



RESEARCH ARTICLE

## Evaluating the Impact of Salinity on Physiological, Chemical, and Biochemical Traits in Wheat (*Triticum aestivum* L.) Genotypes

Abbas Shoukat<sup>1,2</sup>, Ali Hamza<sup>2</sup>, Muhammad Khuram Imtiaz<sup>2</sup>, Mohibe ul Hussnain Asad<sup>2</sup> and Mustafa Khan<sup>2</sup>

<sup>1</sup>Institute of Plant Nutrition and Soil Science, Kiel University, Hermann-Rodewald-Str. 2, 24118 Kiel, Germany

<sup>2</sup>Institute of Soil and Environmental Sciences, University of Agriculture, Faisalabad 38040, Pakistan

\*Corresponding author: [ashoukat@plantnutrition.uni-kiel.de](mailto:ashoukat@plantnutrition.uni-kiel.de) ; [abbasshoukat3854@gmail.com](mailto:abbasshoukat3854@gmail.com)

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### ABSTRACT

The effect of salinity on growth, yield and physiological parameters of five bread wheat genotypes was studied. The experiment set in a controlled environment to observe the umma'n response in wheat plants using salinity treatments of 0, 10 and 15 dS/m. The increase in salt concentration led to a decrease in the length and dry weight of plants and spikes. Under control conditions, SARC-8 had the maximum shoot length whereas SARC-5 had the maximum root length. All genotypes showed significantly less shoot and root growth at higher salinity (EC = 15 dS/m) and SARC-8 showed the highest resilience with respect to root dry weight. Grain yield also drastically decreased under salt stress. Unaj-2017 produced maximum grain yield under moderate salinity (EC = 10 dS/m). The study results indicate that salinity stress hinders growth parameters and yield, but these variables differ across genotypes. It is essential to grow salt-tolerant varieties of wheat in areas with salinity problems.

**Key words:** Genotypes, Wheat, Salinity, Grain yield, Dry Weight.

### INTRODUCTION

The presence of high salinity in soil is harmful for the growth of plants. Excessive sodium and chlorine ions disrupt the structure of the soil which leads to the toxicity of ions. This reduces the yield and biomass of plants (Munns, 2005). About one-fifth of the whole world land is saline. Pakistan is at present tenth in the list of countries with salt stressed areas (Qadir et al., 2005). The improper drainage, poor water management, water-logging, and use of poor quality water are the major causes of salinity (Rengasamy, 2010).

Salinity affects plants when poisonous ions such as sodium and chloride accumulate and the osmotic potential of the soil solution increases, which reduces the availability of water for plant uptake (Rahnama et al., 2010). Salt stress has morphological, biochemical and physiological effects on plants which result in reductions in root and shoot growth, hindered photosynthesis and impeded nutrient uptake (Munns and Tester, 2008). Salt stress also causes the formation of reactive oxygen species (ROS), including hydrogen

peroxide. This can harm the proteins, lipids, and nucleic acids of plants, which can disturb the metabolic process (Shoukat et al., 2025).

The developing world in particular faces challenges from the damaging consequences of salinity in agricultural soils as increasing populations and limited arable lands make it essential to raise the yield of crops per unit area (Epstein 2001). Pakistan produces a lot of wheat, but salinity stress may negatively affect crop yield and quality. Wheat (*Triticum aestivum* L.) is an important staple food crop and provides second to human protein intake. This is of great importance for food security in Pakistan (Pakistan Bureau of Statistics, 2014). Wheat is a moderately salt tolerant crop; however, the responses of different cultivars to various salinity levels vary (Flowers, 2005).

The purpose of this study was to evaluate the salt tolerance of different wheat genotypes through the physiological and biochemical and chemical responses. This will help to know the effect of salinity on wheat and identify the genotypes with more salt stress resistance which is necessary for improving wheat production in saline affected areas.

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## MATERIALS AND METHODS

Pot experiment was conducted to study the salt tolerance characteristics of different bread wheat (*Triticum aestivum* L.) cultivars. The study was conducted under controlled environment conditions at the University of Agriculture Faisalabad, Pakistan. Five wheat genotypes were tested under three salinity levels for their response towards salt stress. We carried out a completely randomized design (CRD) experiment with two-factor factorial treatments, replicated thrice.

### Soil Collection, Preparation, and Pre-Sowing Analysis.

Soil samples were collected from the research area and passed through a 2mm sieve at the University of Agriculture, Faisalabad. Every pot received 12 kg of soil, with five plants placed in each pot. Irrigation with tube well water was done weekly. Weed removal, hoeing, thinning and other agronomic practices were performed till harvesting.

Before sowing, a soil test was conducted to check the parameters. The soil pH was determined as 8.42 and EC (Electrical Conductivity) as 2.29 dS/m. The saturation percentage was 22.53% and the SAR was 13. The texture of the soil was sandy loam and the concentration of soluble calcium ( $Ca^{2+}$ ) and magnesium ( $Mg^{2+}$ ) was 14.45 meq/L. So it also shows soluble sodium ( $Na^+$ ) is 3.05 meq/L. The experiment was based upon these values.

### Treatment and Salinity Imposition Plan

The three treatments in the experiment were: Treatment 1 (T1) – control where salinity was not applied; Treatment 2 (T2) – with salinity of 10 dS/m; and Treatment 3 (T3) – with salinity of 15 dS/m.

According to Completely Randomized Design (CRD) all the treatments were replicated thrice. Salt was added in three doses to achieve the required salinity levels. The initial application was performed prior to planting, while the second and third applications were carried out 15 and 30 days after planting respectively. The quantity of sodium chloride (NaCl) required was calculated using the following formula.

$$\text{Amount of sodium chloride (g/kg)} = \frac{\text{Equiv. Wt of NaCl} \times \text{Saturation Percentage}}{100 \times 1000} \times \text{TSS}$$

(Handbook 60: U.S. Salinity Lab. Staff., 1954).

### Fertilizer and Seed Application

The recommended doses of fertilizers applied in the experiment were nitrogen (130 kg/ha), phosphorus (90 kg/ha), and potassium (60 kg/ha). Urea, SOP and DAP were used for the application of nitrogen, potassium and phosphorus respectively. The urea dosage was applied in three splits (1.039 g/pot). The first split was at the time of pot filling after which second at the time of the second irrigation and the third split at the time of third irrigation was done. SOP (0.64 g/pot) and DAP (1.04 g/pot) were applied at the

time of pot filling after mixing them with the soil. Requisite amount of irrigation was given in order to maintain the field capacity of soil in each pot. The other fertilizers were applied solely at the time of sowing which was done in November 2017.

The wheat seed utilized in the study was procured from the Saline Agriculture Research Centre (SARC) University of Agriculture Faisalabad, as well as the Wheat Research Institute Ayub Agriculture Research Institute (AARI) Faisalabad. The trial utilized five wheat genotypes: Unaj-2016, Ujala-2017, SARC-2 and others.

### Harvesting and Sample Processing

The plants were harvested after reaching full maturity, approximately four months after sowing. Both roots and shoots were carefully collected using scissors. Following harvest, the roots and shoots were placed in paper bags, and the wheat grains were separated from the spikes and stored in plastic bags for further analysis.

The collected samples were initially air-dried and then oven-dried at  $65^{\circ}\text{C} \pm 5^{\circ}\text{C}$  until a constant weight was achieved. The dry matter of both the roots and shoots was subsequently determined.

### Growth and Yield Parameters Assessment

Various growth and yield parameters were measured upon physiological maturity. Length of Plant was measured using a meter ruler placed at the base of the stem and readings were taken in cm. Three days after irrigation of the harvested plants, root length was measured by a meter rod.

We measured the shoot and root dry weights after drying the sample in an oven at a temperature of  $65^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 24 hours and weighing it with an electronic balance. The tillers and spikes per plant were recorded manually, while averages were calculated on five plants per pot. Spike lengths were measured with the help of a meter rod, and the average spike length was taken per pot.

### Yield Assessment Parameters

To assess yield parameters, grains were manually separated from the spikes, and 100 grains were weighed on an electronic balance. Biomass of each pot was determined after harvesting with help of electronic balance. The yield of straw was difference between biomass and grain yield. The separated grains were weighed after harvesting to measure grain yield per pot.

### Chlorophyll content (SPAD value)

After three months of sowing, when the plants completed their vegetative growth, then chlorophyll contents were determined with the help of the chlorophyll meter. To measure the amount of chlorophylls, present in the leaves, average of three readings (from leaf tips to leaf blades) was taken.

### Plant Sample Preparation and Analysis

Sampling of plant shoots was carried out as per the standard process. The samples were dried in an oven as at  $65^{\circ}\text{C} \pm 5^{\circ}\text{C}$  for 72 hrs. Once dry, the shoot samples were ground in a grinding mill.

A flame photometer was used for measurement of sodium ( $\text{Na}^+$ ) and potassium ( $\text{K}^+$ ) concentrations in shoots. The samples of the various plants were digested wet by mixing 0.1g sample with a di-acid mixture ( $\text{HNO}_3$ :  $\text{HClO}_4$  in 3:1 ratio) and heating on a hot plate. After complete digestion, the sample volume was made up to 50mL in a flask. We made standard solutions with sodium chloride and potassium chloride salts. A Sherwood 410 Flame Photometer was used to analyze the samples which were recorded as absorbance.

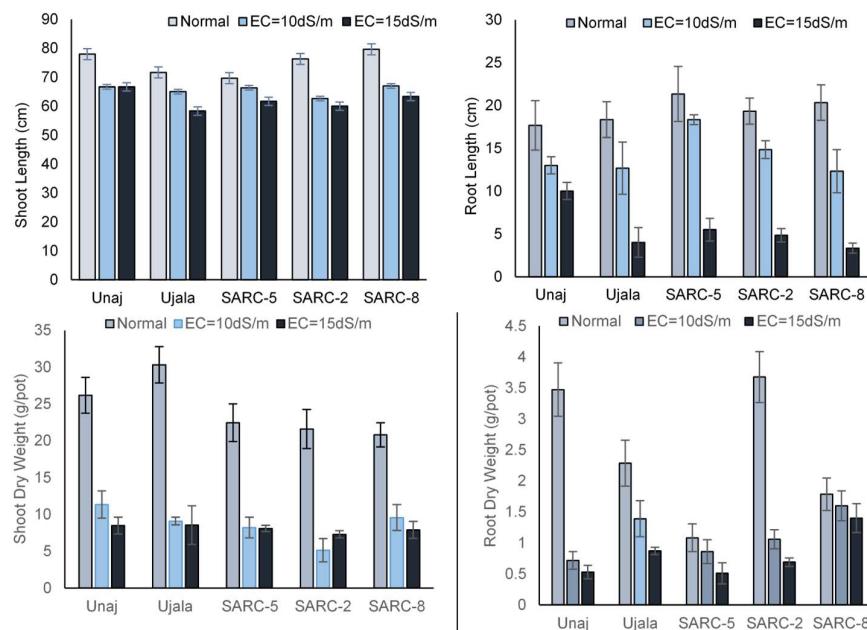
### Statistical Analysis

The data collected was statistically analyzed by using completely randomized design (CRD) factorial arrangements. These treatments and genotypes were compared by the least significant difference (LSD) test to check their significant differences.

## RESULTS

### Shoot and Root Length (cm)

Increasing salinity levels led to a significant change in the shoot, root lengths of wheat genotype (Fig. 1). In control conditions, the shoot lengths ranged from 58.3 cm in SARC-5 to 79.6 cm in SARC-8. The variety Ujala-2016 and SARC-2 differed in shoot length insignificantly. The SARC-5 variety exhibited the longest root length (21.3 cm), while the Unaj-2017 variety had the shortest root length (17.6 cm). According to Illahi et al. (2001) and Sharma et al. (2000), the findings are consistent with the work which says that water deficiency (osmotic stress) and ion toxicity result in retardation of



**Fig. 1:** Effect on shoot and root length of various wheat (*Triticum aestivum* L.) genotypes of various salinity levels.

**Fig. 2:** Effect on shoot and root dry weight (g/ pot) of various wheat (*Triticum aestivum* L.) genotypes of various salinity level.

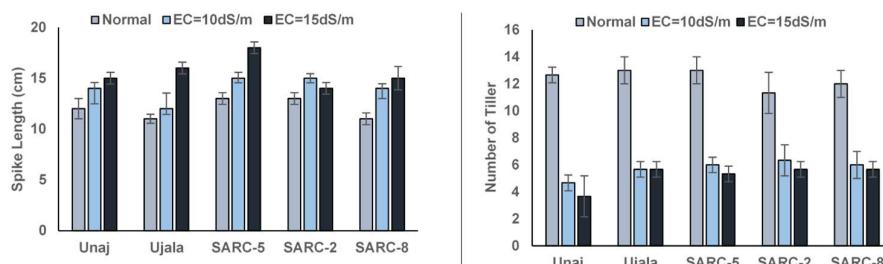
shoot and root length due to salt stress.

When salinity is moderate ( $\text{EC} = 10 \text{ dS/m}$ ), the shoot lengths of SARC-2 and SARC-8 ranged from 62 cm to 67 cm. Highest levels ( $\text{EC} = 15 \text{ dS/m}$ ) shoot lengths of all genotypes reduced significantly and the least reduced in SARC-8 (4%) while the most reduced in SARC-5 (15%). The root length decreased in a similar trend with increased salinity, with SARC-5 exhibiting the longest root length of 18.3 cm at  $\text{EC} = 10 \text{ dS/m}$  and the shortest, with SARC-8 at 12.3 cm. At electrical conductivity ( $\text{EC}$ ) of 15 dS/m, root lengths reduced between 43% in Unaj-2017 and 83% in SARC-8.

### Shoot and Root Dry Weight (g/pot)

The weight of the shoot experienced considerable change due to changing salinity levels. In the control conditions, the shoot dry weight ranged between 5.1 to 30.3 g, having the highest weight (30.3 g) for Ujala-2016 and the lowest (20.8 g) SARC-8. Salt stress ( $\text{EC} = 10 \text{ dS/m}$ ) shoot dry weight ranged from 5.1 g to 11.3 g. Highest dry weight was recorded in Unaj-2017 (11.3 g) and lowest in SARC-2 (5.15 g) (Fig. 2). The decrease in shoot dry weight can be linked to osmotic stress, decreased photosynthesis and reduced nutrient uptake (Ciek & Cakilar 2002; Farouk, 2000).

Salinity also significantly affected root dry weight. The root dry weight in control conditions was recorded 1.08 g (SARC-5) to 3.67 g (SARC-2). In salt-stress situations, where the salt electrical conductivity was 10 dS/m, root dry weight ranged from 0.71 g to 1.6 g, SARC-8 having the maximum dry weight (1.6 g) while Unaj-2017 had the minimum 0.71 g. At  $\text{EC}$  equals fifteen dS/m, we had the lowest root dry weight on SARC-5 and highest on SARC-8. The salt level in the roots will reduce significantly. Water deficiency due to salinity reduces turgor pressure on the cell which declines photosynthesis level according to Ashraf et al. 2008.



#### Spike Length and Number of Spikes under Salinity Stress

As salinity increased, all five wheat genotypes did not exhibit much changes in spike length. Under normal conditions, Ujala-2016, SARC-8 varieties have the lowest spike length of 11 cm while SARC-5 and SARC-2 have the longest spike length at 18 cm. The spike length of the genotypes of EC=10 dS/m did not show significant differences and varied from 12 cm to 15 cm. When the salinity of the test container hit EC = 15 dS/m, the plant spike length ranged between 14 cm (SARC-5) to 18 cm (Unaj-2017), and the distance did not show a significant difference statistically (Fig. 3). The previous findings of Khaliq et al. (2005) have the same pattern and show that salinity has very little effect on spike length but it makes the spikes unfertile.

Salinity had a significant effect on the number of spikes per plant. In the control, Ujala-2016 has highest spike numbers (13) while SARC-2 has lowest number of spikes (11). SARC-2, SARC-5 and SARC-8 had 4 to 6 spikes with EC = 10 dS/m indicating improving quality of seeds. At EC = 15 dS/m, Unaj-2017 had minimum spikes per plant at 3 while Ujala-2016, SARC-8, and SARC-5 recorded 5 spikes each. These results accord with earlier reports that salinity reduces the number of spikes due to reduction in photosynthesis (Richard et al., 1987; Ahmad et al., 2005).

#### Grain Yield (g/pot)

The salinity of soil did not significantly affect five wheat cultivars. Under control, yield of grains varied from 5.14 g/pot (SARC-8) to 14.61 g/pot (Ujala-2016) (Fig. 4). The findings were in agreement with previous studies (Francois et al., 1986) which showed that yield of grains decreased under salty environment mainly due to decreased vegetative growth and germination. Grain yield ranged from 6.3 g/pot (SARC-5) to 12.45 g/pot (Unaj-2017) at an electrical conductivity of 10 dS/m. The reduction in grain yield of all genotypes (55% to 60%) was noticed under EC=15 dS/m. The findings are in line with the results of Akram et al. (2002) and Shafi et al. (2010) who reported the salinity inhibits photosynthesis and retards growth finally diminishing the grain yield.

#### Straw Yield (g/pot)

The straw yield of five bread wheat cultivars is shown under different salinity levels. Salt stress did not significantly affect straw yield. Under normal conditions the straw yield of all the genotypes ranges

from 6.59 to 42.03 g/pot. Ujala-2016 produced maximum straw yield of 42.03 g/pot while SARC-8 produced minimum straw yield of 13.42 g/pot. Not all wheat could tolerate saline soil and water. Amirul-Alam et al. (2015) state that salinity reduces respiration yield due to closure of stomata affecting photosynthesis. The straw yield at 10 dS/m EC ranged from 10.52 – 13.60 g/pot, being maximum in Ujala-2016 (13.60 g/pot) and minimum in Unaj-2017 (10.41 g/pot). At an EC of 15 dS/m, the highest straw yield (10.09 g/pot) was produced by SARC-5 while Unaj-2017 had the lowest (6.59 g/pot). According to Shoukat et al., 2024, the presence of salinity in wheat and barley results in the fewer number of harvestable tillers and spikes.

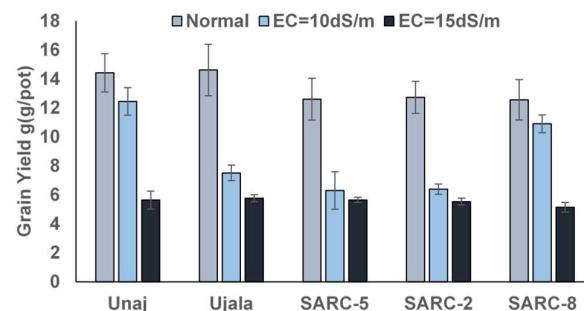


Fig. 4: Effect on grain yield (g/pot) of various wheat (*Triticum aestivum* L.) genotypes of various salinity levels.

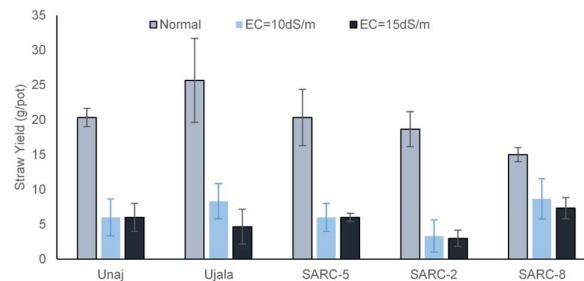
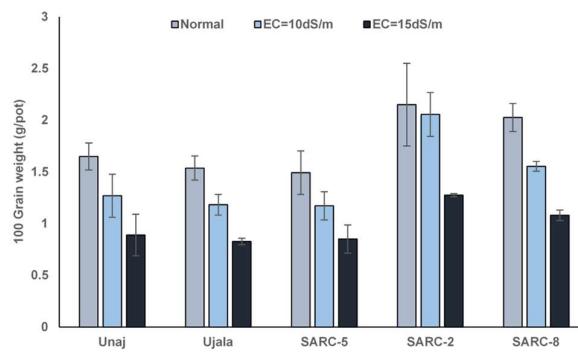


Fig. 5: Effects on straw yield (g/pot) of various wheat (*Triticum aestivum* L.) genotypes of various salinity levels.

#### 100 Grain Weight (g/pot)

As the salt in the soil increased, the weight of 100 grains of five varieties of bread wheat got affected as shown in the figure and data presented in (Fig. 6). The 100 grain weight of the different genotypes ranges from 0.82 to 2.02 g/pot respectively. The highest 100 grain weight was recorded in SARC-8 (2.02 g/pot) while the lowest was SARC-5 (1.49 g/pot). Treatment-2 (EC=10

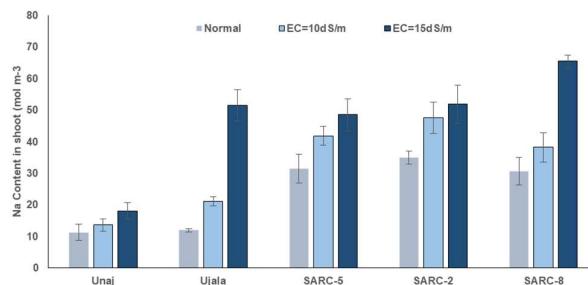
dS/m) produced 100 grain weights from 2.05 to 1.17 g/pot, with SARC-2 and SARC-5 yielding the highest and lowest, respectively (Fig. 5). In treatment-3 (EC = 15 dS/m) except SARC-5 and SARC-2 all-genotype showed reduced 100 grain weight by 46% whereas SARC-5 and SARC-2 showed reduced by 42%. According to Ashraf et al. (2016), higher salinity significantly lowered the 100 grain weight. Similarly, Arif et al. (2016) concluded that the salinity reduces the 100 grain weight of wheat significantly.



**Fig. 6:** Effect on 100 grain weight (g/ pot) of various wheat (*Triticum aestivum* L.) genotypes of various salinity levels.

#### Na<sup>+</sup> Content in shoot (mol m<sup>-3</sup>)

Figure depicted the Na<sup>+</sup> concentration in the shoots of five bread wheat varieties subjected to different salinity levels. The concentration of Na<sup>+</sup> in the shoot was greatly affected by salt stress. SARC-8 had the highest concentration of Na<sup>+</sup> (65.53 mol m<sup>-3</sup>) in treatment-3 (EC = 15 dS/m) and Unaj-2017 had the lowest concentration (11.30 mol m<sup>-3</sup>). The different wheat genotypes accumulated varying amounts of Na<sup>+</sup> in shoots at the various salinities (Fig. 7). This is in agreement with Tavakoli et al. (2011) who observed an increased accumulation of Na<sup>+</sup> in wheat shoots upon salinity. Ainnie & Staden (2010) suggested that salt tolerance as well as sodium exclusion is associated with the constant Na<sup>+</sup> concentration in wheat shoot and leaves.



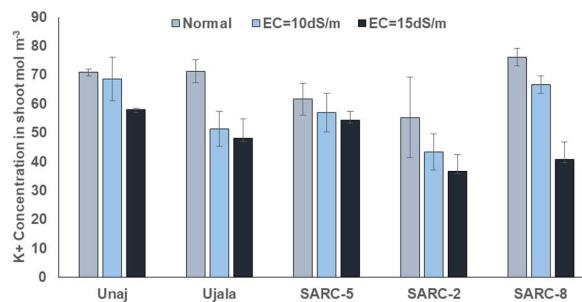
**Fig. 7:** Effect on Na<sup>+</sup> concentration (mol m<sup>-3</sup>) in shoot of various wheat (*Triticum aestivum* L.) genotypes of various salinity levels.

In treatment-2 (EC = 10 dS/m), the concentration of Na<sup>+</sup> in shoots varied from 13.63 to 47.56 mol m<sup>-3</sup>. SARC-2 showed the highest concentration of Na<sup>+</sup> in shoots at

47.56 mol m<sup>-3</sup> while Unaj-2017 showed the lowest concentration Na<sup>+</sup> in shoots at 13.63 mol m<sup>-3</sup>. SARC-8 had the highest Na<sup>+</sup> concentration of 65.53 mol m<sup>-3</sup> while Unaj-2017 had the lowest Na<sup>+</sup> concentration of 18.12 mol m<sup>-3</sup> at EC 15 dS/m. Yadav and Singh (2004) also stated that exclusion of sodium is vital for the salt tolerance of wheat. High concentrations of Na<sup>+</sup> in wheat shoots as stated by Tiessen 1994 can disrupt nutrient balance, cause ion toxicity, and cause osmotic imbalance. A study by (Haq et al., 2002). It highlights the effect of salt on transport rates. Its subjects were salt-sensitive and salt-tolerant plants. The impact was on the Na<sup>+</sup> and Cl<sup>-</sup> concentrations in shoots, leaves, and roots.

#### K<sup>+</sup> Concentration in shoot (mol m<sup>-3</sup>)

Fig. 4.13 shows that K<sup>+</sup> concentration in the shoots of five bread wheat varieties was significantly influenced by increasing soil salinity. Under control conditions, K<sup>+</sup> concentration ranged from 36.7 to 71.21 mol m<sup>-3</sup>, with Ujala-2016 having the highest (71.21 mol m<sup>-3</sup>) and SARC-2 the lowest (55.3 mol m<sup>-3</sup>). Wheat genotypes exhibited varied responses to salinity (Fig. 8). These findings are consistent with Sairam et al. (2002), who reported that higher salinity levels reduce K<sup>+</sup> concentration in shoots due to the antagonistic effect between Na<sup>+</sup> and K<sup>+</sup>. Under salt stress (EC = 10 dS/m), K<sup>+</sup> concentration ranged from 43.37 to 68.53 mol m<sup>-3</sup>. Unaj-2017 showed the highest concentration (68.53 mol m<sup>-3</sup>), while SARC-2 had the lowest (43.37 mol m<sup>-3</sup>). The reduction in K<sup>+</sup> concentration with respect to control conditions was 18%, 32%, 11%, 33%, and 46% for Unaj, Ujala, SARC-5, SARC-2, and SARC-8, respectively. These results align with Saqib et al. (2010), who found that higher salinity increases Na<sup>+</sup> and Cl<sup>-</sup> levels in shoots but reduces K<sup>+</sup> concentration. In treatment-3, K<sup>+</sup> concentrations ranged from 58 to 38 mol m<sup>-3</sup>, with Unaj-2017 showing the highest and SARC-8 the lowest.

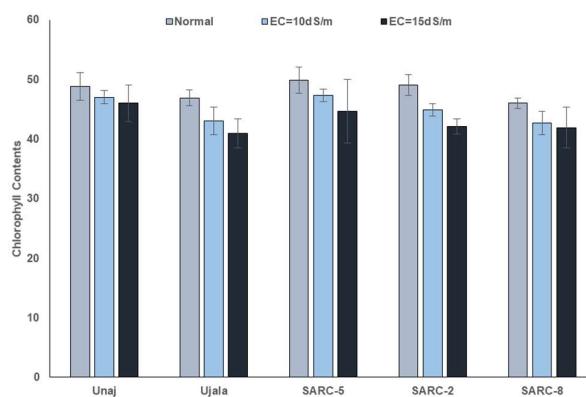


**Fig. 8:** Effect on K<sup>+</sup> Concentration (mol m<sup>-3</sup>) in shoot of various wheat (*Triticum aestivum* L.) genotypes of various salinity levels.

#### Chlorophyll contents

Figure indicates that the chlorophyll content of the wheat varieties did not affect Soil salinity (Fig. 9). SARC-5 had the highest chlorophyll content (49.83%) SARC-8 was lowest (46%). There was no significant difference in chlorophyll content between Unaj-2017 and SARC-2.

Chlorophyll content under salt stress ( $EC = 10$  dS/m) ranged from 42.7% and 47.33%. The highest chlorophyll content was shown by SARC-5 (47.33%) followed by the SARC-8 which exhibited the lowest chlorophyll content (42.7%). The highest chlorophyll content (46%) was noticed in Unaj-2017 and the lowest in Ujala-2016 (41%) at  $EC = 15$  dS/m. According to Zhao et al. (2007), chlorophyll content decreases with increasing salinity due to the accumulation of sodium and decrease of potassium in the shoots, which also reduces the leaf area and dry biomass. Similarly, salinity was found to reduce chlorophyll content by Al-aghabary et al. (2005).



**Fig. 9:** Effect on chlorophyll contents in shoot of various wheat (*Triticum aestivum L.*) genotypes of various salinity levels.

## DECLARATIONS

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**Data Availability:** All the data is available in the article.

**Ethics Statement:** The article is purely a manuscript, and nothing were harmed.

**Author's Contribution:** All authors contributed equally to this work.

**Generative AI Statement:** The authors declare that no Gen AI/DeepSeek was used in the writing/creation of this manuscript.

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