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Effect of Salinity on Antioxidant, Proline and Ion Content in *Luffa acutangula*

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ABSTRACT

Increased human activities, improper irrigation and agriculture practices have led to the rise in the level of salt in agricultural land. This further leads to substantial decline in productivity of crops and vegetables. Other than effecting germination, salinity affects every aspect of vegetable crop development including their morphology, physiological function and yield. Although efforts have been made to understand the mechanisms of salt tolerance in vegetable crops, less attention has been paid to these than to the staple crops. The present study showed reduced antioxidative enzymes, higher K influxes and lower Na^+ influx to provide mitigation effect of salt stress. Salt stress also leads to aggravation of some Protein, proline levels and the cytosolic Na exclusion which cumulatively conferred some level of protection.

Key words *abiotic stress, salinity, antioxidant enzymes, osmolytes, cucurbits*

Agricultural productivity is often controlled by various biotic/abiotic factors that results in diminution of quality/quantity of the crop. Although wild populations and elite varieties have developed mechanisms to overcome these factors, however their application in agricultural practices are limited due to low yield and non availability of seeds. Hence, presently the major problems faced by cultivated crops are drought, water, and salt stress. Of these stress, soil salinity may be contributed due to weathering of parent rocks and minerals in the soil, seawater intrusion into coastal areas, rain water and wind borne materials from lake and other land surface (Plaut et al 2013). These factors may directly/indirectly increase the salinity of the soil and thereby affect the physiology, growth, yield, nutritional superiority and magnitude of crops. The concentration of salt may even stimulate water and ionic imbalance stress thereby further adding to reduced nutritional management collectively due to altered stomatal conductance, photosynthesis and diminutive growth (Yadav et al 2011).

Generally the response of plants to salt stress may be dependent on the growth stage of plant and the severity, interval/amount of exposure to salt stress. At the initial stage of salts stress the plants respond to osmotic shock by showing physiological alteration like interruption of membranes, impairment in the ability to detoxify ROS, difference in the antioxidants ripostes and osmolytes (Gupta and Huang 2014). The ROS production is either aggravated or mitigated by various enzymatic (CAT, SOD, POD, APX, GR, MDHA, DHAR) and non enzymatic antioxidant (ascorbates, glutathione, caratenoids, sugars and sugar alcohol, polyamine, proline, glycine betaine etc) defense mechanisms (Gupta and Huang 2014, Sofo et al 2015, Hanin et al 2016).

Despite the number of studies to ascertain mechanisms of salt tolerance, neither the metabolic site at which salt stress damages the plant nor the adaptive component of the salt tolerance to completely elucidated (Hanin et al 2016). Further salinity tolerance is unlikely to be determined by a single gene/ gene product, and may result from the expression of a number of genes. These in turn may be dependent on their expression and interaction with other biotic / abiotic tolerant genes and the time, stage, extent and dosage of exposure to these agents thereby making it an extremely complex research area.

Among the vegetables consumed by Indians, Luffa (family Cucurbitaceae) is distributed mainly in the tropical regions of the world. *Luffa acutangula* (L.) Roxb (ridged gourd) are widely cultivated in the plains and low hills of the country (Chandra, 1995) and thus may be influenced by salt stress. As a result of the long history of cultivation of Luffa in India under varied climatic, geographical and environmental conditions, a large numbers of variants have been developed from the cultivars through introgression and selection (Prakash et al 2014). The current agricultural practices have included the developed cultivars with high yield, while the wild genetic resources which harbor valuable genes for adaptation to diverse agro-ecological zones, and resistance to diseases, pests and stress environments have slowly been discontinued. Studies on change in morphological, physiological, ion accumulation (Balkaya et al 2016, Ismail 2015, Jafari et al 2015) in cucurbits are reported. Such studies are scarce in *Luffa acutangula.*

The present study hence deals with the preliminary analysis of some of the oxidative response (enzymic and non-enzymic) and mineral elements among two varieties of *Luffa acutangula* (L.) Roxb cultivated in India. In this study, we attempted to comprehend the different influences of various concentrations of single salt (NaCl) at seedling stage of *Luffa acutangula*. The effort helped us to understand the riposte of antioxidants displayed and the mineral ion ratio that may mitigate the effect of salt stress.

MATERIALS AND METHODS:

Two varieties of *Luffa acutangula*, viz., Mumbai local (MLV: Namdeo Umaji, Mumbai) and F1 Hybrid (JLV: Seedco quality, Aurangabad) was surface sterilization, imbibed (48 hours), treated at 50, 100 and 200 mM NaCl (48 hours at room temperature). Control and treated seeds were germinated, (garden soil and sand at 2: 1 ratio) along with half strength Hoaglands media in growth chamber (Green house, D. Y. Patil University, Belapur, Navi Mumbai) at temperature of 25°C and relative humidity of 65-75 %. The analyses were performed after 96 hours of seedling growth. Leaf tissues were collected, weighed and homogenized in an ice-chilled pestle and mortar with respective extraction buffer. All the assays were performed in triplicates. Enzyme activity was expressed as enzyme U/minute/g sample.

Table 1. The effect of salt stress on antioxidative enzymes in *Luffa acutangula* varieties.

Treatments	Catalase U/min/gm		SOD U/min/gm		POD U/min/gm		GR U/min/gm	
	JLV	MLV	JLV	MLV	JLV	MLV	JLV	MLV
Control	0.020	0.095	0.004	0.002	0.003	0.036	0.002	0.014
50 mM	0.005	0.001	0.021	0.001	0.002	0.002	0.002	0.0032
100 mM	0.033	0.007	0.006	0.004	0.002	0.014	0.011	0.008
200 mM	0.006	0.004	0.033	0.000	0.006	0.004	0.013	0.011

Catalase activity was assayed (Luck 1974) with slight modifications. Reaction mixture (1.9ml water, 1ml 0.059M H_2O_2) was allowed to stand (4-5 minutes) before addition of 0.1 ml of plant extract. One enzyme unit was calculated as the amount of enzyme required to decrease the absorbance at 240nm by 0.05 units per minute. The SOD level was measured according to Kakkar *et al.* (1984). The assay mixture contained 0.5ml of plant extract, 1ml of 125mM sodium carbonate, 0.4ml of 25 ìM NBT, 0.2ml of 0.1mM EDTA. After adding 0.4 ml of 1mM hydroxylamine hydrochloride, absorbance was measured at 560nm. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute. The method proposed by Reddy *et al*. (1995) was adopted for assaying the activity of peroxidase (POD). For glutathione reductase assay David and Richard (1983) procedure was followed. The H_2O_2 and proline content was measured spectrophotometrically by Loreto and Velikova 2001. Determination of mineral elements and protein content was done according to Mala et al (2016) and Lowry's method (1951).

All the parameters studied were analyzed statistically using Sigma Stat Statistical Package (Version 14.0). One way ANOVA with $P<0.05$ was considered significant; Post-hoc Fischer analysis was done to test the concentration and varieties of statistical significance.

RESULTS AND DISCUSSION

Currently information about the effects of salt stress on *Luffa acutangula* growth and development are scarce. Salt stress caused substantial decreases in the growth of both plant varieties. These results are parallel to what had been already observed in different crops such as pearl millet (Sneha et al 2014), onion (Correa et al 2013), Potato (Habib et al 2016). The level of catalase was higher than SOD, POD and GR in both the control plants (Table 1). Catalase of both plants significantly changed under increasing salt stress conditions. In control, catalase was higher in MLV, but JLV had higher catalase than MLV at 50 and 100 mM. In general both the varieties show a decrease in catalase level at 50 mM and 200 mM treatment. In JLV plants, in comparison of control (0.004 U /min/ gm/) there in an increase in SOD activity at 50 mM and 200 mM salt treatment. However, in comparison with the control of MLV a remarkable decrease in SOD activity was observed with increase in salt concentration (Table 1).

Peroxidase enzyme was seen (ten times) higher in control MLV than JLV plants (Table 1). With increase in salt concentration the level of POD decreased, however at 200 mM treatment JLV had slightly higher POD levels than MLV. In untreated plants, MLV had higher glutathione reductase than in JLV (Table 1). An increase in GR activity with increase in NaCl concentration from 50 mM to 200 mM was displayed only by JLV while MLV showed a decrease in the value. Higher activity of GR was observed in the both plants under 200 mM NaCl.

Like humans plant researchers are also utilizing H_2O_2 as one of the candidate biochemical biomarker for stress. However, levels of H_2O_2 in natural plants are also poorly established, as it spans different order of magnitude within species (Cheeseman 2006). Our study attempted to compare the levels of H_2O_2 at early morning stage for both the varieties in presence and absence of salt and its correlation

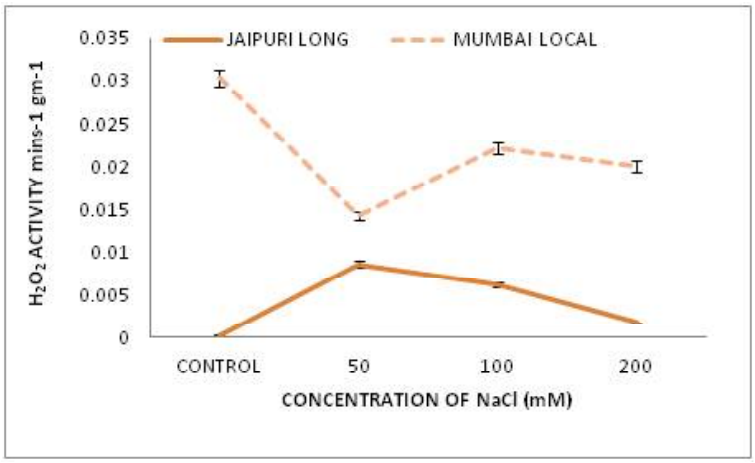

Fig. 1. Effect of salt on levels of H_2O_2 in MLV and JLV

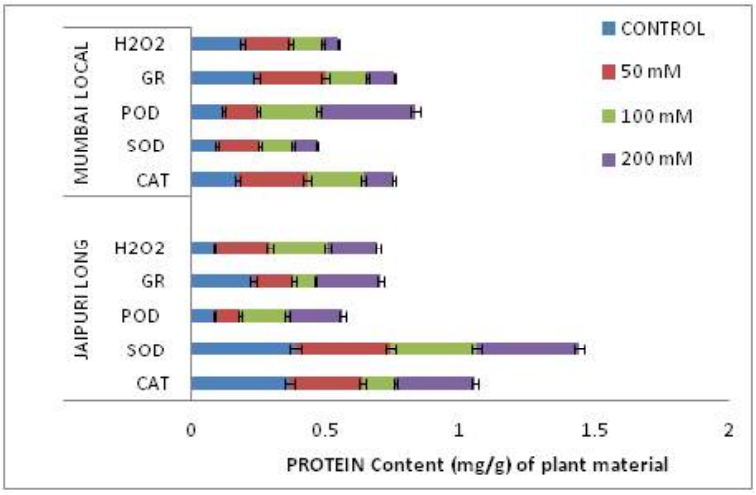

Fig. 2. Amount of protein content in enzyme preparations of JLV and MLV in control and salt treated leaf.

with the levels of antioxidants enzymes synthesized in the plants. The analysis showed control and salt treated MLV had more H_2O_2 than JLV. However with increasing salt stress, JLV plants showed decreasing H_2O_2 (Fig1).

The present study indicates reduced CAT and SOD activity in MLV than JLV, however increased POD, GR and H_2O_2 levels. This may reflect that JLV has better ROS scavenging capacity and less injured lipids of plasma membrane under stress condition than MLV. Previous study on plants with higher levels of antioxidants, either constitutive or induced have revealed greater resistance to oxidative damage (Weisany et al 2012). However this study indicates the effect of varying salt concentration on Luffa to have a gradual difference in the activity of enzymes as compared with control which have led to denaturation/ inactivation of enzymes. It may be possible that levels of CAT and SOD in JLV are constitutively high and are not induced due to salt stress, while in MLV the levels of SOD and CAT were lower indicating that MLV possibly has a different mechanism to tackle the ROS generated by salt stress. This is evident by increase in POD and GR levels. Also due to less CAT in MLV the accumulation of H_2O_2 is higher in MLV which may be responsible to cause oxidative damage to the plant.

The constant decreases in CAT and SOD enzyme at all the three levels i.e, between varieties; salt concentration and salt with variety were observed to be significant ($P \leq 0.05$). The changes in POD/GR were scored to be insignificant statistically ($P \leq 0.05$), thus it can be concluded that the changes in CAT/SOD are directly proportional to salt concentration and appears to be primary response of these Luffa varieties to mitigate the induced salt stress., while the POD/GR appears to be activated in response to induced metabolic changes caused by other factors like SOD, CAT and /or other phytometabolites/ionome variation under salt stress.

The decrease in protein content both for CAT and SOD was remarkably higher in MLV than in JLV (Fig 2). However for POD assay, the protein level increases with an increase in salt concentration for JLV and MLV varieties. While in GR enzyme, the protein content decreased in JLV with increase in level of salt treatment from 50 mM to 200

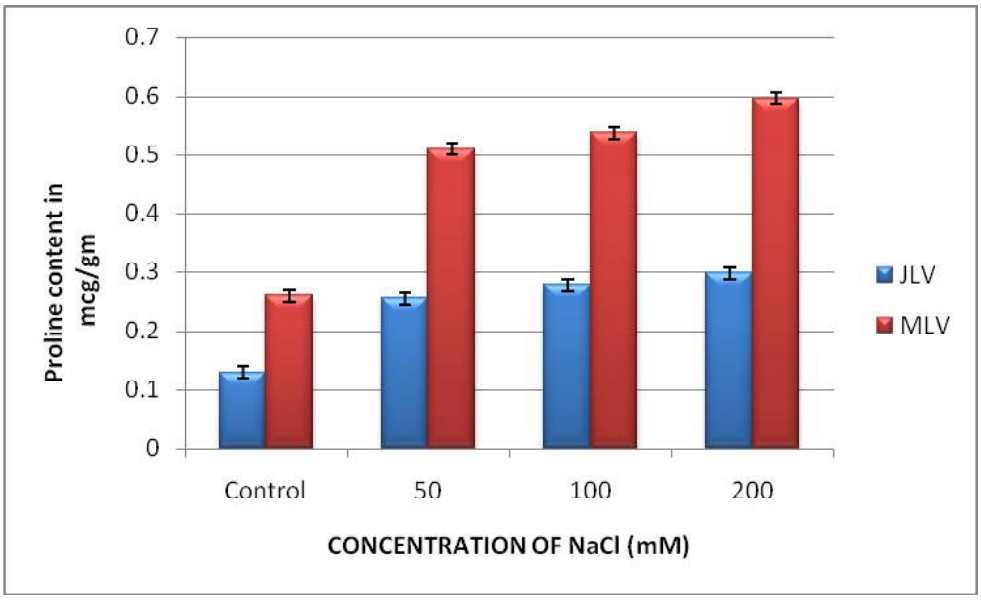

Fig. 3. Effect of salt on amount of proline (mcg/gm) in JLV and MLV at control and salt treated leaf.

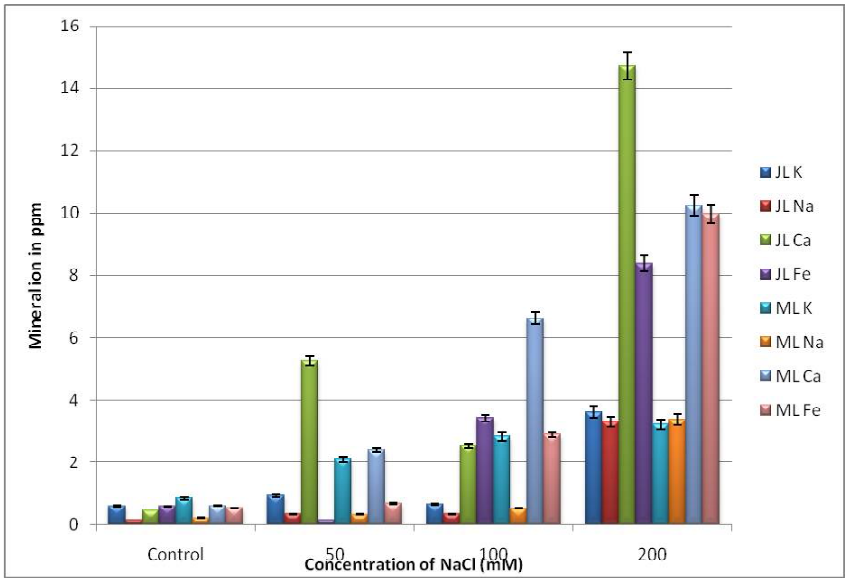

Fig. 4. Analysis of effect of salt on amount of Na^+, K^+, Ca^+ and Fe^+ ions in JLV and MLV in control and salt treated samples.

mM respectively. These results also strongly suggest that at higher salt stress, the ROS generation is affecting the activities and level of different antioxidant enzymes in the two varieties.

Among the compatible solutes / osmolytes, proline is synthesized and accumulated in varying concentrations by the plant. Both plant varieties exhibited increased proline content as the salt concentration increased. The leaf tissues gave a higher response to the salt stress by means of proline content (Fig 3). Previous studies demonstrated elevated levels of proline in Walnut (Yasar and Esra, 2015), Rice cultivars (Joseph et al, 2015), Potato (Jaarsma et al, 2013). In the present study only a two fold increase in proline content was seen in both the varieties at 200mM treatment indicating that both the varieties are synthesizing but not accumulating proline at a very high concentration to combat the salt stress. It may be possible that these varieties are utilizing proline as a source of Nitrogen or Carbon to stabilize the membrane proteins and thus for rapid recovery from stress.

Salt stressed condition leads to decreased nutrient uptake and lowers the movement / translocation of mineral nutrients like Mg^+, K^+, Ca^+ etc. Na^+ ions. Fig 4 shows the amount of these ions accumulated in leaf of JLV and MLV. It is generally observed that salt competes with the K^+ and Ca^+ binding sites in transporters thereby severely disturbing the plant metabolic and nutritional repertoire. Several studies report on mechanisms of Na^+ influence on K^+ uptake (Gupta and Huang 2014). In this study, MLV showed more K^+ accumulation at all salt concentration. Both the varieties had a decrease in K^+/Na^+ ratio to a minimum of 1 at 200mM (Fig 5). The amount of Ca^+ accumulated in JLV was more than in MLV; however as the salt stress increases the Ca^+/Na^+ ratio decreased like K^+/Na^+ ratio indicating the competition of Na with the other ions or decrease in fluxes for accumulation of K^+ and Ca^+ ions or removal of Na^+ ion. Amount of Fe accumulation increased in JLV with increase in salt stress.

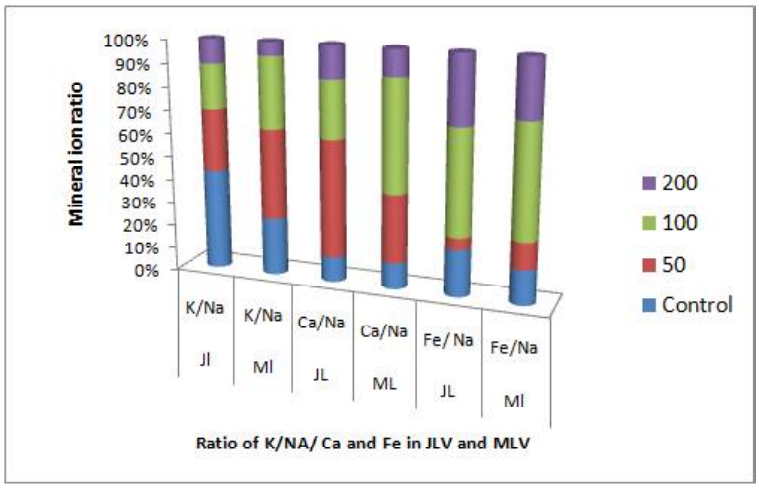

Fig. 5. Effect of salt treatments on ratio of K^+/Na^+, Ca^+/Na^+ and Fe^+/Na^+ in JLV and MLV in control and salt treated plants.

CONCLUSION

The present study showed reduced antioxidative enzymes like catalase and SOD, higher K influxes and lower Na^+ influx or higher Na^+ eflux, thereby maintaining a higher K/Na ratio to provide mitigation effect of salt stress to the MLV. To some extent the salt stress lead to aggravation of POD, GR, Protein, proline levels and the cytosolic Na exclusion which cumulatively conferred some level of protection due to salt stress to the MLV. Further studies with effect of exogenous osmolytes, K and H_2O_2 application will provide more information on the metabolic and nutrient management in response to the cultivated and hybrid varieties of *Luffa acutangula*.

LITERATURE CITED

Balkaya, A. Yýldýz, S. Horuz, A and Doðru S. M. 2016. Effects of salt stress on vegetative growth parameters and ion accumulations in cucurbit rootstock genotypes. *Journal of Crop Breeding and Genetics* **2**(2):11-24.

Chandra, U. 1995. Distribution, domestication and genetic diversity of *Luffa* gourd in Indian subcontinent. *Indian Journal of Plant Genetic Resources*, **8** (2): 189-196.

Cheeseman, J. M.2006. Hydrogen peroxide concentrations in leaves under natural conditions, *Journal of Experimental Botany*, **57**(10): 2435–2444, doi:10.1093/jxb/erl004

Correa, N. S. Bandeira, J. M. Marini, P. Borba, I. C. G. Lopes, N. F and Moraes D. M. 2013. Salt stress: antioxidant activity as a physiological adaptation of onion cultivars, *Acta Bot. Bras*. **27** (2): 394-399.

David, M. and Richard, J. S. 1983. In: Methods of enzymatic analysis, Bergmeyer, J and Grab M. (Eds), Verlag Chemie Wenhein Deer Field, Beach Floride, Pp 358.

Gupta B and Huang B. 2014. Mechanism of Salinity Tolerance in Plants: Physiological, Biochemical, and Molecular Characterization**.** International Journal of Genomics, Volume 2014 (2014), Article ID 701596, http://dx.doi.org/10.1155/2014/701596

Habib, H. S. Kausar, H and Saud, H. M. 2016. Plant Growth-Promoting Rhizobacteria Enhance Salinity Stress Tolerance in Okra through ROS-Scavenging Enzymes. *BioMed Research International*. doi.org/10.1155/2016/6284547

Hanin M, Ebel C, Ngom M, Laplaze L and Masmoudi K 2016. New Insights on Plant Salt Tolerance Mechanisms and Their Potential Use for Breeding. *Front. Plant Sci*. **7**:1787. doi: 10.3389/fpls.2016.01787

Ismail, A. A.Z. 2015. Effect of salinity levels and application stage on cucumber and soil under greenhouse condition. *Intl J Agri Crop Sci*. **8** (1): 73-80.

Jaarsma R, de Vries R. S. M, de Boer, A. H. 2013. Effect of Salt Stress on Growth, Na+Accumulation and Proline Metabolism in Potato (Solanum tuberosum) Cultivars. *PLoS ONE* **8**(3): e60183. doi:10.1371/journal.pone.0060183

Jafari, S. R. Arvin, S. M. J, Kalantari, K. M. 2015. Response of cucumber (*Cucumis sativus* L.) seedlings to exogenous silicon and salicylic acid under osmotic stress. *Acta Biologica Szegediensis* **59(**1):25-33.

Joseph , E. A. Radhakrishnan, V. V, Mohanan, K. V. 2015. A Study on the Accumulation of Proline - An Osmoprotectant Amino Acid under Salt Stress in Some Native Rice Cultivars of North Kerala, India. *Universal Journal of Agricultural Research,* **3***:* 15-22. doi: 10.13189/ujar.2015.030104

Kakkar, P. Das, B. and Viswanathan, P.N. 1984. A Modified Spectrophotometric Assay of Superoxide Dismutase. *Indian Journal of Biochemistry and Biophysics*, **21**:130-132.

Loreto, F. and Velikova, V. 2001. Isoprene Produced by Leaves Protects the Photosynthetic Apparatus against Ozone Damage, Quenches Ozone Products and Reduces Lipid Peroxidation of Cellular Membranes. Plant Physiology, **127**:1781-1787. http://dx.doi.org/10.1104/pp.010497

Lowry, O. H. Rosebrough, N. J. Farr, A. L. and Randall, R. J. 1951. Protein Measurement with the Folin Phenol Reagent *J. Biol. Chem*. **193**:265–275.

Luck,H .In: Methods in Enzymatic Analysis 2 (Ed Bergmeyer). Academic Press New York,1974; p 885.

Mala, P. Rutuja , G. and Sunita, S. 2016. Comparative Evaluation of Essential minerals and Heavy metal ions in *C. grandis* landraces. *International Journal of Pharmaceutical and Chemical Science*s. 5(4): 224-230.

Plaut , M. Edelstein & M. Ben-Hur (2013): Overcoming Salinity Barriers to Crop Production Using Traditional Methods, *Critical Reviews in Plant Sciences*, **32** (4): 250-291. http://dx.doi.org/10.1080/07352689.2012.752236

Prakash, K. Pati, K. Lalit, A. Pandey, Verma, M. 2014. Population structure and diversity in cultivated and wild Luffa Species. *Biochemical Systematics and Ecology* **56:**165-170.http://dx.doi.org/10.1016/j.bse.2014.05.012

Reddy, K. P. Subhani, S. M. Khan, P. A. and Kumar, K. B. 1995. Effect of light and benzyladenine and desk treated growing leaves, Changes in the peroxidase activity. *Cell physiol*. **26**: 984-994.

Sneha, S. Rishi, A. Chandra, S. 2014. Effect of Short Term Salt Stress on Chlorophyll Content, Protein and Activities of Catalase and Ascorbate Peroxidase Enzymes in Pearl millet. *American Journal of Plant Physiology.* **9**:32-37

Sofo, A. Scopa, A. Nuzzaci, M. and Vitti, A. 2015. Ascorbate Peroxidase and Catalase Activities and Their Genetic Regulation in Plants Subjected to Drought and Salinity Stresses. *Int. J. Mol. Sci.*, ***16*** **:**13561-13578; doi:10.3390/ijms160613561

Weisany, W. Sohrabi, Y. Heidari, G. Siosemardeh, A and Golezani, K. G. 2012. Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (Glycine max L.). *Plant Omics Journal*. **5**(2):60-67.

Yadav, S. Irfan, M. Ahmad, A and Hayat, S. 2011. Causes of salinity and plant manifestations to salt stress: A review. *J. Environ. Biol*. **32**:667-685.

Yasar, A and Esra S. 2015.The effect of salt stress on growth, chlorophyll content, proline and nutrient accumulation, and K/NA ratio in Walnut. *Pakistan Journal of Botany*. **44**(5): 1513-1520.

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