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A study on the salinity stress effects on the biochemical traits of seedlings and its relationship with resistance toward sensitive and tolerant flax genotypes

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ABSTRACT

Flax (*Linum usitatissimum*) is an important oilseed and medicinal plant that numerous breeding lines and cultivars have been produced by plant breeders up to now. The present study was conducted to evaluate the biochemical factors changes affected by NaCl oxidative stress in different genotypes of flax. For this purpose, an experiment was conducted as a factorial arrangement based on a completely randomized design with three replications in greenhouse conditions. The experimental factors included three genotypes (Tabare, Golchin, and 375Ha) as the first experimental factor and salinity stress (120 mM NaCl) time course at three levels (0, 24, and 48 hours after salinity treatment) as the second factor. The measured biochemical factors in the leaf were catalase, guaiacol peroxidase, malondialdehyde, hydrogen peroxide, proline, and superoxide dismutase (SOD). The results showed that salinity stress had a significant effect on the content of measured biochemical parameters. The levels of catalase, guaiacol peroxidase, hydrogen peroxide, and proline were statistically significant in the tolerant flax genotypes. Furthermore, the interaction of genotype and time after applying stress had a significant effect on the catalase, hydrogen peroxide, and proline content. The amount of catalase and proline in the 24 hours after stress was more than the 48 hours, indicating the key role of these factors at the beginning of the stress. On the other hand, the amount of guaiacol peroxidase and SOD increased significantly in 48 hours after stress. Generally, the content of the antioxidant factor increased significantly under salinity stress, especially in 375Ha tolerant genotype. This indicates the importance of these enzymes in salt stress tolerance in order to more accurately evaluate the genotypes sensitive and tolerant to the flaxseed in the seedling stage. These results confirmed the slat tolerance of 375Ha genotype in the seedling stress and therefore can be a promising line for regions with slat stress conditions.

1. INTRODUCTION

Oil seeds are among the most significant energy and protein resources for human consumptions. Preparing edible oils from the oil seeds is one of the essentials of producing agricultural products in order to provide food. Considering the fact that more than 90 percent of the edible oil in Iran is imported, conducting research in this regard is among the requirements of agriculture [1]. One of the most important oil and medicinal plants in the world is oil Flax [2]. Flax or *Linum usitatissimum* is an oil plant whose seed has 40%–45% oil and 23%–30% protein. In addition to extracting oil, its pressed cake with 42%–46% protein could be used as a protein resource in livestock diet [3]. Its oil in the normal Flax genotype is used in the industry as a drying oil due to having 5% Linolenic unsaturated fatty acid [4]. Oil Flax is a herbaceous and annual plant that belongs to the Linaceae family and its origin is reported to be west Mediterranean [5]. Canada, China, the U.S., India, and Ethiopia are the largest producers of Flax in the world. Walnut and Flax seeds are among that have the highest rates of Omega 3 fatty acids among the plant foods [6].

On the one hand, salinization of agricultural soils is a huge problem in world agriculture [7]. Salinity stress leads to changes in different

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plants traits that include a range of changes in morphological, physiological, and biochemical traits. Salinity stress resistance is not a constant property and it might not be the same in all growth stages in all plant species and also, different genotypes of the plant show different reactions toward salinity stress [8].

The lack of balance between the energy absorption and consumption in the photosynthesis organs, that is the leaf, during the salinity stress occurrence leads to the formation of ROS and if the plant cannot control it, the stress takes place in the cell membrane and the symptoms of oxidative damages could be observed [9]. In this regard, the antioxidant factors are considered as the first factors coping with free radicals [10]. Plant physiological traits, such as closure of stomata, change in the patterns of growth regulators, and accumulation of metabolites, are also some typical examples of adaptation with the stress condition [11]. Hence, studying the influences of salinity stress by the use of enzymes could help with the quicker and more accurate identification of resistive genotypes of a plant. Plants adapt themselves with the osmatic stress by the accumulation of some soluble materials, such as proline, glycine betaine, polyols, and trehalose, during salination stress [12,13]. In a study, Nasir Khan et al. [7] reported that the presence of NaCl in the culture of 2-month-old Flax seedlings had led to a decrease in the growth and physio-biochemical parameters, except for the leakage of proline, glycine betaine, thiobarbituric acid, H2O2 content, superoxide dismutase (SOD) and catalase, and Na contents. By studying the influence of different NaCl concentrations (0, 25, 50, 75, and 100 mM) on two Flax species of NL-260 and NL-97, Patel et al. [14] reported that there was an increase in the protein, proline, and peroxidase amount, by the increase in the NaCl concentration. The general results showed a contradictory behavior in two cultivars of Flax, considering their salinity tolerance capacity. Salinity stress had led to the inhabitation of peroxidase enzyme and lesser accumulation of proline in NL-260, comparing to NL-97.

Considering the fact that Flax oil seed is currently one of the most valuable oil seeds in the world and also the increasing demand for high-quality edible oils and the need for crop diversification in the salt-affected lands, producing crops that are resistant to salinity stress seems necessary for maintaining food production and achieving food security in the future. However, preparing salinity-tolerant crops is a difficult affair due to the lack of complete understanding of molecular basis for salinity tolerance and lack of access to the salinity tolerance genes.

Studying and identification of the biochemical basis of salinity tolerance in the plants is one of the main methods in producing salinity-tolerant cultivars in the initial steps of breeders. Hence, the objective in this study is to assess the sources tolerating salinity in sensitive and resistive Flax genotypes.

2. MATERIALS AND METHODS

This experiment was carried out to study the biochemical traits of Flax seedlings under salinity and normal stresses in three Flax genotypes of Tababre (sensitive), Golchin (semi-sensitive), and 375Ha (resistive), prepared from Takato Sari Company, and their salinity tolerance was determined in previous studies, in Ardabil Azad Islamic University. To achieve this, an experiment was carried out in the factorial format based on Completely Randomized Design with three replications in pots. The experiment factors included the above-mentioned genotypes and salinity stress time (0, 24, and 48 hours after applying 120 mM NaCl).

All pots were irrigated similarly during the first 2 weeks and after ensuring successful deployment, salinity treatment was applied. To achieve this, similar pots and soil (1 peat moss + 1 peat+ perlite) were used to plant healthy and disinfected seeds and they were kept at the light condition of 16 hours of light and 8 hours of darkness and at 25°C ± 2°C. Samples were taken from the plant leaves and the collected samples were frozen by liquid nitrogen immediately and kept in the freezer at −80°C for biochemical analyses.

After applying the stress, the enzymes in the leaves of the studied genotypes were measured in both stress and lack of stress conditions. Each stress was extracted and measured based on the related instructions. Oxidative catalase [15], SOD [16], peroxidase [17], hydrogen peroxide [18], malondialdehyde [19], and proline [20] were identified and measured based on the methods.

In order to statistically analyze the data, subsequent to testing the normality of the data using Kolmogorov–Smirnov and Shapiro–Wilk, initially the analysis of variance (ANOVA) and mean comparison of the genotypes for the traits were carried out through Duncan method using SAS software ver. 9.4 and the diagram were drawn by Microsoft Office 2016 Excel.

3. RESULTS AND DISCUSSION

The ANOVA results for the traits suggested that salinity stress had a very significant effect on the biochemical factors components. Catalase, guaiacol peroxidase, hydrogen peroxide, and proline had a significant difference with each other in the studied genotypes. On the one hand, the interaction of the genotypes during the sampling after the stress application had a significant influence on the catalase, hydrogen peroxide, and proline. (Table 1) Patil et al.

Table 1: ANOVA (mean square) of biochemical factors of flax genotypes in various times after salinity stress application.

Change sources	Df	Catalase	Guaiacol Peroxidase	Malondialdehyde	Hydrogen Peroxide	Proline	SOD
Genotype	2	124.77**	5.41**	35.72ns	0.0091**	22.09**	0.05ns
Stress Time	2	1761.76**	43.97**	7240.76**	0.5598**	489.07**	55.06**
Genotype × Time	4	111.14**	0.17ns	37.84ns	0.0134**	10.36**	0.06ns
Error	18	5.60	0.78	14.31	0.0015	1.56	0.11
Changes Coefficient (%)		13.21	15.71	9.04	11.03	10.82	9.36

ns and ** are significant and insignificant at 1%.

[21] also reported the significant influence of various NaCl levels on proline, protein, and peroxidase activity in Flax.

Based on the mean comparison results, the catalase component had a significant increase in NaCl stress at 24 and 48 hours after stress applied to all genotypes. However, this increase was greater at 24 hours, comparing to 48 hours. This increase at 24 hours was significantly higher in tolerant and semi-tolerant genotypes, comparing to the sensitive genotype (Fig. 1).

The catalase rate at 24 hours after the stress application increased considerably in Golchin and 375Ha genotypes and this could show the role of this enzyme in salinity stress tolerance, especially in tolerant Flax cultivars. Catalase is an important antioxidant enzyme that plays a significant role in salinity stress condition in omitting H_2O_2 and other harming factors [22]. The gradual increase of catalase rate due to the increase of salinity stress if 2,000, 4,000, and 6,000 mg/l have been reported in three Flax genotypes of Sakha3, Giza8, and Ariane [23]. This increase was higher in the tolerant genotype of Ariane, comparing to the other two genotypes of Sakha3 and Giza8. Nasir Khan et al. [7] had reported the increase in the catalase in Flax due to the NaCl stress of 150 mM.

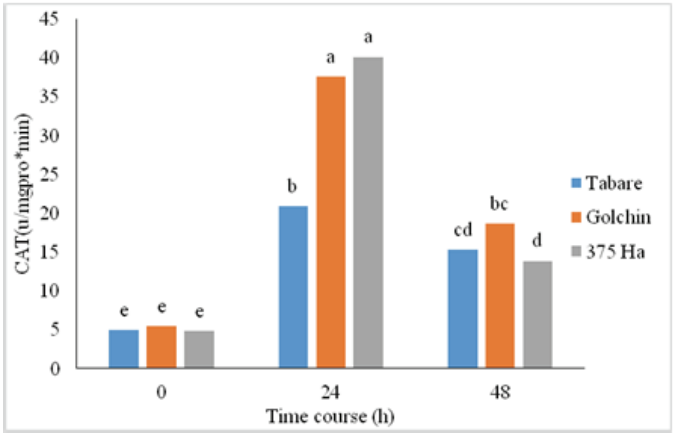

Figure 1: The interaction between genotype and salinity stress on catalase component.

The guaiacol peroxidase mean compassion results suggested that the components of this enzyme were significantly high in tolerant genotype of 375Ha, comparing to the two sensitive (Tabare) and semi-sensitive (Golchin) genotypes. (Fig. 2) In rice also, this enzyme rate was reported to be different between tolerant and sensitive cultivars [24]. Peroxidase plays a significant role in the tolerance of stresses in Organic Plants and they are widespread in Organic Plants. In the meanwhile, guaiacol peroxidase is an important peroxidase that is known as one of the oxidizing enzymes of phenol compounds and plays an important role in inhibiting free oxygen radicals [24].

Guaiacol peroxidase components were higher under salinity stress, comparing the lack of stress and by the increase in the time after the stress, it had a significant increase so that the highest rate of this enzyme after 48 hours after stress (7.7 mgpro*min) was more than double the amount comparing to the lack of stress condition (3.3 mgpro*min). (Fig. 3) Studies have shown that to deal with the toxic effects of oxidative stress, the plants used a series of mechanisms and using peroxidase and catalase enzyme system are two main systems in order to prevent cellular damages due to the stress [25]. In accordance with the results in this study, other studies also show that this enzyme functions as a defense mechanism in plants under stress condition [26].

There was no significant difference observed in the studied genotypes in malondialdehyde rate. However, there was a significant difference found in the NaCl stress condition at 1 percent. This significant difference is shown in Figure 4. As it could be observed, as time passes, the malondialdehyde rate increases and it reaches its maximum after 48 hours after stress application. The malondialdehyde components show the cellular damage rate in the plant. The salinity stress in Flax leads to an increase in malondialdehyde rate and as a result, the increase in the cellular damage in the studied cultivars [23]. There is a direct

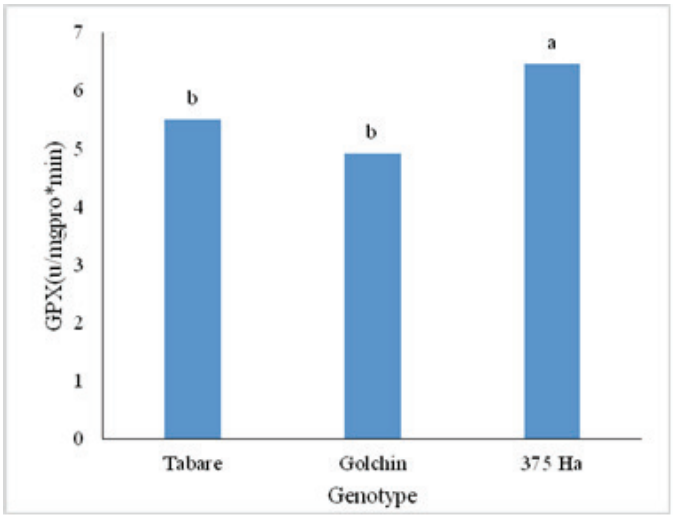

Figure 2: Guaiacol peroxidase components of flax genotypes under salinity stress.

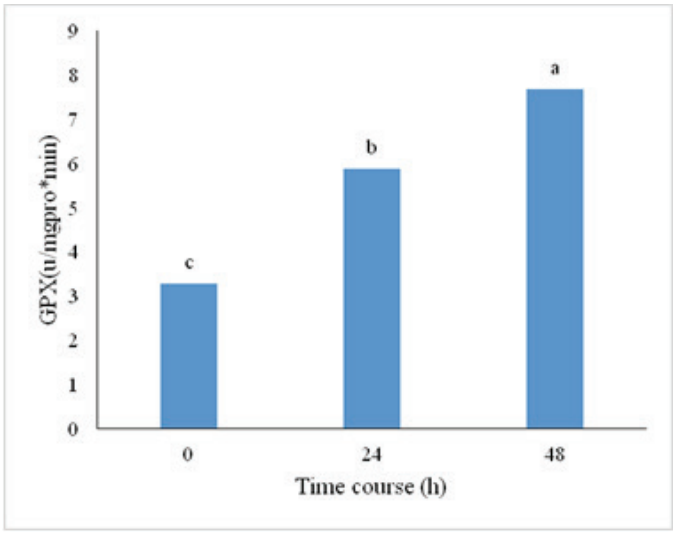

Figure 3: The influence of sampling timing after salinity stress on guaiacol peroxidase.

relationship between lipid peroxidation and membrane damages due to the salinity stress and this rate is lower in the more tolerant cultivars, comparing to the sensitive cultivars [23].

As it could be observed from the diagram of mean comparison related to the interaction of genotypes and stress time on the hydrogen peroxide (Fig. 5), 48 hours after the salination stress application, H_2O_2 rate had a significant increase and this increase is higher in sensitive genotypes, comparing to the tolerant genotypes. Increase in hydrogen peroxide due to salinity stress has been reported in many plants [7,23,26,27]. Hydrogen peroxide is capable of reacting to superoxide and forming more hydroxylated radicals, that in turn leads to higher peroxidation of lipid [26,28].

The proline components showed a significant increase in salinity stress, comparing to the lack of stress. This increase was higher after 24 hours, comparing to 48 after stress, in all studied genotypes. On the one hand, 48 hours after the stress, proline components were higher in the tolerant genotype, comparing to the two other genotypes. Also, 24 hours after stress, the proline rate was lower in the sensitive genotype, comparing to the other two genotypes. (Fig. 6) The increase in the proline components in response to salinity stress of NaCl has been reported in previous studies in Flax [21,29].

Increase in proline in this study, especially 24 hours after the stress, shows the importance of this material in salinity stress condition in the Flax plant. Increase in the leaf proline in salinity stress condition in Flax has been also reported by Moadei Dehnavi et al. [27], According to the researchers, this increase is due to the fact that proline omits the free oxygens produced in stress and protects the larger molecules [30] and helps with preserving the shape and natural structure of macromolecules in stress conditions [31].

The superoxide enzyme components showed a significant increase in the salinity stress condition and by the increase in time after the stress. This increase in the salinity stress condition 48 hours after the stress was double that of the rate of 24 hours after the stress and more than four times higher than lack of stress condition. (Fig. 7) According to the results in this research, different levels

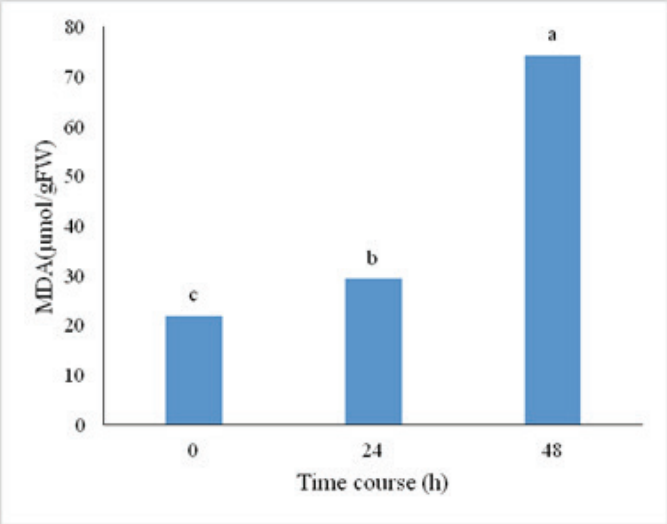

Figure 4: The influence of sampling timing after salinity stress on malondialdehyde.

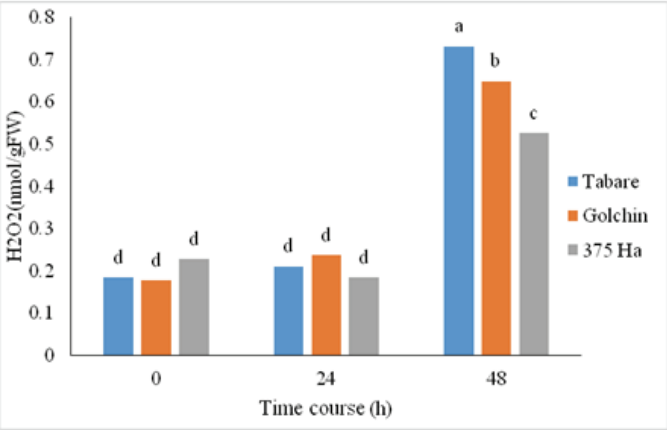

Figure 5: The influence of the interaction between genotype and salinity stress on the hydrogen peroxide components.

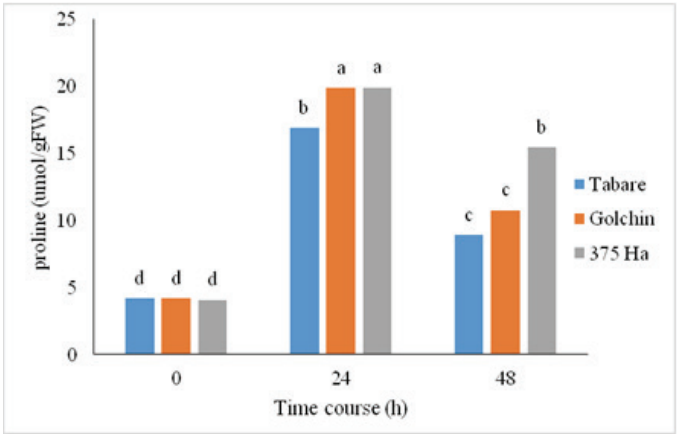

Figure 6: The influence of the interaction between genotype and salinity stress on the proline components.

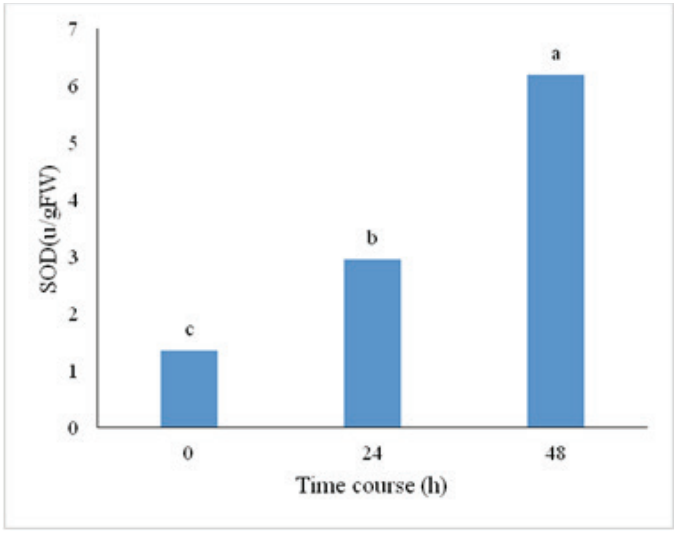

Figure 7: The influence of the interaction between genotype and salinity stress on the SOD.

of salinity stress led to an increase in the SOD enzyme activity in three cultivars of Flax [23]. In this regard, El-Beltagi et al. [32] had reported an increase in SOD enzyme due to the salinity stress in Flax. It is claimed that this enzyme has a great role in increasing the salinity stress tolerance in the plants [8].

In this study, the rates of biochemical components involved in stress in three different genotypes of Flax 24 and 48 hours after NaCl stress were studied. The results suggested that all the studied biochemical factors have a role in salinity tolerance in Flax. Increase in catalase enzyme 24 hours after the stress accompanied a lower level of hydrogen peroxide and its decrease in catalase enzyme 48 hours after stress accompanied with a high level of hydrogen peroxide. This could be due to the neutralizing influence of catalase enzyme on H_2O_2. Also, the results from this study showed that as the time passed, the MDA rate increased under salinity stress and H_2O_2 rate accumulated 48 hours after stress. Increase in MDA shows the damage to the membranes and increase in H_2O_2 is also not a good sign for tolerating salinity stress and its increase is toxic to the plant. However, on the other hand, by the increase in these two factors, guaiacol peroxidase and SOD components had a significant increase by the increase of time after the stress. This could mean that an increase in these factors leads to an increase in antioxidant enzymes of guaiacol peroxidase and SOD. In the study of El-Beltagi et al. [32] also, the rates of these two enzymes had an increase in salinity stress, especially in tolerant genotypes. In general, considering the results of this study, it could be concluded that the salinity stress leads to component change of biochemical factors in different flax genotypes and this change benefits the plant in tolerant genotypes while it leads to cellular damage in sensitive genotypes.

REFERENCES

1. Iran Nezhad H, Hosseini M. Effect of planting date on grain yield of three oilseed Flax varieties in Varamin. J Agric Sci 2005;4:111–20.
2. Yu Y, Wu G, Yuan H, Cheng L, Zhao D, Huang W, et al. Identification and characterization of miRNAs and targets in flax (*Linum usitatissimum*) under saline, alkaline, and saline-alkaline stresses. BMC Plant Biol 2016;16:124.
3. Reynolds JD. Spacing trials with dried peas. Agriculture (London) 1955;56:527–37.
4. Billgili U, Sincik M, Uzan A, Acikgoz, E. The influence of row spacing seeding rate an seed yield and yield components of forage turnip. J Agron Crop Sci 2003;189:250–4.
5. Dash PK, Cao Y, Jailani AK, Gupta P, Venglat P, Xiang D, et al. Genome-wide analysis of drought induced gene expression changes in flax (*Linum usitatissimum*). GM Crops Food 2014;5:106–19.
6. Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M. Flax and flaxseed oil: an ancient medicine & modern functional food. J Food Sci Technol 2014;51:1633–53.
7. Nasir Khan M, Siddiqui MH, Mohammad F, Naeem M, Khan MMA. Calcium chloride and gibberellic acid protect linseed (*Linum usitatissimum* L.) from NaCl stress by inducing antioxidative defense system and osmo protectant accumulation. Acta Physiol Plant 2010;32:121–32.
8. Sairam RK, Srivastava GC. Water stress tolerance of wheat *Triticumaestivum* L.: Variation in hydrogen peroxide accumulation and antioxidant activity in tolerant and susceptible genotype. J Agron Crop Sci 2001;186:63–70.
9. Blokhina O, Virolainen E, Fagestedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: a review. Ann Bot 2003;91:179–94.
10. Dirk I, Montago MV. Oxidative stress in plants. Taylor and Francis Publication, London, UK, 2002. ISBN: 9780415272148.321.
11. Singh A, Saini ML, Behl RK. Seed germination and seedling growth of citrus (*Cytrus* species) root stocks under different salinity regimes. Indian J Agric Sci 2004;74:246–8.
12. Ghoulam C, Foursy A, Fares K. Effects of salt stress on growth, inorganic ions and proline accumulation in relation to osmotic adjustment in five sugar beet cultivars. Environ Exp Bot 2002;47:39–50
13. Sakamoto A, Murata N. The role of glycine betaine in the protection of plants from stress: clues from transgenic plants. Plant Cell Environ 2002;25:163–71.
14. Patel SH, Panagopoulou A, Morreale SJ, Paladino FV, Mar-garitoulis D, Spotila JR (in press) Post-reproductive migration of an adult male loggerhead from Crete revealed by satellite telemetry. In Proceedings from the32nd Annual International Sea Turtle Symposium 2015.
15. Dhindsa RS, Plumb-Dhindsa P, Thorpe TA. Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. J Exp Bot 1981;32:93–101.
16. Fridovich I. Biological effects of superoxide radical. Arch Biochem Biophys 1986;247:1–11.
17. Chance B, Maehly AC. Assay of catalase and peroxidase. Academic Press, New York, NY, 1995.
18. Alexieva V, Sergiev I, Mapelli S, Karanov E. The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant Cell Environ 2001;24:1337–44.
19. Heath RL, Packer L. Photo peroxidation in isolated chloroplast. I. Kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 1969;125:189–98.
20. Bates LS, Waldern RP, Teave ID. Rapid determination of free proline for water stress standies. Plant Soil 1973;39:107–205.
21. Patil A, Kinoshita K, Nakamura H. Hub promiscuity in protein-protein Interaction Networks. Int J Mol Sci 2010;11:1930–43.
22. Bassuany FM, Hassanein RA, Baraka DM, Khalil RR. Role of stigma sterol treatment in alleviating the adverse effects of salt stress in flax plant. J Agric Technol 2014;10:1001–20.
23. El-Bassiouny H, Sadak MS. Impact of foliar application of ascorbic acid and α-tocopherol on antioxidant activity and some biochemical aspects of flax cultivars under salinity stress. Acta Biol Colomb 2015;20:209–22.
24. Moradi Talavat MR, Alemi Said Kh, Karamlachab A, Hasanvand H. Response of antioxidant enzymes, membrane lipidation and cell death of rice cultivars to salinity stress. Process Plant Function 2017;6:293–301.
25. Sridevi S, Venkatesan A. Effect of salinity on mineral nutrient distribution along growing seedlings of *Bruguiera conjugata* Lamk. J Phytol 2009;219–29.
26. Pourakbar L, Moqsoumi Holasso S. Effect of salinity on antioxidant enzymes in roots and aerial parts of corn (Zea mays L.). J Appl Biol 2015;28:5–22.
27. Movahedi Dehnavi M, Niknam N, Behzadi M, Mohtashemi R, Bagheri R. Comparison of physiological responses of flax (*Linum usitatissimum* L.) to drought stress and salinity and salicylic acid spraying. Iran Plant Biol 2017;9:39-62.
28. Chen J, Zhu C, Lin D, Sun ZX. The effect of Cd on lipid peroxidation, hydrogen peroxide content and antioxidant enzyme activities in Cd-sensitive mutant rice seedlings. Can Plant Sci 2007;87:49–57.
29. DeLacerda CF, Cambraia J, Oliva MA, Ruiz HA. Osmotic adjustment in roots and leaves of two sorghum genotypes under NaCl stress. Braz J Plant Physiol 2003;15:113–8.
30. Rahdari P, Tavakoli S, Hosseini SM. Studying of salinity stress effect on germination, proline, sugar, protein, lipid and chlorophyll content

in purslane (*Portulacaoleracea* L.) Leaves. J Stress Physiol Biochem 2012;8:182–93.
31. Koc E, İslek C, Ustun AS. Effect of cold on protein, proline, phenolic compounds and chlorophyll content of two pepper (*Capsicum annuum* L.) varieties. Gazi Univ J Sci 2010;23:1–6.
32. El-Beltagi HS, Salama ZA, El Hariri DM. Some biochemical markers for evaluation of flax cultivars under salt stress conditions. J Nat Fibers 2008;5:316–30.

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