Received: 03 Oct 25 | Revised: 17 Nov 25 | Accepted: 29 Dec 25

RESEARCH ARTICLE

Trends in Biotechnology and Plant Sciences

Evaluation of Genetic Diversity in Barley Under Salt Stress Conditions

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Abstract

Rising salinity is a negative environmental stress with a big influence on agriculture across the world. As the population of the world is increasing day by day. Salt tolerance mechanisms are of increasing interest for improving crops. Among all the cereal crops, barley is considered to be the most saline tolerant. However, the salinity tolerance varies among different cultivars. Under various salinity levels, a hydroponic experiment was performed at the Institute of Soil and Environmental Science, University of Agriculture Faisalabad to assess the performance of barley cultivars. The experiment was carried out with twelve barley genotypes at two levels of salinity (100mM and 200mM NaCl) and a control (Haider-93, B-05011, B-15006, Joo-83, B-15003, B-15005, B-9008, B-14003, B-15002, B-9006, B-14011, B-14007). For a period of six weeks, plant growth (height, root and shoot fresh and dry weight) and physiological traits (membrane stability index, relative water content) of salt-stressed plants including chlorophyll content and ionic concentration Na+ and K+ in roots and shoots were studied. A CRD or Completely Randomized Design with factorial arrangement and three replications were used. According to results, the increase in salinity caused a significant reduction in the growth and physiological parameters of the plant. It also reduced the chlorophyll content as well as K+ concentration. However, the concentration of Na+ increased with growing salinity. Among the different genotypes, the B-05011, B-14003, and B-9006 were found most salt-tolerant, while B-15006, B-15003, and B-15005 were recorded most salt-sensitive. We can use the tolerant genotypes identified from this study for further cultivation in saline soils and breeding programs.

KEYWORDS

Salinity, Yield, Sodium, Barley, Potassium, K^+/Na^+.

Citation: Tasawar Z and Rabaz M, 2025. Evaluation of genetic diversity in barley under salt stress conditions. Trends in Biotechnology and Plant Science, 3(4): 40-52. https://doi.org/10.62460/TBPS/2025.099

1 | INTRODUCTION

Salinity is a significant abiotic stress that adversely affects agricultural productivity worldwide. It disrupts soil structure, causing ionic imbalance and toxicity, which limits plant growth and yield. Approximately 15% of the global land area is affected by salinity, and around 20% of irrigated regions are impacted by salt stress, with Pakistan ranking eighth globally, where 26% of its irrigated land is affected. The main causes of salinity in Pakistan are improper irrigation, poor drainage, and waterlogging. Salt-affected soils are classified into three categories: saline, sodic, and saline-sodic soils, based on electrical conductivity (EC), sodium adsorption ratio (SAR), and exchangeable sodium percentage (ESP) (Rengasamy, 2010). These soils are common in semi-arid and arid regions, affecting 830.5 million hectares worldwide, with 6.35 million hectares in Pakistan (Qadir *et al*., 2005).

Salinity affects plant growth by reducing water availability due to osmotic stress and increasing ion toxicity from excess Na^+, Cl^-, and B. Salt stress impairs physiological processes such as photosynthesis, respiration, and nutrient uptake, which decreases plant biomass and yield (Yamaguchi and Blumwald, 2005; Munns, 2008). Different mechanisms of salt tolerance exist, including osmotic tolerance, ion exclusion, and tissue tolerance (Deinlein *et al*., 2014; Roy *et al*., 2014). Salt-tolerant plants can manage high Na^+ concentrations, maintain K^+/Na^+ ratios, and mitigate oxidative stress caused by reactive oxygen species (ROS) (Apel and Hirt, 2004; Navrot *et al*., 2007). These mechanisms vary among plant species and cultivars, with some plants exhibiting greater tolerance to high salinity levels (Munns & Tester, 2008).

Barley (*Hordeum vulgare* L.) is one of the most salt-tolerant crops, capable of growing in a wide range of environments, from arid regions to higher altitudes. It is an important crop for both human and animal consumption, as well as for brewing. Barley's salt tolerance is mainly associated with Na^+ exclusion and maintaining K^+ concentrations under saline conditions (Forster *et al*., 2000; Kader and Lindberg, 2005). High K^+/Na^+ ratios in barley genotypes have been linked to better salt tolerance, with tolerant genotypes exhibiting efficient K^+ transport and reduced Na^+ accumulation (Shabala *et al*., 2010). This makes barley a promising crop for saline-affected areas, and breeding salt-tolerant varieties is essential for enhancing agricultural productivity in such regions (Munns *et al*., 2006; Katerji *et al*., 2000).

This study aims to investigate the genetic variability in salt tolerance among different barley genotypes under salt stress conditions, focusing on their physiological and biochemical responses.

2 MATERIALS AND METHODS

2.1. Experimental setup

A hydroponic experiment was conducted at the wire house, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, to assess the performance of barley genotypes under varying salinity conditions. The wire house maintained controlled environmental conditions conducive for the experiment.

2.2 Seed source and growth conditions

Twelve barley genotypes (*Hordeum vulgare* L.) were used in this study, sourced from Ayub Agriculture Research Institute (AARI), Faisalabad. Seeds were initially sown in iron trays with a 2-inch sand layer and irrigated with distilled water. After 2-3 days, seedlings were transferred to a greenhouse and transplanted into 100 L tubs containing half-strength Hoagland's solution (Johnson *et al*., 1957). The nutrient solution, aerated by pumps, was composed of macro-nutrients ($Ca(NO_3)_2$, KNO_3, $MgSO_4$, 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), with pH maintained at 6.5 ± 0.5 (Kronzucker *et al*., 2006). The solution was replaced every 8 days.

2.3. Salinity treatments

Salinity stress was applied by using NaCl at two different concentrations, 100 mM and 200 mM, introduced in three increments starting three days after transplanting the nursery into the tubs. The three treatments tested included: the control group (T1), which received no salt; T2, which was exposed to 100 mM NaCl; and T3, subjected to 200 mM NaCl.

2.4. Harvesting and sample preparation

After 42 days of salinity treatment, plants were harvested. Roots and shoots were separated using scissors, and their fresh weights were measured. Samples were then collected in paper bags for further analysis. For dry weight determination, samples were sun-dried and then oven-dried at 70°C until a constant weight was achieved.

2.5. Agronomic parameters

Shoot and root lengths were measured using a meter rod. After harvesting, the fresh weights of both the shoots and roots were determined using an electronic balance. For dry weight determination, both shoot and root samples were oven-dried at 65°C ± 5°C until they reached a constant weight, and the dry weights were then recorded.

2.6. Physiological parameters

Chlorophyll content was measured using a Minolta SPAD-502 meter. Fully expanded young leaves were sampled and hydrated to full turgidity. The fresh weight, turgid weight, and dry weight were recorded after drying at 65°C. The Membrane Stability Index (MSI) was calculated by incubating leaf discs at 40°C and recording the electrical conductivity (C1) before boiling. After boiling, the samples were re-measured for electrical conductivity (C2) (Sairam, 1994). The MSI was calculated using the formula:

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

2.7. Ionic content analysis

Sodium (Na+) and potassium (K+) concentrations in plant tissues were determined using a Sherwood 410 Flame photometer after samples were prepared according to the "Ion Extraction Method" with reagent-grade salts (NaCl, KCl).

2.8. Statistical analysis

Data were analyzed using the statistical methods outlined by Steel *et al*. (1997). Treatment effects were assessed using Duncan’s Multiple Range (DMR) test (Duncan, 1955) for mean separation.

3 RESULTSNAD DISCUSSUIN

3.1. Shoot fresh weight

Fig. 1 depicts the shoot weights of different barley varieties under different salt stress concentrations. Salt stress significantly affected the shoot fresh weight of barley genotypes, and as salt concentration went up, the shoot fresh weight fell down. Barley genotypes have different reactions concerning shoot length when exposed to salt stress.

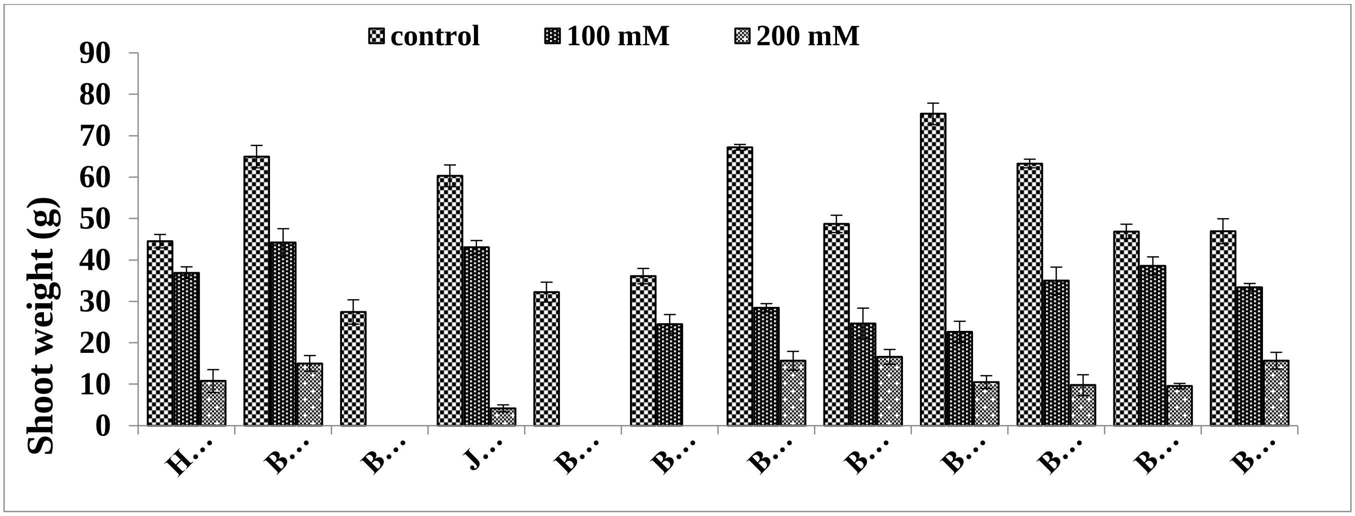

Fig. 1: Impact of salt concentration on the weight of barley shoots the effects of salinity concentration on shoot fresh weight (g) of different barley (*Hordeum vulgar* l.) genotypes (2 genotypes could not grow at 100 mM salt stress).

In control condition B-15002, B-9008 and B-05011 produced the maximum shoot fresh weight of 64.93 g, 67.18 g and 75.27 g respectively. The minimum weight was observed in B-15006, B-15003 and B-15005 that was, 27.44 g, 32.22 g and 36.10 g respectively. Under 100 mM of NaCl, the B-05011, B-14011, and Jon-83 genotypes produced the most shoot fresh weight, respectively 44.24 g, 38.55 g, and 43.02 g. Meanwhile, the B-15002, B-15005, and B-14003 genotypes produced the least shoot fresh weight, respectively 22.66 g, 24.52 g, and 24.62 g. The maximum growth of barley genotypes B-14003, B-9008 and B-05011 at 200 mM salt concentration were 16.58 g, 15.67 g and 14.99 g respectively while the minimum shoot fresh weight at this concentration was shown by B-9006, B-14011 and Jon-83. These genotypes had 9.78 g, 9.54 g and 4.17 g respectively which was much lower when compared with other studied barley genotypes.

According to Ashraf, *et al*. (1999) who measured the salt tolerance of twelve cultivars of Brassica growth and yield reduced with salinity in all cultivars. According to Akhtar, osmotic pressure created due to deficiency of water causes under salinity conditions that also decrease the weight of shoot. According to Munns *et al*. (1995), the weight of Roth cultivars decreased due to higher salinity. Rashid *et al*. (1999) demonstrate that salinity in wheat reduces shoot weight. According to Toselli and Casenave (2003), the drop in shoot fresh weight happened because of ion toxicity that prevented germination, compared to salt tolerance of six genotypes of canola in a similar experiment. As per Li *et al*.

(2006), the result showed the accumulation of particular ion in the leaves caused a decrease in fresh weight of the shoot.

3.2. Root fresh weight

Salt stress affects root fresh weight severely. This result is evident from the results compiled in Fig. 2. As the salt stress increased, the fresh weight of the roots was significantly decreased. Yet, there was notable variation in the application of salt pressure in different barley genotypes. The root fresh weight of different barley genotypes data indicated that root fresh weight significantly reduced with increased salt stress.

The genotypes B-15002, B-9008 and B-9006 weight showed superiority at control condition over the other weights of genotypes. B-15006, B-15003 and B-14011 showed minimum growth of 2.22 g, 1.73 g and 3.3 g respectively. The application of 100 mM NaCl stress showed maximum root fresh weight of 4.78 g, 3.57 g and 3.90 g for B-05011, B-9006 and Haider-93 genotypes while B-9008, B-14011 and B-14003 genotypes showed minimum root fresh weight of 2.41 g, 2.23 g and 2.32 g compared to other barley genotypes. At the concentration of 200 mM, the barley genotypes B-14003, B-9008 and B-14007 showed good results 1.94 g, 2.18 g and 2.26 g while the lowest root fresh weight 0.66 g, 0.52 g and 0.58 g was recorded for B-05011, Haider-93 and Jon-83 as compared to other barley genotypes.

According to Levitt (2004), when present under saline conditions, decreased root fresh weight may be the result of a reduction in water availability, osmotic potential at root surface, or due to specific ion toxicity and nutrient imbalance. The fresh weight of roots was reduced under saline conditions. This could be due to a decrease in the availability of water. Osmotic stresses can delay the germination of seeds and the establishment of seedlings. Under osmotic stresses, there is a decline in water uptake during imbibition. Also, salt stress may cause excessive uptake of ions (Demiral *et al*, 2005). Zynali and Hamdi (2002) suggested NaCl added to growing media led to less root fresh weight of cuttings. The two barley cultivars experienced severely reduced photosynthetic rates because of the diminished root weight under saline conditions. The level of salt had a serious effect on plant growth and a decrease in their root fresh weight. As per Kingsbury *et al*. (1984) also same results were reported that salt stress reduces the plant roots fresh weight.

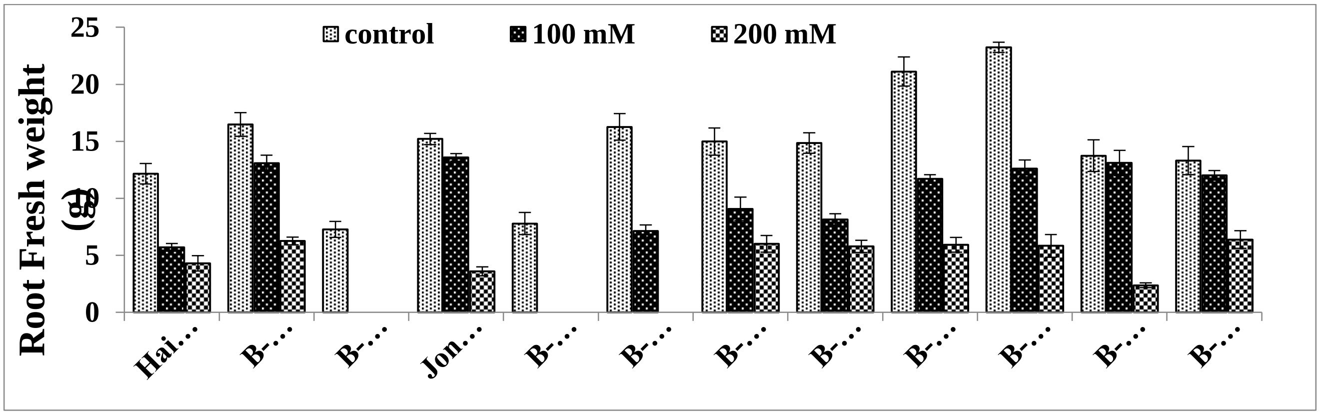

Fig. 2: Effects of Salinity on the Root Fresh Weight of Barley Genotypes.

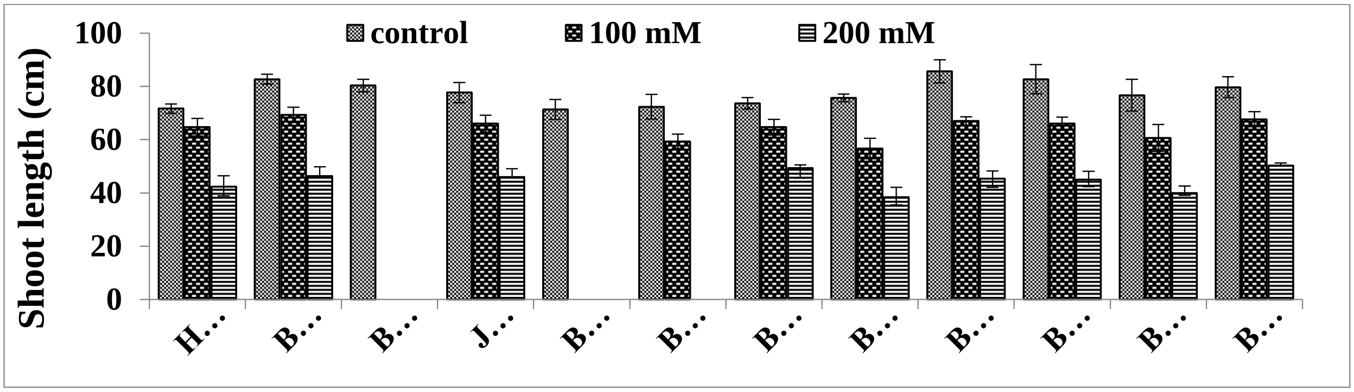

Fig. 3: Effects of salinity on shoot length of different Barley (*Hordeum vulgar* L.) genotypes.

3.3. Shoot Length

The data shown in Fig. 3 shows the effect of salt stress on shoot length of different barley genotypes. Shoot length was greatly affected by salt stress, and it was observed that with increasing salt stress, the shoot length of barley genotypes decreased significantly. Salt stress effects greatly differed among various barley genotypes, signifying significant variation in response thanks to the application of salt stress. According to the data, the length of the various barley genotypes has been mentioned which indicates that the shoot length is significantly reduced with increasing salt stress.

In the control condition, which was not salt-stressed the barley genotypes B-15002, B-9006 and B-05011 gave 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 gave minimum shoot length respectively. In the second treatment, the B-05011, B-14007, and B-15002 genotypes exhibited better results of 69.33 cm, 67.66 cm and 67 cm. Conversely, the B-14003, B-15005, and B-14011 genotypes showed minimum shoot lengths of 56.66 cm, 59.33 cm and 60.66 cm, respectively. During the final treatment, the barley genotypes were greatly impacted by the application of salt stress, which was done at a dosage of 200 mM NaCl. However, the varieties of B-14007 and B-9008, with lengths of 50.33 cm and 49.33 cm, respectively, had the highest shoot length. However, B-14003 had the lowest shoot length at 38.33 cm, compared to other barley varieties.

The decrease in above-ground growth could be due to both osmotic stress and salt toxicity due to higher osmolality as stated by Illahi *et al*. (2001) Under salty conditions, Sharma and Garg (2000) talked about how cell extensibility and growth factors are hampered. According to Mahmood *et al*. (2009), the increase in salinity level of the soil decreases the turgor pressure of the plant affecting the metabolic processes and thus cell enlargement and decrease takes place in growth related parameters.

3.4. Root length

Fig. 4 shows the roots lengths of different types of barley against different salt stress concentrations. Salt stress had a considerable impact on root length (Table-4), and increasing salt concentration decreased the root length of barley genotypes. Barley types did not all show the same response to salt stress with respect to their root length. The root length of barley genotypes was maximum of B-15002, B-9006 and Jon-83 which was 58.33 cm, 58.33 cm and 56 cm relatively with other genotypes, While minimum root length was B-15006, Haider-93 and B-15005 which was 37.66 cm, 41 cm and 40.33 cm at control conditions. While B-15005, Haider-93 and B-14007 genotypes lowest results were 38.33 cm, 36 cm and 40 cm, B-14011, B-15002 and B-9008

rose sharply to 70 cm, 62 cm and 50 cm. Barley genotypes were most affected when salt stress increased to 200 mM. The maximum root length was found to be 27.67 cm, 30.67 cm, and 28 cm in B-15002, B-9008, and B-05011 at 200 mM while minimum root length 20.33 cm, 25 cm and 24 cm in B-14007, B-14011 and Haider-93. Other barley genotypes had higher root lengths than the mentioned genotypes. The findings are in agreement with Jamil *et al*. (2005) observation that the canola varieties root length decreased as salinity levels increased. Higher salt stress levels will cause the length of root to decrease because it delays germination or emergence of seedling. A growth slower, increase in salinity can restrain the root length and thus imbalance the nutrition uptake by the plants. Sun *et al*. (2005) said that the length of roots was significantly shortened due to increased salt stress and imbalance of the nutrients composition. The roots of the seedlings were harmed due to increasing levels of salt concentration, which reduced the length of the roots and prevented elongation of roots described by Warner *et al*. (2004). The increase in osmotic pressure of soil solution and an imbalance of nutrients is responsible for this reduction. Rooting substance that has greater salinity level causes stress and thus many elements that required by plants becomes inaccessible hence causing decrease in plants length of roots (Lopez el at summarized. (2002).

3.5. Shoot dry weight

Fig. 5 and Table-5 shows that shoot dry weight was maximum in 25 mM. As salt stress increased, it was discovered that the shoot's dry weight decreased significantly. As more salt is added, growth parameters fail to increase. The amounts of salts affect the concentration of shoot dry weight in barley genotypes. The study states that data shoot dry weight of distinct barley genotypes explains that shoot dry weight significantly reduced with increasing salt stress.

B-9006 and B-9008 and B-15002 are the genotypes that observed the maximum shoot dry weight (5.98 g, 6.34 g and 6.38 g). The weights of B-15006, B-15003 and B-14011 were lower at 2.22 g, 1.73 g and 3.3 g respectively. When the salt concentration rose to 100mM, barley genotypes B-05011, B-9006 and Haider-93 performed well with maximum shoot dry weight 4.78 g, 3.90 g and 5.98 g as compared to other genotypes while B-14011, B-9008 and B-14003 had minimum shoot dry weight 2.23 g, 2.41g and 2.32g compared to other barley genotypes. The maximum shoot dry weight at 200 mM NaCl stress was 2.26 g, 2.18 g and 1.94 g for genotypes B-14007, B-9008 and B-14003. Whereas, other barley genotypes B-05011, Haider-93 and Jon-83 showed minimum shoot dry weight of 0.66 g, 0.52 g and 0.58 g respectively.

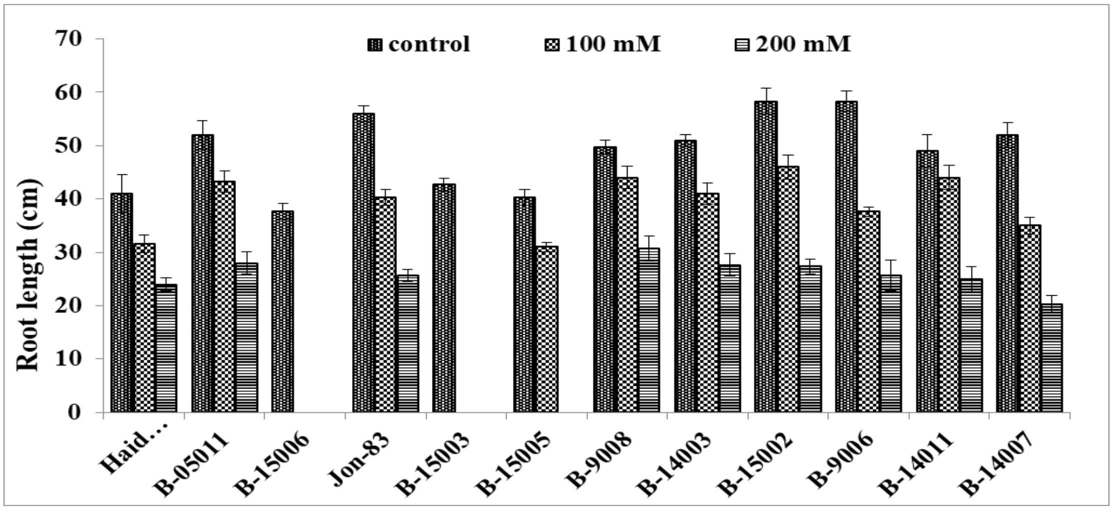

Fig. 4: Effects of salinity on Root length of different Barley (*Hordeum vulgar* L.) genotypes.

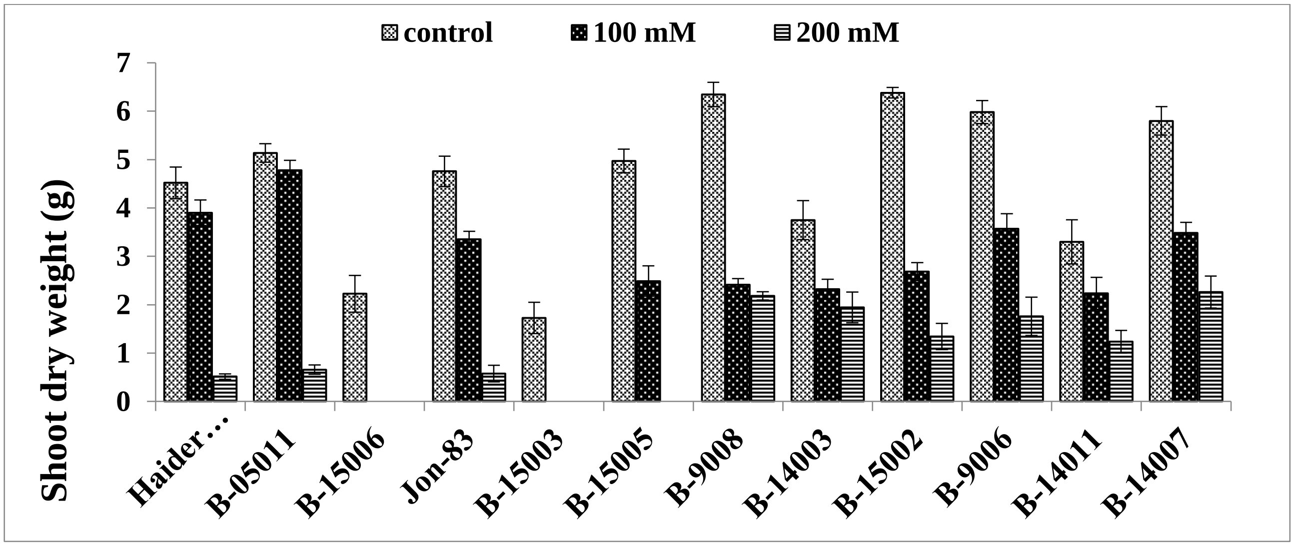

Fig. 5: Effects of Salinity on the Shoot Dry Weight of Barley (*Hordeum vulgare* L.) genotypes.

Similarly, Ciek and Cakilar (2002) study that osmotic stress and salt stress significantly decrease the shoot dry weight in the plants. Under salinity conditions, the turgor pressure of a plant cell is decreased and stomata closes which lowered the rate of photosynthesis and finally dry weight decreased. According to Farouk (2000), less production of leaf area and leaf number causes a reduction in dry weight. Yadiv *et al*. (2001) reported that high salt treatment reduced CO2 uptake in leaves which reduced the dry weight. When salinity decreases, it means water and mineral nutrients are not being available as much as before. This leads to a decrease in water potential of a rooting medium. It is a growth inhibiting factor as reported by Lessani and Marschner. According to Tevitt (1998), weight loss could be due to less photosynthesis, increased ion toxicity due to salts, and turgor pressure loss under salinity. Osmotic stress may be responsible for reduction in plant germination and loss of weight. Moreover, Kumar (2002) argued that it could be because of toxic ions accumulation and cellular organelles impairment.

3.6. Root dry weight

Data in Fig. 6 and Table-6 show the effects of salt stress on the root dry weight of some barley genotypes. Adding salt stress greatly impacted the root dry weight measurements and as salt stress increased, root dry weight of barley genotypes significantly decreased. Nonetheless, there was considerable diversity regarding the response in various barley genotypes under the influence of salt stress.

In the control condition of the experiment, where barley genotypes did not experience salt stress, the maximum value for root dry weight was for B-9006, Haider-93 and B-15002 which were 1.24 g, 1.07 g and 1.12 g. Less root weight was of B-15003, B-15006 and B-14011 which were 0.33 g, 0.43 g and 0.53 g respectively. For the second treatment, barley genotypes B-14007, B-9006, and Jon-83 were found to have the highest root dry weight of 0.80 g, 0.82 g, and 0.8 g respectively. On the other hand, B-9008, B-15005 and B-14011 were found to have the lowest dry weight of root which were 0.47 g, 0.49 g and 0.39 g respectively. At a concentration of 200 mM, the barley genotypes B-15002, B-05011 produced better root dry weights of 0.50 g, 0.43 g, and 0.52 g while B-14011, B-14003, and Jon-83 gave the minimum root dry weights of 0.31 g, 0.14 g and 0.25 g.

Neuman (2001) found similar results in which canola species displayed reduced seedling emergence and less weight of roots. Similar was the trend which salinity lent to the weight of root as a result of presence of higher salt concentration (Huang and Redman, 2010). High salt stress concentration can lead to water deficiency, turgidity of cells, and other adverse effects that ultimately reduce the rate of photosynthesis and the dry weight of roots. According to Hameed *et al*. (2006), there was an improved rate of evapo-transpiration in canola varieties under higher salt stress because salt concentration in the root zone ultimately reduces dry root weight. Higher salinity negatively affects cell division due to limited carbon mobility in leaves. As a result, there is a downfall in the rate of photosynthesis and dry weight of shoot (Farhoudi, 2010). This means that due to the concentration of salt stress, the cells can lose their turgor pressure. Due to the reduced turgor pressure, the rate of photosynthesis and dry weight of root decreases. The reproductive process can also be affected by the impact on metabolic processes that cause greater transport of toxic ions and imbalanced nutrition. (He and crammer (2010).

3.7. Membrane Stability Index

Barley membrane stability index (MSI) showed a distinct variety-wise pattern under each concentration of the stress (Fig. 7). Salt stress had a significant impact on the membrane stability index. According to the average Data Membrane Stability Index, it was significantly lowered with the rise of NaCl stress in various 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 %. B-9008 2-20011 and B-14007 barley genotypes showed 56.72%, 58.26% and 51.16% maximum Membrane Stability Index at 200 mM, whereas 2-9006, B-14011 and Jon-83 were found to have less Membrane Stability Index 43.94%, 45.17% and 45.85% as compared to other barley genotypes.

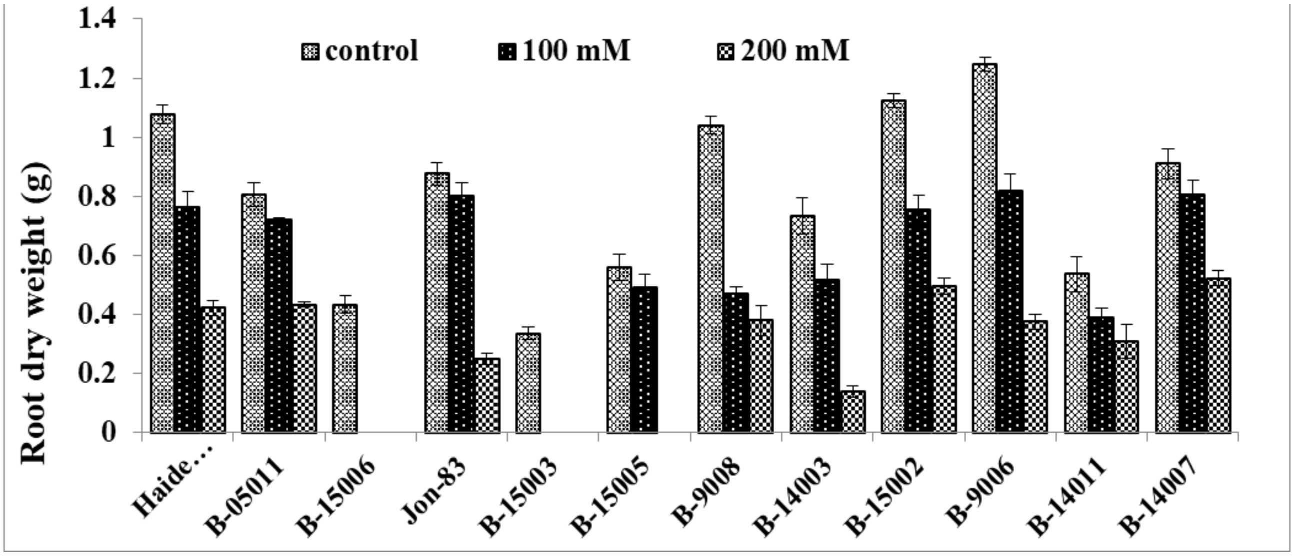

Fig. 6: Effects of Salinity on the Root Dry Weight of Barley (*Hordeum vulgare* L.) genotypes.

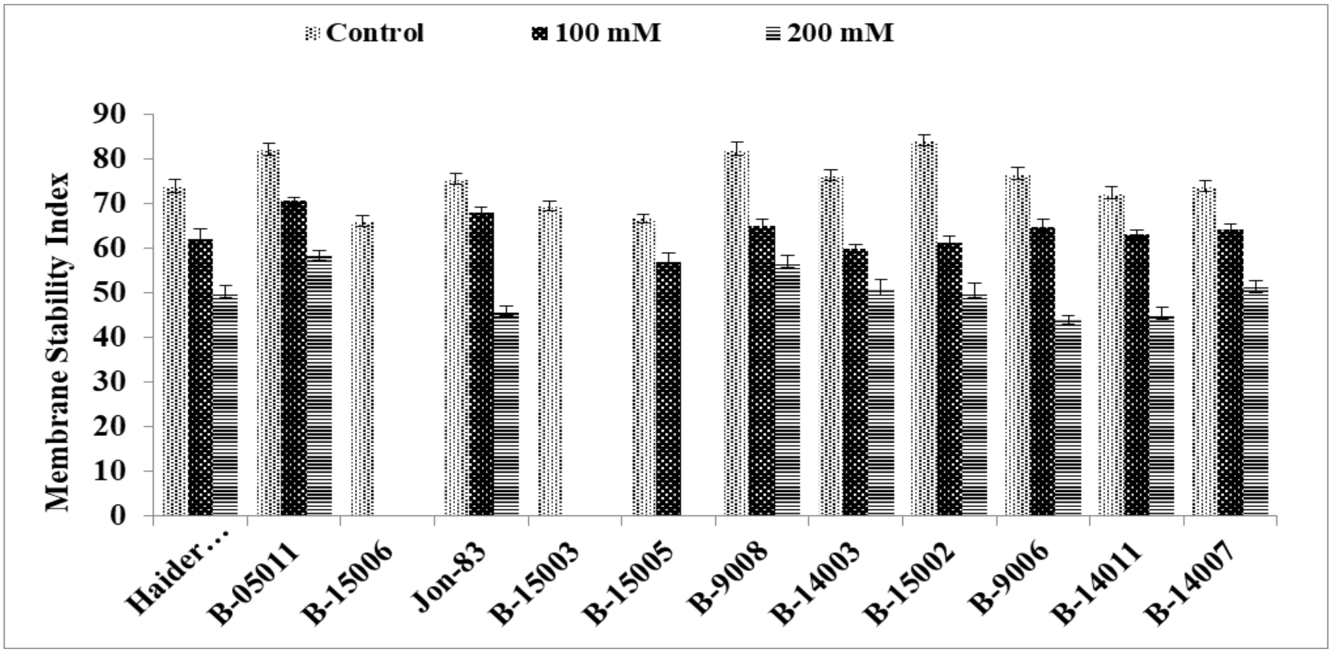

Fig. 7: Effects of Salt on the Membrane Stability Index of Barley (*Hordeum vulgare* L.) genotypes.

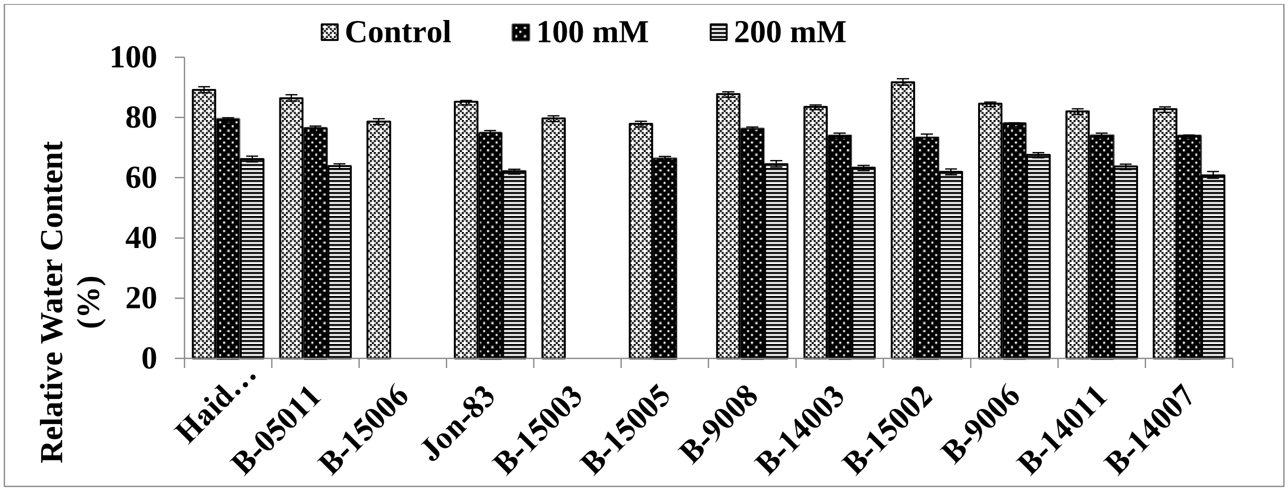

Fig. 8: Impacts of Salinity on the Relative Water Content of different Barley (*Hordeum vulgare* L.) genotypes.

According to Jamil *et al*. (2016), all the growth parameters were affected by salt stress and it is noted that the stability of the membrane was influenced by the increase in salinity that has an inversely proportion with growth related parameters. As the salt levels go up, the plant cells face an ordeal. The high concentration of salinity causes lesser water uptake in the plants. This cause loss of turgidity of the cells. The cells appear flaccid. Due to the effect of salt stress the membrane gets affected. With a gradual increase of salt stress it causes injury. Hence membrane damage of plant takes place.

3.8. Relative water content

Fig. 8 and Table 4 show the RNA contents of the plants and their comparison. As salinity stress increased, the relative water content was significantly decreased in the plant. All barley varieties' relative water content relies on the salts' concentration. The data showed that the different barley genotypes relative water content decreased when the salt stress increases.

Under control condition B-9008, Haider-93 reached a maximum relative water content of 87.73%. Under similar conditions, B-15002 reached a content of 89.17%. Lastly, B-150021 saw the highest content of 91.75%. Varieties B-

15005, B-15006 & B-14011 showed lesser values of 77.86 percent, 78.69 percent and 82.03 percent. In the second stress treatment who were subjected to 100 mM NaCl stress the barley genotypes B-05011, B-9006 and Haider-93 exhibited maximum performance relative water contents 76.36 %, 77.95 % and 79.47 % respectively while on the other hand, 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. When salt stress increased to 200 mM, three barley genotypes showed higher relative water content. In particular, B-9008 showed 64.53 % relative water content while B-9006 and Haider-93 showed 67.61 % and 66.25 % respectively. Genotypes B-14007, B-15002 and Jon-83 showed low relative water content among the barley genotypes studied.

According to Khosravinejad *et al*. (2008), salinity does affect the relative water contents and also it may also affect the chlorophyll contents and photosynthetic pigments. When the concentration of NaCl is increased, the relative water content of the plant gets reduced. As the concentration of salts increases in the root medium, the water potential in the root medium becomes lower than the plant and hence water moves out from the plant. And also when salt stress occurs, the evapotranspiration rate of the plant becomes high than its water uptake and hence cause deficiency of water in plants which affects the shoot and root ratio as relative water content is reduced it decrease the growth of plant and it increase the shoot and root ratio. Akhter *et al* in 2010 stated that the direct response of the plant against salinity leads to loss of turgor pressure of cell causing a reduction in leaf area.

3.9. Chlorophyll contents

The information condensed in Fig. 9 examines how salt stress affects the chlorophyll contents of various barley genotypes. Use of salt stress had a considerable influence on chlorophyll contents. With the implementation of salt stress, a large disparity in barley genotypes was found in their variations to their responses.

The barley genotypes Haider-93, B-9008, and B-14007 demonstrated the highest chlorophyll contents at the control condition, where no salt stress was applied, at 43.30, 48.73 and 47.83 per cent respectively. The minimum chlorophyll content showed that B-15006, B-15003 and Jon-83 obtained lower chlorophyll which was 35.40 %, 33.33 % and 38.40 %. At low concentration (100mM) of NaCl solution barley genotypes B-05011, B9008 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 %. When the concentration reaches 200 mM, the maximum chlorophyll content of barley genotypes was noted in B-14007, B-9008, and Haider-93 with values of 35.3%, 36.93%, and 37.16%. Conversely, the minimum chlorophyll content of B-05011, B-14007 and Jon-83 with values of 32.9%, 35.3%, and 31.63% were noted as compared to other barley genotypes.

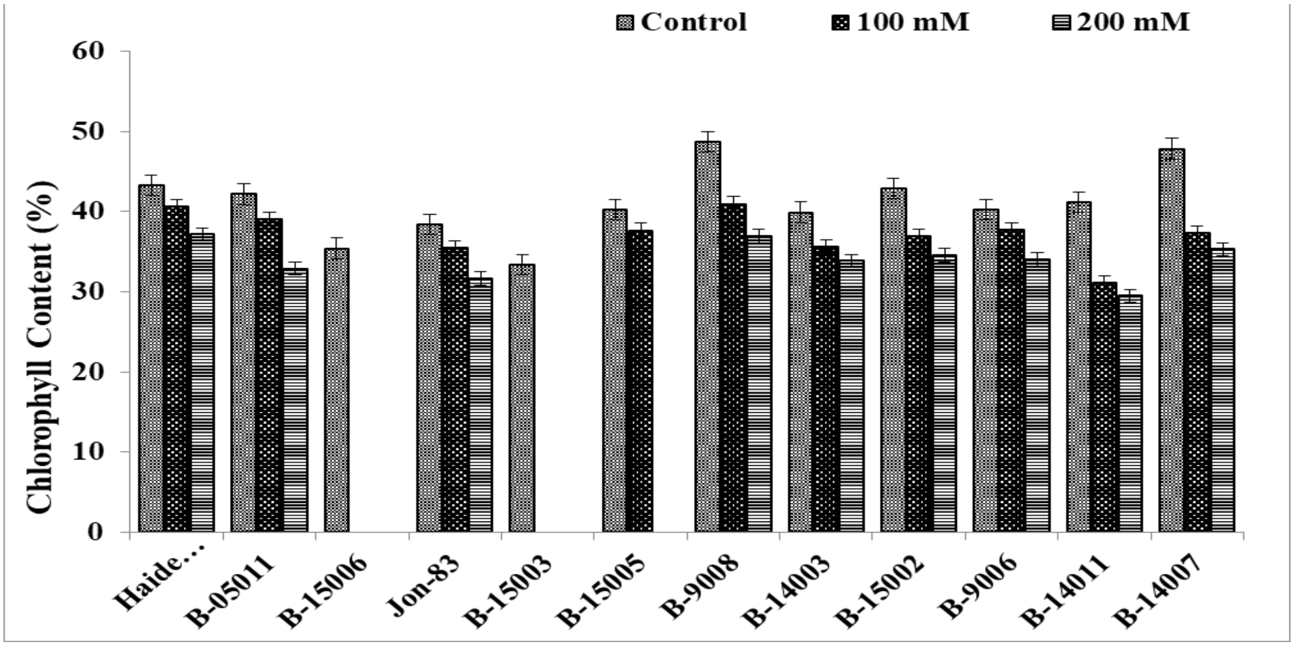

Fig. 9: Assessment of Salt stress on the Chlorophyll Contents of Barley (*Hordeum vulgar* L.) genotypes.

The outcomes of the study showed that rate of germination varied greatly among the oat genotypes as per Zhao *et al*. (2007). There was a decline in chlorophyll levels, a decrease in dry biomass and leaf area, and an increase in

Na+ and decrease in K+ levels.

All parameters reduced by application of salt treatment. High salt stress conditions negatively affect all metabolic functions of the plant. When salinity level increases, stomata closing occurs which effects gases exchange, and causing water stress in plants. Ultimately, this will affect the photosynthetic rate of plant. As salinity levels rise, the amount of chlorophyll gradually declines. Chlorophyll content was reported to decrease with increased salinity levels in tomato plants (Al-aghabary *et al*. 2005).

3.10. Na^+ Concentration in shoot

Fig. 10 examines the sodium ion concentrations of different barley types under various salt stress levels. As salt stress increases, the concentration of sodium ions in barley genotypes also increases. The plant's sodium content improved due to increases in potassium transport channel use for sodium uptake. Three genotypes of barley, B-9008, B-05011 and B-9006, recorded minimum Na+ concentrations of 21.38, 20.91 and 17.76 moles per m3. As for the high Na+ concentrations, this was seen on Haider-93, B-14003 and B-15006, which was 25.84, 27.53 and 27.72 moles per m3 in that order. Under increased NaCl stress, barley varieties B-9008, B-05011 and Jon-83 showed the least interference with Na+ concentration, which was 45.53 moles per m3, 44.53 moles per m3 and 47.26 moles per m3 respectively. On the other hand, barley varieties B-15005, B-14003 and B-15002 showed maximum Na+ concentration of 61.78 moles per m3, 58.33 moles per m3 and 59.85 moles per m3 respectively. In the final treatment, when the salt stress was raised to 200 millimolar, barley genotypes B-9008, B-05011 and Jon-83 showed minimum Na+ concentration 76.46 moles per m3, 78.47 moles per m3 and 80.25 moles per m3 when compared with other varieties. The highest concentrations of sodium ions were found on B-14003, B-14011 and B-15002 which were 99.58 moles per m3, 98.88 moles per m3 and 105.37 moles per m3 respectively.

According to Tavakoli *et al*. (2011) findings of the study that most plants accumulate Na+ in the shoots when grown in salt stress conditions. It is supported by Ainnie and Staden (2010). It was reported by Yadav and Singh (2004) that salt tolerance is associated with the exclusion of Na+ ion and maintenance of almost uniform concentration of this ion in leaves of all ages. According to Tiessen (1994), a high concentration of sodium typically perturbed the nutrient balance that caused specific ion toxicity, while disturbing the osmotic regulation. The ability of cells to accumulate Na+ in the vacuole is positively correlated with the salt tolerance of plants. Salt tolerant plants are the opposite of salt sensitive plant. They have a low rate of transport of ions like Na+ and Cl to the leaves. Salt tolerant plant can compartmentalize these ions in their vacuoles. This prevents built-up of Na and Cl in the cytoplasm or cell walls which prevents salt toxicity as reported by Haq *et al*. (2002).

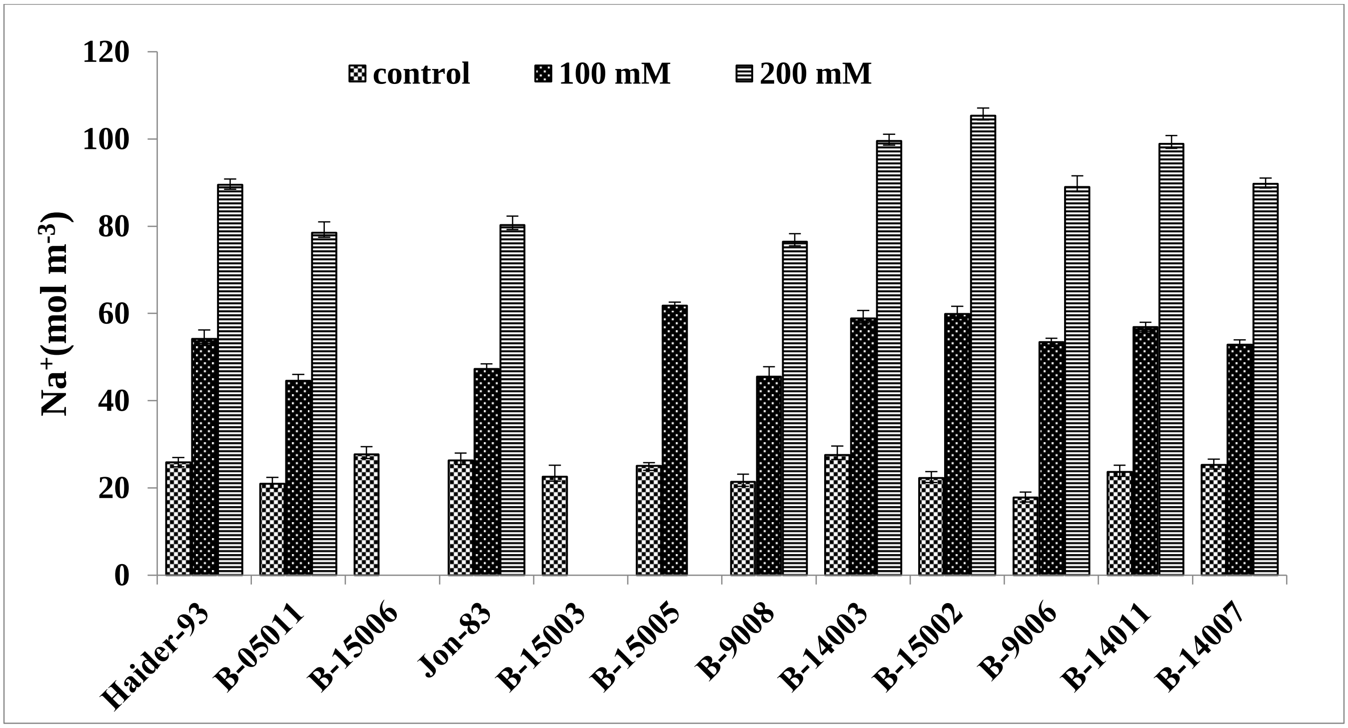

Fig. 10: Effects of Salinity on the Na^+ Concentration in shoot of different Barley (*Hordeum vulgare* L.) genotypes

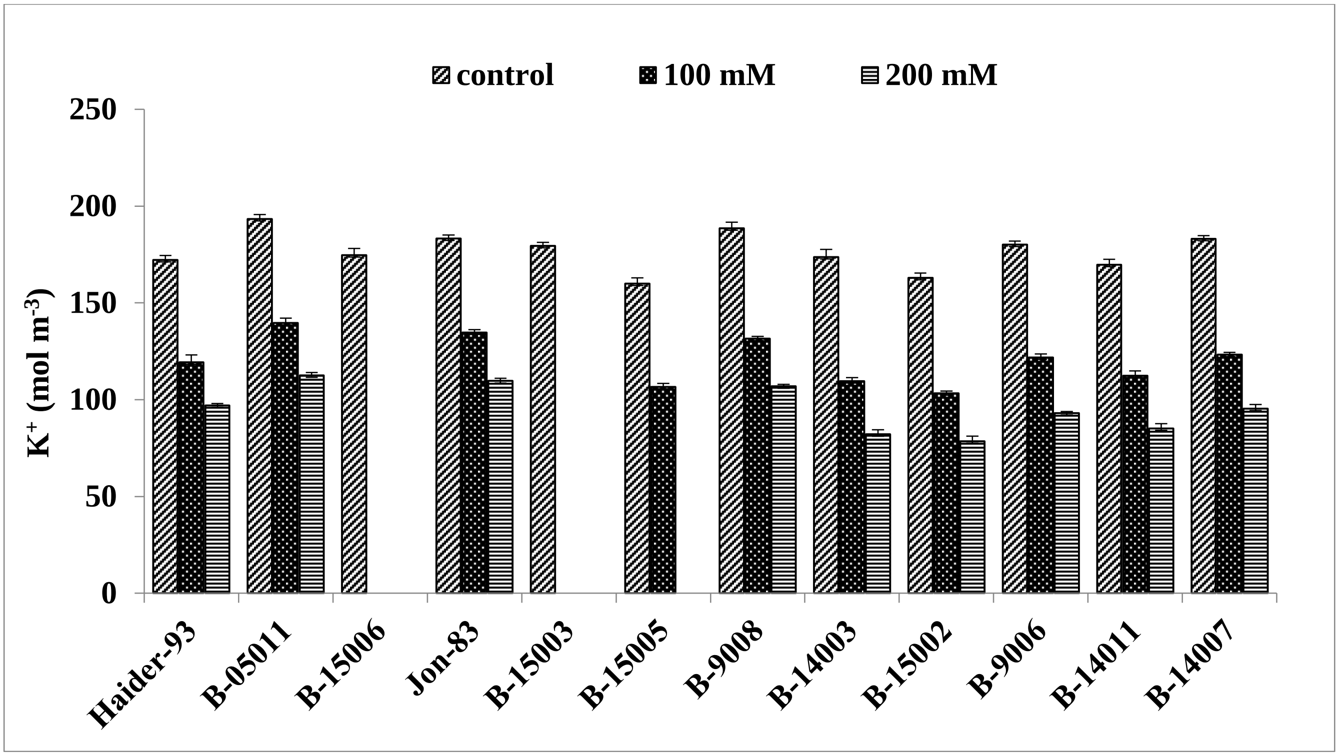

Fig. 11: Effects of Salinity on the K^+ Concentration in the shoot of different Barley (*Hordeum vulgare* L.) genotypes.

3.11. K^+ Concentration in shoot

The concentration of Na+, K+ in the root of different barley genotypes was considerably reduced under salt stress, in contrast to (Fig. 11 and table – 11). With increasing salt concentration, the K+ levels of barley types drop. Each type of barley showed a different response to the parameters measured Due to competitive nature of K+ and Na+ due to high similaritiesin their ionic size, K+ uptake in roots of plants has good correlation with shoot K+ concentration and the Na+ accumulation in the shoots.

Three types of barley genotypes B-9008, B-05011 and Jon-83 when subject to control condition showed lesser concentration of K+, 188.59 moles per m3, 193.32 moles per m3 and 183.23 moles per m3 respectively. While the lesser K+ contents were found in B-15005, B-14011 and B-15002,159.94 moles per m3, 169.11 moles per m3 and 163.01 moles per m3 respectively. When 100 mM salt stress was applied as the second treatment, the barley genotypes B-9008, B-05011 and Jon-83 displayed superior K+ Concentration 131.59 moles per m3, 139.68 moles per m3 and 134.71 moles per m3 as compare to other barley varieties. The minimum K+ values were found in B-15005, B-14011, and B-15002 at 106.64 moles per m3, 112.34 moles per m3, and 103.36 moles per m3 respectively. For the last treatment of 200 mM NaCl stress application maximum K+ Concentration shown by barley genotypes B-9008, B-05011 and Jon-83 are 106.92 moles per m3, 112.56 moles per m3 and 109.67 moles per m3 relative to other barley varieties. B-14003, B-14011 and B-15002 had minimum K+ levels, measuring 82.17 mole per m3, 85.16 mole per m3 and 78.51 mole per m3 respectively.

The findings of the current experiment correspond with those of Sairam *et al*. (2002) who reported that with increasing salt stress, Na+ content increased, and K+ contents of various wheat cultivars increased. There is an antagonistic effect between sodium and potassium that is present between the two and has more ratio. According to Chartzoulakis *et al*. (2002), as the salinity concentration increases, the levels of Na^+ and Cl^- increase in root tissues. Most affected aerial parts of the plant have higher concentrations of Na+ and Cl-. Potassium concentration is less at high salinity levels.

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Salinity causes significant reduction in the growth and physiological performance of the barley. It also reduced the chlorophyll content and K+ concentration whereas concentration of Na+ increased with growing salinity. Among the different genotypes, the B-05011, B-14003, and B-9006 were found the most tolerant whereas B-15006, B-15003, and B-15005 appeared to be the most sensitive.

Funding: This study was not supported by any public, commercial, or non-profit funding agency.

Conflicts of Interest: The authors confirm no conflicts of interest.

Authors' Contribution: Both authors contributed equally.

Generative AI Statement: The authors declare that this manuscript has been written without the use of generative artificial intelligence tools.

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