Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Central European Journal of Experimental Biology, 2013, 2 (3):20-25
(http://scholarsresearchlibrary.com/archive.html)

Central European Journal of Experimental Biology
Scholars Research Library
ISSN: 2278–7364

Salinity Stress: Effects on Growth, Biochemical Parameters and Ion Homeostasis in *Solanum lycospersicum* L. (Cv. Dan eka)

***Gumi[1], A.M., Aliero[1], A.A., K. Shehu[1] and A. Danbaba[2]**

[1]Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto
[2]Department of Mathematics, Usmanu Danfodiyo University, Sokoto

---

ABSTRACT

The response of Solanum lycospersicum (L.) to varied concentrations of salinity stress was investigated. Plant growth, biochemical parameters, cytotoxic ion sequestration and ionic balance were determined. The plant exhibited a decline in number of leaves, length of leaf and dry matter accumulation measured. The number of flowers increased at 50 mM NaCl concentration. Free proline content increases with increasing NaCl concentration and differ significantly ($P < 0.05$) while Glycine Betaine (GB) content did not differ significantly ($P > 0.05$). Salinity stress increased cytotoxic ions (Na^+ and Cl^-) and Ca^{2+} with a corresponding decrease in K^+ concentrations. The ionic balance (Na^+/K^+) was low due to high content of K^+ levels the plant accumulated ranging from 77.00 to 65.00 mg Kg^{-1}. It can be concluded that the osmolyte (Pro and GB) accumulations, low Na^+/K^+ ratio and high number of flowers are a possible indicator of salt tolerance in the S. lycospersicum genotype studied.

Key words: cytotoxic ions; salt tolerance; *Solanum lycospersicum*; Salinity stress

---

INTRODUCTION

Soil salinity is a major and ever-present threat to crop yields, especially in countries where irrigation is an essential aid to agriculture. The United Nations environment program estimates that approximately 20% of agricultural land and 50% of crop land in the world is salt stressed [49]. Soil salinity is detrimental to plant growth and adversely affect plant metabolism and cause important modification in growth, development and gene expression of plants [14]. These modifications may lead to the accumulation or depletion of certain metabolites; resulting in the imbalance in the levels of relatively small sets of cellular proteins which could increase, decrease, appear or disappear after salt treatment. The entry of NaCl into the root cells, its symplasmic transport past the casparian band and its transfer into the transpiration stream are the primary steps of salt accumulation in plants. The pathways for the uptake of Na^+ into the root symplasmic space have not yet been established in much detail. It has been generally assumed that non selective K^+ ion channels allow entry into the cell [9]. However, more recent studies suggest that Na^+ is taken up with K^+ by a high affinity K^+ uptake carrier [8][7]. All plants have evolved a cellular mechanism of salt stress survival by either avoiding or tolerating the salt stress. Plants are either dormant during salt stress or there must be cellular adjustment to tolerate the saline environments. Cellular adjustment mechanisms can be categorized as those that functions to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effect caused by these stresses.

The chemical potentials of the saline solutions, initially establish a water potential imbalance between the apoplast and symplast that leads to turgor decrease which if severe enough can cause growth reduction [20]. The cellular response to turgor reduction is osmotic adjustments. The cytosolic and organellar machinery of halophytes (salt tolerant) and glycophytes (salt sensitive) is equivalently Na^+ and Cl^- sensitive; so osmotic adjustment is achieved in

these compartments by accumulation of compatible osmolytes and osmoprotectants [20][21]. Free polyamines degraded via Diamine oxidase (DAO) and polyamine oxidase (PAO), can contribute to proline accumulation through γ-aminobutyric acid production [3]. However Na^+ and Cl^- are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity [50][12].

Tomato (*Solanum lycopersicum* L.) is a short lived perennial cropped as annuals. It belongs to the family Solanaceae (nightshade family) and is typically cultivated for its edible fruits. The leaves, stems and green unripe fruits of tomato plant contain small amount of the poisonous alkaloid tomatine [6]. The levels of tomatine are generally too small to be dangerous; so foods such as fried green tomato are safe to eat. Ripe tomato does not contain any detectable tomatine [10].

The majority of crop plants are relatively salt sensitive and are unable to tolerate high level of salinity [24]. Despite bulk data available on the effect of salt on agricultural crops [31][22][17][38][36][39][40][27], not much has been done on tomato [33][43][13][14][46] especially on tomato varieties grown in Sokoto agro climatic zone of Nigeria. This research aimed at investigating the responses of tomato (*Solanum lycopersicum* L.) to varied salt concentration on its growth and biochemical parameters with a view to establishing an insight on the salt tolerance mechanism of tomato.

MATERIALS AND METHODS

This study was conducted in Biological Garden, Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto - Nigeria. The seeds of tomato (*Solanum lycospersicum* cv. Dan eka) were obtained from a local market at Sokoto metropolis, Nigeria.

Plant Growth Condition

The seeds of *S. lycospersicum* were collected and surface sterilized by soaking in 5% sodium hypochlorate for 15 minutes and washed 3 times with sterile distilled water. The seed were first sown in a nursery bed and then uniformly germinated seedlings (10 days old) were selected and transferred to a polythene bag containing a mixture of river sand and manure (3:1 ratio). Sodium chloride (NaCl) was weighed and dissolved in irrigation water to make variant concentration of 50 mM, 75 mM and 100 mM of salt concentrations which were used to water the plants. The solutions were then stored in air tight cans to prevent evaporation which will in turns increase solution concentrations. The seedlings of tomato were divided into four groups: the first represented the control where no NaCl was added to the nutrient solution, the second, third and fourth groups received 50, 75 and 100 mM of NaCl treatments respectively, added to the nutrient solution. Each treatment was replicated three (3) times and each replicate consist of three (3) plants. The seedlings were exposed to varied salt concentration for 21 days.

Morphological Characterization:

After 21 days of salt treatment, the seedlings were harvested and floral count (number of flowers), number of leaves, length of leaves (cm) and dry mass DM (g/plant) of plants were determined. For dry mass determination, shoots and roots were left in desiccators at 80^{o}C for 2 days and parameters computed according the formula of Hunt [45].

Elemental Analysis:

Dried plant material (0.2 g) was ashed in a muffle furnace at 500^{o}C for three (3) hours. The ashes were digested with 5 ml of 7N nitric acid (HNO_3). After appropriate dilution, the filtrate was assayed for Na^+, K^+, and Ca^{2+} using atomic flame emission spectrometry. Cl^- was measured using titrimetric method [48].

Determination of Free Proline Content

Extraction and determination of free proline was performed according to the methods of Bates *et al.* [30]. Ground samples (1g) of plant material were extracted with 3% sulphosalicylic acid and filtered through Whatmann filter paper and the extract (2 ml) were held for 1 hour in boiling water by adding 2 ml ninhydrin and 2 ml glacial acetic acid, after which cold toluene (4 ml) was added. Proline content was measured spectrophotometerically at 520 nm and calculated as $Umolg^{-1}$DW against standard proline.

Determination of Glycine Betaine Content

Extraction and determination of betaine was carried out according to the method of Grieve and Maas [5]. Betaine was extracted by stirring finely ground-dried sample with demineralised water at 100^{o}C for 1 hour. Betaine content was determined spectrophotometrically after reaction with potassium iodide (KI-I_2) at 365 nm.

Statistical Analysis

The results were expressed as mean of three replicates and the data were subjected to one way analysis of variance (ANOVA) test. Differences between means were determined by Duncan's Multiple Range test using MINITAB statistical software.

RESULTS

The results for the physiological and biochemical responses of tomato (*Solanum lycospersicum*) to different salt concentrations are summarised in Table 1 and Figures 1 and 2.

EFFECTS OF NaCl ON MORPHOLOGICAL CHARACTERS OF TOMATO

At 75 mM and 100 mM NaCl, the floral number was decreased (Fig.1). At 50 mM NaCl, highest number of flowers was recorded averaging 10.11 per plant. The number of leaves per plant of tomato was markedly affected by salinity stress in a concentration dependent manner (Fig.1). The control has the highest number of leaves (66.78 per plant) and 100 mM has the least number of leaves (24.22 per plant). However, the results differ significantly ($p<0.05$). The length of leaves (cm) of *S. lycospersicum* decreased with increasing salt concentration. The results differ significantly ($p<0.05$). The dry matter accumulation DM (g plant^{-1}) was reduced by increasing concentration of salt. The control shows the highest dry matter accumulation (4.21 g plant^{-1}) while 100 mM NaCl shows the least dry matter accumulation (0.96 g plant^{-1}). The results differ significantly ($p<0.05$).

Table 1: Effect of different salt concentrations on ionic content (mgKg^{-1}DW) of Tomato (*Solanum lycospersicum*)

Treatments (mM)	Na^+	K^+	Ca^{2+}	Cl^-
0	2.33^a	73.00^a	0.75^a	37.75^a
50	15.00^b	77.00^b	1.25^b	53.55^b
75	26.00^c	67.00^c	1.05^c	42.85^c
100	33.00^d	65.00^c	1.20^{bc}	56.25^b
LSD (0.05) =	**1.445**	**2.500**	**0.002**	**0.03**

Values are means of triplicate determinations. Values with different superscript in the same column are significantly different ($p<0.05$).

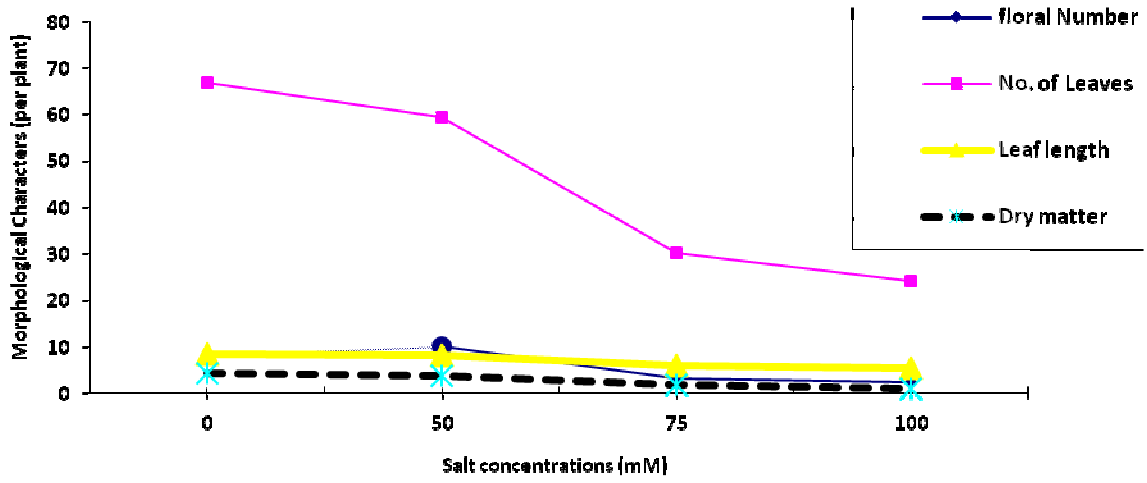

Figure 1: Effects of salt stress on morphological characters of tomato (*Solanum lycospersicum* L.) after 21 days of salt stress episode.

EFFECTS OF NaCl ON PROLINE AND GLYCINE BETAINE CONTENT OF TOMATO

The free proline content of tomato measured as **(**µMol g^{-1}) increased with increasing concentration of salt. The control had 1.16µMolg^{-1} while 100 mM and 75 mM had 1.53µMolg^{-1} each (Fig.2). The results differ significantly ($p<0.05$) but means comparisons shows no significant difference between the salt treated groups (50 mM, 75 mM and 100 mM), but revealed a significant difference ($p<0.05$) between the control and salt treated groups. Salt stress episode shows no significant effects ($p>0.05$) on glycine betaine content of tomato. Higher values of 1.41 µMol g^{-1} and 1.43 µMol g^{-1} were obtained in 50 mM and 100 mM NaCl treatments respectively (Fig.2).

EFFECT OF SALT STRESS ON ION HOMEOSTASIS OF TOMATO

Table 2 summarised the accumulation of ions (both cytotoxic and non cytotoxic) by *S. lycospersicum* under salt stress episode. The results differ significantly ($p<0.05$). Sodium ion (Na^+) increase with increasing concentration of salt, potassium ion (K^+) content was also salt concentration dependent with 50 mM showing the highest K^+ levels (77.00 mg Kg^{-1}DW). Calcium and chlorine ions were not salt concentration dependent as they increased and decreased randomly but still the control has the least content while 100 mM has the highest content of Ca^{2+} and Cl^-.

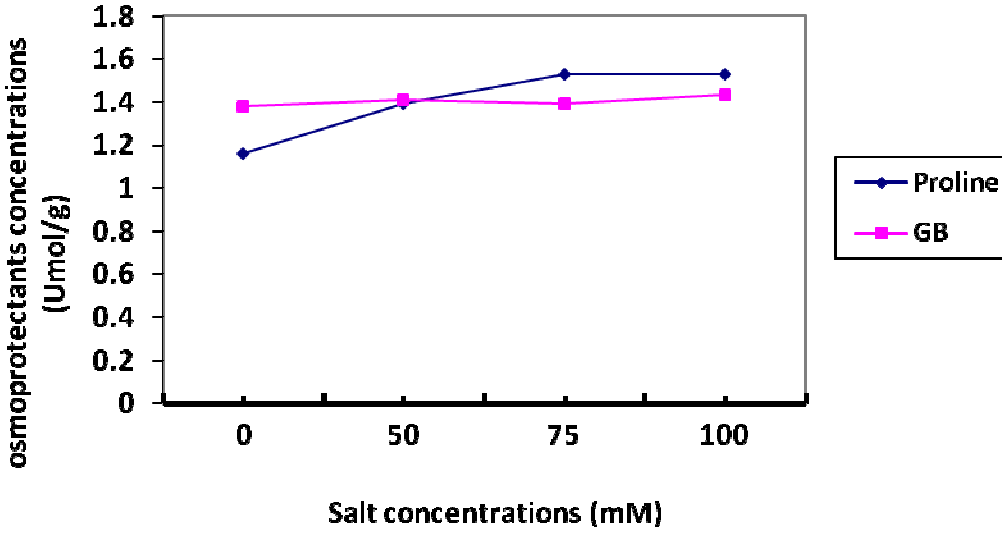

Figure 2: Effects of salt concentrations on proline and GB content of *Solanum lycospersicum* after 21 days of salt stress episode.

DISCUSSION

In this study, salt stress inhibited dry matter accumulation of *S. lycospersicum* which corroborates the findings of Amini *et al.* [14] on tomato; Reddy *et al.* [40] on *Jatropha curcas* and on maize (*Zea mays*) by Mansour *et al.* [39]. The number of flowers was affected by different salt concentration, and 50 mM treatment shows the highest number of flowers. This trend may be due to the fact that salinity stress induces changes in proteomics of tomato [14][46]. Proteins and other macro biomolecules play a key role in flower formation and fruit quality. Farnandez-Garcia *et al.* [43] reported that salt stress (below 70 mM concentration) improve fruits quality and nutritional content of tomato. These findings explain why the 50 mM of salt produced the highest number of flowers than the rest of the treatments (control inclusive).

Increase in salt stress significantly affected the number and length of leaves in a concentration dependent manner (Fig. 1). The chemical potential of saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which if severe enough can cause growth reduction [20]. Growth inhibition of salt stress was also reported on tomato by Brewer *et al.* [42] and on *Zea mays* by Mansour *et al.* [39] and also on soybean by Amirjani [41]. As salinity is first perceived in the root, it is likely that a root-derived signal, presumably abscisic acid is formed which directly or indirectly down-regulates leaf expansion rate [25][28].

In this study, the content of proline increases with increasing salt concentration. The accumulation of osmolyte compounds is often proposed as a solution to overcoming the negative consequences of water deficits in crop production which has been proposed as an adaptive mechanism for drought and salt tolerance. Indeed, osmolyte accumulation (OA) in plant cell results in a decrease of the cell osmotic potential and help in the maintenance of water absorption and cell turgor pressure, which might contribute to sustaining physiological processes, such as stomatal opening, photosynthesis and expansion growth [2] [26] [35] [1]. The content of free proline accumulation among the treated plants did not differ significantly but they all differ from the control treatment (0 mM NaCl). This infers that higher proline accumulated in the stressed plants than in the unstressed plant and hence free proline accumulated in response to salinity stress. This finding agrees with the reports of Ashraf [31]; Mansour [36]; Mansour *et al.* [39] and Manikandan and Design [27]. Proline accumulation in response to environmental stresses has been considered by a number of investigators as an adaptive trait concerned with stress tolerance [1]. The increased proline level above the required level is used in protein synthesis as considered by some authors as a part of an adaptive strategy to tolerate salinity [44]. The glycine betaine (GB) content of *S. lycospersicum* was observed to increase with increasing salt concentration. High GB accumulation is suggested to be involved in osmotic

adjustment, since it has been proven that high concentration of GB or Pro are not required for their protective effects under salinity [36]. The observed high GB content is in accordance with previous reports [36][37][32][39].

The amount of inorganic ions such as $Na^{+,}$ K^+, Ca^{2+} and Cl^- increased with increasing salt concentrations except in K^+ which increased at one time and then decreased at higher salt concentration in order to sustain the osmotic potential and maintain water influx into the plant. The concentration of Na^+ increased from 2.33 mg Kg^{-1} to 33.00 mg Kg^{-1} in 0 mM and 100 mM NaCl respectively. In contrast, the concentration of K^+ content in *S. lycospersicum* grown under 75 mM and 100 mM NaCl was significantly lower than those of the control and 50 mM NaCl. Under salt stress, Na^+ competes with K^+ for uptake into roots through common transport systems and does this effectively since the Na^+ in saline environments is usually considerably greater than K^+ [11][15]. These findings can be attributed to the competitive interactions between K^+ and Na^+ ions and the inhibition of K^+ uptake by high concentration of Na^+ as reported by Bernstein [29].

A high cytosolic K^+/Na^+ ratio is important for maintaining cellular metabolism. In the present study, the levels of Na^+ gradually increased with increasing concentration of salt, while K^+ levels somehow decreased with increasing concentration of salinity stress (though higher at 50 mM NaCl). High levels of Na^+ inside the cell inhibits the uptake of K^+ thereby increase Na^+/K^+ ratio which in turns affects plant metabolism [15]. The metabolic toxicity of Na^+ is largely due to its ability to compete with K^+ for binding sites essentials for cellular function. More than fifty (50) enzymes are known to be activated by K^+ and Na^+ cannot substitute in this role [23]. Moreover, protein synthesis requires high concentration of K^+ for the binding of tRNA to ribosome [16] and probably other aspects of ribosome functions [47]. The maintenance of low cytosolic Na^+ concentration and Na^+/K^+ homeostasis is an important aspect of salinity tolerance and that salt tolerant lines show lower Na^+/K^+ levels [34]. Based on the Na^+/K^+ ratio observed in this study, the *S. lycospersicum* variety studied could be classified as a salt-tolerant line.

In this study, NaCl increased Ca^{2+} and Cl^- concentrations of *S. lycospersicum*. Many studies have confirmed that NaCl stress may be partially alleviated by increased Ca^{2+} -supply to the growth medium [4]. Depending on the concentration ratio, Na^+ and Ca^{2+} may displace each other from the plasma membrane, it is obvious that Na^+ may affect cellular Ca^{2+} -homeostasis, whereas Ca^{2+} may reduce Na^+ -toxicity. Also Ca^{2+} affects K^+/Na^+ selectivity at plasma membrane [19]. From the results of this study, the concentration of Ca^{2+} is too low to alter the accumulation of Na^+ in the plant tissues; hence there is no correlation between Na^+ accumulation and increase in Ca^{2+} concentration. These observations corroborate the findings of Cramer *et al.* [19] on maize (*Zea mays*) cultivars. The high cytoplasmic concentrations of Cl^- recorded in salinity stressed treatments of *S. lycospersicum* may likely accounts for the principal cause of salt-induced growth reduction as observed by Zidan *et al*. [22] and Cramer *et al*. [18].

In general, the results of this study demonstrated that all the growth parameters evaluated decrease with increasing salt concentration, except number of flowers which was high in 50 mM NaCl as a result of protein induced changes caused by the slight increase in salinity stress which in turns promotes flower induction. In contrast, Biochemical characteristics and ionic content of *S. lycospersicum* was observed to increase with increasing salt concentration except potassium ion (K^+) content which as expected is in accordance with most previous data.

CONCLUSION

The high osmolyte accumulations (proline and glycine betaine), low Na^+/K^+ ratio and high number of flowers are the possible indicators of salinity tolerance in the *S. lycospersicum* genotype studied.

Acknowledgements
We are grateful to Mal. Aliyu Gandu of Biological garden, UDUS and Mal. Ahmad Modi, of soil chemical laboratory, faculty of Agriculture, UDUS for their technical support. We equally appreciate Timothy Emanuel and Zainab Muhammad Hassan, both Botany undergraduate students for their help in field work.

REFERENCES

[1] A Blum. *Plant Growth Regulation*, **1996**, 20, 135-148.
[2] A Blum; J Mayer; G Gozlan. *Plant, Cell and Enviroment*, **1983**, 6, 219-225.
[3] A Bouchereau; A Aziz; F Larher; J Martin-Tanguy. *Plant Science*, **1999**, 140, 103-125.
[4] A Lauchli. *The American Society of Plant Physiologist Symposium Series*, **1990**, 4, 26-35.
[5] C Grieve; EV Maas. *Physiology of Plants*, **1984**, 16, 167-171.
[6] D Barceloux. *Disease-a-month*, **2009**, **55**(6), 391-402
[7] DP Schachtman; A Fairhead; O Babourina. *Plant Physiology*, **1995**, 108, 38-39.

[8] DP Schachtman; JI Schroeder. *Nature*, **1994**, 370, 655-658.
[9] DP Schachtman; ST Tyerman; BK Terry. *Plant Physiology*, **1991**, 97, 598-605.
[10] DR Pittenger. In Vegetables that contains natural toxins, California Master Gardener Handbook. ANR Publications, USA, **2002**, pp 643-4.
[11] DW Rains. In: plant under stress. Eds. J.H. Flowers, M.B. Jones, Cambridge University Press, London, **1989**, pp. 181-197.
[12] E Blumwald; GS Aharon; MP Apse. *Biochemica et Biophysica Acta*, **2000**, 1465, 140-151.
[13] F Amini; AA Ehsanpour. *American Journal of Biochemistry and Biotechnology*, **2005**, 1 (4)**,** 204-208.
[14] F Amini; AA Ehsanpour; QT Hoang; JS Shin. *Russian Journal of Plant Physiology*, **2007**, 54, 464-471.
[15] FJM Maathuis; TJ Flowers; AR Yeo. *Journal of Experimental Botany*, **1992**, 43, 1219-1223.
[16] G Blaha; U Stelzl; CMT Spahn; RK Agrawal; J Frank; KH Nierhaus. *Methods in Enzymology*, **2000**, 317, 292-309.
[17] GR Cramer. In Handbook of plants and crop stress. Ed. M. Pessarakli, Mercel Dekker, New York, **1993**, 449-459.
[18] GR Cramer; GJ Alberico; C Schmidt. *Australian Journal of Plant Physiology*, **1994**, 21, 663-674.
[19] GR Cramer; J Lynch; A Lauchli; E Epstein. *Plant Physiology*, **1987**, 83, 510-516.
[20] HJ Bohnert; DE Nelson; RG Jensen. *Plant and cell*, **1995**, 7, 1099 - 1111.
[21] HJ Bohnert; RG Jensen. *Trends in Biotechnology*, **1996**, 14, 89-97.
[22] I Zidan; A Shavi; I Ravina; PM Neumann. *Journal of Plant Nutrition*, **1992**, 15, 1407-1419.
[23] IS Bhandal; CP Malik. *International Review of Cytology*, **1988**, 10**,** 205-24.
[24] J Levitt. In Response of plant to environmental stresses: Water, radiation, salt and other stresses, Academic Press, New York, **1980**.
[25] JB Passioura. *Australian Journal of Plant Physiology*, **1988**, 15, 687-693.
[26] JM Morgan. *Annual Review Plant Physiology*, **1984**, 35, 299-319.
[27] K Manikandan; R Designh. *Recent Research in Science and Technology*, **2009**, 1(2), 48-51.
[28] K Zhao; R Munns; RW King. *Australian Journal of Plant Physiology*, **1991**, 18, 17-24.
[29] L Bernstein. *American Journal of Botany*, **1995**, 48, 909-918.
[30] LS Bates; P Waldren; ID Tare. *Plant and Soil*, **1973**, 103, 875-883.
[31] M Ashraf. *Plant and Soil*, **1989**, 119, 205-211.
[32] M Ashraf; PJC Harris. *Plant Sciences*, **2004**, 166**,** 3-16.
[33] MJ Sanchez-Blanco; MC Bolarin; JJ Alarcon; Torrecillas. *Physiology of Plant*, **1991**, 83, 269-274.
[34] MK Chattopadhyay; BS Tiwari; G Chattopadhyay. *Physiology of Plants*, **2002**, 116, 192-199.
[35] MM Ludlow; RC Muchow. *Advances in Agronomy*, **1990**, 43, 107-153.
[36] MMF Mansour. *Biology and Plants*, **1994**, 36, 429-434.
[37] MMF Mansour. *Biology and Plants*, **2000**, 43**,** 491-500.
[38] MMF Mansour; EJ Stadelmann. *Physiology of Plant*, **1994**, 91, 389-394.
[39] MMF Mansour; KHA Salama; FZM Ali; AF Abou Hadid. *Genetics and Applied Plant Physiology*, **2005**, 31(1-2), 29-41.
[40] MP Reddy; N Kumar; DV Sudheer; K Meenakshi; B Girish. *Biologia*, **2008**, 63**,** 378-382.
[41] MR Amirjani. *American journal of plant physiology,* **2010**, 5, 350-360.
[42] MT Brewer; JB Moyseenko; AJ Monforte; E Van der Knaap. *Journal of Experimental Botany*, **2007**, 58, 1339-1349.
[43] N Fernandez-Garcia; V Martinez; A Cerda; M Caryajal. *Journal of Horticultural Sciences and Biotechnology*, **2004**, 79**,** 995-1001.
a. Wolf; WD Jeschke; W Hartung. *Journal of Experimental Botany*, **1990**, 41, 593-600.
[44] PD Hare; WA Cress; J Van Staden. *Plant, Cell and Environment*, **1998**, 21, 535-553.
[45] R Hunt. In Plant Growth Analysis, Eduard Arnold, London, **1981**, pp 5
[46] RA Shibli; M Kushad; GG Yousef; MA Lila. *Plant Growth Regulation*, **2007**, 51, 159-169.
[47] RG Wyn Jones; J Gorham; E McDonnell. In: salinity tolerance in plants, Eds. R.C. Staples, H. Gary, H. Toenniessen, Wiley, New York, **1984**, 189-203.
[48] S Ranganna. In Manual of Analysis of fruits and vegetable product, McGraw-Hill, New Delhi, **1977**, pp 10.
[49] TJ Flowers; AR Yeo. *Australian Journal of Plant physiology*, **1995**, 22, 875-884.
[50] X Niu; RA Bressan; PM Hasegawa; JM Pardo. *Plant Physiology*, **1995**, 109, 735-742.