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Effect of Pretreatment of H_2O_2 on Seed Germination and Vegetative Growth of Chickpea (*Cicer arietinum* L.)

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Abstract: Seed priming is a simple pre-germinating strategy to improve seed performance and alleviate the negative effects associated with stress exposure. Seed priming technique was used in different plants to improve seed germination and tolerance on stress exposure. The aim of this study was to evaluate the effects of H_2O_2 on seed germination and vegetative growth of different plants. Overall the tolerance index was improved under salt treatment when the seeds were pretreated with H_2O_2.

Key words: Pretreatment · H_2O_2 · Chickpea · Germination · Vegetative

INTRODUCTION

Salinity refers to the occurrence of various salts in soil or water in concentration that may interfere with the growth of plants. It comprises of chloride, sulfates and bicarbonates of sodium, calcium, magnesium and potassium [1]. The problem of water-logging and salinity in Pakistan is typical for irrigated agriculture where adequate drainage is not provided. Soil salinity in Pakistan is a product of climatic conditions, original soil chemistry, land use, irrigation practices and the shallow depth of the water table [2]. In Pakistan substantial salinization is reported in all the provinces [1]. Overall the total area of Pakistan is 79.61 million ha out of which 21.87 m ha is cultivated and 6.18 m ha is salt affected. Total area of Punjab is 20.63 m ha out of which 12.27 m ha is cultivated and 2.67 m ha is salt affected, the total area of Sindh is 14.09 m ha out of which 5.65 m ha is cultivated and 2.11 m ha is salt affected, the total area of Balochistan is 34.72 m ha out of which 2.11 m ha is cultivated and 1.35 m ha is salt affected, the total area of KPK is 10.17 m ha out of which 1.84 m ha is cultivated and 0.05 m ha is salt affected [1].

Among various strategies, pre-sowing treatment and priming of seeds are easy, low cost low risk and effective approaches to overcome the environmental stress problems [3, 4]. Priming is a controlled hydration process followed by re drying that allows metabolic activities to proceed before radical protrusion [5-7]. The adverse effects of salt-stress can be alleviated by various measures, including seed priming (a.k.a. pre-sowing seed treatment). The general purpose of seed priming is to partially hydrate the seed to a point where germination processes are begun but not completed [4]. Various priming strategies include osmopriming, halopriming, hormonal priming or hydropriming, etc. which involving treatment of seeds with osmotica, inorganic salts, hormones or water, respectively, to induce pre-germination changes. These changes usually have profound effects on germination rate and uniformity in emergence of seedlings, specifically under stressful conditions [4, 8].

Exogenous H_2O_2 treatments simultaneously enhanced multi-resistance to heat, chilling, drought and salt stresses in maize seedlings [9]. Uchida *et al*. [10] and Azevedo Neto *et al.* [11] demonstrated that the pretreatment with H_2O_2 in nutrient solution induces acclimation to salt stress in rice and maize seedlings, respectively.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of Lahore College for Women University. The chickpea seeds of wild type were collected from National Agriculture Research Centre, Islamabad.

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Experiment: 1 at Germination Stage

Sterilization: Surface sterilization of seeds was done by washing seeds with 2% mercuric chloride solution for 5 minutes and then the seeds were rinsed with distilled water thrice. Plastic Petri dishes with a tight-fitting lid were used for the experiment. Petri dishes were first washed and then oven dried at 1500°C. 20 seeds were grown in each Petri dish. Seeds were hand sorted to eliminate broken, small and infected seeds. Seed were allowed to germinate in laboratory conditions on filter paper (Whatman No. 1) in Petri dishes. 5 treatments were applied and there were 3 replicates per treatment. In first treatment, distilled water was applied (control set). In second treatment, seeds were first presoaked in distilled water for 8 hours and then distilled water was applied. In third treatment, seeds were first soaked in H_2O_2 for 8 hours and then distilled water was applied. In fourth treatment, seeds were first soaked in distilled water for 8 hours and then 150mM NaCl was applied. In fifth treatment seeds were first soaked in H_2O_2 and then 150mM NaCl was applied. Seeds were allowed to germinate in laboratory conditions. Seeds were considered to be germinated with the emergence of the radical. The germinating seeds were counted at regular intervals. The lengths of radical and plumule of the germinated seeds were measured and recorded after 10 days.

Experiment: 2 At Vegetative Stage: Same sterilization procedure was applied as in experiment1.In this experiment seeds were grown in plastic pots of 5.12g weight filled 3/4th with soil. 1 seed was sown in each pot at a depth of 2 cm. Same treatments were applied on alternate day as in experiment 1 and there were 3 replicates per treatment. After 14 days plants were harvested. Root length and shoot length were measured. Samples were stored after weighing at -20°C in freezer till further analysis.

Relative Water Content of Leaves: The fresh weight of flag leaf from each treatment was recorded. The leaves were then immersed in distilled water in beakers and left for 24 h. Thereafter, fully turgid leaves were weighed again. The leaves were dried in oven for 72 h at 70°C, until constant weight of leaves was obtained. Relative water content (RWC) of flag leaf was calculated.

RWC = [(fresh mass - dry mass)/ (saturated mass - dry mass)] × 100.

Proline Content of Leaves: Proline was measured from the upper 2nd and 3rd leaves. Freeze-dried grinded material approximately (0.1g) was homogenized with 4mL. sulfosalicylic acid (3.0%) in mortar and placed overnight at 5°C. Suspension was centrifuged at room temperature at 3000 rpm for 5min. Supernatant was mixed with 4 mL.acidicninhydrin reagent. Reaction mixture was mechanically shaken; the contents in the tubes were heated in boiling water bath for 1h. Thereafter content in the tubes were cooled and the mixture was extracted with 4 mL.of toluene in a separating funnel. The absorbance of toluene layer was recorded at 520 nm with a spectrophotometer. Preparation of Ninhydrin reagents.

Ninhydrin (2.5g) was dissolved in 6 M orthophosphoric acid. 40.5mL phosphoric acid (6M) was prepared by mixing 28.5 mL orthophosphoric acid 85% with 12mL distilled water. Glacial acetic acid (180mL) was added to reagent.

Chlorophyll and Carotenoids Test: The chlorophyll and Carotenoids tests were taken on spectrophotometer model UV 300, Company O.R.I., ReinbekerWeg 75 Hamburg, Germany. The readings of samples were taken on different wavelengths i.e. 480, 510, 645, 663. Following formula was applied on the spectrophotometer reading.

Chl.a (mg g^{-1} f.wt) = [12.7 (O.D 663) - 2.69 (O.D 645)] x V/1000 x W

Chl.b (mg g^{-1} f.wt) = [22.9 (O.D 645) - 4.68 (O.D 663)] x V/1000 x W

Carotenoids (mg g^{-1} f.wt) = [7.6 (O.D 480) - 1.69 (O.D 510)] x V/1000 x W

W = weight of the fresh leaf (g)

V = volume of the extract (ml)

RESULTS

The data representing the effect of pretreatment of H_2O_2 on germination percentage of chickpea under salt stress (Fig. 1) differ non-significantly ($p<0.05$). The data regarding the relative water content of leaves (%age) is given in Fig. 2. This data also revealed non-significant ($p<0.05$) effect of different treatments.

It was observed that the 150mM NaCl induced a significant decrease in the contents of pigment fractions (chlorophyll a and b) and consequently of the total chlorophyll content as compared with control plants. The chlorophyll b and total chlorophyll content of the leaves of chickpea exhibited a little increase when

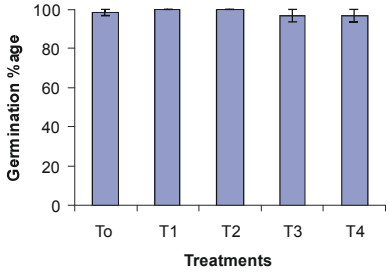

Fig. 1: The effect of pretreatment of H_2O_2 on germination percentage of chickpea

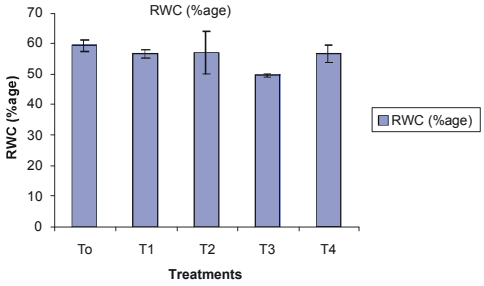

Fig. 2: The effect of pretreatment of H_2O_2 on relative water content (%age) of chickpea at vegetative growth

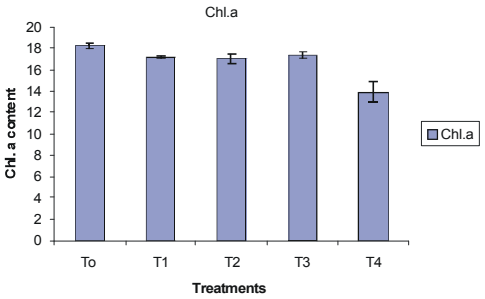

Fig. 3: The effect of pretreatment of H_2O_2 on chlorophyll a content (mg/l) of chickpea at vegetative growth

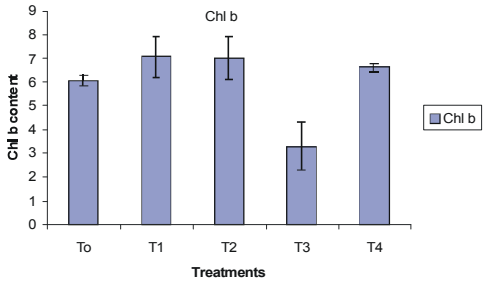

Fig. 4: The effect of pretreatment of H_2O_2 on chlorophyll b content (mg/l) of chickpea at vegetative growth

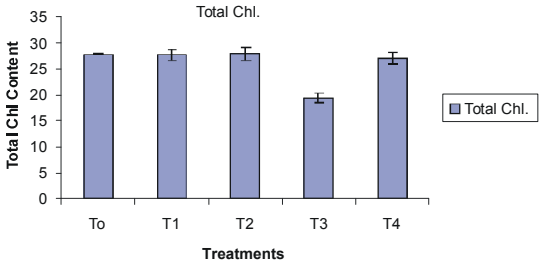

Fig. 5: The effect of pretreatment of H_2O_2 on total chlorophyll content (mg/l) of chickpea at vegetative growth

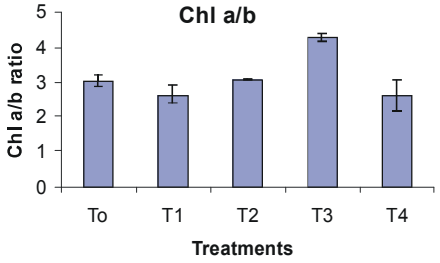

Fig. 6: The effect of pretreatment of H_2O_2 on chlorophyll a/b ratio (mg/l) of chickpea at vegetative growth

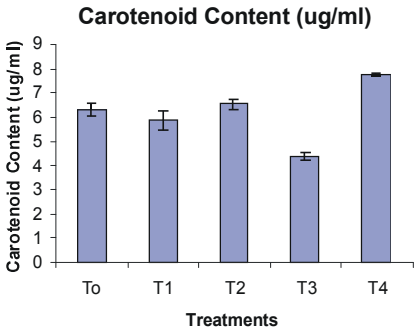

Fig. 7: The effect of pretreatment of H_2O_2 on carotenoid content (ug/ml) of chickpea at vegetative growth.

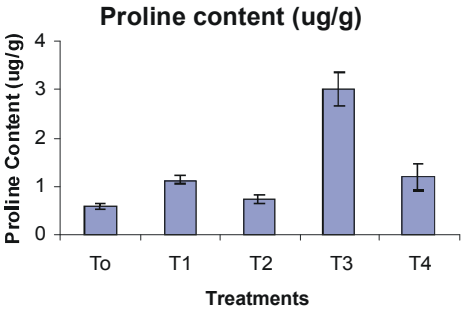

Fig. 8: The effect of pretreatment of H_2O_2 on proline content of chickpea at vegetative growth.

chickpea seeds were pretreated with H_2O_2 as compared to the plants which were not pretreated with H_2O_2 while the pigment contents of the rests of the treatments differ non-significantly (Fig. 3, 4 and 5). The chlorophyll a/b ratio was maximum under NaCl treatment; the rests of the treatments differ non-significantly ($p<0.05$) as shown in Fig. 6.

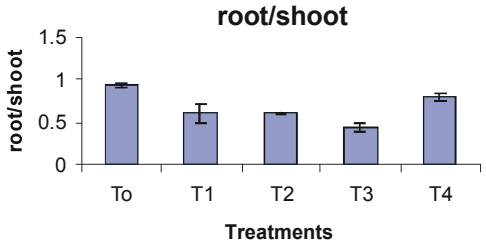

Fig. 9: The effect of pretreatment of H_2O_2 on root/shoot ratio of chickpea at vegetative growth

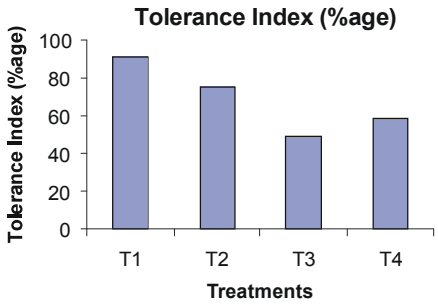

Fig. 10: The effect of pretreatment of H_2O_2 on tolerance index (%age) of chickpea at seed germination stage

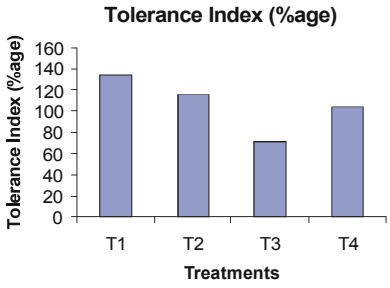

Fig. 11: The effect of pretreatment of H_2O_2 on tolerance index (%age) of chickpea at vegetative stage

The content of carotenoid pigment decreased significantly ($p<0.05$) under NaCl treatment (Fig. 7). The carotenoid content increased significantly ($p<0.05$) when seeds were pretreated with H_2O_2 and NaCl was applied. Remaining treatments differ non-significantly ($p<0.05$).

Proline content of leaves was found to be maximum under NaCl treatment and H_2O_2 pretreated seeds contain less proline content under saline as compared to plants not treated with H_2O_2. Rests of the treatments differ non-significantly in terms of proline content (Fig. 8).

The data revealing the root/shoot ratio was found to be maximum under control treatment. The root/shoot ratio significantly decreased ($p<0.05$) in all treatments as compared to control. In case H_2O_2 pretreated seeds under salt stress, the values of root/shoot ration was found to be higher as compared to the plants under same treatment not pretreated with H_2O_2 (Fig. 9).

The tolerance index (%age) at seed germination of chickpea was found to be maximum under control treatment and minimum under NaCl treatment. There were significant differences among rests of the values (Fig. 10). The tolerance index (%age) at vegetative growth of chickpea was found to be maximum under control treatment and minimum under NaCl treatment. There were significant ($p<0.05$) differences among rests of the values (Fig. 11). Overall the tolerance index was improved under salt treatment when the seeds were pretreated with H_2O_2.

Graphs in figure 1-11 shows the effect of pretreatment of H_2O_2 on chickpea of different parameters.

DISCUSSION

The subject of this work has arisen from the idea that oxidants used in the breaking of seed dormancy [12] and might also be used in alleviating the harmful effects imposed by environmental stresses during germination and vegetative growth. The hypothesis was proven by the findings obtained. Pre-treating chickpea seeds with H_2O_2 in a harmless dose and duration may have mediated "the vigilance" in the seed to an environmental stress and so, the seed, by moving the mechanisms of defense, might have succeeded germination and seedling growth in the conditions of salinity.

The data representing the effect of pretreatment of H_2O_2 on germination percentage of chickpea in Fig. 1 differ non-significantly. The reason of this non-significant difference is that the chickpea seeds used in this experiment might be tolerant to salinity at germination stage.

The data revealing the relative water content (Fig. 2) differ non-significantly because the plant might was able to maintain its water budget through the accumulation of osmotica like proline or through any other mechanism.

A decrease in photosynthetic pigment content of chickpea leaves under salt was observed (Fig. 3 4, 5,6). Many environmental factors control chlorophyll synthesis in plant. Existing these factors as limiting factors cause to disordering the synthesis of chlorophyll. NaCl stress decreased total chlorophyll content of the plant by increasing the activity of the chlorophyll degrading enzyme: chlorophyllase [13], inducing the destruction of the chloroplast structure and the instability of pigment protein complexes. The decrease in chlorophyll contents under saline conditions is reported by Iqbal *et al.* [14] and Ashraf *et al*. [4]. Results of various studies showed that a, b chlorophyll contents, total chlorophyll and carotenoid reduced in leaf of wheat plants

which were grown in saline soil. And this reduction is due to chlorophyllase activity enzymes in plant affected by salinity. A and b chlorophylls rate decreased due to salinity stress, chlorophyll reduction can attributed to changing Nitrogen metabolism direction to forming compounds such as proline which is used to regulate osmosis [15]. Khan *et al.* [16] reported that there is significant correlation between total chlorophyll and alfalfa yield under saline conditions. They suggested that a, b and total chlorophyll decrease due to increasing salinity in alfalfa cultivars. a and b chlorophylls content reduce in respond to salinity stress , this may be for forming protolytic enzymes such as chlorophyllase which is responsible to decompose chlorophyll and damaging photosynthetic structures, is other cause at this reduction [17]. The chlorophyll a/b ratio increased during salinity which implies that stimulation of Chlorophyll a accepted from NaCl is smaller than that of chlorophyll b. contrasting results were obtained in effects of salt stress on the contents of chlorophyll and organic solutes in *Aeluropuslittoralis var. sinesis Debeaux* [18]. And the effect of salinity stress on nutrient uptake and chlorophyll content of tropical turfgrass [19]. It is therefore proven that soil salinity had negative effects on the growth and photosynthetic metabolism of chickpea which can be alleviated by applying H_2O_2 to seeds before sowing.

Salinity treatments caused the increased proline content in chickpea plant (Fig. 7). Proline is believed to protect plant tissue against stress by acting as a nitrogen-storage compound, osmo-solute and hydrophobic protectant for enzymes and cellular structures [20]. Many plants accumulate proline as a non-toxic and protective osmolyte under salinity, including mangrove [21], maize [22], sorghum [23] and canola [24]. Some authors have, however, argued that excessively high levels of proline accumulation may be a response to leaf damage when exposed to high NaCl concentration and that a higher level of proline accumulation is associated with salt sensitive traits in tomato [25] and sorghum [26]. The results could be interpreted as follows. Firstly, salinity stress limits the uptake of CO_2[27], resulting in decreasing carbon reduction by Calvin cycle [28], which leads to non-availability of $NADP^+$ for acceptance of electrons during photosynthesis. These reactions are summarised in Fig. 13. The excess accumulation of proline may therefore be a result of metabolic changes induced by high salinity. The present results also agrees with the observations of Delauney and Verma [29], who stated that excess proline accumulation in response to high salinity functions by other than osmotic adjustment.

The root/shoot ratio was decreased in NaCl treatment (Fig. 9). The contrasting results were found by Snapp and Shennan [30]. The root/shoot ratio was increased when seeds were presoaked in H_2O_2 and NaCl was applied. The reason can be that H_2O_2 increases tolerance to salinity in chickpea.

The effect of pretreatment of H_2O_2 on tolerance index (%age) of chickpea at seed germination and vegetative stage was shown in Fig. 10 and Fig. 11. The tolerance index was maximum in seeds presoaked in distilled water and minimum in NaCl(150mM) treatment. The tolerance index in H_2O_2 pretreated seeds was increased as compared to NaCl treatment. Hence the data proved that the H_2O_2 pretreatment increases tolerance index in Chickpea.

Thus it can be concluded that H_2O_2 pretreatment increases tolerance of chickpea to salinity at germination and vegetative stages by lowering the degradative effects of salt on pigments and by improving root to shoot ratio under stress condition. Extension of the present research may be carried out in field.

REFERENCES

1. Anonymus.2004.http://www.fao.org/docrep/x5871e/x5871e03.htm
2. Sattar, S., T. Hussnain and A. Javaid, 2010. Effect of salinity on cotton (*Gossypium arboretum*). The Journal of Animal and Plant Science, 20(2): 87-89.
3. Wahid, A. and A. Shabbir, 2005. Induction of heat stress tolerance in barley seedlings by pre-sowing seed treatment with glycinebetaine. Plant Growth Reg., 46: 133-141.
4. Ashraf, M. and M.R. Fooland, 2005. Pre-sowing treatment a short gun approach to improve germination under saline and non-saline conditions. Advances in Agronomy, 88: 223-271.
5. Khan, A.A., 1992. Preplant physiological conditioning. Hortic Rev., 13: 131-81.
6. Sivritepe, N., H.O. Sivritepe and A. Eris, 2003. The effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. Sci. Hort., 97: 229-237.
7. Sivritepe, H.O., N. Sivritepe, A. Eris and E. Turhan, 2005. The effects of NaCl pre-treatment on salt tolerance of melons grown under long-term salinity. Sci. Hort., 106: 568-581.
8. Parera, C.A. and D.J. Cantliffe, 1991. Improved germination and modified imbibtion of shrunken-2 sweet corn by seed disinfection and solid matrix priming. J. Am. Soc. Hortic. Sci., 116: 942-945.

9. Gong, M., B. Chen, Z.G. Li and L.H. Guo, 2001. Heat-shock-induced cross adaptation to heat, chilling, drought and salt stress in maize seedlings and involvement of H_2O_2. J. Plant Physiol., 158: 1125-1130.
10. Uchida, A., A.T. Jagendorf, T. Hibino, T. Takabe and T. Takabe, 2002. Effect of hydrogen peroxide and nitric oxide on both salt and heat stress tolerance in rice. Plant Sci., 163: 515-523.
11. Azevedo Neto, A.D., J.T. Prisco, J. Eneas-Filho, J.V. R. Medeiros and E. Gomes-Filho, 2005. Hydrogen peroxide pre-treatment induces stress acclimation in maize plants. J. Plant Physiol., 162: 1114-22.
12. Fontaine, O., C. Huault, N. Pavis and J.P. Billard, 1994. Dormancy breakage of *Hordeumvulgare* seeds:e ffects of hydrogen peroxide and scarification on glutathione level and glutathione reductaseactivity. Plant Physiology and Biochemistry, 32: 677-683.
13. Rao, G.G. and G.R. Rao, 1981. Pigment composition and chlorophyllase activity in Pigeon pea (*Cajanusindicus Spreng*.) and gingelly (*Sesamumindicum* L.) under NaCl salinity. Indian J. Exp. Biol., 19: 768-770.
14. Iqbal, N., M.Y. Ashraf, J. Farrukh, J.M. Vicente and A. Kafeel, 2006. Nitrate reduction and nutrient accumulation in wheat (*Triticumaestivum* L.) grown in soil salinization with four different salts. Journal Plant Nutrition, 29: 409-421.
15. Dela-Rosa, I.M. and R.K. Maiti, 1995. Biochemical mechanism in glossy sorghum lines for resistance to salinity stress. J. Plant Physiol., 146: 515-519.
16. Khan, M.G., M. Silberuus and S.H. Lips, 1998. Response of alfalfa to potassium, calcium and nitrogen under stress induced by sodium chloride. Biol Plant., 40: 251-259.
17. Sabater, B. and M.I. Rodriguez, 1978. Control of chlorophyll degradation in detached leaves of Barley and Oat through effect of kinetic on chlorophyllase levels. Physiol. Plant., 43: 274-276.
18. Liu, Z.H., S.L.R.B.L.R. and Z.K.F, 2007. Effects of salt stress on the contents of chlorophyll and organic solutes in *Aeluropus Littoralis var. sinesis Debeaux.* Zhi Wu Sheng Li Fen Zi Sheng Wu Xue Xue Bao, 33(2): 165-77.
19. Greenway, H. and R. Munns, 2011. Mechanism of salt tolerance of halophytes. Ann. Rev. Plant Physiol., 31(1): 149-190.
20. Greenway, H. and R. Munns, 1980. Mechanism of salt tolerance in nonhalophytes. Annu. Rev. Plant Physiol., 31: 149-190.
21. Parida, A., A.B. Das and P. Das, 2002. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, Bruguieraparviflora, in hydroponic culture. Plant Biol., 45: 28-36.
22. Cicek, N. and H. Cakirlar, 2002. The effect of salinity on some physiological parameters in two maize cultivars. Bulgaria J. Plant Physiol., 28(1-2): 66-74.
23. De Lacerda, C.F., J. Cambraia, M.A. Oliva and H.A. Ruiz, 2005. Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. Environ. Exp. Botany, 54: 69-76.
24. Bandeh-hagh, A., M. Toorchi, A. Mohammadi, N. Chaparzadeh, G.H. Salekdeh and H. Kazemnia, 2008. Growth and osmotic adjustment of canola genotypes in response to salinity. Journal of Food, Agriculture and Environment, 6(2): 201-208.
25. Bolarin, M.C., A. Santa-Cruz, E. Cayuela and F. Perez-Alfocea, 1995. Short-term solute changes in leaves and roots of cultivated and wild tomato seedlings under salinity. J. Plant Physiol.,147: 463-468.
26. De Lacerda, C.F., J. Cambraia, M.A. Oliva and H.A. Ruiz, 2005. Changes in growth and in solute concentrations in sorghum leaves and roots during salt stress recovery. Environ. Exp. Botany, 54: 69-76.
27. James, R.A., A.R. Rivelli, R. Munns and S.V. Caemmerer, 2002. Factors affecting CO_2 assimilation, leaf injury and growth in salt stressed durum wheat. Funct. Plant Biol., 29: 1393-1403.
28. Lawlor, D.W. and C. Cornic, 2002. Photosynthetic carbon assimilation and associated metabolismin relation to water deficits in higher plants. Plant Cell Environ., 25: 275-294.
29. Delauney, A.J. and D.P.S. Verma, 1993. Proline biosynthesis and osmoregulation in plants. Plant J., 4: 215-223.
30. Snapp, S.S. and C. Shennan, 1992. Salinity effects on root growth and death dynamics of *Lycopersicon esculentum.* New Phytologist, 121: 71-79.