International Journal of Botany Studies
ISSN: 2455-541X; Impact Factor: RJIF 5.12
Received: 01-02-2021; Accepted: 20-03-2021: Published: 05-03-2021
www.botanyjournals.com
Volume 6; Issue 2; 2021; Page No. 71-75

Comparative adaptations in halophytes (*Salicornia Europaea* L., *Puccinellia Distans* (JACQ.) parl., and *Atriplex olivieri* MOQ.) exposed to salt stress

Iclal Durdu[1], Peyami Battal[2], Mehmet Emre Erez[3*]
[1] Ministry of Education, Kayseri, Turkey
[2] Health Services Vocational School, Gazi University, Ankara, Turkey
[3] Department of Molecular Biology and Genetics, Van Yüzüncü Yıl University, Van, Turkey

Abstract
Halophytic species show greatly different adaptation mechanisms in response to high salinity. The present study examines the effects of extreme salt (NaCl) application on three different halophytic plants (*Salicornia europaea* L., *Puccinellia distans* (Jacq.) Parl., and *Atriplex olivieri* Moq.). In order to evaluate the effects of salt stress, ten-day old halophyte seedlings were subjected to 0, 200, 400, 600 and 800 mM NaCl concentrations for two months. Growth, leaf water potentials, stoma situations and proline contents were evaluated. Chlorophyll and carotenoid content, soluble carbohydrates and hormone levels were also analyzed. *Puccinellia distans* and *Atriplex oliveri*'s growth was suppressed more than *Salicornia europea*; up to 400 mM salt concentration. Physiological changes also included; significant proline accumulation, carbohydrates and phytohormone adjustments and changes in osmotic potential, protein profiles and stomata size. The results suggested that possesses strategies, commonly present in halophytic plants, to cope with high salt levels differ with halophytic plant species. In addition, *Salicornia europea* can be used for halophytic adaptation studies as a model plant.

Keywords: halophytes, salt stress, adaptation, *Salicornia europea*

Introduction
Salt tolerance is the ability of plants to cope with salt stress and survive their life cycle at soluble salt concentrations. Approximately 9.5 million hectares of the world's soil are primarily saline soil, without secondarily salinized soil in cultivated land. On the other hand, fresh water resources are limited and soil salinity increases with mistreat irrigation. Under these circumstances, researchers prompt the regarding of halophytes adaptation mechanism. [1]. Many plants improve adaptation mechanisms either to prohibit salt from their cells or to tolerate its proximity with the cells. [2] Plants exhibit overabundance of physiological, biochemical and molecular mechanisms to cope with salt injuries. [3]
Abscisic acid is responsible for the modification of salt-stress-induced genes. Salt stress regulates polyamine biosynthesis and catabolism by acting as a cellular signal in hormonal pathways thereby regulating abscisic acid (ABA) in response to stress. [4] ABA-inducible genes are supposed to play a significant role in the mechanism of salt tolerance in rice [5]. As well-known function, ABA also triggers stomatal closure by rapidly altering ion fluxes in guard cells under environmental stress conditions.
Exposure of plants to salinity results in increased generation of reactive oxygen species, as by-products, which damage the cellular components. [6] Reactive oxygen species cause chlorophyll degradation and membrane lipid peroxidation, reducing membrane fluidity and selectivity [7]. In addition, proteins play an imminent role in plant stress response since they are directly involved in the acquisition of an enhanced stress tolerance [8].
Salicornia species are edible plants with plenty of minerals, and it can be used as a source of soda ash and soap making. Seeds are also used for to produce animal feedstuff and as a biofuel, feedstock on coastal land where the conventional crops cannot be grown and able to accumulate and store salt within their leaves and stems which allows them to survive.
Halophytes are plants growing on or surviving in saline conditions and their adaptation mechanisms are different from species to species. Therefore, in order to successfully understand salt tolerance in plants, the mechanism at each level must be studied individually. Thus, this study was undertaken to provide information on tolerance mechanism of three different halophytes grown under higher salinity levels.

Materials and Methods
Plant materials and salt treatments
Plant seeds used in study were collected from halophytic habitats, healthy and vigorous seeds were chosen. Seeds were surface sterilized and germinated in sterile petri dishes. Germinated seeds transferred to the pots (120 x 90 mm) with nonsterilized soil mixed with sand (soil; sand 3; 1 v/v). Pots were kept in phytotron at + 28 °C at 16 h daylight/8 h moonlight. Plants were exposed to salinity by adding NaCl (200, 400, 600, 800 mMol L^{-1}.) to their irrigation water for two months. Control groups were irrigated with tap water at same volume (100 ml/d). Each treatment was performed in triplicate under same conditions. At the end of the growing period, plants were harvested for analysis and samples were kept in deep freezer at -80 °C until analyses.

Examination of stomata samples and leaf osmotic potential
When plants were harvested from the pots, leaves were preserved in formalin; acetic acid; ethanol (FAA: 1; 1;9) for microscopic examination [9].Leaf osmotic potential was measured with Wescor 5520 osmometer. osmotic potentials were performed as Mega Pascal.

Determination of chlorophyll and carotenoid content
Fresh leaves were homogenized with a pestle in a mortar. Chlorophyll and carotenoids were extracted from leaves with 80 % acetone and absorbance values were measured at 450, 645 and 663 nm wavelengths for total chlorophyll content and carotenoids. The amount of total chlorophyll and carotenoids was calculated according to [10].

Quantification of proline contents
The method developed by [11] was used for the quantification of proline content. 0.5 g of plant material was homogenized using 10 ml of 40 % aqueous methanol. Homogenates were mixed with 2 mL of acid ninhydrin (1.25 g of ninhydrin + 30 mL of glacial acetic acid + 20 mL of 6 M phosphoric acid). The sample was heated for one hour at 100 °C in a water bath. Then 4 mL of cold toluene were added. This solution was mixed and read at 520 nm and the results were expressed as mol g-1 fresh weight.

Determination of sugars
2 gr of sample was homogenized with 40 mL of methanol. The mixture was incubated on magnetic stirrer at 65 °C for 1 h.. and centrifuged at 4 °C, 1300 rpm for 30 min. Methanol was removed by rotary evaporator. Extract was passed through Sep-Pack C18 cartridge. Filtrated by 0.45 µm membrane and injected into HPLC [12].

Extraction and determination of phytohormone levels
The analysis of phytohormones (Gibberellic acid and abscisic acid) was done according [13]. Battal *et al*. (2001). One gram of sample was mixed with 20 ml cold methanol and sonicated at Ultra Tissue Lysis. Methanol was removed at rotary evaporator. The extracts were dissolved in K_2HPO_4 buffer (pH=8.5) and centrifuged at 10.000 g for 1 h at 4°C. Samples were mixed well with PVPP and filtered through Whatman filter paper. The filtrates were load to Sep-Pack C 18 cartridges and extracts were injected into HPLC. [13].

Preparation of whole-cell proteins and SDS-PAGE analysis
One gram of fresh sample was mixed with 25µl of denaturing buffer (pH 6.8) containing 0.006 M Tris-HCl, 2.5 % glycerol, 0.5 % SDS and 1.25 % β-mercaptoethanol and boiled for 5min. After centrifugation for 3 min at 10.000 g. the supernatants were transferred into an eppendorf tube [14]. Electrophoresis was performed with discontinuous buffer system in UVP vertical electrophoresis Unit. The gel was scanned with densitometer and the molecular weight of each band was determined using one dimensional analysis of software (Lab Image ver.6.)

Data analysis
All data were evaluated by one-way ANOVA using SPSS 9.0 for Windows as well as the least significance difference and Post-hock significant difference tests. The experiments were repeated with three replicates for each treatment and comparisons with $P<0.05$ were considered significantly different

Results
The NaCl treatment did not show any effect on shoot length of *Salicornia europea*. However exposure of 400 mM and further salt treatments decreased the shoot length of *Puccinellia distans* significantly. Shoot length of *Atriplex olivieri* was also decreased by increasing salt treatment concentration compare to control group. (Figure 1A). 200 mM of NaCl treatment did not cause a remarkable change in osmotic potential values whereas 400 mM and 600 mM salt treatments were decreased leaf osmotic potential significantly especially in *Atriplex olivieri* and *Puccinellia distans* plants (Figure 1. B). These data suggested that halophytes exhibits little or no growth stimulation at low salinity levels, while higher salinities reduced growth [15]. High salt stress set up osmotic homeostasis with water potential and ion distribution. This dispersion of homeostasis occurs not only at cellular level but also in whole plants levels [16]. 400 mM salt treatment had no significant effect on chlorophyll content of *Puccinellia distans.* Nevertheless, the chlorophyll content was decreased by 600 mM salt treatment. Chlorophyll content of *Atriplex olivieri* was stabile up to 600 mM salt treatment but it was also reduced with 800 mM salt treatment. Finally chlorophyll content of *Salicornia europea* showed uniform values in all salt treatment concentrations (Figure 1.C). Also 200-600 mM salt treatments were significantly decreased carotenoid content of *Puccinellia distans*. On the other hand carotenoid content of Salicornia europea remained unchanged at all salt treatments compared to control group (Figure 1.D). Salt stress has direct and indirect effects on the chlorophyll content and photosynthetic efficiency of plants. The direct effects are achieved by regulating the activity and expression levels of enzymes involved in chlorophyll biosynthesis and photosynthesis, plants accommodate leaf morphology under saline conditions with reduced photosynthetic machinery, as well as reduction of chlorophyll content [16]. Salinity causes significant decreases in *Chl-a*, *Chl-b* and carotenoid in leaves of *Bruguieria parviflora* [3].

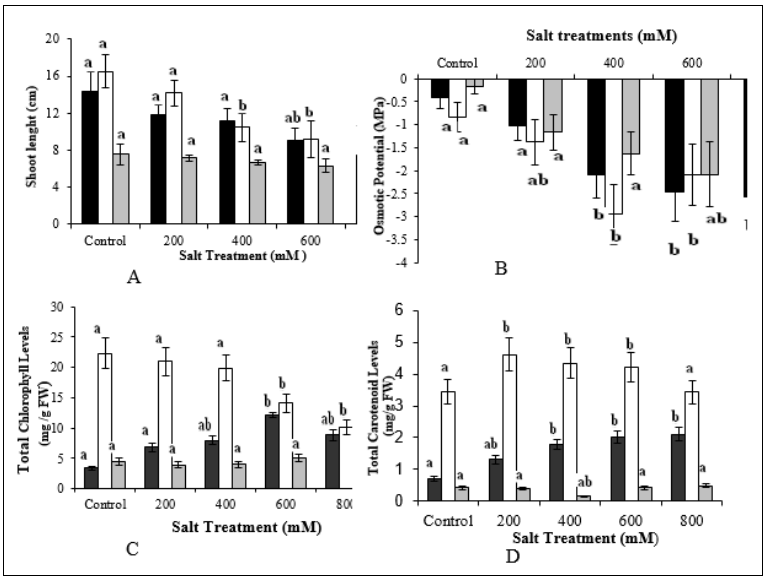

Fig 1: The effects of salt treatments on shoot lengths (A), osmotic potential (B), total chlorophyll (C) and caretonoid (D) of halophyte plants. Means ± S.E., n =5. Bars with different letters are significantly different at P<0.05. (■ *Atriplex olivieri,* □ *Puccinellia distans,* □ *Salicornia europea*)

The comparison of stoma size of control plants with 800 mM salt treatment groups of halophyte plants were shown in Fig. 3.A,B,C. Stoma size did not show prominent differences in *Puccinellia distans* but the stoma size of *Atriplex olivieri* and *Salicornia europea* significantly decreased in 800 mM salt treatment compared to control group (Figure 2). These adverse stress factors result in an enhanced stomata closure, a decreased photosynthesis rate and a reduced plant growth. The plant ability to cope with stress underlies plant salinity tolerance. The plant has to behave physiologically embarrassment for growth on saline substrate with regulation of CO_2 and H_2O exchange by stomata [17].

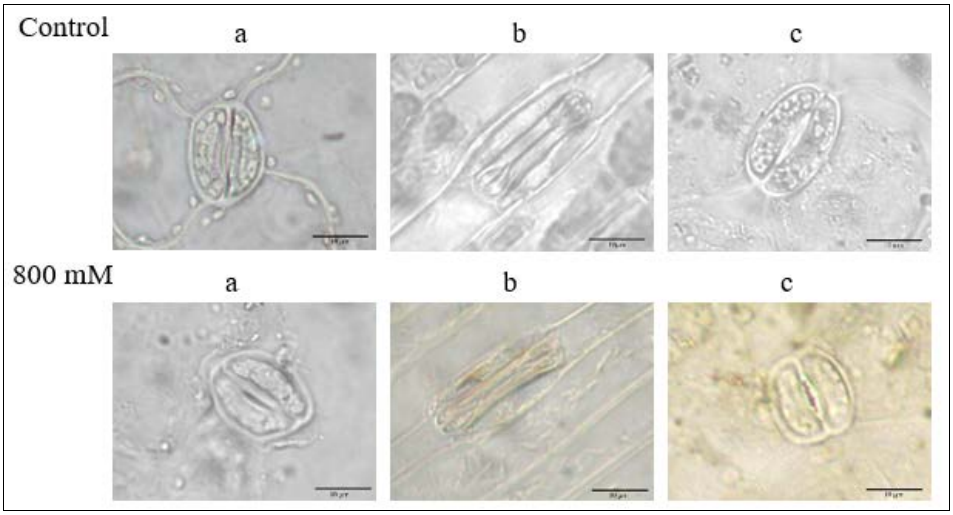

Fig 2: The effects of 800 mM salt treatments on stoma size a: *Atriplex olivieri* b: *Puccinellia distans* c: *Salicornia europea*

Free proline content in leaves of *Atriplex olivieri* plants exposed to 600 mM salt treatment reached to the highest level and showed six times higher accumulation than leaves that of the leaves of control group. In *Puccinellia distans*, proline accumulation was started at 200 mM salt treatment and the highest proline accumulation in *Puccinellia distans* was determined in 800 mM NaCl treated group. Free proline content in leaves of *Salicornia europea* did not change in all salt treatments (Figure 3A). Proline accumulation increase salt resistance of economic crops [18]. Proline can preserves protein structure and activity with reducing enzyme denaturation by inactivating hydroxyl radicals and other reactive chemical species. Accumulation of free amino acids like as proline reduces osmotic potential in cytoplasm and provide to maintaining homeostasis under environmental stress [19].

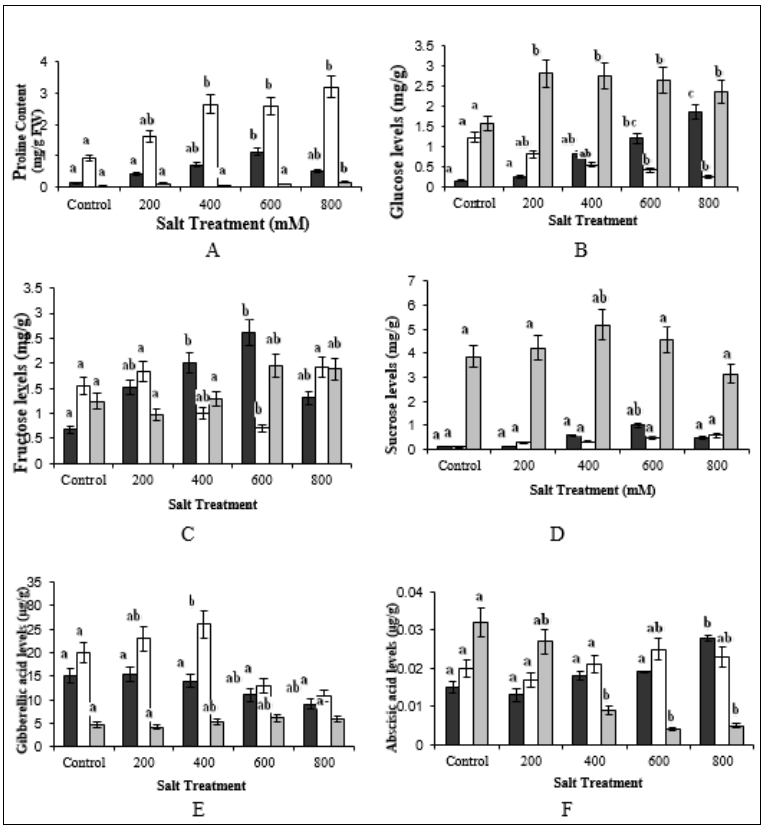

Fig 3: The effects of salt treatments on Proline (A), glucose (B), fructose (C), sucrose (D) Gibberellic acid (E), Abscisic acid (F) of halophyte plants. Means ± S.E., n =5. Bars with different letters are significantly different at P<0.05. (■ *Atriplex olivieri,* □ *Puccinellia distans,* □ *Salicornia europea)*

Exposure of 800 mM NaCl, *Atriplex olivieri* crucially increased glucose level. Although increment of salt treatment significantly increase glucose levels of *Salicornia europea*, also significant decreases were observed with increasing of salt concentration in *Puccinellia distans* compared to non-salinized control groups (Fig.6.A). NaCl treatment enhanced total sugar and proline accumulation, prevents toxic and nutrient deficiency effects of salinity Nevertheless in some plant species salinity may increase the but decrease in others [20] [21].

GA3 levels of Puccinellia distans reached to maximum value in 400 mM salt treatment but decreased with increasing NaCl concentration. However, no significant difference was determined between the control and salt treatments in leaves of Salicornia europea (Fig.7.A). GA3 altered the pattern of distribution and accumulation of ions in shoots and roots under salt stress [22].

Salt treatments altered leaf ABA concentrations in all halophytic species. Salt treatment in Atriplex olivieri, especially 800 mM NaCl treatment led to a significant increase on ABA concentration. Salt stress increases biosynthesis and accumulation of abscisic acid in higher plant [23]. The increased ABA content was accompanied by an improvement in K^+/Na^+ ratio leading to increased salt tolerance.

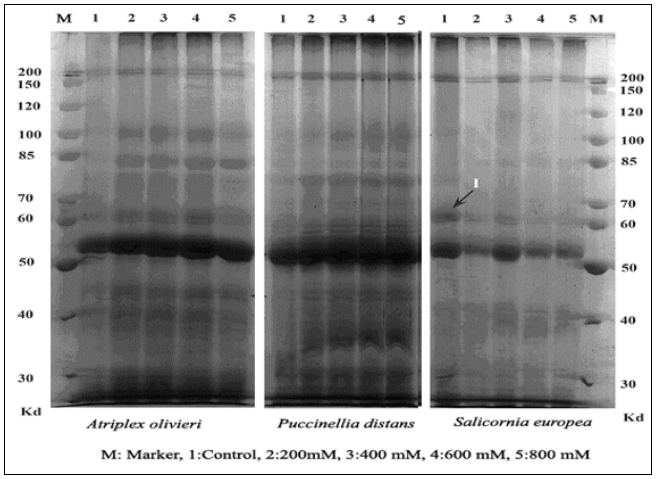

Fig 4: Protein profiles in leaves of halophyte plants in response to different NaCl concentration.

The protein band at molecular weight of 52 kDa (Kilo Dalton) was considered as a structural protein and its concentration was altered with different salt treatments. These protein band's concentrations were increase with increment of salt treatment in *Atriplex olivieri* and *Puccinellia distans* plants. However, in *Salicornia europea*, the band was found to be different in the concentration of 200 mM, 400 mM and 800 mM salt applications. The double protein bands in molecular weights of 58/59 kDa and 43/44 kDa were observed in all salt treatments obviously in *Puccinellia distans*, however they were not visible in control group. The protein band in molecular weight of 84 kDa was determined only in the salt treatments of *Atriplex olivieri*. Also molecular weight of 60 kDa protein band was seen in control group of *Salicornia europea* plant. Nevertheless the concentration of band was decreased with increasing salt treatments (Figure 4). In barley, salinity induces six new proteins in roots and also SDS–PAGE analysis in peanut (*Arachis hypogaea* L.) display that plants exposed to NaCl show induction (127 and 52 kDa) or repression (260 and 38 kDa) in the synthesis of a few polypeptides.

In the present study demonstrated that *Salicornia europea* is a highly salt-tolerant species that is able to survive and grow at salinities up to 800 mM salt treatment. Adaptation mechanism of plants response to salt stress is either to exclude salt from cells or to tolerate with physiological progresses such as photosynthesis and protein synthesis [24]. Scientific articles about various salt-tolerance mechanisms can be found in and many reports emphasize the important role of halophytes in improving saline soil conditions and the cultivation of salt-tolerant crops [25] [26].

Conclusion

Regarding to the fluctuated data provided from three different halophytic plants, bring to mind the question of whether halophytes require saline conditions for their existences. It was also suggested that the physiological mechanism leading to salt tolerance in halophytes are regulated in such a way that allow them competitive advantages to salt stress over other plants not only with single adaptation mechanism but also with their genetics. However adaptation mechanisms vary from plant to plant species. This study has also demonstrated that *Salicornia europea* show the best tolerance mechanism, comparison with the *Puccinellia distans* and *Atriplex olivieri*. Combining with all studies about halophyte adaptation mechanism, in this study we aimed to force the halophyte plants to understand their tolerance limit and this will generate crop plants able to tolerate and grow in high salt concentrations.

Acknowledgements

This study was supported by a giant from the Scientific Research Projects Presidency of Yüzüncü Yıl University. (YYU-FBE-082)

References

1. Kefu Z, Hai F, Ungar IA. Survey of halophyte species in China. Plant Science. 2002; (163):491-498.
2. Parida AK, Das AB. Effects of NaCl stress on nitrogen and phosphorous metabolism in a true mangrove *Bruguiera parviflora* grown under hydroponic culture. J. Plant Physiol. 2004; (161):921-928.
3. Parida A, Das AB, Das P. NaCl stress causes changes in photosynthetic pigments, proteins and other metabolic components in the leaves of a true mangrove, *Bruguiera parviflora*, in hydroponic cultures. J. Plant Biol. 2002; (45):28-36.
4. Shevyakova NI, Musatenko LI, Stetsenko LA. Effects of abscisic acid on the contents of polyamines and proline in common bean plants under salt stress," Russian Journal of Plant Physiology. 2013; (60):200-211.
5. Gupta S, Chattopadhyay MK, Chatterjee P, Ghosh B, Sen Gupta DN. Expression of abscisic acid-responsive elementbinding protein in salt tolerant indica rice (*Oryza sativa* L. cv. Pokkali). Plant Mol. Biol. 1998; (137):629-637.
6. Van Breusegem F, Dat JF. Reactive oxygen species in plant cell death. Plant Physiology. 2006; (141):384-390.

7. Verma S, Mishra SN. Putrescine alleviation of growth in salt stressed *Brassica juncea* by inducing antioxidative defense system. Journal of Plant Physiology. 2005; (162):669-677.
8. Sobhanian H, Aghaei K, Komatsu S. Changes in the plant proteome resulting from salt stress: Toward the creation of salt-tolerant crops. J. Proteomics. 2011; (74):1323-1337.
9. Zwieniecki MA, Boyce CK, Holbrook NM. Hydraulic limitations imposed by crown placement determine final size and shape of *Quercus rubra* L. leaves. Plant Cell Environ. 2004; (27):357-365.
10. Witham FH, Blaydes DR, Devlin RM. Experiments in Plant Physiology. Van Nostrand Reinhold, New York, 1971.
11. Bates LS, Waldren RP, Teare ID. Rapid determination of free proline for water stress studies. Plant Soil. 1973; (39):205-207.
12. Karkacier M, Erbas M, Uslu MK, Aksu M. Comparison of different extraction and detection methods for sugars using Amino–Bonded phase HPLC. Journal of Chromatographic Science. 2003; (41):331-333.
13. Battal P, Tileklioğlu B. The Effects of Different Mineral Nutrients on the levels of cytokinins in Maize (*Zea mays* L.). Turk. J. Bot., 2001; (25):123-130.
14. Laemlii UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature, 1970; (227): 680-685.
15. Naidoo Kift. Responses of the salt marsh rush Juncus kraussii to salinity and waterlogging Aquatic Botany. 2006; (84):217-225.
16. Koyro HW. Effect of Salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). Environ. Exp. Bot., 2006; (56):136-146.
17. Koyro HW. Study of potential cash crop halophytes in a quick check system task. Veg. Sci. 2003; (38):5-17.
18. Naidu BP, Paleg LG, Aspinall D, Jennings AC, Jones GP. Amino acid and glycine betaine accumulation in cold-stressed wheat seedlings. Phytochem., 1991; (30):407-409.
19. Sairam RK, Tyagi A. Physiology and Molecular Biology of Salinity Stress Tolerance in Plants. Current Science. 2004; (86):407-421.
20. Agastian P, Kingsley SJ, Vivekanandan M. Effect of salinity on photosynthesis and biochemical characteristics in mulberry genotypes. Photosynthetica, 2000; (38):287-290.
21. Sivritepe N, Sivritepe HÖ, Eriş A. The Effects of NaCl priming on salt tolerance in melon seedlings grown under saline conditions. Scientia Horticulturae. 2002; (97):229-237.
22. Iqbal M, Ashraf M. Gibberellic acid mediated induction of salt tolerance in Wheat plants: Growth, ionic partitioning, photosynthesis, yield and hormonal homeostasis. Environmental and Experimental Botany. 2010; (69):225-232.
23. Taylor IB, Burbidage A, Thompson AJ. Control of abscisic acid synthesis. J Exp Bot. 2000; (51):1563-1574.
24. Parida AK, Das AB. Salt tolerance and salinity effects on plants:a review. Ecotooxicology and Environmental Safety. 2005; (60):324-349.
25. Abd El-Hack ME, Samak DH, Noreldin AE *et al*. Towards saving freshwater: halophytes as unconventional feedstuffs in livestock feed: a review. Environmental Science and Pollution Research. 2018: 25(32):31971-31986.
26. Ozfidan konakci C, Uzilday B, Ozgur R *et al*. Halophytes as a source of salt tolerance genes and mechanisms: a case study for the Salt Lake area, Turkey. Functional Plant Biology, 2016; 43(7): 575-589.