Screening of barley (*Hordeum Vulgare* L) genotypes against salt stress under hydroponic conditions

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Abstract: A solution culture-based study was conducted in the wire house to observe the behavior of varieties of *Hordeum Vulgare* L. against various salt stress concentrations. For this study twelve varieties were selected. Three Salinity levels (0mM, 100mM and 200mM) were used. NaCl was used for imposing salt stress. Crop duration was 42 days. In this study the data on followed parameters like, root length, shoot length and membrane stability index were recorded. The data on Na^+ and K^+ were identified by ion extraction method and then run samples on flame photometer. The study was organized and analyzed by CDR technique having 3 replications. By using standard procedures, the collected information of study was statistically organized. Among twelve genotypes B-05011, B14003 and B-9006 were salt tolerant, While B-15006, B-15003 and B-15005 were most salt sensitive as compared to other barley genotypes.
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Introduction

Salinity is an important abiotic stress world-wide that causes reduction in agricultural crops yield output against higher salinity concentrations (Aslam et al., 2019). About 7% of the total area and 20% of irrigated area in the world is salt affected respectively. Salinity is observed in those areas which have high temperature, low intensity rainfall, presence of salty parent material, poor practices of management, poor quality irrigation water and high evapo-transpiration resulting in rise of salts particularly in arid and semi-arid areas. Worldwide more than 75 countries have wide areas of salt affected lands. These soils are commonly present in semiarid and arid region of the world. Globally almost 830.5 million hectares land is salt affected. In Pakistan 6.35 million hectares land is salt affected (Qadir *et al*., 2005). Saline soils are those soils having ECe > 4 dSm^{-1}, SAR< 13 and ESP< 15. Sodic soils have ECe < 4 dSm^{-1}, SAR> 13 and ESP> 15. Saline sodic soil are those soils have ECe > 4 dSm^{-1}, SAR> 13 and ESP> 15 (Rengasamy, 2010). Salinity stress limits the productivity of crops (Tahir *et al*., 2018) and has adverse effects on germination, plant strength and crop yield (Safdar et al., 2019). Salinity induced many biochemical, morphological, physiological, biochemical and molecular changes in plants are due to salt stress (Ahmad et al., 2019). Under stressed conditions a range of variation is occurring in plants (Safdar et al., 2019).

Barley (*Hordeum vulgare* L.) is an important cereal crop in the world. In semi-arid tropics of Asia, Africa, and South America it is considered as main staple food or feed crop. The grain is generally used as human food, animal fodder and also used as raw material for beer production. Barley is normally grown in the arid and semi-arid regions (Baik and Ullrich, 2008). Variability exists in tolerance to salinity among barley varieties. Seed germination is affected in barley by salt stress. The extent of adverse effects of salt stress on barley crop greatly differs and depends on type of cultivar. Barley is considered as salt tolerant among crops (Forster *et al*., 2000). Hirich (2014) conducted an experiment on local variety of chickpea (*Cicerarietinum* L.) for check the salt tolerance in this variety against the high saline irrigation water. The irrigation water was used with different salinity levels. After results it was concluded that there was a negative relationship between saline irrigation water and growth parameters of Chickpea. Due to salinity decreased in seed yield, water uptake and water productivity due to increase in salinity. Proline, soluble sugars and Na^+/K^+ ratio increased due to increase in Saline water stress.

Shelden *et al*., (2013) conducted an experiment to identify genetic variation in root growth in the cereal crop barley (*Hordeum vulgare* L.) in response to the early phase of salinity stress. Seminal root elongation was examined at various concentrations of salinity in seedlings of eight barley genotypes consisting of a landrace, wild barley and cultivars. Salinity inhibited seminal root elongation in all genotypes, with considerable variation observed between genotypes. Relative root elongation rates were 60–90% and 30–70% of the control rates at 100 and 150 mM NaCl, respectively. The screen identified the wild barley genotype CPI71284–48 as the most tolerant, maintaining root elongation and biomass in response to salinity. Root elongation was most significantly inhibited in the landrace Sahara. Root and shoot Na^+ concentrations increased and K^+ concentrations decreased in all genotypes in response to salinity. However, the root and shoot ion concentrations did not correlate with root elongation rates, suggesting that the Na^+ and K^+ concentrations were not directly influencing root growth, at least during the early phase of salt stress.

Higher salt stress concentration is present in the environment of the developing countries which plays major role in disturbing the yield related potential of crops and also ecosystem of soil. The recent research was organized in order to screen the barley (*Hordeum vulgare* L.) genotypes at various salinity levels in solution culture to identify response of different barley genotypes and to classify them according to their salinity tolerance. So that best varieties can be selected according to level of salinity in the soil in order to get maximum production at the particular environment.

Materials and methods

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.

Seed Source

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

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.

TREATMENTS

The following treatments were used:

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

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 the plant samples were collected in separate paper bags.

Shoot length (cm)

The shoot length was measured with the help of measuring rod. The shoots were measured by placing them on the meter rod.

Root Length (cm)

The root length was measured with the help of measuring rod. The roots were measured by placing them on the meter rod.

Membrane stability index (MSI):

The leaf membrane stability index (MSI) of intact 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:

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

Determination of Na^+ and K^+

Samples were prepared by following procedure of "Ion Extraction Method". After the preparation of samples sodium and potassium contents were determined by using Sherwood 410 Flame photometer with the help of standard solutions using reagent grade salts of NaCl and KCI.

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

Shoot length

Data summarized in Figure 1 and Table-1 explained the effect of salt stress on the shoot length of several barley genotypes. Shoot length was considerably influenced with application of salt stress and it was found that with increasing salt stress shoot length of barley genotypes significantly reduced. However, there was considerable variation with respect to response in different barley genotypes was found with application of salt stress. The data shoot length of different barley genotypes explained that shoot length significantly reduced with increasing salt stress.

At control condition where no salt stress was applied the barley genotypes B-15002, B-9006 and B-05011 showed maximum shoot length of 85.66 cm, 82.66 cm and 82.66 cm respectively, while B-15003, Haider-93 and B-15005 71.33 cm, 71.67 cm and 72.33 cm showed minimum shoot length respectively. In the second treatment with increasing salt stress to 100 mM NaCl B-05011, B-14007 and B-15002 genotypes showed better results 69.33 cm, 67.66 cm and 67 cm while B-14003, B-15005 and B-14011 showed minimum shoot length 56.66 cm and 59.33 cm and 60.66 cm respectively. While in the last treatment where salt stress was applied at the rate of 200 mM NaCl growth of barley genotypes were severely affected. However, genotypes B-14007 and B-9008, 50.33 cm and 49.33 cm have maximum shoot length respectively while B-14003 showed minimum shoot length 38.33 cm, as compared to other barley genotypes.

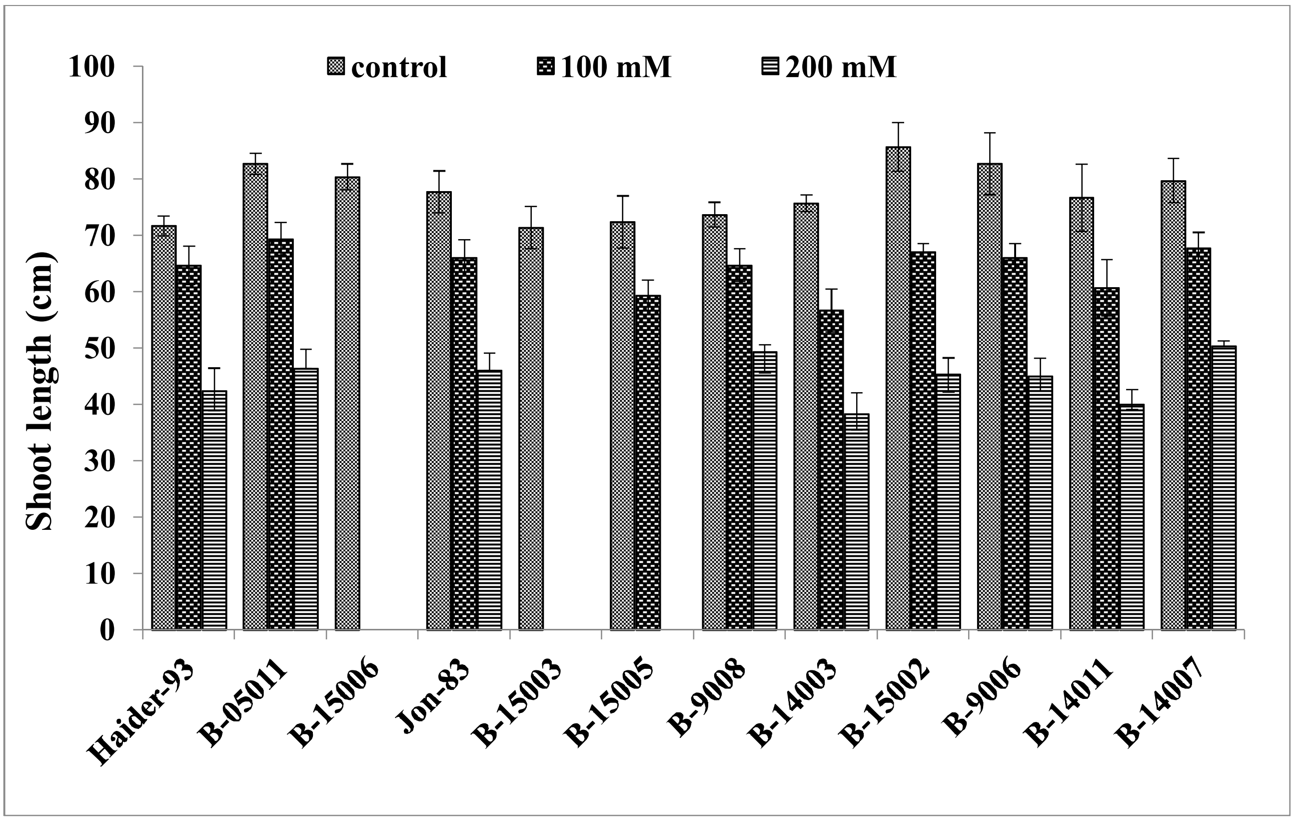

Figure-1: Effects of various salinity levels on shoot length of different Barley (*Hordeum vulgar* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Table-1 Analysis of Variance table for Shoot length

Source	DF	SS	MS	F	P
Treatment (T)	2	34858.7	17429.4	590.04	0.0000
Variety (V)	11	22613.9	2055.8	69.60	0.0000
T×V	22	13073.7	594.3	20.12	0.0000
Error	70	2067.8	29.5		
Total	107	72661.6			

Grand Mean 54.852 CV 9.91

Such reduction in shoot might be due to the water stress and salt toxicity caused by higher salinity concentrations as reported by Illahi *et al*. (2001). Under salinity extensibility of cell and growth factors are significantly reduced as explained by Sharma and Garg (2000). Higher salinity levels effect the metabolic processes of plants turgor pressure was decreased which ultimately cause reduction in enlargement of cell and reduction takes place in the growth-related parameters as explained by Mahmood *et al*. (2009).

Root Length

Figure 2 describes the root length for different varieties of barley against various salt stress concentrations. The root length was significantly affected by salt stress (Table-2) and concentration of salts increases root length of barley genotypes decreases. However, barley genotypes showed variability responses with respect to root length under salt stress. The mean data root length justifies that it was considerably decreased with enhancing NaCl stress in different barley genotypes.

The barley genotypes B-15002, B-9006 and Jon-83 have maximum root length 58.33 cm, 58.33 cm and 56 cm comparatively with other genotypes, while minimum root length was observed in B-15006, Haider-93 and B-15005 which was 37.66 cm, 41 cm and 40.33 cm at control conditions. At 100 mM NaCl stress B-9008, B-14011 and B-15002 genotypes performed better 44 cm, 46 cm and 44 cm while Haider-93, B-15005 and B-14007 showed minimum results 31.66 cm, 31 cm and 35 cm. With increasing salt stress to 200 mM barley genotypes were most affected. At 200 mM maximum root length was observed in B-15002, B-9008 and in B-05011 27.67 cm, 30.67 cm and 28 cm while B-14007, B-14011 and Haider-93 showed minimum root length 20.33 cm, 25 cm and 24 cm as compared to other barley genotypes.

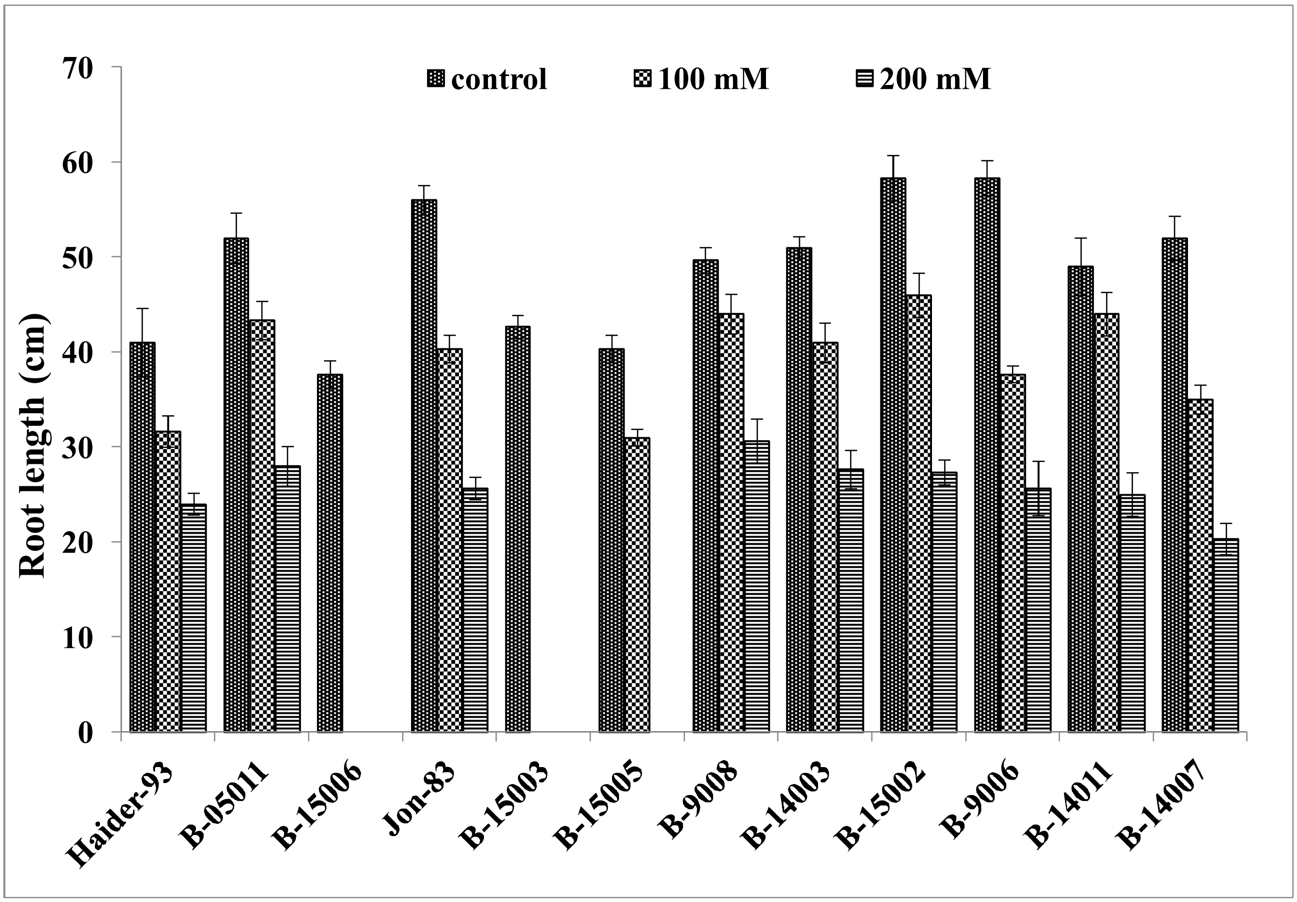

Figure 2: Effects of various salinity levels on Root length of different Barley (*Hordeum vulgar* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

The results are according to Jamil *et al*. (2005) who observed that under higher salinity levels canola varieties showed decrease in the root length. Jeannette (2002) observed that length of root decreased under higher salt stress levels because it delays seedling emergence and germination. The decrease in root length with increase in salinity may be due to the reduction in growth rate and imbalanced nutrition. Sun *et al.* (2005), described that because of higher salt stress and imbalance in composition of nutrients considerably decreased the length of roots. Under high salt stress conditions, the first emergence stage was affected. Due to higher salinity concentration seedling roots were affected that's way length of roots are decreased so elongation of roots does not take place described by Warner *et al.* (2004). This reduction is due to increase in soil solution Osmotic pressure and the imbalances in needed elements. Higher salinity levels in rooting medium cause stress and thus elements required by plants becomes unavailable hence cause decrease in plant's roots and shoots length indicated by Lopez *el at.* (2002).

Table-2: Analysis of Variance table for Root length

Source	DF	SS	MS	F	P
Treatment (T)	2	15675.1	7837.53	758.18	0.0000
Variety (V)	11	11743.0	1067.55	103.27	0.0000
T×V	22	3219.6	146.35	14.16	0.0000
Error	70	723.6	10.34		
Total	107	31369.0			

Grand Mean 33.833 CV 9.50

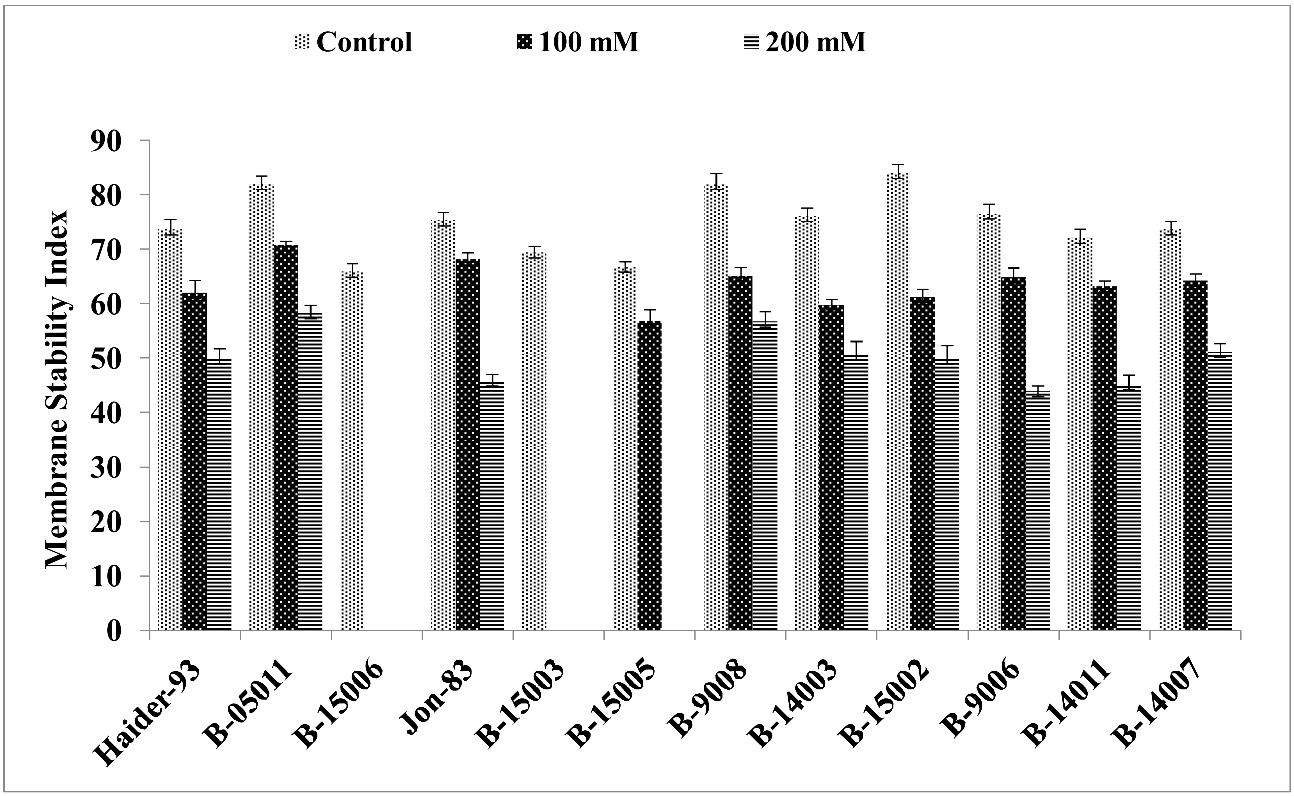

Figure 3: Effects of Salt concentration on the Membrane Stability Index of different Barley (*Hordeum vulgare* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Membrane Stability Index

Figure 3 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 is according to Jamil *et al.* (2012) who observed that all growth parameters are affected 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.

Table 3: 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

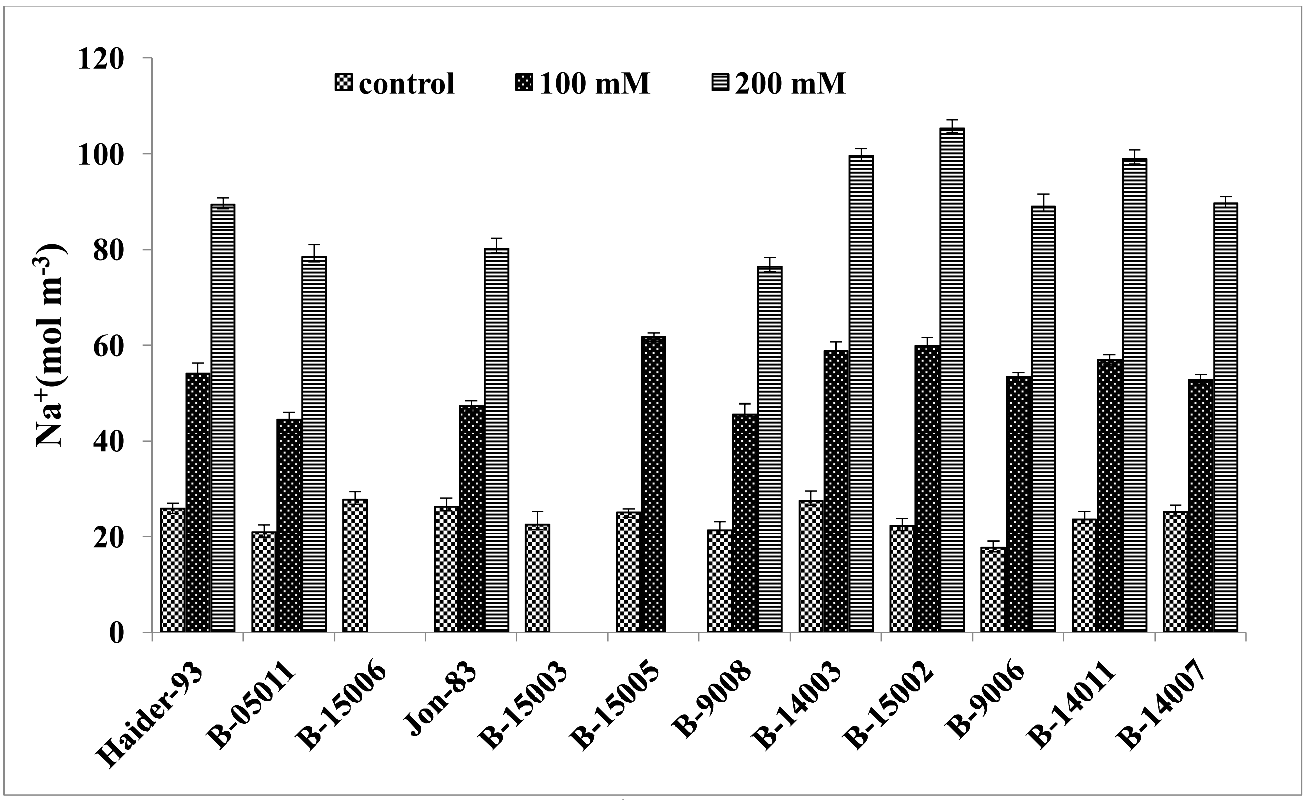

Figure 4: Effects of Salinity concentration on the Na^+ Concentration in shoot of different Barley (*Hordeum vulgare* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Na^+ Concentration in shoot

Figure 4. explore the Na^+ concentration of different varieties of barley against various salt stress concentrations. With increasing salt stress, Na^+ concentration of barley genotypes also increases. Na^+ uptake increased by potassium transport channel and ultimately Na^+ contents improved in the plant.

At control condition barley genotypes B-9008, B-05011 and B-9006 showed minimum Na^+ concentration 21.38 moles per m^3, 20.91 moles per m^3 and 17.76 moles per m^3 While higher Na^+ concentrations were observed on Haider-93, B-14003 and B-15006 which was 25.84 moles per m^3, 27.53 moles per m^3 and 27.72 moles per m^3. With enhancing NaCl stress to 100 mM barley genotypes B-9008, B-

05011 and Jon-83 indicated minimum Na^+ Concentration 45.53 moles per m^3, 44.53 moles per m^3 and 47.26 moles per m^3 While higher Na^+ concentrations were observed on B-15005, B-14003 and B-15002 which was 61.78 moles per m^3, 58.33 moles per m^3 and 59.85 moles per m^3. In the last treatment where salt stress was increased to 200 mM barley genotypes B-9008, B-05011 and Jon-83 showed minimum Na^+ concentration 76.46 moles per m^3, 78.47 moles per m^3 and 80.25 moles per m^3 as compared to other varieties. While the higher Na^+ Concentrations were observed on B-14003, B-14011 and B-15002 which was 99.58 moles per m^3, 98.88 moles per m^3 and 105.37 moles per m^3.

The outcome of this study is according to Tavakoli *et al.* (2011) who stated that most plants accumulate Na^+ in their tissues of shoot when grown in salt stress conditions. Ainnie and Staden (2010) noted that salt tolerance is related to exclusion of Na^+ ion and maintenance of almost uniform concentration of this ion in leaves of all ages. Yadav and Singh (2004) reported that salt tolerance is related to exclusion of Na^+ ion and maintenance of almost uniform concentration of this ion in leaves of all ages. High sodium content generally disrupted the nutrient balance, thereby causing, specific ion toxicity despite disturbing the osmotic regulation stated by Tiessen (1994). The capacity of the cells to compartmentalize Na^+ in the vacuole was positively related to plant salt tolerance. Salt tolerant plants differ from salt sensitive plant in having a low rate of Na^+ and Cl^- transport into leaves and the ability to compartmentalize these ions in vacuoles to prevent a built-up in the cytoplasm or cell walls, and thus avoid salt toxicity reported by Haq *et al.* (2002).

Table 4: Analysis of Variance table for Na^+ Concentration in shoot

Source	DF	SS	MS	F	P
Treatment (T)	2	33947	16973.4	2399.92	0.0000
Variety (V)	11	37191	3381	478.05	0.0000
T×V	22	3517.4	1598.8	226.06	0.0000
Error	70	495	7.1		
Total	107	106849			
Grand Mean 45.238	CV 5.88				

K^+ Concentration in shoot

In contrast to the Na^+, K^+ concentration in the root of different barley genotypes were remarkably reduced with under salt stress (Figure 5 and Table-5). As concentration of salts increases K^+ Concentration of barley genotypes decreases. However, each barley genotype showed various responses with respect to measured parameters. Na^+ and K^+ has antagonistic effect between them, thus at control conditions maximum K^+ concentration in barley genotypes were determined and minimum K^+ concentration was observed at high salinity as K^+ Concentration decreased with increase salt concentration.

At control condition barley genotypes B-9008, B-05011 and Jon-83 showed lesser concentration of K^+ 188.59 moles per m^3, 193.32 moles per m^3 and 183.23 moles per m^3 While minimum K^+ contents were observed in B-15005, B-14011 and B-15002 which was 159.94 moles per m^3, 169.11 moles per m^3 and 163.01 moles per m^3. In the second treatment where 100 mM salt stress was applied, barley genotypes B-9008, B-05011 and Jon-83 showed higher K^+ Concentration 131.59 moles per m^3, 139.68 moles per m^3 and 134.71 moles per m^3 when related with other varieties of barley. While minimum K^+ contents were observed in B-15005, B-14011 and B-15002 which was 106.64 moles per m^3, 112.34 moles per m^3 and 103.36 moles per m^3. In the last treatment where 200 mM NaCl stress was applied, barley genotypes B-9008, B-05011 and Jon-83 showed maximum K^+ Concentration 106.92 moles per m^3, 112.56 moles per m^3 and 109.67 moles per m^3 when related to other varieties of barley. While minimum K^+ levels was observed in B-14003, B-14011 and B-15002 which was 82.17 moles per m^3, 85.16 moles per m^3 and 78.51 moles per m^3.

The present results of study are according to Sairam *et al.* (2002) who stated that Na^+ content increased and K^+ contents increased in various wheat cultivars at increased salt stress. Sodium and Potassium has antagonistic effect present between them and has more ratio of sodium and potassium. At increased salinity concentration the levels Na^+ and Cl^- concentration higher in roots and plants aerials parts are affected most while potassium concentration is less at high salinity levels reported by Chartzoulakis *et al.* (2002) in six olive cultivars.

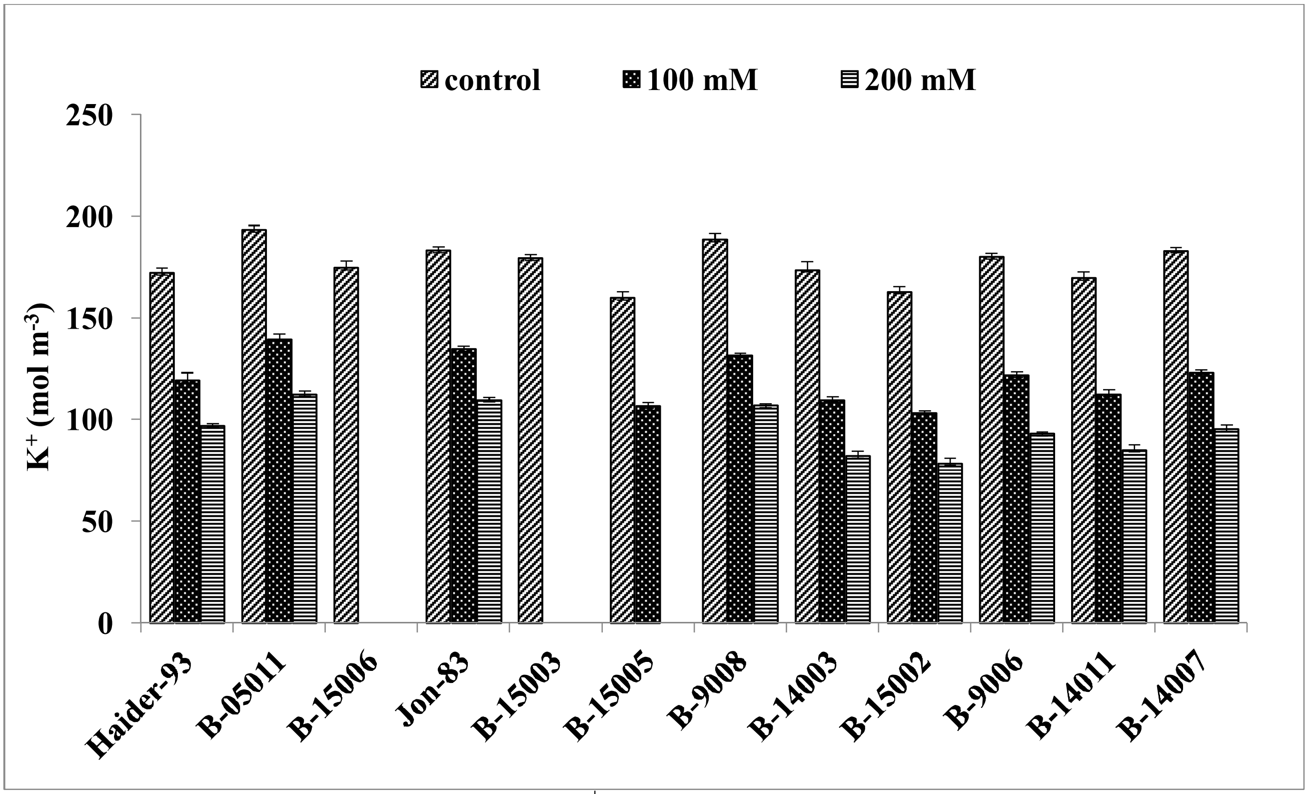

Figure 5: Effects of Salinity concentration on the K^+ Concentration in the shoot of different Barley (*Hordeum vulgare* L.) genotypes (Two genotypes could not grow at 100 mM salt stress).

Table 5: Analysis of Variance table for K^+ Concentration in shoot

Source	DF	SS	MS	F	P
Treatment (T)	2	212502	106251	7866.84	0.0000
Variety (V)	11	94587	8599	636.66	0.0000
T×V	22	50318	2287	169.34	0.0000
Error	70	945	14		
Total	107	358355			

Grand Mean 116.21 CV 3.16

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