may be suggested that increasing levels of proline helps to protect membranes from oxidation instead of osmotic regulation as an initial response to stress. Free proline has been found to act as a protein stabilizer, a metal chelator, an inhibitor of lipid peroxidation, and O_2^- free radical scavenger[28][29]. The osmolyte, Proline levels in leaves were elevated under salt stress (Table 2, Fig 2A). The effect was moderate up to 25 mM NaCl during the entire period of exposure. Proline content showed greater improvements at higher concentration i.e., 50 mM NaCl. An increase in proline in salt stress plants has been reported by a number of researchers [27][30][31].

Total Flavonoid content: Flavonoids are among the most bioactive plant secondary metabolites outperforming well known antioxidants, such as ASC and α- tocopherol in scavenging ROS formed under adverse environmental conditions [32]. The most abundant flavonoids, flavonols accumulate in their glycosylated form after an inductive light treatment and absorb UV-B light in the 280-320 nm region and are therefore regarded as effective UV filters [33]. The amount of total flavonoids was found to increase in stressed plants (Table 2, Fig 2B).

Table 2: Levels of Proline and Total Flavonoid in leaves of Brinjal plants subjected to 25 mM and 50 mM NaCl stress

Sample	Proline (µg/g FW tissue)	Total Flavonoid content (mg/g FW tissue)
Control	470 ± 147	101.3 ±5.13
Sample A	930 ± 304	138.3 ±45.2
Sample B	1173 ± 175	98.66 ±1.52
Sample C	2855 ± 426	469.33 ±53
Sample D	2366 ± 321	287 ± 50.8



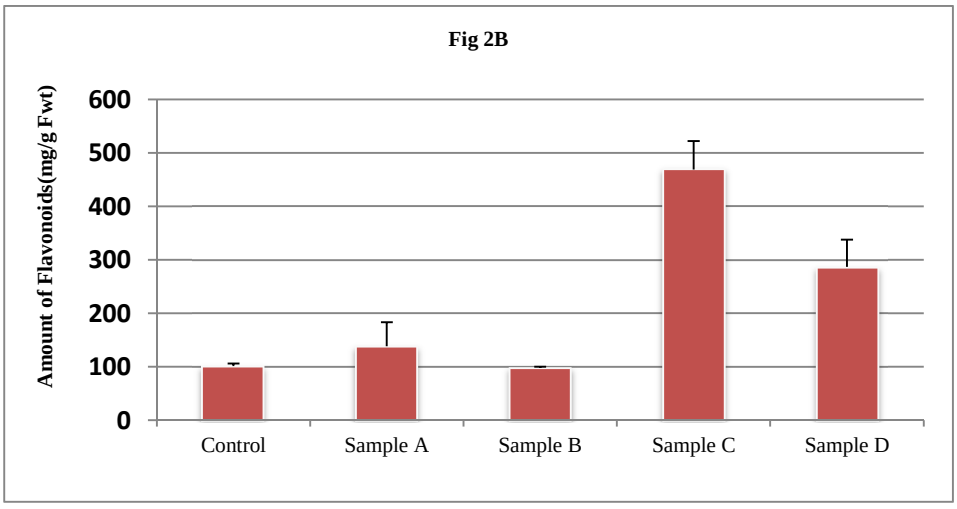


Fig 2: Proline (2A) and Flavonoid (2B) content of leaf tissue of Brinjal after treatment with 25 mM NaCl, 3DAS (Sample A) and 5DAS (Sample B); 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D). Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$)

Response of Ascorbate and Reduced Glutathione to salinity stress: The intense generation of reactive oxygen species (ROS) is one of the reasons of disturbances in physiological process in plants exposed to abiotic factors. In these cases, the development of the oxidative stress could be considered as a disturbance in the balance between ROS production and functioning of the antioxidant system in the plant cell. Plant adaptation to various stressors depends largely on both the functioning of antioxidant enzymes, such as superoxide dismutase (SOD), guaiacol peroxidases (POX), catalase (CAT), ascorbate peroxidise (APX), glutathione reductase (GR) and others; and on the accumulation of low molecular weight antioxidant[34]. The latter are more efficient in countering ROS than antioxidant enzymes [35][36]. The impact of the oxidative stress depends on the interaction of several factors that determine the antioxidant status of the plant. Ascorbate (ASC), the key antioxidant in plants reacts directly with hydroxyl radicals, superoxide and singlet oxygen [37]. As a powerful reducing agent, ASC maintains chloroplastic α-tocopherol and metalloenzyme activity and acts as a reductant in enzymatic reactions and in nonenzymatic free radical scavenging of superoxide and H_2O_2[38]. ASC also plays an essential role in plant growth and development and has been implicated in many physiological responses [39]. ASC levels in leaves of stressed seedlings decreased

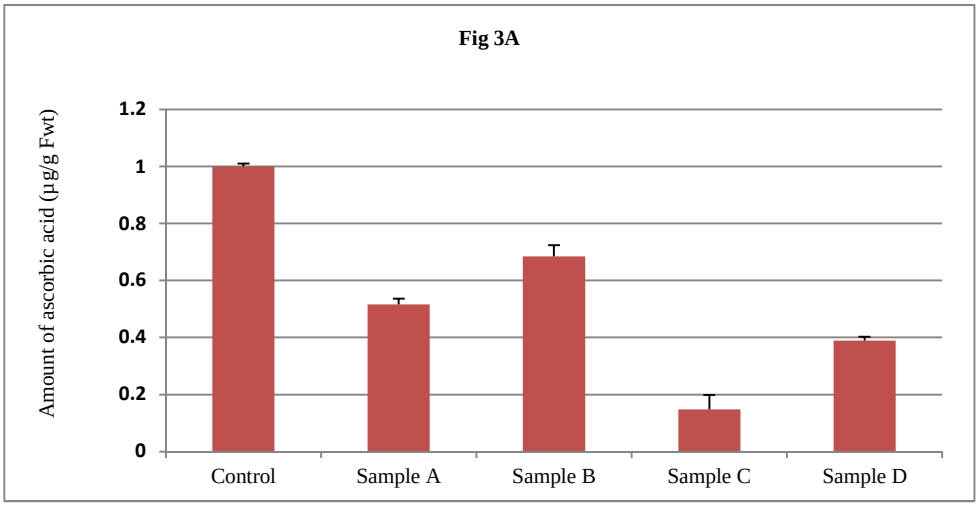
progressively with exposure time and concentration (Table 3, Fig 3A), indicating effective scavenging of ROS in Brinjal. This can be accounted for on the basis of ascorbic acid oxidase activity that stimulates the oxidation of ascorbic acid, inhibiting oxidation of the cell materials and promoting survival. The basal and induced levels of ASC appear to differ in plant species depending on the necessity generated by the plant's environment and physiological situation. As ASC is easy to recycle by dehydration reaction with tocopherol and it does not interfere with light absorption, plants use it more widely as a scavenger than flavonoids. Both oxidized forms of ASC are relatively unstable in aqueous environments while dehydroascorbate (DHA) can be chemically reduced to GSH to ASC[40]. The regeneration of reduced ASC is extremely important because the fully oxidized DHA has a short half life and would be lost unless it is reduced back. Improved tolerance to oxidative stress in *N. tabacum* and *Populus canescens* plants has been found to be associated with higher foliar concentration of ASC. Elevated levels of ASC have been reported under UV-B stress in higher foliar ASC contents and improved tolerance to oxidative stress in *C. auriculata* seedlings [41]. It was also reported that high light condition and drought significantly increases the ASC content in *P. asperata* seedlings [42]. Salt stress severely reduced growth of wheat (*Triticum aestivum L.*). Foliar spray with ASC improved the growth of non stressed plants, but did not alleviate the adverse effect of salt stress on plants .This indicates that foliar spray with applied ASC protected the photosynthetic machinery from the damaging effects of salt stress, it did not improve the growth of the wheat cultivars under saline conditions[43].

GSH plays an important role in the response of plants to environmental stresses [44]. In addition, GSH is actively involved in the cyclic transfer of reducing equivalents in the ascorbate/glutathione pathway[38], regulation of sulfate transport, signal transduction, conjugation of metabolites, detoxification of xenobiotics[45], redox regulation of the cell cycle[46]and the expression of stress-responsive genes[47]. The increase of GSH in leaf tissues (Table 3, Fig 3B) is considered to be responsible for generating ASC via the ascorbate-GSH cycle. Increase concentration of GSH has also been observed with the increasing Cd concentration in *P. sativum*[48], *Sedum alfredii*[49], *L. purpureus*[27]and *V.mungo*[50]. They also observed that plants with low levels of GSH were highly sensitive to even low levels of Cd^{2+} due to limited phytochelatin synthesis[45]. Reduced glutathione (GSH) protects the plant cells from the oxidative damage based on its redox buffering action and abundance in the cell. Involvement of GSH in the ascorbate-glutathione cycle results in its conversion to the disulfide form (GSSG), especially when plants are subjected to environmental stress that alters glutathione biosynthesis. It catalyses the reduction of the oxidized form of glutathione utilizing NADPH, and is thus important for maintaining the GSH pool [50][52]. Increase in cellular GSH levels by improving GSH biosynthetic capacity or through the manipulation of glutathione reductase activity that converts GSSG back to GSH was found to show an enhanced resistance to oxidative stress as well as to abiotic stresses in plants. A central nucleophilic cysteine residue is responsible for the high reductive potential of GSH, which scavenges cytotoxic H_2O_2 and reacts non- enzymatically with other ROS, such as O_2, O_2, and OH [38].

Table 3: Levels of Ascorbate and Reduced glutathione content in leaves of Brinjal plants subjected to 25 mM and 50 mM NaCl stress

Sample	Ascorbic acid (µg/g FW tissue)	Reduced glutathione (µg/g FW tissue)
Control	1.00 ± 0.01	20.15 ± 4.5
Sample A	0.5I6 ± 0.02	23.28 ± 0.1
Sample B	0.684 ± 0.04	24.84 ±1.18
Sample C	0.148 ± 0.05	27.63 ±3.35
Sample D	0.389 ± 0.014	58.44 ±3.90

Fig 3A



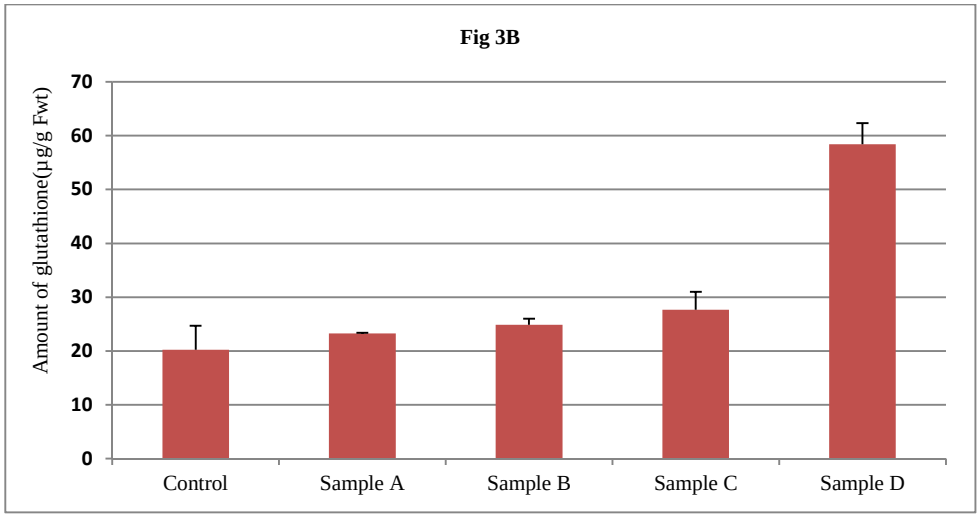


Fig. 3: ASC (3A) and GSH (3B) content of leaf tissue of Brinjal after treatment with 25 mM NaCl, 3DAS (Sample A) and 5DAS (Sample B) interval; 50 mM NaCl 3DAS (Sample C) and 5DAS (Sample D). Data plotted are mean ± SE of duplicates of three separate replicates, mean values were compared by one way ANOVA ($P \leq 0.05$)

CONCLUSION

In conclusion, the Brinjal plant is tolerant to salt up to 50 mM NaCl. Higher levels were found to be detrimental to growth. The antioxidant system in leaves involves the non-enzymatic components GSH, ASC and proline. The response of plants to salt stress is based on the transcriptional action of many defense proteins, and research has not discovered the basis for them all. Osmotic stress and ion toxicity are the problems stemming from salt stress, and the resulting decrease in chemical activity causes cells to lose turgor. Cell growth depends on turgor to stretch the cell walls, and lack of turgor implies danger for cell survival. The plant's defense against this salinity attack requires osmotic adjustment, and, to a certain degree this can be done through synthesis of intracellular solutes.

REFERENCES

[1] MRI Mondal, MS Islam, MAB Jalil, MM Rahman, MS Alam & MHH Rahman. Krishiprojuktihatboi (Handbook of Agro-technology), 5th edition. Bangladesh Agricultural Research Institute,**2011**, 407-408.
[2] C Sahi, A Singh, K Kumar, E Blumwald, A Grover. *Functional and Integrative Genomics* 6, **2006**, 263-284.
[3] RG Allen, LS Pereria, D Raes& M Smith. *FAO Irrigation and Drainage*, **1998,** Paper No. 56.
[4] B Choudhary, K Gaur; the Development and Regulation of Bt Brinjal in India (Eggplant/Aubergine), **2009**, ISAAA Brief No.38. ISAAA: Ithaca, NY.
[5] C Collonnier , I Fock, V Kashyap, G L Rotino, M C Daunay, Y Lian, I K Mariska, M V Rajam, A Servaes, G Ducreux, D Sihachakr, Applications of biotechnology in eggplant, Plant Cell, Tissue and Organ Culture 65, **2001**, 91-107.
[6] RL Levine, D Garland, CN Oliver, A Amici, I Climent, AG Lenz. *Methods in Enzymology*, **1990.**
[7] RK Sairam ,KV Rao, GC Srivastava. *Plant Science*, 163, **2002**, 1037-104.
[8] R Storey, RG Wyn-Jones. *Science Letter*, 4, **1975** , 161-168.
[9] CR Naik, CL Joshi. Ineffectual role of proline metabolism in salt stressed sugarcane leaves, Proceedings of the Indian Academy of Sciences 92,**1983**, 265-269.
[10] PB Kavi Kishor, S Sangam, RN Amrutha, PS Laxmi, NR Naidu, KRSS Rao, S Rao, KJ Reddy, P Theriappan, N Sreenivasulu. *Current Science* 88, **2005**, 424-438.
[11] SV Chadalavada, B Rajendrakumar, VB Reddy, AR Reddy. *Biochemical and Biophysical Research Communications* 201, **1994**,957-963.
[12] P Sivakumar, P Sharmila, P PardhaSaradhi. *Biochemical and Biophysical Research Communications*,**2000**, 279, 512-515.
[13] G Mackinney. *J. Biol. Chem.*,**1941**, 140, 315-322.
[14] DL Arnon, Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris, Plant Physiol*., 24, **1949**, 1-15.
[15] KS Murty and SK Majumder. *Curr. Sci.*, 31, **1962**, 470-471.
[16] LS Bates, RP Waldren and ID Teare. *Plant Soil*, 39, **1973**, 205-207.
[17] C Chang, M Yang, H Wen, J Chern. *J. Food Drug Analysis*, 10, **2002**, 178-182.

[18] S Sadasivam and A Manickam, Vitamins. In: Sadasivam S., Manickam A. (Eds.) *Biochemical methods*, 2nd Edition. New Age International (P) Limited, New Delhi, **1997**,185-186.
[19] E Beutler, O Duron and BM Kelly. *J. Lab. Clin. Med.*, 61, **1963**, 882.
[20] AK Parida, AB Das and P Das. *J. Plant Biol*., **2002,** 45, 28-36.
[21] RP Souza, EC Machado, JAB Silva, AMMA Lagoa and JAB Silveria. *Envir. Exp. Bot.*, 51, **2004**, 45-56.
[22] J Zhang and MB Kirkham. New Phytol., 132, **1996**, 361-373.
[23] MA Turan, V Kalkat, S Taban. *Int.J.Agric.Res*., 2(5), **2007**, 483-488.
[24] J Cheruth, G Ragupathi, K Ashot, M Paramasivam, S Beemarao, P. Rajaram. *Acta physiol. Plant.*, 30 (3), **2008**, 287-292.
[25] VD Taffouo, JK Kouamou, LMT Ngalangue, BAN Ndjeudji, A Akao. *Int. J. Bot.*, vol. 5(2)**, 2009**, 135-143.
[26] VD Taffouo, OF Wamba, E Yombi, GV Nono, A Akoe. *Int. J. Bot.*, Volume 6(1), **2010**, 53-58.
[27] MR Dsouza, VR Devaraj. *Acta. Physiol. Planta*, 32, **2010,** 341-353.
[28] M Ashraf and MR Foolad. Environ. Exp. Bot., 59, **2007**, 206-216.
[29] M Trovato, R Mattioli and P Costantino. *Rend Lincei-SciFis*., 19, **2008**, 325-346.
[30] E Bandeoglu, F Eyidogan, M Yucel, HA Oktem. *Plant Growth Regul* 42, **2004**, 69–77
[31] F Eyidogan, MT Oz. *Acta Physiol Plant*, 29, **2007**, 485–493
[32] T Lovdal , KM Olsen, R Slimestad, M Verheul, C Lillo. *Phytochemistry*, 71,**2010**, 605-613.
[33] A Solovchenko and M Schmitz-Eiberger, *J. Exp. Bot.*, 54, **2003**, 1977-1984.
[34] JM Cheeseman, *Plant Stress*, 1, **2007**,4-15.
[35] LN Kurganova, AP Veselov, TA Goncharova and V Sinitsyna Yu. *Russ. J. Plant Physiol*., 44, **1997**,630-635.
[36] O Blokhina, E Virolainen and KV Fagerstedt, *Ann. Bot.*, 91, **2003**,179-194.
[37] SS Gill and N Tuteja. *Plant Physiology and Biochemistry*, 48, **2010**, 909-930.
[38] G Noctor and CH Foyer. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 49, **1998**, 249-279.
[39] DM El Hariri, MS Sadak and HMS El-Bassiouny. *International Journal of Academic Research*, 2(6): **2010**, 101-109.
[40] CH Foyer and B Halliwell. *Planta*, 133, **1976**, 21-25.
[41] S Agarwal. *Biol. Plant*., 51, **2007**, 157-160.
[42] Y Yang, C Han, Q Liu, B Lin and J Wang. *Acta Physiol. Plant.*, 30, **2008**, 433-440.
[43] A Ameer, GS Markhand , AR Mahar , SaeedAbro and Nisar Ahmed Kanhar. *Pak.J.Bot.*, **2009**, 1303-1310.
[44] JM Ruiz and E Blumwald. *Planta*, 214, **2002**, 965-969.
[45] C Xiang, BL Werner, EM Christensen and DJ Oliver. *Plant Physiol.*, 126, **2001**,564-574.
[46] R Sanchez-Fernandez, M Fricker, LB Corben, NS White, N Sheard, CJ Leaver. *Proc. Natl. Acad. Sci. USA*, 94,**1997**, 2745- 2750.
[47] PM Mullineaux and T Rausch. *Photosynth. Res*., 86, **2005**, 459-474.
[48] A Metwally, VI Safronova, AA Belimov and KJ Dietz. *J. Exp. Bot.*, 56, **2005**, 167-17.
[49] RL Sun, Q X Zhou, FH Sun and C X Jin. *Environmental and Experimental Botany*, 60 (3), **2007**, 468-476.
[50] AS Molina, C Nievas, MVP Chaca, F Garibotto, U González, S M Marsá, C Luna, M S Giménez, and F Zirulnik. *Plant Growth Regul.*, 56, **2008**, 285-295.
[51] ASV ChalapathiRao and AR Reddy., Glutathionereductase: a putative redox regulatory system in plant cells. In: Sulfur Assimilation and Abiotic Stresses in Plants, N.A. Khan, S. Singh, S. Umar (Eds.), Springer, The Netherlands ,**2008**,111-147.
[52] AR Reddy and AS Raghavendra, Photooxidative stress. In: Physiology and Molecular Biology of Stress Tolerance in Plants, KV MadhavaRao, AS Raghavendra, KJ Reddy (Eds.), Springer, The Netherlands, **2006**,157-186.