Effect of Sodium Sulfate on Growth Parameters, Biochemical Parameters, Lipid Peroxidation (LPO), and Different Antioxidant Parameters of Indian Mustard (Brassica Juncea) var. Goldi

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Abstract

Soil salinity is one of the important constraints limiting crop production in arid and semi-arid regions. Here our experiment was conducted to determine the effect of different concentrations (0, 4Mm hos/cm, 8Mm hos/cm and 12Mm hos/cm) of salt (Na2SO4) on growth parameters, biochemical, reactive oxygen species (ROS) and different antioxidants (enzymatic and non-enzymatic scavengers) parameters such as catalase (CAT), Superoxide Dismutase (SOD), Ascorbic acid (ASA) and proline parameters of Brassica juncea (var. Goldi), along with nontreated control plants in measurement periods at 65 and 75 DAS. Salt treatment showed a significantly negative effect on plant length (cm), leaf area (cm2), fresh and dry mass (gm), and chlorophyll content (mg-1 FM) at 65 DAS. In the leaves of salt-stressed plants, Lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), ascorbic acid (ASA), and proline activities significantly increased with increasing salt concentrations as compared to the control plants at 75 DAS. Our results suggest that Na+ is detoxify and show less impact of salt stress in mature and younger leaves. This work understanding how a plant responds at different salinity levels. This work may be helpful to know the insights of susceptibility/ resistance of crop growth under salt stress.

Keywords: Salinity, Brassica juncea, Reactive Oxygen Species (ROS), Na2SO4 and Antioxidants.

Introduction

Salinity is one of the most important abiotic factors and a wide-spread agricultural problem in semi-arid regions, which renders fields unproductive and limits plant growth (Banzai et al. 2002: Khan 2003; and Athar et al. 2009). Salt stress can affect plant survival, biomass, plant height, and plant morphology and affect the capacity of a plant to collect water and nutrients (Parida and Das; 2005). In India, about 30 million hectares of coastal land remains barren and uncultivable because of saline soil. High levels of soil salinity can cause water deficit, ion toxicity, and nutrient deficiency. Salinity can also affect plant growth because of the high concentration of salts in the soil solution interferes with the balanced absorption of essential nutritional ions by plants. Saline soils of Iran are form from the accumulation of various chloride and sulfate salts dominated by Na2SO4 Jafari (1993) & Szabolic (1992). Abiotic stresses are the main factors for reducing crop yield (Munns and Tester, 2008). Sulfur (S) is a structural constituent of several coenzymes and prosthetic groups, such as ferredoxin, which are also use for N assimilation. Accumulation of N maintains high chlorophyll content and enzyme activity (Lawlor et al. 1989). ROS formation also induce due to high salinity levels with in plant cells, and its results over-accumulation cause oxidative damage of membrane lipids, proteins, and nucleic acids (Pérez-López et al., 2009; Gill and Tuteja, 2010). Several experiments have documented the mitigation effect of Si application under salt stress on many plants. Conceicao et al. (2019) examined the protective role of Si in sunflower when grown under salinity conditions. The Oleiferous Brassica is the third most important source of vegetable oil in the world. Brassica is the Latin name of a genus that is taxonomically place within the Brassicaceae (Cruciferae), which is one of the tenth most economically important plant Family in the world. Indian mustard (Brassica Juncea (L.) Czern & Coss) is a rabi crop extensively grown as rain-fed as well as under irrigated conditions. Mustard is high in Vitamin A and C and iron. Per 100 g, the leaf is contain 24 calories.

Material and Methods

The experiments was conduct in the research laboratory of the department of Botany, Mohammad Ali Jauhar University, Rampur India, during the winter session of 2018-19. This study was Perform with Indian mustard seeds (var. Goldi). Series of pots (12cm) was fill with an equal amount of soil (kg-1), and then the equal number of sterilized seeds was sown and addeddifferent salt concentrations, i.e. (0, 4Mm hos/cm, 8Mm hos/cm and 12Mm hos/cm) of salt (Na2SO4) in the soil and mixed thoroughly. Each treatment was represents by three pots. Samples were randomly collect at 65 and 75 days after sowing (DAS) and determined the effect of three levels of Na2SO4 on growth parameters (Plant length and leaf area), biochemical parameters such as (fresh and dry mass and chlorophyll). The estimation of total chlorophyll was done in the matured leaf by spectrophotometer using the method of Arnon (1949). Identify the level of lipid peroxidation (LPO) was measured in the term of malondialdehyde (MDA), as described by Rao and Sresty (2000). The estimation of Enzymatic antioxidants also identified, such as catalase (CAT), was measured in the samples based on a previously established protocol by Hosetti and Frost (1994) and the superoxide dismutase (SOD) activity was estimate by Bauchamp and Fridovich (1971). Non- enzymatic antioxidants such as ascorbic acid (ASA) concentration was identify by using a slightly modified method of Luwe et al., (1993). Proline (Pro) content was measured using ninhydrin reagent and toluene extraction (Bates et al., 1973).

Statistical Analysis

All the statistical analysis was perform using computing. The data represented the average of the three replicates have been analyzed statistically and represented in figures. The effect of salinity levels and time (days) on all parameters was determined using one-way ANOVA and two-way ANOVA.

Results and Discussions

Salt Stress

Sodium sulfate (Na_2SO_4) is the dominant salt in alkaline soils, Therefore sodium exists in the soil solution as well as on clay surfaces. Consequently, in plants the salinity stresses in two ways: higher concentrations of salts in the soil, make it roots harder for extract water (osmotic stress), and secondly high salt level within the plant may be harmful (specific ion toxicity) (Munns and Tester et al., 2008). Salt stress has a main impact on development and plant growth and Processes of seedling growth. Vegetative growth is also adversely affected by high salt concentration, which ultimately causes low plant growth (Sairamand and Tyagi, 2004).

Effect of Salinity (Na2SO4) on Growth Parameters

Plant length (Root and shoot length)

Plant height (cm) was negatively associated with different salt concentrations (0, 4Mm hos/cm, 8Mm hos/cm and 12Mm hos/cm) at 65 DAS (Fig.a). Shoot and root growth increased of mustard plants at 4 Mm hos/cm salt treatment as compared to the control plants. The toxic effects observed on the leaves were mainly necrosis at high salt (Na_2SO_4) concentration (12Mm hos/cm). In cultivar (Brassica juncea) at low (4 Mm hos/cm) and moderate (8 Mm hos/cm), salinities showed minimum plant death. The plant death was observed at a high (12 Mm hos/cm) salinity level as compared to control. Growth has been consider as the result of different physiological mechanisms and its reduction after salt treatment (Munns 2002). Sign of leaf necrosis has often been correlated with high sodium (Karakas et al., 2000), Kapoor and Srivastava (2010) on Vigna mungo L. They found that increasing the concentrations of salts developed a decline in the lengths of the plants.

Leaf area

Generally, the results showed a reduction in leaf area with increasing salinity levels (0, 4Mm hos/cm, 8Mm hos/cm, and 12Mm hos/cm) at 65 DAS (Fig.b). Salinity had no significant effect on leaf area (cm2). In fig. b result showed that a higher level of salt (Na2SO4) decrease the leaf area throughout the experiment compared with the control plants. The result showed a negative relationship between salinity stress and vegetative growth parameters. These findings are in agreement with the results which have been report for pistachio rootstocks (Karimi et al., 2014). These results also agree with what Mathur et al. (2006) reported that the stress of the moth bean plant (Vigna aconitifolia L.) with increasing concentrations of sodium chloride led to a decrease in leaf area.

Biochemical Parameters

Fresh and Dry Mass

Fresh and dry mass (gm) significantly affected by salt treatment. In cultivar mustard (var. Goldi) showed more reduction in both weights of root and shoot, which treated with 8 Mm hos/cm and 12 Mm hos/cm salt (Na2SO4) at 65 DAS. In fig.c at 4Mm hos/cm, Na2SO4 treatment shows an increase in both fresh and dry weight of mustard as compared to the untreated plants. Many studies worked on the same trends, the study by Jamil et al., (2007) on radish plants (Raphanus sativus L.), a work by Rui et al., (2009) on Bruguiera L., and finally a experiment by Memon et al., (2010) on Brassica compestris L. The increase in fresh weight of the shoot system may be due to the ability of the plant to increase the size of its sap vacuoles.

Chlorophyll and Carotenoids

Study of using different concentrations (0, 4Mm hos/cm, 8Mm hos/cm and 12Mm hos/cm) of salt (Na2SO4) on the mustard plants at 65 DAS. Notify the chlorophyll content, including chlorophyll 'a'; 'b', and total chlorophyll. The results showed in fig.d and e, inverse relationship between salt concentration and chlorophyll & carotenoids content. Whenever the salt concentration increased, chlorophyll & carotenoid content decreased, respectively. At 4Mm hos/cm treatment showed no reduction of chlorophyll & carotenoids content as compared to control plants. Taffouo et al. (2010), on Vigna subterranean (L.) Demonstrate that salt stress ofsodium chloride caused a decrease in total chlorophyll content. Also, Mustard and Renault (2006) registered a reduction of carotenoids content in seedlings of dogwood, which were stress with salt treatment.

Lipid peroxidation (LPO)

In this study, seeds were grown with different salt concentrations (0, 4Mm hos/cm, 8Mm hos/cm and 12Mm hos/cm) to study the effect of salinity on lipid peroxidation (LPO) at 75 DAS. The LPO was estimated as thiobarbituric acid (TBA) reactive substances. In fig. f , LPO significantly increased with increasing concentration of sodium sulfate (Na2SO4). At 12Mm hos/cm showed higher enhancement of LPO as compared with non-treated plants. Under salt stress, the LPO increase can be correlated with the accumulations of ions and active oxygen species (AOS) production (Hernandez et al., 2001 and Misra and Gupta, 2006).

Enzymatic Antioxidants

Catalase (CAT)

Effect of salt stress had no significant role on catalase (CAT). In the leaves of Brassica juncea (var. Goldi), CAT activity for control and salt-stressed plants changed during the experimental period at 75 DAS (fig.g). In the leaves of salt stress, plants of mustard CAT activity increase mainly at 8Mm hos/cm and 12Mm hos/cm treatment as compared with 4Mm hos/cm. Our result is agreement with Rout and Shaw (2001), who suggested that CAT is the important H2O2 scavenging enzyme, leading to salt tolerance. These enzymes also important in salt tolerance in barley (Liang et al., 2003).

Superoxide Dismutase (SOD)

In our experiment, in the leaves of Brassica juncea SOD activity also increased with increasing concentration of salt (Na2SO4) at 75 DAS. In fig. h showed that SOD activity in salt-stressed plants greater than control plants. At 12 Mm hos/cm showed higher enhancement of SOD activity as compared with others. SOD is one of the most ubiquitous enzymes and plays a key role in cellular defense mechanisms against ROS. It has been show that different concentrations of salinity increase SOD activity in salt-tolerant cultivars (Gosset et al., 1994 and Hernandez et al., 2000).

Non-Enzymatic Antioxidants

Ascorbic acid (ASA)

In our study also observed that different concentration (0, 4Mm hos/cm, 8Mm hos/cm, and 12Mm hos/cm) of salt significantly increased the activity of ascorbic acid (ASA) in the leaves of Brassica juncea (var. Goldi) at 75 DAS. In fig.i, the highest positive effect was observed with 12Mm hos/cm salt treatment as compared to the untreated plants. Ascorbic acid plays main roles in plant growth, functioning in cell division, cell wall expansion, and other developmental processes (Foyer et al., 2003). ASA is a key component, and an essential requirement of a tolerance.

Proline (Pro)

The enhanced proline activity was observ in this study. The amount of proline content in the leaves of Brassica juncea (var. Goldi) increased with increased different salt (Na2SO4) concentrations (0, 4Mm hos/cm, 8Mm hos/cm and 12Mm hos/cm) at 75 DAS. Proline is an essential amino acid that is ubiquitous in all the plants. In fig.h , the maximum proline accumulation was record at 12Mm hos/cm treatments. The content of proline increased has also been identify earlier in response to salt stress in Brassica juncea (Hayat et al., 2007; Yusuf et al., 2008).

Conclusion

Several studies have revealed that the Increasing concentration of salt (Na2SO4) have the strongest negative effect on the plant growth parameters. At a lower (4Mm hos/cm) dose of salt showed a positive impact on growth parameters as compared to the 8Mm hos/cm and 12Mm hos/cm experiments. A significant correlation was present between sodium sulfate and ROS (LPO), enzymatic & non- enzymatic ROS scavengers. Our work might help grow mustard cultivar under salinity conditions.

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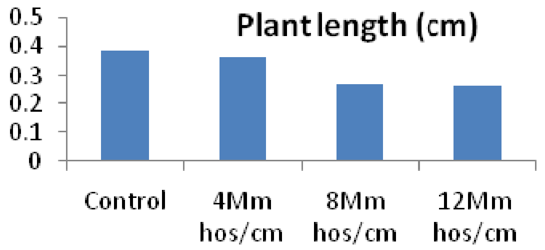


Figure.a

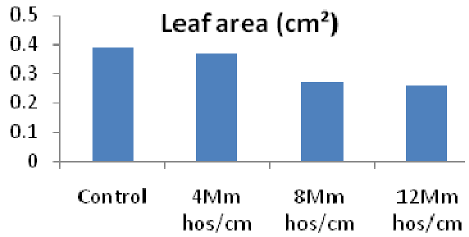
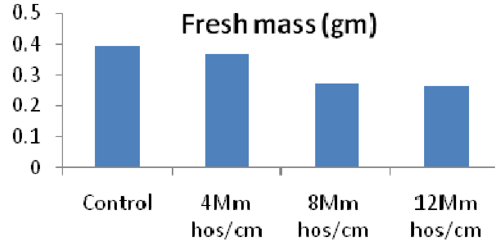


Figure.b



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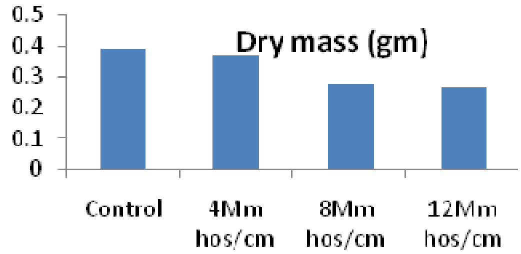

Figure.d

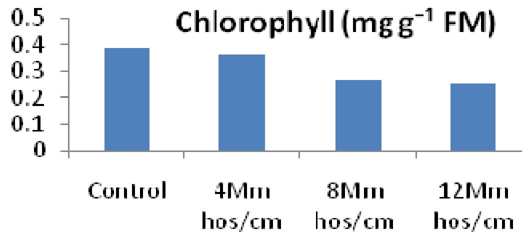

Figure. e

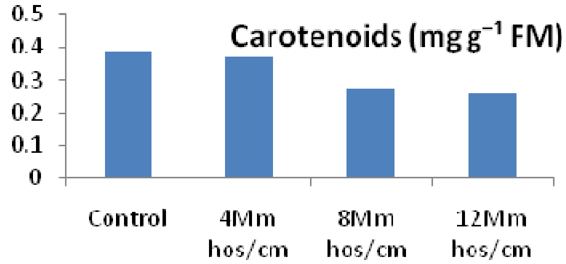

Figure. f

Salt stress show a decreasing effect on growth and biochemical parameters of mustard (Brassica juncea L.. var. Goldi) at 65 DAS.

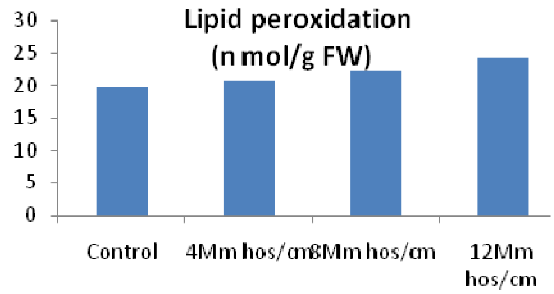

Figure. g

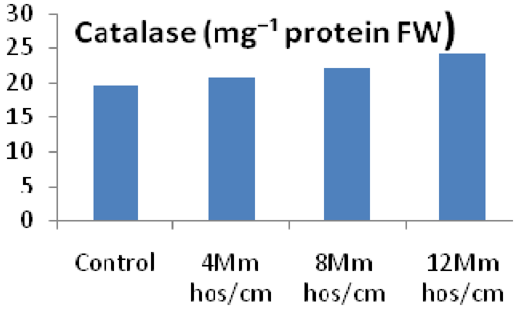

Figure. h

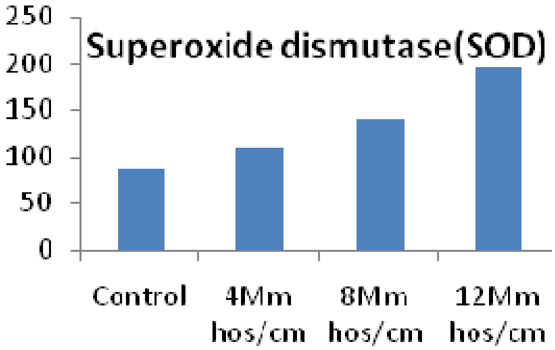

Figure. I

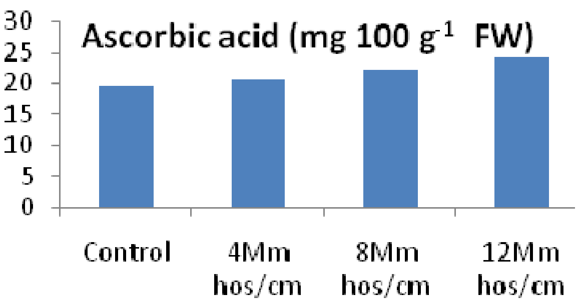

Figure. j

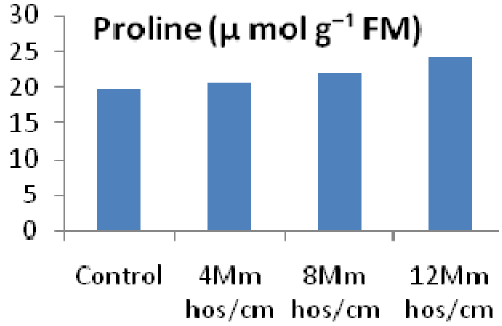

Figure. K

Salt stress show an increasing effect on lipid peroxidation (LPO) and different antioxidant Parameters of mustard (Brassica juncea L.. var. Goldi) at 75 DAS.

They are showing the effect of different concentrations (0, 4Mm hos/cm, 8Mm hos/cm, and 12Mm hos/cm) of salt on plant height (cm) of mustard.

References

1. Athar HR, Khan A and Ashraf M. (2009). Inducing salt tolerance in wheat by exogenously applied ascorbic acid through different modes. J. Plant Nutr. 32:1799-1817.

2. Banzai T, Hershkovits G, Katcoff DJ, Hanagata N, et al., (2002). Identification and characterization of MRNA transcripts differentially expressed in response to high salinity using differential display in the mangrove. Plant Sci.162:499 505.
3. Khan, NA. (2003). NaCl-inhibited chlorophyll synthesis and associated changes in ethylene evolution and antioxidative enzymes in wheat. Biol. Plant 47: 437-440.
4. Parida, A.K and Das, A.B. (2005). Salt tolerance and salinity effects on plants. Ecotoxicol. And Environ. Safety 60:324-334.
5. Jafari. M. (1993). Investigation tolerant of some of Iran wide-range land grasses plant to salt stress, institute of forest & range researches of Iran publication house. Num: 67.
6. Szabolic, I. (1992). Salinization of soil and water and its relation to desertification. Desertification Contr. Bull. 21:32-37.
7. Lawlor DW, Kontturi M and Young AT. (1989).By flag leaves of wheat occur photosynthesis, about protein, ribulose biphosphate carboxylase activity, and nitrogen supply. J. Exp . Bot. 40:43-52.
8. Arnon, DJ., and Stout, PR. (1949). In isolated chloroplasts, Copper enzymes, and in beta Vulgaris. Plant physiology. 14: 371-375.
9. Pérez-López U., Robredo A., et al. (2009). The salinity caused oxidative stress in two barley cultivars are mitigated by elevated CO2. Physio. Plant, 135: 29-42.
10. Gill S. S., Tuteja N. (2010). Abiotic stress tolerance in crop Plants, Reactive oxygen species and antioxidant machinery. Plant Physio. Biochem., 48 -: 909–930. 10.
11. Munns, R., and M. Tester (2008). Mechanisms of salinity tolerance, Ann. Rev. Plant, Biol. 59:651–681.
12. Smith, J.H.C., Benitez, A. (1955). Chlorophylls analysis in plant materials. In: Peach, K., Tracey, M.V. (Eds.).-. In: Modern Methods of Plant Analysis, vol. 4. Springer- Verlag, Berlin, pp. 142–196.
13. Rao and Sresty, (2000). In the seedlings of pigeon pea (Cajanus Cajan (L.) Millspaugh) antioxidative parameters in response to Zn and Ni stresses plant Sci.157 113-128.
14. Conceicao, S. S., Oliveira Neto, C.F.D., Marques, E. C., Barbosa, A.V.C., Galvao, et al., (2019). Silicon modulates the activity of antioxidant enzyme and nitrogen compounds under salt stress sunflower plants. Agronomy and Soil Science, 65, 1237-1247.
15. Hosetti, B.B., and Frost, S. (1994). Catalase activity in useless water, Water Res. 28: 497-500.
16. Bates, L. S., Teare, I. D., et al., (1973). For water-stress studies rapid determination of free proline. Plant and Soil. 39: 205-207.
17. Beauchamp, C., and Fridovich, I. (1971). Superoxide Dismutase: Improved Assays and an Assay Applicable to Acrylamide Gels Anal Biochem. 44: 276.
18. Luwe MWF, Takahama U, Heber U (1993). Role of ascorbate in detoxifying ozone in the apoplast of spinach (Spinacia oleracea L.) leaves, Plant physiology, vol.101:969-976.
19. Munns, R. (2002). Comparative physiology of salt and water stress. Plant, Cell & Environment, 25(2), 239-250.
20. Kapoor, K., Srivastava, A. (2010). Salinity tolerance of Vigna mungo (var. Pu-19), for Assessment of using ex vitro methods. Asian J. Biotech. 2 (2), 73–85.
21. Jamil, M., Rehman, S.U., Lee, K.J., Kim, J.M., Rha, H.K. (2007b). Growth reduced ps2 photochemistry and chlorophyll content by salinity in radish. Sci. Agric. 64(2), 111–118.
22. Rui, L., Wei, S., Mu-xiang, et al., (2009). In Burguiera seedlings leaf anatomical changes under salt stress. Sub-trop. Bot. 17 (2), 169–175.
23. Memon, S.A., Hou, X., Wang, L.J. (2010). Morphological analysis of Pak Choi. EJEAFChe 9 (1), under salt stress responses, 248–254.
24. Karakas B. Bianco RL, Rieger M. (2000). Association of leaf marginal scorch with sodium accumulation in salt-stressed peach, Hort Sci. 35:83-84.
25. Mathur, N., Singh, J.et al. (2006). Genotypes at different salinity levels in moth bean, Biomass production, productivity, and physiological changes. Am. J. Plant Physiol. 1 (2), 210–213.
26. Taffouo, V.D., Akoe, A. et al., (2010). Three Bambara groundnuts (Vigna subterranean (L.) verdc.) responses growth, yield, water status, and ionic distribution, grown under saline conditions. Int. J. Bot. 6 (1), 53–58.
27. Mustard, J., Renault, S.(2006). The response of red-osier dogwood seedling to NaCl during the onset of bud break. Can. J. Bot. 84 (5), 844–851.

28. Foyer, C.H. and Noctor, G. (2005). A re-evaluation of the concept of oxidative stress in a physiological context, and oxidant and antioxidant signaling in plants:. Plant Cells Environ. 28: 1056–1071.
29. Hayat S, Ali B, Hassan SA, Ahmad A.(2007). Effect of salinity induced changes in Brassica juncea. Turk J, Biol. 31:141_146.
30. Yusuf M, Hasan SA, Ali B, Hayat S, et al./ (2008). In Brassica juncea salinity-induced changes due to effect of salicylic acid. J Integ Plant Biol. 50:1_7.
31. Hernandez, A. Jimenez, A.R. Barcelo, F.Sevilla. (2001). Antioxidant system and o2-/H2O production in the apoplast of Pisum L. leave: its relation with NaCl induced necrotic lesions in minor veins. Plant Physiology, 127 -, pp .817-831.
32. Misra and Gupta (2006). In Catharanthus roseus seedlings effect of salinity and different nitrogen sources on the activity of antioxidant enzymes and indole alkaloid content, Journal of Plant Physiology, 163.11-18.
33. Rout, N.P., Shaw, B.P. (2001). Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci. 160, 415-423.
34. Liang, Y., Chen Q., Liu, Q., et al. (2003). Antioxidant enzymatic activity increases by exogenous silicon (Si) and reduces lipid peroxidation in roots of salt-stressed barley (Hordeum vulgare L.). J. Plant Physiology. 160, 1157-1164.
35. Gosset, D.R., Millhollon, E.P., Lucas, M.C., (1994). Antioxidant response to NaCl stress in salt-tolerance and salt-sensitive cultivars of cotton. Crop Sci. 34, 706- 714.
36. Harnandez, J.A., Jimenez, et al. (2000). Tolerance of Pea (Pisum sativum L.) to long term stress is associated with induction of antioxidant defenses. Plant Cell Environ. 23, 853-862.
37. Karimi HR, MalekiKuhbanani R. (2014). Evaluation of hybrid of P. Atlantica x P. Vera cv. "Badami-Rize-Zarand" as pistachio rootstock to salinity stress according to some growth indices, ecophysiology and biochemical parameters. J Stress PhysiolBiochem 10 (3): 5-17.
38. Munns, R., and M. Tester (2008). Mechanisms of salinity tolerance, Ann. Rev.PlantBiol.59, 651–681, DOI: 10.1146 annurev.arplant.59.032607.092911.
39. Sairam, R.K. & A. Tyagi (2004). In plants molecular and physiological biology of salinity stress tolerance Current Science. 86: 407-421