

Effect of salt stress on growth, proline, glycinebetaine and photosynthetic pigment concentrations on cowpea plant

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Abstract: Soil salinity has existed long before human and agriculture but the problem has been aggravated by agricultural practices such as irrigation. In this study, effect of applied NaCl and Na₂SO₄ on growth, proline, glycinebetaine and photosynthetic pigment concentrations of cowpea plant (*Vigna unguiculata* L. Walp) was investigated. The experiment was arranged in a completely randomized design with three replications under greenhouse condition. The soil used was irrigated with NaCl and Na₂SO₄ at the rates of 0, 50, 100 and 150 mM NaCl and Na₂SO₄ respectively. Growth of the cowpea plants was inhibited by salinity. Application of NaCl and Na₂SO₄ significantly decreased photosynthetic pigment (such as chlorophyll a, chlorophyll b, total chlorophyll and carotenoid). However, it could stimulate traits that could lead to the survival of cowpea plant in saline environment due to proline and glycinebetaine accumulations that increased as a result of salt stress.

[Oyetunji O.J. and Imade F.N. **Effect of salt stress on growth, proline, glycinebetaine and photosynthetic pigment concentrations on cowpea plant.** *Nat Sci* 2014;12(12):156-161]. (ISSN: 1545-0740). <http://www.sciencepub.net/nature>. 21

Key words: Salinity, concentrations, *Vigna unguiculata* (L.) Walp., salt, stress, applications.

1. Introduction

Salinity is defined as the presence of minerals at high levels (cations: K, Mg, Ca, Na and anions: NO₃, HCO₃, SO₄, Cl) in water and soil (Zahedi *et al.* 2012). Salinity is an environmental stress that limits growth and development in plants. It is considered the most injurious factor affecting crop production and agricultural sustainability in arid and semi arid region of the world reducing the value and productivity of the affected land (Munns 2002).

Soil salinity has existed long before human and agriculture but the problem has been aggravated by agricultural practices such as irrigation. Salt stress can be a major challenge to plant. It limits agriculture all over the world particular in irrigated farmland (Rausch *et al.* 1996). Today, about 20% of the world cultivated land and nearly all irrigated land are affected by salinity (Peleg *et al.* 2011). The total global area of salt affected soil has recently been estimated to be approximately 830 millions hectare (Martinez-Beltran and Manzur 2005). Their genesis may be natural or accelerated by the extension of irrigated agriculture, the intensive use of water combined with high evaporation rates and human activity (Lambers, 2003) and this has been a major constraint on crop productivity (Witcombe *et al.* 2008).

Plants have improved complex mechanisms systems for adaptation to osmotic and ionic stress caused by high salinity, under the salt stress. The adaptation is generally associated with osmoregulation adjustment by using some osmotic regulators, such as

potassium, soluble sugar, proline and betaine (Munns 2005; Hong-Bo *et al.* 2006).

The role of the osmotic regulators in cell osmotic adjustment, membrane stabilization and detoxification of injurious ions in plants exposed to salt stress is widely reported (Ashraf and Foolad 2007). However, the significance of proline accumulation in osmotic adjustment is still debated and varies according to the species (Rodriguez *et al.* 1997).

Cowpea is the most economically important indigenous African legume crop and has a wide variety of uses as a nutritious component in the human diet as well as livestock feed (Langyintuo *et al.*, 2003). It is cultivated as a dry land crop under different climatic conditions ranging from semi-arid to sub humid (Murillo-Amador *et al.*, 2006).

The objective of the study is to investigate the response of cowpea plant (*Vigna unguiculata* L. Walp) to a high NaCl and Na₂SO₄ treatment from 0 to 150 mM on some morphological character, photosynthetic pigment, proline and glycinebetaine accumulation.

2. Material and Methods

The experiment was carried out in pots on two varieties (TVu 11711 and TVu 15245) of cowpea plant (*Vigna unguiculata* L. Walp.) in the green house at the Department of Botany, Faculty of Science, University of Ibadan. Cowpea seeds were planted in pots of 14 cm diameter and 18.5 cm depth; each pot was filled with 3.0 kg soil. Four seeds of each cultivar were sown in each pot. The experiment was arranged in a completely randomized design with three replications. When the

seedling were well established (after 10 days,) that is, when the first trifoliate leaf had reached its full size and the second trifoliate leaf was starting its development, thinning was carried out, leaving two plants per pot. Salt treatment commenced 5 days after plants were thinned. These involved the application of NaCl and Na₂SO₄ with varied equimolar concentration (0 mM, 50 mM, 100 mM, and 150 mM). The treatments were applied twice a week except for the control (0 mM) that was watered regularly. The plants were sprayed 15 days after sowing with an insecticide containing Lambda-cyhalothrin 2.5 EC to prevent incidence of pest and insect infestation once in two weeks.

Morphological characters such as plant height, number of leaves, leaf length, leaf width and leaf area were monitored. Plant height, leaf length and leaf width were determined using a meter rule (cm) while the number of leaves were counted and recorded, approximately four weeks of treatment. The calculation of the individual plant species leaf area were effected with the formula $A = (L \times W \times CF)$ proposed by Montgomery (1911) cited in Jonathan *et al.* (2013), where L is the maximum leaf length, W is the maximum leaf width, and CF is the coefficient factor. According to Jolaoso (1988) cited in Jonathan *et al.* (2013) the coefficient factor used for the calculations of leaf area for Cowpea is 2.7.

The contents of photosynthetic pigments such as Chlorophyll a, Chlorophyll b, Total Chlorophyll and carotenoid were determined from the method of Lichtenthaler and Wellbum (1985) cited in Alikhani *et al.* (2011).

Free proline in the leaves and stem was determined following the method of Bates *et al.* (1973) cited in Kaymakanova and Stoeva (2008) and Glycinebetaine was determined following the method of Grieve and Grattan (1983) cited in Sarwar *et al.* (2006).

Data were subjected to analysis of variance, using Statistical analysis system (SAS). The Duncan's Multiple Range test at 95% level of probability was used to test the differences among mean

3. Results

The results in Table 1 shows that salinity had significant effect on plant height, leaf area and numbers of leaves in the two accessions. Plant height decreased significantly with increased in the concentrations of the salts applied in both accessions. The height of the crop was inversely proportional to the concentrations of the two salt treatments. Application of 150 mM of NaCl resulted in stunted growth (27.00 cm) of TVu 15245.

Although this was not significantly different from pot treated with 100 mM of NaCl. Similarly, TVu 11711 treated with 150 mM Na₂SO₄ had the shortest plants (37.67 cm) but not significantly different from those treated with 100 mM Na₂SO₄. The reason behind this may be that under salinity, the osmotic pressure in soil solution exceeds the osmotic pressure in plants cells due to the presence of higher concentrations of salts, and thus reduces the ability of plants to take up water and minerals. The leaf area decreased with increased in salts concentrations. TVu 15245 had the largest leaf area (253.08 cm²) in the control, while the smallest (118.83 cm²) was recorded in the same TVu 15245 accession treated with 150 mM NaCl. No significant difference was observed in TVu 11711 treated at different levels of Na₂SO₄ and NaCl. Although significant difference was observed in TVu 15245 treated at different levels of Na₂SO₄. According to Pettigrew (2008), the reduction in the leaf area could be as a result of deficiency of potassium under saline condition.

TVu 15245 had the highest (50.00) mean number of leaves in the control pot (0 mM NaCl) and the least (11.00) mean number was recorded in the same accession treated with 100 mM NaCl. However, there was no significant different in both accession treated with different concentration of NaCl and Na₂SO₄ except for TVu 11711 that shows significant differences between 50 and 150 mM Na₂SO₄. Our findings are in agreement with those of Hadi *et al.* (2012) and Amador and Dieguez (2007) who found that salinity induced reduction in number of leaves.

Effect of varying concentrations of NaCl and Na₂SO₄ on photosynthetic pigments of two accessions of cowpea is presented in Table 2. The results show that salinity had significant ($P \leq 0.05$) effect on pigment contents of the two accessions of cowpea. Generally, the pigments contents decreased with increased in salt concentrations. There was no significant difference in chlorophylls a, b, total chlorophyll and carotenoid component of TVu 11711 treated with 0-100mM NaCl. Similarly, TVu 15245 accession treated with 0-50 mM NaCl was not significantly different with respect to these pigments. There is no significant difference in chlorophylls a, b and carotenoid of TVu 11711 treated with 0 - 50 mM Na₂SO₄ while there was significant difference in chlorophyll a, b, total chlorophyll and carotenoid constituents of TVu 15245 treated with 0 - 150 mM Na₂SO₄.

Both NaCl and Na₂SO₄ applications reduced chlorophyll a, chlorophyll b, total chlorophyll and

Table 1. Effect of varying concentrations of NaCl and Na₂SO₄ on plant height (cm) leaf area (cm²) and number of two accessions of *Vigna unguiculata* (L.) Walp. after four weeks of treatment

Salt	Concentration (mM)	Plant height		Leaf area		Numbers of leaves	
		TVu 11711	TVu 15245	TVu 11711	TVu 15245	TVu 11711	TVu 15245
NaCl	0	175.5 ^a	186.67 ^a	197.82 ^a	253.08 ^a	43.00 ^a	50.00 ^a
	50	42.33 ^b	48.67 ^b	169.67 ^{ab}	170.46 ^b	15.00 ^b	16.00 ^b
	100	37.33 ^b	29.67 ^c	156.15 ^{ab}	142.93 ^{bc}	15.00 ^b	11.00 ^b
	150	37.75 ^b	27.00 ^c	167.96 ^b	118.83 ^c	12.50 ^b	12.00 ^b
Na ₂ SO ₄	0	161.00 ^a	207.67 ^a	232.56 ^a	245.03 ^a	45.00 ^a	44.33 ^a
	50	58.00 ^b	56.67 ^b	157.28 ^b	163.59 ^b	17.00 ^b	21.33 ^b
	100	43.00 ^{bc}	36.67 ^b	148.46 ^b	147.68 ^{bc}	15.67 ^{bc}	17.67 ^b
	150	37.67 ^c	30.67 ^b	122.49 ^b	119.48 ^c	14.00 ^c	13.33 ^b

Values represent means of three replicates. Values followed by the same letter(s) in the same column are not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

Table 2. Effect of NaCl and Na₂SO₄ on production of photosynthetic pigments of *Vigna unguiculata* (L.) Walp..

Salt	Conc. (mM)	Chl a		Chl b		Tchl		Carotenoid	
		TVu 11711	TVu 15245	TVu 11711	TVu 15245	TVu 11711	TVu 15245	TVu 11711	TVu 15245
NaCl	0	21.79 ^a	14.79 ^a	11.17 ^a	8.02 ^a	32.96 ^a	22.81 ^a	3.71 ^a	3.48 ^a
	50	19.42 ^a	15.20 ^a	11.08 ^a	7.76 ^a	30.5 ^a	22.96 ^a	4.13 ^a	3.36 ^a
	100	14.65 ^{ab}	9.20 ^b	11.02 ^a	6.29 ^a	25.67 ^{ab}	15.49 ^b	1.87 ^{ab}	1.63 ^b
	150	11.98 ^b	3.63 ^c	8.60 ^a	2.81 ^b	20.58 ^b	6.44 ^c	1.10 ^b	0.72 ^c
Na ₂ SO ₄	0	21.85 ^a	16.49 ^a	10.55 ^a	8.24 ^a	32.40 ^a	24.74 ^a	3.96 ^a	3.40 ^a
	50	20.01 ^a	13.23 ^b	10.21 ^a	7.66 ^a	30.23 ^b	20.89 ^b	4.25 ^a	2.84 ^b
	100	11.4 ^b	9.35 ^c	9.21 ^a	5.69 ^{ab}	20.61 ^c	15.04 ^c	1.34 ^b	1.93 ^c
	150	9.39 ^b	3.73 ^d	7.38 ^a	2.58 ^b	16.77 ^d	6.31 ^d	1.04 ^b	0.84 ^d

Each value is a mean of three replicates. Values in the same column with the same letter(s) were not significantly different at $P \geq 0.05$ using Duncan's Multiple Range Test (DMRT).

Chl a: Chlorophyll a, Chl b: Chlorophyll b, and Tchl: Total chlorophyll

carotenoids contents. These results are in agreement with those reported by Iqbal *et al.* (2006), who reported that chlorophyll content was decreased under saline conditions. Also Ashrafuzzaman *et al.* (2000) found out that increased concentration of NaCl and Na₂SO₄ reduced chlorophyll a, b and total chlorophyll. Similar findings were also reported by Jamil *et al.* (2012), A reduction in carotenoids content due to salinity stress was observed in *Brassica juncea*, in mulberry and in *Aegiceros corniculatum* (Parida and Das 2005 cited in Mane *et al.* 2011). From the present investigation decrease in carotenoid content in the leaves of *V. unguiculata* indicated that the higher concentration of salt possibly acts as inhibitory and thus unable to effectively prevent chloroplast from photo-oxidative damage. In contrast at lower levels of salt, carotenoids performs protective role for chloroplast and acts as accessory pigments.

The effect of the treatments on the proline and glycinebetaine accumulations in both leaves and stem of control and salt stressed cowpea plants were presented in Figures 1 (a, b, c and d) Under salt-stressed conditions the proline and glycinebetaine concentrations were higher than in control plant.

Moreover, the contents of proline and glycinebetaine in leaves of the salt-treated plants were significantly increased in comparison to the untreated plants ($P \leq 0.05$). In leaf, proline and glycinebetaine concentrations increased with salinity and peaked at 150 mM NaCl and Na₂SO₄ (Figures 1a and b). Stem followed the same pattern but concentrations of proline and glycinebetaine were significantly lower than in leaf (Figures 1c and d). However, the proline content was not affected under non salt-stressed control conditions, irrespective of cultivar or salt type. Proline and glycinebetaine accumulation in salt stressed plants is a primary defense response to maintain the osmotic pressure in a cell (Turan *et al.* 2009). Researchers (Desingh and Kanagaraj, 2007; Koca *et al.* 2007; Veeranagamallaiah *et al.* 2007) have shown that the role of proline in osmotic adjustment, is to protect the cell structure and function in plants in salt-tolerant and salt-sensitive cultivars of many crops The present study shows that the salt treatments induced an increase in proline and glycinebetaine concentration in cowpea plants. Similar result has been reported by Cha-Um and Kirdmanee (2009) and Turan *et al.* (2009).

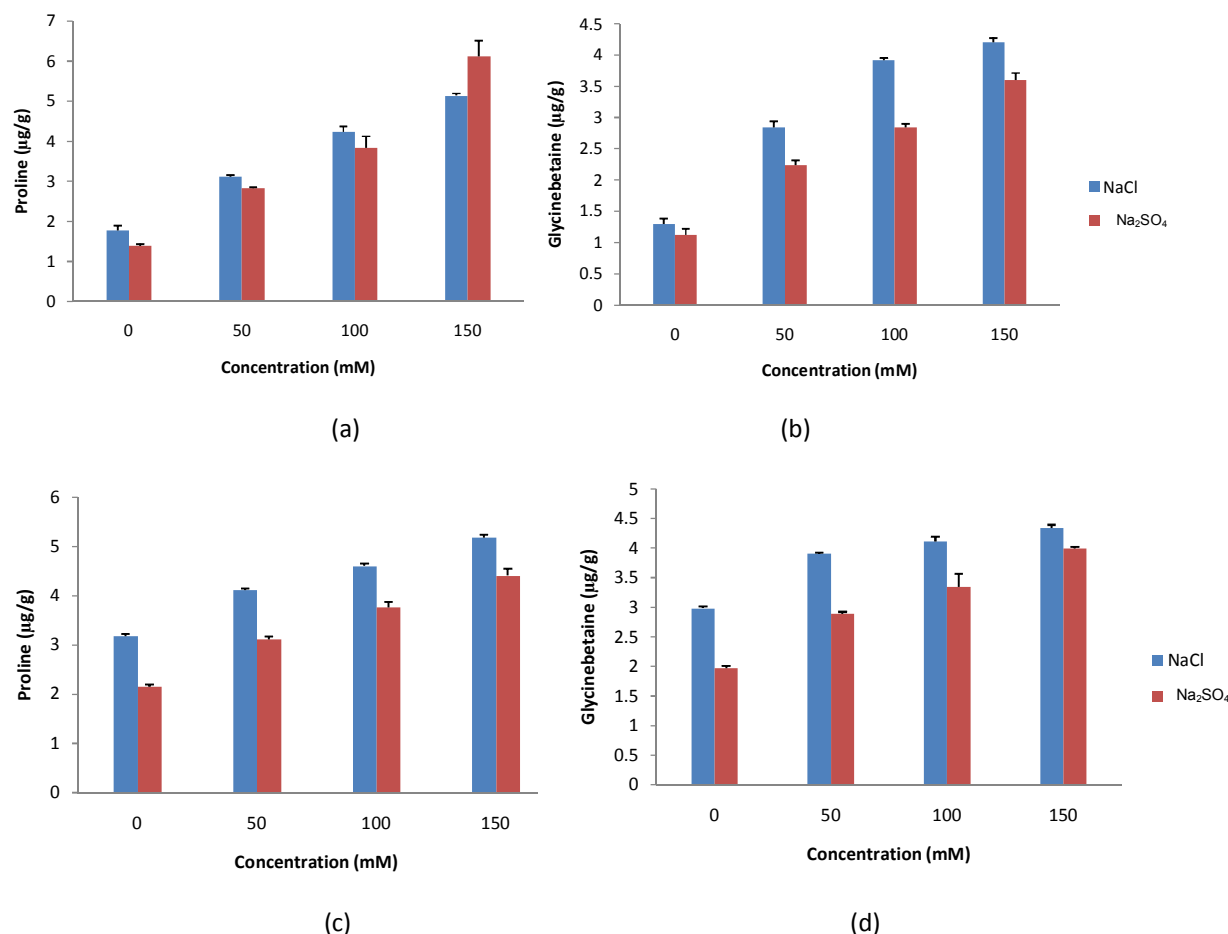


Figure 1. Effect of NaCl and Na₂SO₄ applications (0, 50, 100, and 150 mM) on the proline and glycinebetaine content of leaf in *V. unguiculata* L. Walp. (a) and (b) accession TVu 11711; (c) and (d) accession TVu 15245.

4. Conclusion

The growth processes in the cowpea plants were suppressed which was a result of the disturbed osmotic processes and the toxic effect of Cl⁻, SO₄²⁻, and Na⁺. The equimolar concentrations of NaCl and Na₂SO₄ suppressed, each to a different extent, the physiological processes in the plants. The present study showed that soil salinity inhibited plant growth. On the other hand, Total chlorophyll concentration was decreased, proline and glycinebetaine concentrations of plant were increased by salinity. The accumulation rate of the two osmolyte in the leaf and stem showed that in Cowpea plant, proline and glycinebetaine play more important role in osmotic adjustment of the leaf and stem respectively. Growing cowpea in saline soil will affect its growth, and photosynthetic pigments. However, it could stimulate traits that could lead to the survival of cowpea plant in saline environment due to proline and glycinebetaine accumulations that increased as a result

of salt stress. Further research should be conducted in field using more accessions in other to authenticate these findings and should include biochemical analysis in addition to morpho-physiological evaluation and at molecular level.

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References

1. Alikhani F, Saboora A, Razavi K. Changes in osmolytes contents, lipid peroxidation and photosynthetic pigment of *Aeluropus lagopoides*

- under potassium deficiency and salinity. *J. Stress Physiol. Biochem.*, 2011; 7(2): 5-9.
2. Ashraf M, Foolad MR (2007). Roles of glycinebetaine and proline in improving plant abiotic stress resistance. *Environ. Exp. Bot.*, 59: 206-216.
 3. Ashrafuzzaman M, Khan MAH, Shohidullah SM, Rahman MS. Effect of salinity on the chlorophyll content, yield and yield components of QPM cv. Nutricia. *Pak. J. Biol. Sci.*, 2000; 3(1): 43-46.
 4. Bates, L.S., Waldren, R.P., Teare, I.D. Rapid determination of free proline for water-stress studies. *Plant Soil* 1973; 39: 205-207.
 5. Cha-um S, Kirdmanee C. Effect of salt stress on proline accumulation, photosynthetic ability and growth characters in two maize cultivars. *Pak. J. Bot.* 2009; 41: 87-98.
 6. Desingh R, Kanagaraj G. Influence of salinity stress on photosynthesis and antioxidative systems in two cotton varieties. *Gen. Appl. Plant Physiol.* 2007; 33: 221-234.
 7. Grieve CM, Grattan SR (). Rapid assay for determination of water soluble quaternary ammonium compounds. 1983; *Plant Soil*, 70: 303-307.
 8. Hadi F, Hussain F, Arif M. Growth Performance and Comparison of Cowpea Varieties under Different NaCl Salinity Stresses. *Greener J. Phys. Sci.*, 2012; 2(1): 044-049,
 9. Hong-Bo S, Xiao-Yan C, Li-Ye C, Xi-Ning Z, Gangh W, Yong-Bing Y, Chang-Xing Z, Zan-Min Z. Investigation on the relationship of proline with wheat anti-drought under soil water deficits. *Colloids Surf. B. Biointerfaces* 2006; 53: 113-119.
 10. Iqbal N, Ashraf MY, Javed, F., Martinez V, Ahmad K. Nitric-perchloric acid wet digestion Nitrate reduction and nutrient accumulation in wheat (*Triticum aestivum* L.) grown in soil salinization with four different salts. *J. Plant Nutr.*, 2006; 29: 409-421.
 11. Jolaoso MA. Effect of rate and methods of N & P fertilizer application on growth and nutrient uptake of intercropped maize & cowpea. Ph.D. Thesis, University of Ibadan, Nigeria. 1988.
 12. Jonathan SG, Oyetunji OJ, Olawuyi OJ, Uwukhor PO. Application of Pleurotus ostreatus SMC as soil conditioner for the growth of soybean (*Glycine max*). *Academia Arena* 2013' 5(1): 1-2.
 13. Kaymakanova M, Staeva N. Physiological reaction of bean plants (*Phaseolus vulgaris* L.) to salt stress. *Gen. Appl. Plant Physiol.*, 2008; 34(3-4): 177-188.
 14. Koca H, Bor, M., Özdemir F, Türkan I. The effect of salt stress on lipid peroxidation, antioxidative enzymes and proline content of sesame cultivars. *Environ. Exp. Bot.* 2007; 60: 344-351.
 15. Lambers H. Introduction, dry land salinity: a key environ. issue in Southern Australia. *Plant Soil*, 2003; 257: 5-7.
 16. Lichtenthaler HK, Wellbum AR. Determination of total carotenoids and chlorophylls a and b of leaf in different solvents. *Biol. Soc. Trans.*, 1985; 11:591-592.
 17. Mane AV, Saratale GD, Karadge BA, Samant JS. Studies on the effects of salinity on growth, polyphenol content and photosynthetic response in *Vetiveria zizanioides* (L.) Nash. *Emir. J. Food Agric.*, 2011; 23(1): 59-70.
 18. Martinez-Beltran J, Manzur CL. Overview of salinity problems in the world and FAO strategies to address the problem. In: Proceedings of the International Salinity Forum, Riverside, California, April 2005, pp. 311-313.
 19. Montgomery EG. Correlation studies in corn. *Nebraska Agricultural Experimental Station, Annual Report*, 1911; 24: 108 - 159.
 20. Munns R. Genes and salt tolerance: bringing them together. *New Phytol.*, 2005; 167: 645-663.
 21. Munns RC. Comparative physiology of salt and water stress. *Plant Cell Environ.*, 2002; 25: 239-250.
 22. Murillo-Amador, B., Troyo-Diequez, E., Garcia-Hernandez, J.L., Lopez-Aguilar, R., Avila-Serrano, N.Y., Zamora-Salgado, S., Rueda-Puente, R.O. and Kaya, C. Effect of NaCl salinity in the genotypic variation of cowpea (*Vigna unguiculata*) during early vegetative growth. *Scientia Horticulturae* 2006; 108, 423-431.
 23. Jamil M, Bashir S, Anwar S, Bibi S, Bangash A, Ullah F, Rha ES. Effect of salinity on physiological and biochemical characteristics of different varieties of rice. 2012.
 24. Parida AK, Das AB. Salt tolerance and salinity effect on plants: A Review. *Ecotoxicol. Environ. Safety*, 2005; 60: 324-349.
 25. Peleg, Z., Reguera, M., Tumimbang, E., Walia, H., Blumwald, E. Cytokinin mediated source/sink modifications improve drought tolerance and increases grain yield in rice under water stress. *Plant Biotechnology Journal*, 2011; in press (DOI: 10.1111/j.1467-7652.2010.00584.x).
 26. Pettigrew, W.T. Potassium influences on yield and quality production for maize, wheat, soybean and cotton (electronic source). *Physiologia plantarum*. 2008; 133 (4): 670-681.
 27. Rausch T, Kirsch M, low R, Lehr A, Viereck R, Zhigang A. Salt stress responses of higher plants: the role of proton pumps and Na⁺/H⁺ antiporters. *J. Plant Physiol.*, 1996; 148: 425-433.

28. Rodriguez HG, Roberts JKM, Jordan WR, Drew MC. Growth, water relations, and accumulation of organic and inorganic solutes in roots of maize seedlings during salt stress. *Plant Physiol.* 1997; 113: 881- 893.
29. Sarwar MKH, Ullah, I, Rahman M, Ashraf M, Zafar Y. Glycin betaine accumulation and its relation to yield and yield component in cotton genotype grown under water deficit condition. *Pak. J. Bot.*, 2006; 38(8): 1449-1456.
30. Steel RGD, Torrie JH. Principles and procedures of statistics, second ed. McGraw Hill, New York. 1980.
31. Turan, M.A., Elkarim, A.H.A, Taban, N., Taban, S. Effect of salt stress on growth, stomatal resistance, proline and chlorophyll concentrations on maize plant. *Afr. J. Agric. Res.*, 2009; 4 (9): 893 – 897.
32. Veeranagamallaiah G, Chandraobulreddy P, Jyothsnakumari G, Sudhakar C. Glutamine synthetase expression and pyrroline-5-carboxylate reductase activity influence proline accumulation in two cultivars of foxtail millet (*Setaria italica* L.) with differential salt sensitivity. *Environ. Exp. Bot.*, 2007; 60: 239-244.
33. Witcombe JR, Hollington PA, Howarth CJ, Reader S, Steele KA. Breeding for abiotic stresses for sustainable agriculture. *Philos. Trans. R. Soc. B: Biol. Sci.*, 2008; 363: 703–716.
34. Zahedi SM, Ansari NA, Azizi M. The study of the effect of salinity stress on the germination and initial growth of cowpea (*Vigna unguiculata* L. Walp). *J. Agric. Technol.*, 2012; 8(7): 2353-2372.

12/20/2014