Screening of barley genotypes on the basis of physiological parameters under salt stress

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Abstract: Salinity is one of the most severe stress factors threatening the agriculture over the globe. The population is increasing at an alarming rate. Therefore significant interest is present in salt tolerance mechanisms and improved performance of crop plants under soil salinity. Barley is considered as one of the most salt tolerant crops however different levels of tolerance are present among barley cultivars. A hydroponic experiment was carried out in wire house of Institute of Soil and Environmental Science, University of Agriculture Faisalabad to evaluate the performance of barley cultivars under different levels of salinity. 12 barley genotypes (Haider-93, B-05011, B-15006, Joo-83, B-15003, B-15005, B-9008, B-14003, B-15002, B-9006, B-14011, B-14007) were grown against two salinity levels (100mM and 200mM) along with control. Crop duration was 6 weeks and physiological (membrane stability index and relative water content), chlorophyll contents were determined. The experiment was arranged according to completely randomized design (CRD) with factorial arrangement having three replications. It was found that physiology, and chlorophyll contents significantly reduced with increasing salt stress. Among twelve genotypes B-05011, B14003 and B-9006 were found salt tolerant, While B-15006, B-15003 and B-15005 were found most salt sensitive as compared to other barley genotypes. The selected tolerant genotype could be grown as natural in saline soil to get better growth (yield as well as good material for breeding programme.
[Iqra Aslam, Meh Gul, Iqra Ghafoor, Rabia Yaseen, M. Usman Gani, H. M. Waseem, Rida Nawaz, Aiman Ali, Misha Iqba. **Screening of barley genotypes on the basis of physiological parameters under salt stress.** *Nat Sci* 2019;17(7):74-79]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 10. doi:10.7537/marsnsj170719.10.

Keywords: salinity, yield, sodium, barley

Introduction

Salts present in rhizosphere above certain limits exert adverse effect on plant biomass and yield due to limited availability of water can decrease yield of many crops (Kahlown and Azam, 2002. Salinity is increasing on a world scale and more than 10% of arable land affected by it and this cause more than 50% reduction in yield of major crops (Wang *et al.,* 2003). In Pakistan out of 22 million hectare cultivated lands, salt affected area is estimated about 6.67 million hectare (Khan, 1998). Adverse effects of salinity on crop growth have two characteristics: (1) the increased osmotic potential of the soil solution with salinity makes the water in the soil less available for plants, and (2) specific effects of some elements (Na^+, Cl^- and B) present in excess concentrations (Tahir *et al.,* 2018; Yamaguchi and Blumwald, 2005). Salt stress has different effects on plants and thus, there are many mechanisms to tolerate it as well which can be grouped into three main categories namely, osmotic tolerance, ion exclusion and tissue tolerance (Roy *et al.,* 2014). The high salt concentration adversely affects physical and chemical soil properties as well as soil microbial activities, thus lowering soil productivity. High toxicity of salt and high osmotic potential results in lowering soil carbon (C) (Wong *et al.,* 2009).

Salinity induced many biochemical, morphological, physiological, biochemical and molecular changes in plants are due to salt stress (Ahmad *et al.,* 2019; Kafi, 2009). Under stressed conditions a range of variation is occurring in plants (Safdar *et al.,* 2019; Munns and Tester, 2008). Salt stress affects the basic metabolic pathways such as respiration and photosynthesis are affected due to salinity. Salinity affects the efficiency of respiratory enzymes (Moradi and Ismail, 2007). The photosynthesis process is inhibited by two ways such as stomatal and non-stomatal factors (Desingh and kanagaraj, 2007). The 65% inhibition in rate of photosynthesis and stomatal conductance will was observed under saline conditions (Saqib *et al*., 2006). Different mechanisms of salinity tolerance in different plants are present like Na^+ efflux maintain ion homeostasis, antioxidant enzyme detoxify cytotoxic toxicity ROS scavenges like catalase (CAT) and super oxide dimutase (SOD) and production of compatible solutes and compartmentation to detoxify the reactive oxygen species (Navrot et at., 2007). The other option to utilize such areas, use of salt tolerant plants could

be a vital option. Introduction of new genetic characters in specific cultivars and selecting cultivars by screening method which are tolerant to salt stress (Munns *et al*., 2006) to get such plants or genotypes that can be grown on salt affected lands (Katerji *et al*., 2000). Salt tolerance in Triticeae including Barley is normally related with Na^+ ion exclusion and ability of plant to maintain sufficient concentration of K^+ ion under saline conditions during growth (Colmer *et al*., 2006). Tavakoli *et al*., (2010) stated that higher tolerance of Barley genotype is related due to higher ratio of K^+/Na^+ in the shoot.

Salt tolerant genotypes were found capable of maintaining higher xylem K^+/Na^+ ratios and more efficient in loading of K^+ into the xylem (Shabala *et al*., 2010). Tavakkoli *et al*., (2011) stated that Na^+ and Cl^- exclusion among barley genotypes are independent mechanisms and different genotypes expressed different combinations of the two mechanisms. High concentrations of Na^+ reduced K^+ and Ca^{2+} uptake and reduced photosynthesis mainly by reducing stomatal conductance. The objective of proposed study was to investigate the difference in genetic potential of different barley (*Hordeum Vulgare* L.) genotypes under salt stress conditions.

Materials and methods

3.1 Work Plan

A hydroponic experiment was conducted at wire house, Institute of Soil and Environmental sciences, University of Agriculture Faisalabad. Rain protected wire house is designed to maintain experiment under more control conditions.

3.2 Seed Source

Seeds of barley (*Hordeum vulgare* L.) genotypes were obtained by Ayub Agriculture Research Institute (AARI) Faisalabad.

3.3 Growth conditions

In this study, twelve different barley (*Hordeum vulgare* L.) genotypes were used. Seeds of barley cultivars were sown in laboratory in iron trays having 2 inch sand layer. Nursery was irrigated with the distilled water. Then nursery was shifted to glass house after 2-3 days. Nursery was transplanted into 3 tubs (100 L) with half strength of hoagland's solution. Solution was kept aerated by aeration pumps. Nutrient solution was comprised of macro-nutrients nutrients Ca $(NO_3)_2.4H_2O$, KNO_3, $MgSO_4.7H_2O$, KH_2PO_4 and micro-nutrients H_3BO_3, $MnCl_2.4H_2O$, $ZnSO_4.7H_2O$, $CuSO_4.5H_2O$, H_2MoO_4. H_2O, Fe-EDTA (Johnson *et al.,* 1957). pH of solution was maintained at 6.5+0.5 throughout the experiment ((Kronzucker *et al.,* 2006). Nutrient solution was changed at interval of 8 days. Nutrients solution was prepared by using distilled water.

Salinity was developed by using NaCl salt after three days of transplanting nursery into tubs. The NaCl salt was added in three installments to achieve the desired levels of 100 mM and 200 mM.

3.4 Treatments

The following treatments were used:

i. T_1 Control
ii. T_2 100 mM NaCl
iii. T_3 200 mM NaCl

3.5 Harvesting

The plants were harvested after 42 days of salinity imposition in hydroponic system and then separated into root and shoot with the help of scissor. After taking root and shoot length and their fresh weight, the plant samples were collected in separate paper bags.

3.6 Chlorophyll contents (SPAD value)

The leaf chlorophyll content was determined before harvesting by using chlorophyll meter (Minolta SPAD. 502 Meter). Average (SPAD) reading was recorded form the measures (from leaf tip to leaf blade).

3.7 Membrane stability index (MSI):

The leaf membrane stability index (MSI) of inact plant, was determined according to the method of Sairam (1994). Leaf discs (100 mg) were thoroughly washed in running tap water fallowed by washing with distilled water thereafter lead discs were heated in 10 ml of double distilled water at 40°C for 30 min. Then the electrical conductivity (C1) was recorded by EC (Electrical conductivity) meter. Subsequently the same samples were placed in boiling water bath (100°C) for 10 min and their electrical conductivity was recorded (C2).

The MSI was calculated as:

$$MSI\ (\%) = [1-(C1/C2)] \times 100$$

3.8 Relative water content (RWC):

Relative water content (RWC) top most fully expanded young leaf was sampled form each replication, mid leaf section of about 5.0 cm^2 was cut with scissors and placed in a vial larger than sample after recording fresh weight and immediately hydrated to fully turgidity. After 4h under normal light and temperature, the samples were taken out of the water and well dried quickly with filter paper and immediately weighed to obtain the fully turgid weight. The samples are oven dried at 65+5°C in hot air oven till constant weight to determined dry weight (Barss and Weatherley, 1962).

3.9 Statistical Analysis

The collected data was evaluated by statistical technique (Steel *et al.,* 1997) and effects of treatments were assessed according to Duncan's Multiple Range (DMR) test (Duncan, 1955).

Results and discussion

Membrane Stability Index

Figure 1 describes the membrane stability Index for different varieties of barley against various salt stress concentrations. The membrane Stability Index was significantly affected by salt stress. The mean data membrane Stability Index justifies that it was considerably decreased with enhancing NaCl stress in different barley genotypes.

Barley genotypes B-9008, B-05011 and B-15002 showed maximum Membrane Stability Index 81.95 %, 82 % and 84.04 % While in B-15005, B-15006 and B-15003 minimum MSI was observed which was 66.78 %, 65.91 % and 69.46 % respectively. On the other hand with increasing salt stress to 100 mM barley genotypes B-05011, B-9008 and Jon-83 genotypes performed better 70.71 %, 65.13 % and 68.14 % while B-15002, B-15005 and B-14003 showed minimum Membrane Stability Index which 61.27 %, 56.84 % and 59.86 %. At 200 mM maximum Membrane Stability Index of barley genotypes was observed in B-9008, B-05011 and in B-14007 56.72 %, 58.26 % and 51.16 % while B-9006, B-14011 and Jon-83 revealed less Membrane Stability Index 43.94 %, 45.17 % and 45.85 % as compared to other barley genotypes.

The outcome of study are according to Jamil *et al.* (2012) who observed that all growth parameters are effected by salt stress and maintaining the stability of membrane was influenced as it has inversely proportion growth related factors. As salt concentration increase plant cell affected as due to high salinity concentration less water uptake in the plants and thus cause loss in the turgidity of the cells and cells become flaccid it affects the membrane of leaf with gradual increase in salt stress it causes injury and hence damages the membrane of plant.

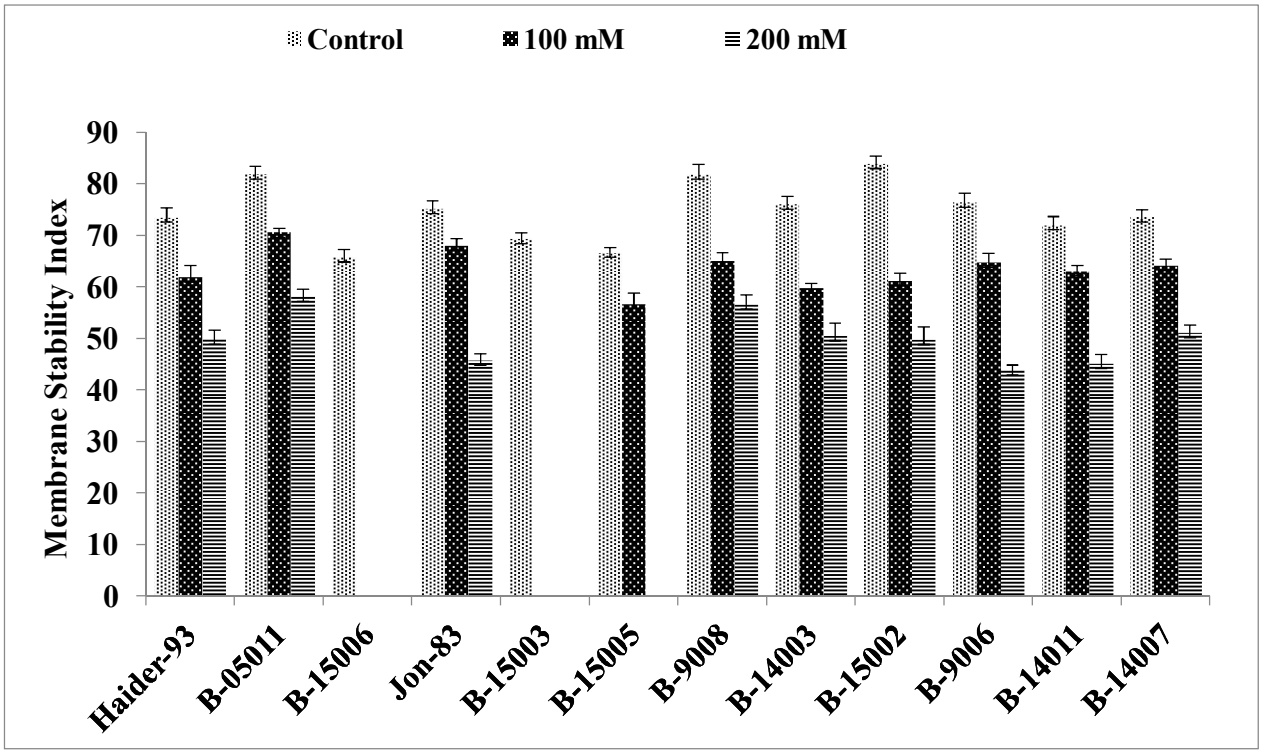

Figure 1: Effects of Salt concentration on the Membrane Stability Index of different Barley (*Hordeum vulgare* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Table 1: Analysis of Variance table for Membrane Stability Index

Source	DF	SS	MS	F	P
Treatment (T)	2	25115.3	1255.7	2084.18	0.0000
Variety (V)	11	27894.9	2535.9	420.88	0.0000
T×V	22	11458.8	520.9	86.45	0.0000
Error	70	421.8	6.0		
Total	107	64940.3			
Grand Mean 55.145		CV 4.45			

Relative Water Content

The results concise in figure 2 and table 2 revealed the relative water content. It was observed that with increasing salt stress the relative water content was significantly decreased. Relative water content of all genotypes of barley depends upon the concentration of salts. The data on relative water content of different barley genotypes explained that relative water content significantly reduced with increasing salt stress.

At control condition B-9008, Haider-93 and B-15002 showed maximum relative water content 87.73 %, 89.17 % and 91.75 % g respectively. While B-15005, B-15006 and B-14011 which was 77.86 %, 78.69 % and 82.03 % showed less results respectively. In the second treatment where 100 mM NaCl stress was applied barley genotypes B-05011, B-9006 and Haider-93 genotypes performed better 76.36 %, 77.95 % and 79.47 % while B-15002, B-15005 and B-14003 had minimum relative water content which was 73.37 %, 66.43 % and 73.98 % comparatively to other barley genotypes. With enhancing salt stress to 200 mM higher relative water content of barley genotypes was observed in B-9008, B-9006 and in Haider-93 64.53 %, 67.61 % and 66.25 % while B-14007, B-15002 and Jon-83 showed lower relative water content 60.84 %, 62 % and 62.17 % as compared to other barley genotypes.

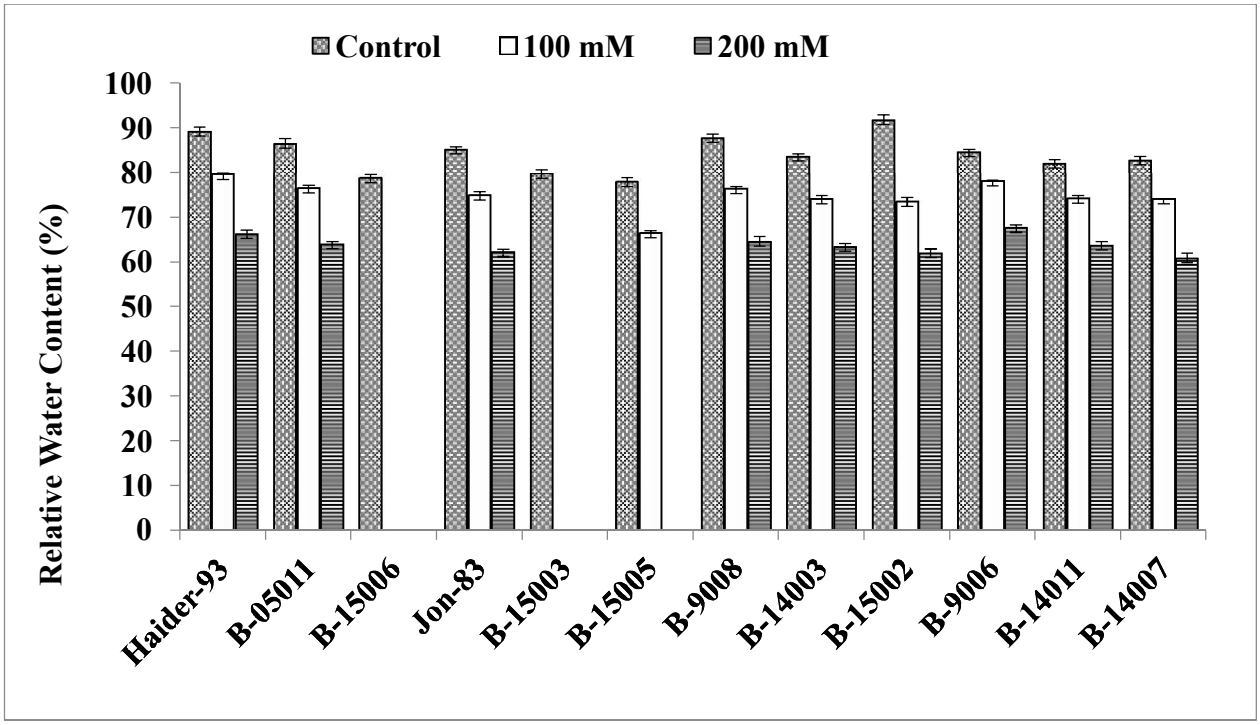

Figure 2: Effects of Salinity concentration on the Relative Water Content of different Barley (*Hordeum vulgare* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Table 2: Analysis of Variance table for Relative Water content

Source	DF	SS	MS	F	P
Treatment (T)	2	23988.1	11994.0	7409.20	0.0000
Variety (V)	11	37917.4	3447.0	2129.37	0.0000
T×V	22	18489.8	840.4	519.18	0.0000
Error	70	113.3	1.6		
Total	107	80521.1			
Grand Mean 64.735		CV 1.97			

Chlorophyll contents

Data summarized in Figure 3 explore the effect of salt stress on the chlorophyll contents of several barley genotypes. Chlorophyll contents was considerably influenced with application of salt stress. However there was considerable variation with respect to response in different barley genotypes was found with application of salt stress.

At control condition where no salt stress was applied the barley genotypes Haider-93, B-9008 and

B-14007 showed maximum chlorophyll contents 43.30 %, 48.73 % and 47.83 % as compared to other genotypes. While minimum chlorophyll contents were observed in B-15006, B-15003 and Jon-83 which was 35.40 %, 33.33 % and 38.40 %. At 100 mM barley genotypes B-05011, B-9008 and Haider-93 genotypes had higher chlorophyll contents 39.07 %, 40.97 % and 40.60 % while B-14011, Jon-83 and B-14003 showed minimum chlorophyll contents 31.10 %, 35.50 % and 35.63 %. At 200 mM maximum chlorophyll contents of barley genotypes was observed in B-14007, B-9008 and in Haider-93 35.3 %, 36.93 % and 37.16 % while B-05011, B-14007 and Jon-83 had minimum chlorophyll contents 32.9 %, 35.3 % and 31.63 % as compared to other barley genotypes.

The outcomes of study are according to Zhao *et al.* (2007) who showed that rate of germination varied greatly among the oat genotypes. Reduction in chlorophyll contents, dry biomass, reduced leaf area, More Na^+ and less K^+ was observed. All parameters reduced by application of salt treatment. Under high salt stress conditions all metabolic functions of plant are affected. Higher salinity levels cause stomata closing which effects gases exchange, water stress in plants and hence effects the photosynthetic rate of plant. Chlorophyll contents reduced gradually as concentration of salts increased. Chlorophyll contents decreased by increased salinity levels in tomato plants reported by Al-aghabary *et al.* (2005).

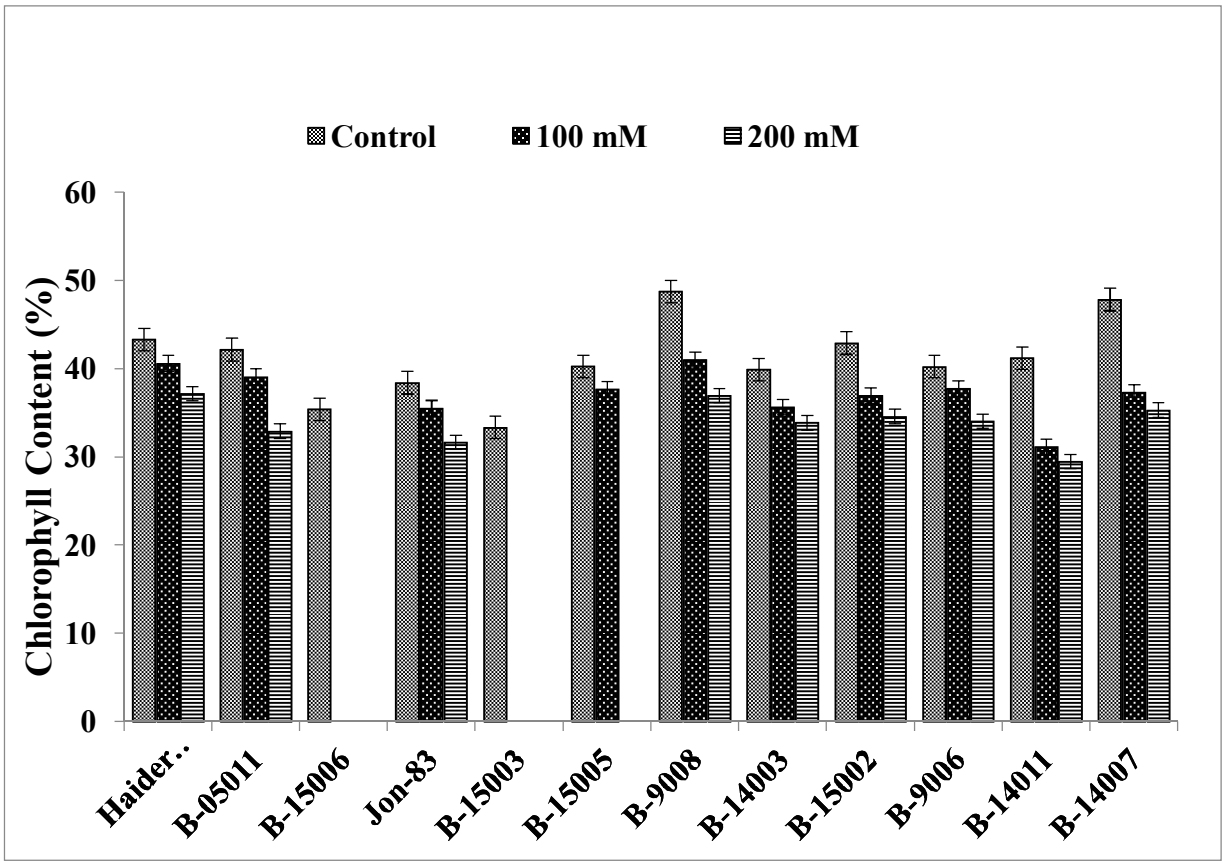

Figure 3: Effects of Salt stress on the Chlorophyll Contents (%) of different Barley (*Hordeum vulgar* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Table 3: Analysis of Variance table for Chlorophyll contents

Source	DF	SS	MS	F	P
Treatment (T)	2	4424.7	2212.36	238.08	0.0000
Variety (V)	11	7338.6	667.14	71.79	0.0000
T×V	22	5279.1	239.96	25.82	0.0000
Error	70	650.5	9.29		
Total	107	17705.2			
Grand Mean 33.587		CV 9.08			

References

1. Muhammad Ahmad, Abbas Shoukat, Maria Anjum, M. Kamran, M. Qusain Saeed, Raza Hussain, Muhammad, Hadi Abbas and Yousuf shafiq. Assessment of tomato genotypes against salinity on the basis of morphological and physiological parameters in hydroponic conditions. *Nat Sci* 2019;17(2):56-70.
2. Al-aghabary, K., Z. Zhu and Q. Shi. 2005. Influence of silicon supply on chlorophyll content, chlorophyll fluorescence, and antioxidative enzyme activities in tomato plants under salt stress. J. Plant Nutr. 27: 2101-2115.
3. Chartzoulakis, K., M. Loupassaki, M. Bertaki and I. Androulakis, 2002. Effects of NaCl salinity on growth, ion content and CO2 assimilation rate of six olive cultivars. Sci. Hort. 96: 235-247.
4. Colmer, T.D., R. Munns and T.J. Flowers 2006. Improving salt tolerance of wheat and barley: future prospects. Anim. Prod. Sci. 45: 1425-1443.
5. Desingh, R. and G. Kanagaraj. 2007. Influence of salinity stress on photosynthesis system in two cotton genotypes grown under saline soils. Physiol. Plant. 33: 221-234.
6. Hamna Safdar, Aniqa Amin, Yousuf Shafiq, Anum Ali, Rabia Yasin, Abbas Shoukat, Maqsood Ul Hussan, Muhammad Ishtiaq Sarwar. A review: Impact of salinity on plant growth. *Nat Sci* 2019;17(1):34-40.
7. Haq, T., J. Akhtar, M.A. Haq and M. Hussain. 2002. Effect of soil salinity on the concentration of Na, K and Cl in the leaf sap of the four Brassica species. Int. J. Agri. Biol. 4: 385-388.
8. Jamil, M., M. Ashraf, S. Rehman, M. Ahmad, and E.S. Rha. 2012. Salinity induced changes in cell membrane stability, protein and RNA contents. Afr. J. Biotechnol. 11: 6476-6483.
9. Kafi, M. 2009. The effects of salinity and light on photosynthesis, respiration and chlorophyll fluorescence in salt-tolerant and salt-sensitive wheat (*Triticum aestivum* L.) cultivars. J. Agr. Sci. Tech. 11: 535-547.
10. Kahlown, M.A., W. Changgui. And M. Azam. 2003. Effects of saline drainage effluent on soil health and crop yield. Agri. Water Mang. 62: 127-138.
11. Katerji, N., A. Hamdy and M. Mastrorilli. 2000. "Salt tolerance classification of crops to soil salinity and to water stress day index". Agri. Wat. Manage. 43: 99-109.
12. Khan, S. 1998. Rethinking rational solutions for the irrigation salinity. Aust J Agr Resour. 129-140.
13. Moradi, F. and A.M. Ismail. 2007. Responses of Photosynthesis, Chlorophyll Fluorescence and ROS-Scavenging systems to salt stress during seedling and Reproductive Stages in Rice. Annal Bot. 99: 1161-1173.
14. Muhammad Tahir, Muhammad Mubashar Zafar, Ali Imran, Muhammad Asad Hafeez, Muhammad Sheraz, Rasheed, Hafiz Saad Bin Mustafa, Asmat Ullah. Response of tomato genotypes against salinity stress at germination and seedling stage. *Nat Sci* 2018;16(4):10-17.
15. Munns, R. and M. Tester. 2008. Mechanisms of salinity Tolerance. Annu. Rev. plant Biol. 59: 651-681.
16. Munns, R., K. Kakar, M. wandrey and R.A. James. 2006. Screening methods for salinity tolerance: a case study with tetraploid wheat. plant soil. 59: 12-18.
17. Roy S and U. Chakraborty. 2014. Salt tolerance mechanisms in Salt Tolerant Grasses (STGs) and their prospects in cereal crop improvement. Bot. Stud. 55:31.
18. Sairam, R.K., K.V. Rao and G.C. Srivastava. 2002. Differential response of wheat genotypes to long term salinity stress in relation to oxidative stress, antioxidant activity and osmolyte concentration. Plant Sci. 163: 1037-1046.
19. Saqib, M., C. Zarb, Z. Rengel and S. Schubert. 2006. Salt resistant and salt sensitive wheat genotypes show similar biochemical reaction at protein level in the first phase of salt stress. J. Soil Sci. 169: 542-548.
20. Shabala, S, T.A. Cuin, J. Pang, W. Percey, Z. Chen, S.Conn, C. Eing and L.H. Wegner. 2010. Xylem ionic relations and salinity tolerance in barley. The Plant J. 61: 839-853.
21. Tavakoli, E., F. Fatehi, S. Coventry, P. Rengasamy and G.K. McDonald. 2011. Additive effects of Na+ and Cl– ions on barley growth under salinity stress. J. Exp. Bot. 62: 2189-2203.
22. Wang, X.S. and J.G. Han. 2007. Abscisic acid concentrations are correlated with leaf area reductions in two salt-stressed rapid cycling Brassica species. plant soil. 79: 25-35.
23. Wong, V. N. L., R. C. Dalal and R. S. B. Greene. 2009. Carbon dynamics of sodic and saline soils following gypsum and organic material additions: a laboratory incubation. Appl. Soil Ecol. 41: 29-40.
24. Yadav, S. and N. B. Singh. 2004. Strategies to enhance yield of rapeseed-mustard in India. Physiol. Plant. 5: 235-245.
25. Yamaguchi, T. and E. Blumwald. 2005. Developing salt tolerant crop plants: challenges and opportunities. Trends Plant Sci. 10: 615-620.
26. Zhao, G.Q., B.L. Ma and C.Z. Ren. 2007. Growth, gas exchange, chlorophyll fluorescence, and ion content of naked oat in response to salinity. Crop Sci. 47: 23-131.

4/20/2019