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# ASSESSMENT OF SALT TOLERANCE IN WHEAT (*TRITICUM AESTIVUM* L.) GENOTYPES THROUGH PHYSIOLOGICAL, BIOCHEMICAL, AND MOLECULAR CHARACTERIZATION

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## Abstract

This study was conducted at National Institute for Genomics and Advanced Biotechnology (NIGAB), National Agricultural Research Centre (NARC), Islamabad, Pakistan, during 2015–2016 cropping season. The objectives were to evaluate physiological, biochemical, and growth responses of wheat genotypes under salt stress, and to assess their genetic diversity using SSR and RAPD markers. In germination experiments, cultivars were tested against 50, 75, 100, 125 and 150 mM salt levels. The research novelty lies in the integrative approach combining morphological, physiological, biochemical, and molecular characterization to identify salt-tolerant wheat genotypes suitable for breeding programs, which has not been extensively reported for these locally important cultivars. A completely randomized design (CRD) with three replications was used for both germination and hydroponics experiments. Salt stress significantly affected seed germination and seedling growth of the four wheat genotypes. At 150 mM NaCl, germination reduction was lowest in Local White (20–30%) and Pasban-90 (30–40%) and highest in Frontana (50–60%) and Chakwal-97 (40%). Similarly, total chlorophyll content was decreased by 23% and 26% in Local White and Pasban-90, respectively, while Chakwal-97 and Frontana showed higher reductions of 36% and 37%. These results showed that Local White and Pasban-90 exhibited comparatively higher tolerance to salt stress. Under 150 mM salt stress, wheat genotypes Local White and Pasban-90 exhibited higher tolerance compared to Chakwal-97 and Frontana. Seedling shoot length was reduced by 20% and 35% in Local White and Pasban-90, respectively, while Chakwal-97 and Frontana showed reductions of 41% and 46%. Root length was declined by 30–31% in tolerant genotypes versus 38–46% in sensitive ones. Fresh weight was decreased by 26% in Local White and Pasban-90 but by 46–49% in Chakwal-97 and Frontana, whereas dry weight reductions were <20% in tolerant and >30% in sensitive genotypes. Physiologically, total chlorophyll content was decreased by 23–26% in tolerant genotypes and 36–37% in sensitive ones; chlorophyll a and b showed similar trends. Relative water content, membrane stability index, water potential, and osmotic potential were minimally affected in Local White and Pasban-90 (reductions 23–30%) but markedly decreased in Chakwal-97 and Frontana (reductions 40–52%). SSR based genetic diversity study showed that these genotypes were genetically divergent. These results demonstrate a strong association between physiological resilience and molecular divergence, supporting the selection of Local White and Pasban-90 as promising parents for salt-tolerance breeding programs.

**Key words:** Genetic diversity; Hydroponics; RAPD markers; SSR markers; Salinity stress; Screening; Wheat genotypes

## Introduction

Water shortage, high salt levels, frost, and high temperature are different evnvironmental constraints that interfere with plant growth and development (Abdullah *et al.,* 2025; Ali *et al.,* 2025; Rafique *et al.,* 2025). Salt stress is a major limiting factor among different abiotic stresses that drastically reduce plant growth and yield (Gurmani *et al*., 2014; Zaman & Qureshi, 2018; Iqbal & Qureshi, 2021). Salinity affects 954 million hectares of land in the world and about 6 Mha cultivated land (40%) in Pakistan (Hafeez *et al.*, 2021). In Pakistan, salt stress is one major problem in irrigated land because of poor drainage practice (Evans *et al*., 2013). Crop plants are sensitive to sodium and chloride ions when found in excess in salt affcted soils (Jamali *et al*., 2015; Shaheen *et al.,* 2023). Salt stress causes hyperosmotic and hyperionic effects which interfere with seedling growth, vegetative and reproductive phase of crop plants (Rani & Merlin, 2012). Osmotic stress reduces plant growth and high $Na^+$ or $Cl^-$ ions accumulation decrease osmolytes accumulation in young organs (Ahmad *et al*., 2013). Crop plants show variation in salt stress tolerance and it is very important to identify tolerant or sensitive genotypes (Hussain *et al*., 2013). Saline stress inhibits seed germination, thereby reducing plant establishment (Khayatnezhad *et al*., 2010; Hussain *et al*., 2013; Souror *et al*., 2014). High salt accumulation in inter and intracellular space drastically decrease the germination percentage primarily due to changes in water relations. Seed germination is often delayed under high salt levels due to embryo damage caused by sodium and chloride ions, inhibition of water uptake, and increased exosmosis (Rahman *et al.,* 2008). Salt tolerant wheat genotypes show sodium and chloride

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compertmentlization at the organ and organelle level compared to salt-sensitive ones.

Wheat is ranked third as staple food in the world and recognized as an important cereal (Gurmani *et al.*, 2014; Shehzad *et al.*, 2022; Shafqat *et al.*, 2023; Safder *et al.*, 2025). It is considered as a foundation of national food security (Abbas & Shafique, 2019; Mehmood *et al.*, 2020). About 9–10 million hectares are sown for wheat annually, making it the dominant rabi crop in the country (Ali *et al.*, 2024). In Pakistan, wheat is used as staple food, and the country ranks fourth wheat producer in the world (Saeed *et al.*, 2012; Iqbal *et al.*, 2018; Shehzad *et al.*, 2023). Wheat contributes a substantial share of the agricultural value-added sector and is central to dietary energy and protein supply for the population (Alamgeer *et al.*, 2022; Dinsa & Balcha, 2024; Batool *et al.*, 2025). Pakistan's agriculture is under serious threat from soil salinity and sodicity. A recent assessment by Food and Agriculture Organization (FAO) reports that approximately 6.67 million hectares of agricultural land nearly 30% of cultivated area are salt-affected (Ahmed, 2024). Salinity and sodicity reduce soil fertility, impair water uptake, disturb ionic balance, and lead to osmotic and oxidative stress in crops (Syed *et al.*, 2020). Salinity significantly reduces wheat plant development and yield. Physiological processes mainly photosynthetic activity is suppressed under salt stress (Manzoor *et al.*, 2015).

Cultivation in salt effected land is possible through identification of salt tolerant genotypes. For this purpose, wheat germplasm screening is done on the basis of morphological, physiological, biochemical and genetic characters. Salt-tolerant lines provide a practical solution for sustaining crop performance on salt-affected soils, where conventional varieties often fail to germinate, maintain growth, or achieve reasonable yields (Melino & Tester, 2024; Rehman *et al.*, 2025). Incorporating such genotypes into breeding programs allows the development of cultivars capable of maintaining ionic balance, efficient water uptake, and physiological stability under saline conditions (Egea *et al.*, 2023). This, in turn, supports stable grain yield, reduces economic losses for farmers, and helps ensure food security in regions where salinity continues to expand due to poor drainage, waterlogging, and climate-related changes in irrigation water quality (Atta *et al.*, 2023). Hence, salt tolerant wheat genotypes show less accumulation of sodium ions and higher $K^+/Na^+$ ratio. While previous studies have documented the physiological and biochemical effects of salinity on wheat, there is limited research on the evaluation of diverse wheat genotypes under local saline conditions to identify high tolerant genotypes. Therefore, this study was conducted to identify and characterize salt-tolerant wheat genotypes that can maintain productivity on salt-affected soils, providing a critical foundation for breeding programs and sustainable agricultural practices.

## Materials and Methods

**Germination experiment:** This experiment was carried out at National Institute for Genomics and Advance Biotechnology, NARC Islamabad Pakistan during 2015–2016 cropping season. In all these experiments complete randomized design (CRD) was used with three replications. Randomization was achieved by assigning treatments to petriplates using a random number table, and plates were rotated daily within the growth chamber to minimize positional effects due to light or temperature gradients. Four wheat cultivars were selected for this experiment on the basis of their acceptability among farmers in Pakistan. Sodium Hypochlorite 1% solution was used for surface sterilization of seeds of wheat cultivars (Pasban-90, Chakwal97, Frontana and Local White). Grains were dipped in 1% sodium hypochlorite solution for 15 min and washed twice with dstilled water (Manzoor *et al.*, 2015). The experimental unit was a single petriplate containing 25 grains. Each treatment had three independent replications, and petriplates were arranged in a completely randomized design (CRD) to minimize positional or environmental effects within the growth chamber. Each replication consisted of 25 grains placed in a 9 cm diameter sterile petriplate lined with Whatman No. 1 filter paper. 5 mL of respective salt solution or distilled water (control) was added to each plate. Plates were incubated in a growth chamber at 25 ± 2°C, with a 16 h light / 8 h dark photoperiod, and relative humidity of 60–65%. Salt stress was imposed using analytical grade NaCl at concentrations of 50, 75, 100, 125, and 150 mM. Solutions were freshly prepared in distilled water before use, and the filter papers were kept moist throughout 15-day experiment to maintain consistent salt levels. Grains germination was monitored for 15 days, and grains were considered germinated when the radicle reached 5 mm in length, following the method of Miri & Mirjalili (2013). Grains that did not reach this threshold by the end of the 15-day period were recorded as ungerminated. Salt stress was maintained continuously by preparing the growth medium (hydroponic solution) with the assigned NaCl concentrations at the start of the experiment and monitoring the salt level daily. The solutions were replenished or replaced as needed to ensure that the salt concentration remained stable for the entire duration of the experiment. This allowed the seedlings to be exposed to consistent salinity throughout the study. Parameters such as root and shoot length (cm), seedling fresh weight and seedling dry weight, germination percentage (G%), and germination index (GI) were recorded according to Singh *et al.*, (2010). Fresh and dry weights were measured from 15-day-old seedlings at the end of the germination experiment. For fresh weight, whole seedlings from each plate were blotted to remove surface moisture and weighed immediately. For dry weight, the seedlings were oven-dried at 70°C for 48 hours until constant weight was achieved. Promptness index and germination stress tolerance index (Ashraf *et al.*, 2008), seedling vigor index (Anon., 2005) were also recorded. Total chlorophyll content was estimated by following method of Arnon (1949).

**Hydroponics experiment:** In germination experiment cultivars were evaluated against different NaCl levels, it was observed that culitvars were most succeptiable to 150 mM NaCl concentrations. The 150 mM NaCl concentration was selected based on previous studies indicating that this level represents severe salinity stress for wheat, allowing differentiation between salt-tolerant and salt-sensitive genotypes. This concentration is physiologically relevant to salt-affected soils in Pakistan, where electrical conductivity can reach levels equivalent to 150 mM NaCl, thus making the results applicable to field conditions. In hydroponics experiment, grains were tested against two treatments control and 150 mM salt stress. Completely randomized design (CRD) with three replications was used as a experiment design. Grains were germinated in sand filled trays. Hydroponic setup comperised of 200 L steel tank internally lined with polyethylene sheet. Hoagland's nutrient solution was filled in the tank (Hoagland & Arnon, 1950) and foam wrapped 10 days old seedling were transferred in holes in Styrofoam sheet floating over nutrient solution. pH of nutrient solution was maintained between 5.5 to 6.0 and air pumps were used for aeration of solution. Salt levels were developed by adding in daily increments of 50 mM NaCl till 150 mM level was reached. Seedlings were grown in individual containers filled with nutrient solution, with 10 seedlings per genotype per replicate and three independent replications arranged in a completely randomized design (CRD). Environmental conditions were maintained at 25 ± 2°C, with a 16 h light / 8 h dark photoperiod, light intensity of 300 µmol $m^{-2}$ $s^{-1}$, and relative humidity of 60–65%. The hydroponic experiment was conducted for 21 days, starting from seedling establishment in the nutrient solution until the harvest of seedlings for growth, physiological, and ionic measurements. Containers were rotated every two days to minimize positional effects and ensure uniform exposure to growth chamber conditions. This setup allowed accurate assessment of growth, physiological, and ionic responses of different wheat genotypes under controlled saline conditions. The following parameters was recorded in this experiment.

**Morphological attributes:** Shoot and root length (cm), leaf area ($cm^2$), number of tillers, number of nodes, fresh and dry weights (g) were recorded at the end of the hydroponics experiment, after 30 days of growth under control and salt stress treatments. Data on morphological parameters were collected by carefully uprooting the seedlings at the end of the experiment. Shoot and root lengths were measured in cm using a ruler from the base to the tip. Leaf area was determined using a leaf area meter in $cm^2$. Number of tillers and nodes were counted manually for each seedling. Fresh weights (g) of shoots and roots were recorded immediately after harvest using an analytical balance, and dry weights (g) were measured after drying samples in an oven at 70°C until constant weight. All measurements were taken for each seedling in all replications to ensure accuracy and consistency.

**Physiological attributes:** Leaf water potential was recorded using method reported by Scholander *et al.*, (1965) and osmotic pressure was measured using osmometer (Capell & Doerffling, 1993). Total chlorophyll content, chlorophyll a and chlorophyll b and cell membrane stability were recorded following Manzoor *et al.*, (2015). Leaf relative water content was measured by method of Weatherly (1950). These physiological parameters were studied because they provide information into how plants maintain water balance, photosynthetic efficiency, and membrane integrity (structural and functional stability of cell membranes, particularly their ability to maintain selective permeability and prevent leakage of cellular solutes) under salinity stress. Ionic concentrations were determined using flame photometry for $Na^+$ and $K^+$, and titrimetric method for $Cl^-$, calibrated with standard solutions of known concentrations.

**Biochemical attributes:** Total soluble protein was estimated using methods of Lowry *et al.*, (1951) and Bradford (1976) and soluble sugar analysis was done by method of Manzoor *et al.*, (2015). Free amino acids were recorded by method of Hamilton & Van Slyke (1943) and proline content was measured following Manzoor *et al.*, (2015). Biochemical analyses were conducted to understand osmotic adjustment and metabolic changes that support growth under high salt conditions.

**Antioxidant analysis:** Suproxide dismutase (SOD) was estimated by using the method of Giannopolitis & Ries (1997). Catalase and peroxidase were estimated by Cakmak *et al.*, (1993) method. Antioxidant enzyme activities were assessed to determine the ability of wheat genotypes to mitigate oxidative stress caused by salt-induced reactive oxygen species, which can damage cellular structures and reduce productivity. Together, these analyses allow identification of genotypes with physiological and biochemical traits associated with enhanced salinity tolerance, which is critical for breeding and sustainable crop production in salt-affected areas.

**Genetic diversity studies:** DNA isolation, purification, and quantification of 4 wheat genotypes under study were carried out following Shahzad *et al.*, (2012). DNA was extracted from 100 mg of young leaf tissue per genotype using a modified CTAB method (Shahzad *et al.,* 2012). The extraction buffer contained 2% CTAB, 100 mM Tris-HCl (pH 8.0), 20 mM EDTA, 1.4 M NaCl, and 1% β-mercaptoethanol. Samples were incubated at 65°C for 30 min, followed by purification with chloroform: isoamyl alcohol (24: 1). DNA was precipitated with isopropanol, washed with 70% ethanol, and dissolved in TE buffer. DNA integrity was verified by 0.8% agarose gel electrophoresis, and purity was assessed spectrophotometrically, with A260/A280 ratios between 1.8 and 2.0. DNA concentration was adjusted to 50 ng/µL for PCR. PCR reactions were performed in a 25 µL volume, containing 50 ng template DNA, 0.2 µM primer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, and 1 U Taq DNA polymerase. Thermal cycling consisted of an initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 45 s, annealing at 36-55°C for 45 s, and 7°C for 1 min, with a final extension at 72°C for 10 min. PCR products were separated on a 1.5% agarose gel in 1X TBE buffer at 100 V for 90 minutes, stained with ethidium bromide, and visualized under a UV transilluminator. A 100 bp DNA ladder was used for size estimation, and the gel was photographed and scanned. A total of 26 RAPD and 53 SSR primers were selected and then their conditions were optimized to find genetic diversity response in 4 wheat genotypes. These primers were selected to provide broad genome coverage and maximize the likelihood of detecting polymorphisms. RAPD primers target random dominant loci, while SSR primers are co-dominant and locus-specific, together allowing a comprehensive assessment of genetic diversity. Only 15 RAPD primers produced polymorphic amplicons and were used for analysis. RAPD primers included OPA 1, OPA 2, OPA 3, OPA 5, OPA 10, OPA 13,

OPA 18, OPB 1, OPB 7, OPB 8, OPB11, OPC 6, OPE 1, OPF 13 and OPJ 13. Only 38 SSR markers produced polymorphic results. For RAPD and SSR data, the scoreable bands were considered as a single locus/allele. The loci were scored as present (1) or absent (0). Pair-wise comparisons of the cultivars based on the proportion of amplification products (alleles) were used to measure the genetic similarity by Dice coefficients. The Dice coefficients were computed by using Simqual sub program in similarity routine of NTSYS-pc version 2.2 (Rohlf, 2005). The resultant similarity matrix data was used to construct a dendrogram by using Sequential Agglomerative Hierarchical Nesting (SAHN) based on unweighted pair-group method with an arithmetic average (UPGMA) to infer genetic relationships and phylogeny among genotypes. Despite the small number of genotypes, both RAPD and SSR markers were combined to increase the resolution and reliability of genetic diversity assessment. RAPD markers detect genome-wide dominant polymorphisms, while SSR markers provide highly reproducible co-dominant, locus-specific variation. The combination strengthens the confidence in identifying genetic relationships and supports the selection of diverse parental lines for breeding programs.

### Statistical analysis

Data were analyzed using Statistix 8.1 software. Two-way ANOVA was performed for each parameter, and significant differences among treatments were determined using Tukey's Honest Significant Difference (HSD) test at $p \leq 0.05$.

## Results

**Germination experiment:** Salt stress negatively affected grains germination and seedling growth of the 4 spring wheat (*Triticum aestivum* L.) varieties. Significant differences ($p \leq 0.05$) were observed among varieties and salt levels for germination percentage (GP), germination index (GI), and seedling vigor index (SVI) (Table 1). Under control conditions, all varieties showed 100% GP for Local White (LW), Pasban-90 (P-90), and Chakwal-97 (Ch-97), and 75% for Frontana (Front). At 150 mM NaCl, GP declined sharply, with LW at 37.5%, P-90 at 33.5%, Ch-97 at 25%, and Front at 30%. Similarly, GI decreased progressively with increasing salinity: LW dropped from 10 (control) to 3.75 (T150), P-90 from 10 to 3.35, Ch-97 from 10 to 2.5, and Front from 7.5 to 3.0.

Seedling vigor index (SVI) also declined under salt stress. LW maintained the highest SVI at 281.5 under 150 mM NaCl, followed by P-90 (270), Ch-97 (160.5), and Front (165), indicating differential tolerance among genotypes. Statistical analysis revealed that LW and P-90 were significantly more tolerant than Ch-97 and Front at higher salt levels ($p \leq 0.05$), while Frontana was the most susceptible variety. Seedling length (SL) decreased with increasing salinity, with reductions at 150 mM NaCl as follows: LW (9.5 cm; 60% reduction), P-90 (10.9 cm; 56%), Ch-97 (6.4 cm; 74%), and Front (5.5 cm; 73%) (Table 2). These results showed that LW and P-90 exhibited better germination and early seedling growth under severe salt stress compared to Ch-97 and Front, highlighting their potential for cultivation in salt-affected soils.

### Hydroponics experiment

**Morphological parameters:** Salt stress of 150 mM drastically reduced plant growth and development in hydroponics experiment. Root and shoot length was significantly reduced under salinity (Table 3). Shoot length in salt stress varied from 17.1 to 21.7 cm with an average 19.4 cm. Root Length varied between 20.9 to 28.6 cm with an average of 24.7cm under salt stress. Salt stress reduce shoot length of Local White, Pasban-90, Chakwal-97 and Fronatan by 20, 35, 41 and 46%, respectively. Similarly, root length showed decline under salt stress viz. 30% in Local White, 31% in Pasban-90, 38% in Chakwal-97 and 46% in Frontana as compared to control. Leaf area for Pasban-90, Local White, Chakwal-97 and Frontana showed sensitivity and showed 16%, 19%, 35% 37% decline, respectively. Fresh and dry weight of plants showed reduction under salt stress (Table 4). Fresh weight of Local White and Pasban-90 reduced upto 26% due to salt stress application. Chakwal-97 and Frontana plants fresh weight showed more sensitivity towards 150 mM salt and reduced by 46% and 49%, respectively. Maximum reduction in dry weight was observed for Chakwal-97 and Frontana (>30%) and Pasban-90 while minimum reduction (<20%) was recorded in Local White. Tillers and node per plant were also significantly affected due to salinity stress. Maximum number of node and tillers per plant were recorded in control condition (Table 4).

### Physiological and biochemicals parameters

**Chlorophyll contents:** Photosynthetic activity is very important for plant survival. Total chlorophyll, chlorophyll a and chlorophyll b contents were decreased under salt stress (Table 5). Total chlorophyll content reduction was observed in Local White and Pasban-90 was comparetively less which were 23 and 26%, respectively than chakwal-97 and Frontana where reduction was 36% and 37% compared to control. Minimum reduction in chlorophyll a content was recorded in Local White (23%) followed by Pasban-90 (27%), whereas maximum reduction was recorded in Chakwal-97 (46%) and Frontana (50%) under salt stress. Chlorophyll b content showed significant reduction in all genotypes which were 30, 33, 40 and 42% reduction, respectively compared to control (Table 5).

**Membrane stability index and relative water contents:** Membrane stability index and relative water content was decreased with increasing salt levels (Table 6). Local white and Pasan-90 showed minimum reduction in membrane stability index which were 23 and 26% compared to control whereas maximum reduction was shown by wheat genotypes Chakwal-97 and Frontana which were 40 and 42% at 150 mM salt stress. The maximum reduction in relative water content was shown by Chakwal-97 (40%) and Frontana (47%), however, minimum reduction was shown by wheat genotypes Local White (25%) and Pasban-90 (29%) compared to control (Table 6).

**Table 1. Effect of salt stress on germination attributes of wheat cultivars in germination experiment.**

| Variety | Treatment | G % | GI | SVI | SL |
|---|---|---|---|---|---|
| LW | CONT | 100 ± 0.00 | 10 ± 0.00 | 1370 ± 1.154 | 23.9 ± 1.34 |
| LW | T50 | 83.5 ± 8.33 | 8.35 ± 0.833 | 810 ± 5.77 | 20.8 ± 2.91 |
| LW | T75 | 75 ± 0.00 | 7.5 ± 0.00 | 682.5 ± 1.154 | 19.2 ± 0.95 |
| LW | T100 | 62.5 ± 0.00 | 6.25 ± 0.00 | 537.5 ± 2.77 | 16.6 ± 1.14 |
| LW | T125 | 62.5 ± 0.00 | 6.25 ± 0.00 | 500 ± 1.00 | 13 ± 2.65 |
| LW | T150 | 37.5 ± 0.00 | 3.75 ± 0.00 | 281.5 ± 2.77 | 9.5 ± 3.6 |
| P-90 | CONT | 100 ± 0.00 | 10 ± 0.00 | 1450 ± 1.154 | 25.1 ± 1.56 |
| P-90 | T50 | 91.61 ± 8.33 | 9.16 ± 0.833 | 1390 ± 1.95 | 21.8 ± 1.12 |
| P-90 | T75 | 75 ± 0.00 | 7.5 ± 0.00 | 892.5 ± 2.77 | 20.7 ± 2.04 |
| P-90 | T100 | 75 ± 0.00 | 7.5 ± 0.00 | 727.5 ± 1.00 | 20.1 ± 1.9 |
| P-90 | T125 | 62.5 ± 0.00 | 6.25 ± 0.00 | 562.5 ± 1.198 | 13.3 ± 2.6 |
| P-90 | T150 | 33.5 ± 4.166 | 3.35 ± 0.4166 | 270 ± 1.154 | 10.9 ± 3.3 |
| Ch-97 | CONT | 100 ± 0.00 | 10 ± 0.00 | 1490 ± 1.167 | 24.4 ± 3.9 |
| Ch-97 | T50 | 66.6 ± 4.166 | 6.66 ± 0.4166 | 712.5 ± 1.21 | 20.1 ± 1.43 |
| Ch-97 | T75 | 62.5 ± 0.00 | 6.25 ± 0.00 | 625 ± 2.73 | 17.1 ± 1.91 |
| Ch-97 | T100 | 62.5 ± 0.00 | 6.25 ± 0.00 | 581.25 ± 2.02 | 10.5 ± 2.2 |
| Ch-97 | T125 | 29.26 ± 4.266 | 2.92 ± 0.4266 | 210 ± 2.41 | 8.2 ± 3.1 |
| Ch-97 | T150 | 25 ± 0.00 | 2.5 ± 0.00 | 160.5 ± 1.52 | 6.4 ± 0.67 |
| Front | CONT | 75 ± 0.00 | 7.5 ± 0.00 | 960 ± 1.251 | 20.8 ± 2.45 |
| Front | T50 | 70.9 ± 4.166 | 7.09 ± 0.4166 | 825 ± 1.31 | 17.8 ± 2.3 |
| Front | T75 | 62.5 ± 0.00 | 6.25 ± 0.00 | 631.2 ± 1.95 | 11.4 ± 1.77 |
| Front | T100 | 62.5 ± 0.00 | 6.25 ± 0.00 | 575 ± 2.67 | 10.7 ± 1.6 |
| Front | T125 | 45.5 ± 8.366 | 4.55 ± 0.8366 | 296.2 ± 1.84 | 9.2 ± 0.89 |
| Front | T150 | 30 ± 0.00 | 3.0 ± 0.00 | 165 ± 1.38 | 5.5 ± 0.15 |

G % = Germination percentage; GI = Germination index; SVI = Seedling vigor index; SL = Seedling length (cm); All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97 Front = Frontana; CONT = Control; T50 = 50 mM stress; T75 = 75 mM stress; T100 =100 mM stress; T125 = 125 mM stress; T150 =150 mM stress

**Table 2. Effect of salt stress on seedling fresh weight, seedling dry weight and total chlorophyll contents of seedling of wheat cultivars.**

| Variety | Treatment | SFW | SDW | TCCS |
|---|---|---|---|---|
| LW | CONT | 2.17 ± 0.93 | 1.15 ± 0.545 | 10.5 ± 1.632 |
| LW | T50 | 1.95 ± 0.17 | 0.91 ± 1.626 | 9.1 ± 1.620 |
| LW | T75 | 1.76 ± 2.41 | 0.88 ± 1.187 | 9.6 ± 1.437 |
| LW | T100 | 1.31 ± 3.55 | 0.71 ± 0.793 | 7.1 ± 1.764 |
| LW | T125 | 1.09 ± 1.92 | 0.56 ± 0.738 | 6.5 ± 2.655 |
| LW | T150 | 0.92 ± 1.26 | 0.37 ± 0.364 | 5.1 ± 1.722 |
| P-90 | CONT | 2.57 ± 3.54 | 1.31 ± 1.542 | 12.7 ± 2.15 |
| P-90 | T50 | 2.11 ± 2.67 | 1.16 ± 0.821 | 12.2 ± 1.719 |
| P-90 | T75 | 1.89 ± 1.22 | 0.98 ± 0.591 | 11.6 ± 0.916 |
| P-90 | T100 | 1.54 ± 1.95 | 0.73 ± 0.121 | 10.5 ± 1.450 |
| P-90 | T125 | 1.07 ± 1.16 | 0.59 ± 0.797 | 8.4 ± 0.9635 |
| P-90 | T150 | 1.01 ± 1.33 | 0.49 ± 0.269 | 6.1 ± 0.566 |
| Ch-97 | CONT | 2.25 ± 2.76 | 1.37 ± 0.717 | 11.3 ± 1.33 |
| Ch-97 | T50 | 1.84 ± 1.33 | 1.06 ± 0.427 | 9.4 ± 1.79 |
| Ch-97 | T75 | 1.36 ± 1.14 | 0.85 ± 0.293 | 8.1 ± 1.05 |
| Ch-97 | T100 | 1.02 ± 1.65 | 0.61 ± 1.174 | 8.9 ± 0.92 |
| Ch-97 | T125 | 0.99 ± 0.11 | 0.29 ± 1.623 | 6.2 ± 1.45 |
| Ch-97 | T150 | 0.86 ± 0.54 | 0.21 ± 0.884 | 4.4 ± 2.43 |
| Front | CONT | 2.18 ± 1.74 | 0.97 ± 0.192 | 9.5 ± 1.02 |
| Front | T50 | 1.76 ± 2.65 | 0.83 ± 0.372 | 8.1 ± 1.350 |
| Front | T75 | 1.58 ± 1.91 | 0.73 ± 1.96 | 7.7 ± 1.783 |
| Front | T100 | 1.42 ± 0.75 | 0.61 ± 0.23 | 6.1 ± 0.810 |
| Front | T125 | 1.02 ± 1.43 | 0.40 ± 1.793 | 4.7 ± 0.159 |
| Front | T150 | 0.78 ± 0.33 | 0.21 ± 2.74 | 3.9 ± 0.394 |

SFW = Seedling fresh weight (g); SDW = Seedling dry weight (g); TCCS = Total chlorophyll contents of seedling (mg/g); All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97 Front = Frontana; CONT = Control; T50 = 50 mM stress; T75 = 75 mM stress; T100 =100 mM stress; T125 = 125 mM stress; T150 =150 mM stress

**Table 3. Effect of salt stress on shoot length (cm), root length (cm) and leaf area (cm$^2$) of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | SL | RL | LA |
|---|---|---|---|---|
| LW | CONT | 30.8 ± 0.62 | 41.9 ± 0.598 | 21.76 ± 1.156 |
| LW | T150 | 24.5 ± 1.03 | 29.1 ± 1.15 | 18.33 ± 0.54 |
| P-90 | CONT | 31.9 ± 0.32 | 40.3 ± 1.129 | 20.86 ± 1.234 |
| P-90 | T150 | 20.7 ± 2.92 | 27.6 ± 0.54 | 16.8 ± 0.76 |
| Ch-97 | CONT | 29.8 ± 1.05 | 40.5 ± 2.91 | 21.4 ± 1.982 |
| Ch-97 | T150 | 17.5 ± 0.47 | 24.9 ± 0.42 | 14.1 ± 0.51 |
| Front | CONT | 28.6 ± 0.37 | 40.8 ± 1.091 | 23.36 ± 1.1 |
| Front | T150 | 17.1 ± 1.56 | 21.9 ± 1.568 | 14.8 ± 0.33 |

SL = Shoot length (cm); RL = Root length (cm); LA = Leaf area (cm$^2$); All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97 Front = Frontana; CONT = Control; T50 = 50 mM stress; T75 = 75 mM stress; T100 =100 mM stress; T125 = 125 mM stress; T150 =150 mM stress

**Table 4. Effect of salt stress on fresh weight (g), dry weight (g) tillers per plant and nodes per plant of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | FW | DW | TPP | NPP |
|---|---|---|---|---|---|
| LW | CONT | 22.8 ± 0.620 | 12.3 ± 0.59 | 13 ± 0.33 | 5 ± 0.883 |
| LW | T150 | 16.85 ± 1.036 | 10.2 ± 0.11 | 2 ± 0.00 | 2 ± 0.33 |
| P-90 | CONT | 21.66 ± 1.760 | 11.3 ± 1.55 | 15 ± 0.881 | 6 ± 0.52 |
| P-90 | T150 | 16.1 ± 1.069 | 9.1 ± 0.23 | 0 ± 0.00 | 1 ± 0.00 |
| Ch-97 | CONT | 20.93 ± 0.320 | 10.63 ± 1.12 | 11 ± 1.20 | 5 ± 0.577 |
| Ch-97 | T150 | 11.16 ± 1.924 | 7.06 ± 0.54 | 1 ±0.53 | 1 ± 0.30 |
| Front | CONT | 20.8 ± 1.052 | 10.03 ± 1.51 | 10 ± 0.93 | 5 ± 0.881 |
| Front | T150 | 10.56 ± 0.471 | 6.13 ± 0.42 | 0 ± 0.00 | 1 ± 0.00 |

FW = Fresh weight (g); DW = Dry weight (g); TPP = Tillers per plant; NPP = Nodes per plant; All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97 Front = Frontana; CONT = Control; T50 = 50 mM stress; T75 = 75 mM stress; T100 =100 mM stress; T125 = 125 mM stress; T150 =150 mM stress

**Table 5. Effect of salt stress on total chlorophyll contents (mg/g), chlorophyll a contents (mg/g) and chlorophyll b contents (mg/g) of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | Total Chl (mg/g) | Chl a (mg/g) | Chl b (mg/g) |
|---|---|---|---|---|
| LW | CONT | 16.5 ± 1.881 | 6.37 ± 0.577 | 5.15 ± 0.37 |
| LW | T150 | 12.7 ± 0.48 | 4.9 ± 1.00 | 3.63 ± 0.153 |
| P-90 | CONT | 16.06 ± 0.34 | 6.51 ± 0.154 | 5.09 ± 0.447 |
| P-90 | T150 | 11.7 ± 1.75 | 4.7 ± 1.245 | 3.39 ± 0.293 |
| Ch-97 | CONT | 15.4 ± 0.67 | 7.15 ± 1.131 | 5.43 ± 0.72 |
| Ch-97 | T150 | 10.16 ± 1.03 | 3.79 ± 0.673 | 3.26 ± 1.00 |
| Front | CONT | 15.56 ± 1.54 | 6.94 ± 0.56 | 5.18 ± 0.36 |
| Front | T150 | 9.8 ± 0.33 | 3.44 ± 0.22 | 3.01 ± 1.00 |

All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97; Front = Frontana; CONT = Control; T150 = 150 mM stress; Total Chl = Total chlorophyll contents; Chl a = Chlorophyll a; Chl b = Chlorophyll b

**Table 6. Effect of salt stress on membrane stability index (%), relative water contents (%), water potential (-MPa) and osmotic potential (-MPa) of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | MSI (%) | RWC (%) | WP (-MPa) | OP (-MPa) |
|---|---|---|---|---|---|
| LW | CONT | 51 ± 0.674 | 47 ± 0.54 | 5.1 ± 0.99 | 9.4 ± 0.431 |
| LW | T150 | 39 ± 1.24 | 35 ± 1.29 | 6.3 ± 0.15 | 11.9 ± 1.174 |
| P-90 | CONT | 50 ± 1.154 | 48 ± 0.54 | 4.7 ± 1.24 | 9.3 ± 0.850 |
| P-90 | T150 | 37 ± 0.890 | 34 ± 0.98 | 5.9 ± 0.78 | 12.1 ± 0.532 |
| Ch-97 | CONT | 50 ± 0.552 | 45 ± 0.48 | 5.6 ± 0.13 | 9.51 ± 0.191 |
| Ch-97 | T150 | 30 ± 0.116 | 27 ± 0.14 | 8.1 ± 0.69 | 14 ± 0.980 |
| Front | CONT | 49 ± 0.832 | 48 ± 0.58 | 5.4 ± 0.97 | 9.1 ± 0.841 |
| Front | T150 | 28 ± 0.756 | 25 ± 1.02 | 7.9 ± 0.48 | 13.8 ± 0.335 |

All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97; Front = Frontana; CONT = Control; T150 = 150 mM stress; MSI = Membrane stability index; RWC = Relative water contents, WP = Water potential; OP = Osmotic potential

**Table 7. Effect of salt stress on ionic relations of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | Na (mg/g d. wt.) | K (mg/g d. wt.) | Cl (mg/g d. wt.) | K/Na ratio |
|---|---|---|---|---|---|
| LW | CONT | 4.8 ± 0.2667 | 23.5 ± 0.600 | 5.5 ± 0.1527 | 4.91 |
| LW | T150 | 7.9 ± 0.881 | 12.01 ± 0.259 | 9.3 ± 0.673 | 1.52 |
| P-90 | CONT | 5.1 ± 0.366 | 23.8 ± 0.331 | 6.1 ± 0.245 | 4.66 |
| P-90 | T150 | 8.5 ± 0.154 | 11.9 ± 0.1543 | 8.6 ± 0.633 | 1.40 |
| Ch-97 | CONT | 5.3 ± 0.55 | 22.9 ± 0.119 | 5.4 ± 0.3444 | 4.32 |
| Ch-97 | T150 | 10.9 ± 0.189 | 9.8 ± 1.05 | 11.6 ± 0.6210 | 0.89 |
| Front | CONT | 5.8 ± 0.457 | 23.6 ± 1.00 | 5.87 ± 1.007 | 4.06 |
| Front | T150 | 11.6 ± 0.142 | 8.7 ± 0.469 | 10.1 ± 1.38 | 0.75 |

All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97; Front = Frontana; CONT = Control; T150 = 150 mM stress; Na = Sodium; K = Potassium; Cl = Chloride

**Table 8. Effect of salt stress on protein contents (µg/g f. wt.), proline contents (µg/g f. wt.) and sugar contents (mg/g d. wt.) of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | PC | PC | SC |
|---|---|---|---|---|
| LW | CONT | 4.37 ± 0.13 | 87 ± 0.328 | 9.56 ± 1.154 |
| LW | T150 | 7.93 ± 0.5 | 160 ± 0.790 | 26.76 ± 0.33 |
| P-90 | CONT | 4.03 ± 0.36 | 90 ± 0.331 | 9.31 ± 0.672 |
| P-90 | T150 | 7.84 ± 0.15 | 157± 0.897 | 25.94 ± 0.178 |
| Ch-97 | CONT | 4.9 ± 0.27 | 85 ± 0.656 | 9.81 ± 0.892 |
| Ch-97 | T150 | 5.61 ± 0.61 | 139 ± 0.178 | 20.31 ± 1.00 |
| Front | CONT | 4.37 ± 0.37 | 88 ± 0.599 | 8.15 ± 0.352 |
| Front | T150 | 5.8 ± 0.91 | 135 ± 0.134 | 19.47 ± 0.923 |

PC = Protein contents (µg/g f. wt.); PC = Proline contents (µg/g f. wt.); SC = Sugar contents (mg/g d. wt.); All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97; Front = Frontana; CONT = Control; T150 = 150 mM stress; f. wt. = Fresh weight; d. wt. = Dry weight

**Table 9. Effect of salt stress on peroxide dismutase (µg/ml f. wt.), catalase (µg/ml f. wt.) and superoxide dismutase (µg/ml f. wt.) of wheat cultivars in hydroponics experiment.**

| Variety | Treatment | POD (µg/ml f. wt.) | Cat (µg/ml f. wt.) | SOD (µg/ml f. wt.) |
|---|---|---|---|---|
| LW | CONT | 67 ± 0.67 | 15.8 ± 1.00 | 107.6 ± 1.02 |
| LW | T150 | 95 ± 1.154 | 46.5 ± 0.00 | 167.4 ± 0.591 |
| P-90 | CONT | 69 ± 0.88 | 16.2 ± 0.33 | 103.9 ± 0.991 |
| P-90 | T150 | 93 ± 0.172 | 42.3 ± 0.871 | 179.1 ± 0.333 |
| Ch-97 | CONT | 64 ± 0.33 | 15.1 ± 0.833 | 105.4 ± 0.79 |
| Ch-97 | T150 | 81 ± 0.916 | 38.1 ± 0.458 | 151.1 ± 1.05 |
| Front | CONT | 61 ± 1.00 | 14.9 ± 0.921 | 101.7 ± 0.98 |
| Front | T150 | 80 ± 2.33 | 39.4 ± 0.395 | 153.3 ± 0.37 |

All figures are means of three repeats ± standard error; LW = Local white; P-90 = Pasban-90; Ch-97 = Chakwal-97; Front = Frontana; CONT = Control; T150 = 150 mM stress; POD = Peroxide dismutase; Cat = Catalase; SOD = Superoxide dismutase contents; f. wt. = Fresh weight

**Water potential and osmotic potential:** Water potential and osmotic potential are main indicators of stress induction. Maximum reduction in water potential was observed with Chakwal-97 and Frontana which were 44 and 46% (Table 6) compared to control, while Local White and Pasban-90 were categorized with minimum reduction which were 23 and 25%. Osmotic potential values under salt stress ranged from 11.9 –MPa to 13.8-MPa compared to control. Local White and Pasban-90 showed minimum reduction in osmotic potential which were 26% and 30% however Chakwal-97 (47%) and Frontana (52%) showed maximum reduction at 150 mM salt stress (Table 6).

**Ionic relations:** Salt stress significantly affected ionic balance in all wheat genotypes ($p \leq 0.05$). Sodium ($Na^+$) and chloride ($Cl^-$) concentrations in leaves were increased with increasing NaCl levels (Table 7). Maximum $Na^+$ accumulation at 150 mM NaCl was observed in Chakwal-97 (10.9 ± 0.32 mg/g d.wt.) and Frontana (11.6 ± 0.28 mg/g d.wt.), representing an average increase of 47% compared to control. Conversely, potassium ($K^+$) content decreased with increasing salinity. Local White and Pasban-90 maintained relatively higher $K^+$ levels under salt stress (12.01 ± 0.21 and 11.9 ± 0.19 mg/g d.wt., respectively), while average $K^+$ was decreased by 54% under 150 mM NaCl. Chloride content ranged from 8.6 ± 0.15 to 11.6 ± 0.22 mg/g d.wt., with Pasban-90 exhibiting the lowest accumulation under stress. The $K^+/Na^+$ ratio was declined under salinity for all genotypes, with Local White and Pasban-90 showing smaller reductions (68% and 69%, respectively), while Chakwal-97 and Frontana exhibited the greatest reduction. All reported values $Na^+$, $K^+$ and $Cl^-$ were means ± standard error (SE) of three replicates, and differences among treatments and genotypes were confirmed statistically using ANOVA ($p \leq 0.05$) (Table 7).

**Osmolytes accumulation:** Osmolytes accumulation such as protein, sugar and proline content increased with increasing level of salt (Table 8). Protein content ranged from 5.61 to 7.93 (µg/g f. wt.) and on the basis of average protein content was increased 53% at 150 mM salt level compared to control.. Maximum protein content was observed for genotype Local white and Pasban-90, whereas minimum protein content was recorded for Chakwal-97 and Frontana. Proline content on average was increased by 68% at salt stress over control and maximum proline content (160 and 157 µg/g f. wt.) in Local White and Pasban-90. Soluble sugar content ranged between 19.47 to 26.76 (mg/g d. wt.) under salt stress (Table 8). Proline content was increased on average 60% under salt stress over control. Pasban-90 and Local White was identified with maximum production of sugar content (Table 8).

**Antioxidant enzymes:** Superoxide dismutase (SOD), peroxide dismutase (POD) and catalase (Cat) activity were also recorded and results of present study depicted that all enzyme activities were increased with increasing salt levels (Table 9). POD activity varied between 80 to 95 (µmol $min^{-1}$ $g^{-1}$ FW) under salt stress and enzyme activity on average was increased 33% at 150 mM salt as compared to control (Table 9). Maximum activity of Cat (46.5 µmol $min^{-1}$ $g^{-1}$ FW) was recorded for Local White and minimum activity (38.1 µmol $min^{-1}$ $g^{-1}$ FW) for Chakwal-97 at 150 mM salt. Cat activity on average was increased by 62% under salt stress over control. SOD activity ranged between 153 to 179 (µmol $min^{-1}$ $g^{-1}$ FW) and maximum activity was recorded for Pasban-90 under salt stress. SOD enzyme activity on average was increased by 58% under salt stress compared to control (Table 9).

**Genetic diversity:** Genetic diversity analysis based on RAPD and SSR markers revealed clear clustering of wheat cultivars according to salt tolerance. The salt-tolerant cultivars, Local White and Pasban-90, clustered together with a genetic similarity of 73.3%, while the salt-susceptible cultivars, Chakwal-97 and Frontana, formed a separate cluster with 83.4% similarity (Fig. 1). The similarity between the tolerant and susceptible clusters was 54%, indicated moderate genetic divergence. Statistical support for these clusters was provided by Dice similarity coefficients and unweighted pair-group method with arithmetic mean (UPGMA) clustering, with bootstrap analysis (1000 replicates) confirming branch stability above 70% for all major clusters. Figure 1 shows the dendrogram generated from combined RAPD and SSR data. Branch labels correspond to cultivar names, and cluster lengths reflect genetic distances derived from the similarity matrix. The clustering method used was UPGMA based on Dice coefficients, which allowed the visualization of genetic relationships and differentiation between salt-tolerant and salt-susceptible genotypes. This analysis confirms that Local White and Pasban-90 have the genetic potential to tolerate moderate salt stress, while Chakwal-97 and Frontana are comparatively susceptible.

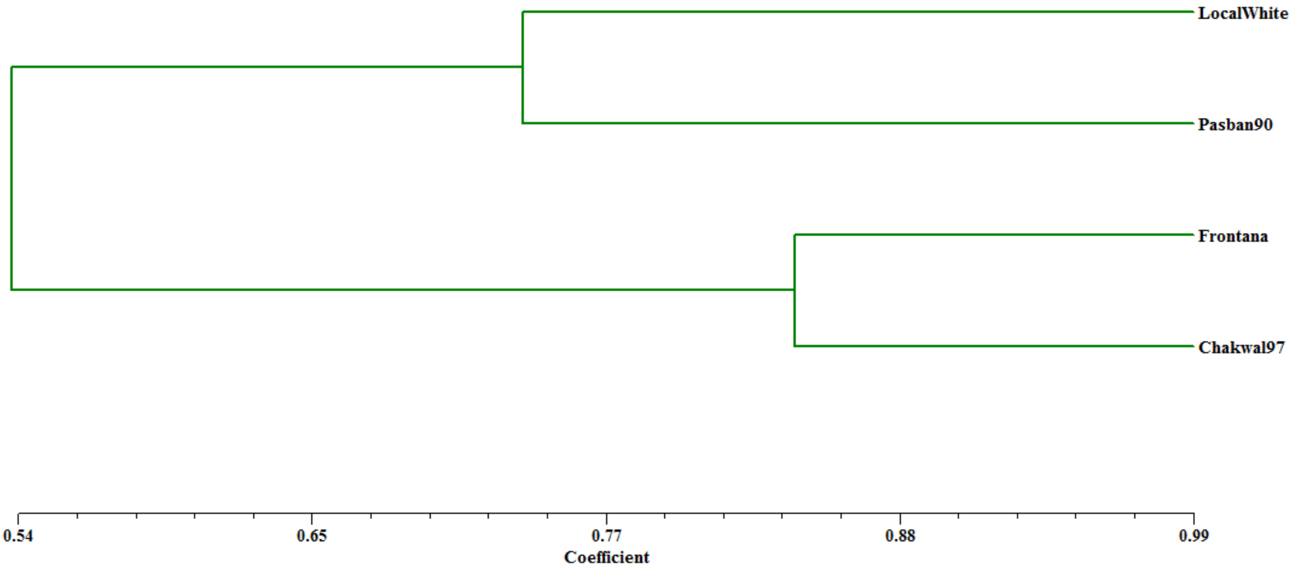

Fig. 1. Genetic relationship between four wheat cultivars as revealed by RAPD and SSR markers analysis.

## Discussion

Radicle and plumule length are mainly significant attributes in saline condition because roots play important role in water absorption and minerals. Shoot provide support and supply nutrients, minerals and food for the rest of the plant. Therefore, seedling length is main evidence about the plants response to salinity (Sourour *et al*., 2014). Seedling chlorophyll content decreased with increasing salt levels and comparable trend was recorded by other authors (Rahman *et al*., 2008). Plants seedling growth stage is very sensitive to salt stress because salinity alters plant water relation and ionic stress. Salt stress was responsible to generate oxidative stress resulting to alter cell ultra-structure. Wheat plants are vulnerable to salt stress at seedling stage as tolerance mechanism is not fully developed (Sourour *et al*., 2014). Plant growth of wheat is inhibited in saline conditions due to varying metabolic activities and activation of antioxidative defense mechanism. Antioxidative defense mechanism has been positively correlated with plant stress tolerance and decrease of chlorophyll content with increasing salt levels (Akca & Samsunlu, 2012).

The findings of current study show that root and shoot length show variability at 150 mM salt level. Wheat genotypes with higher sensitivity for salt show considerable decline in shoot elongation compared to salt tolerant genotypes. Local white and Pasban-90 show minimum reduction in shoot length at 150 mM. Root length of all wheat genotypes were less affected under salt stress. Likewise, wheat plant shoot and root length were reduced with increasing salt levels (Hussain *et al*., 2013). Fresh and dry weight adversely effected at 150 mM salt and shows variability according to genotype's tolerance. Similar findings were reported by Gurmani *et al*., (2014) when fresh and dry biomass were clearly decreased with increasing salt levels and wheat genotypes showed variation (Hussain *et al*., 2013). Fresh and dry weight of all wheat genotypes were reduced because of reduction in water and osmotic potential. Leaf area of Local White and Pasban-90 was comparatively less effected under salt stress compared to Frontana and Chakwal-97. Salt stress effects on leaf area were less negative compared to other morphological attributes. Tiller number and node per plant showed considerable reduction under salt stress. Our findings are similar to Hussain *et al*., (2013) observation. They reported similar results in which number of tiller showed reduction with high salt levels. This is not surprising as plant water and nutrient absorption was decreased as a result plant shoot length, leaf area, fresh weight, dry weight, tiller per plant and node per plant were decreased.

Physiological processes slowed down under salt stress due to limited availability of water and nutrients. Salt stress imposed negative effect on photosynthetic machinery and photosynthetic pigments. Total chlorophyll content, chlorophyll a and chlorophyll b content showed significant reduction under salt stress. Total chlorophyll content was significantly affected due to salt stress (27%) compared to control. Local White and Pasban-90 exhibited minimum reduction in chlorophyll a and chlorophyll b content under salt stress. Wheat genotypes were exposed to high salt levels as it influenced photosynthetic pigments accumulation in leaves and enhance chlorosis (Jamali *et al*., 2015). Plant water relation parameters show sensitivity towards increasing salt levels and found a negative relation between salt stress and water attributes. Local White and Pasban-90 showed minimum changes in water and osmotic potential while for Chakwal-97 and Frontana water and osmotic potential were significantly affected in salt stress. Salt stress alters water and osmotic potential and change cell turgidity (Jamali *et al*., 2015). Membrane stability is an important parameter to screen salt tolerant wheat genotypes. Pasban-90 and Local White had higher membrane stability under salt stress compared to control, while Chakwal-97 and Frontana showed sensitivity of membrane to salts. Similar findings were reported by Jamali *et al*., (2015), they found wheat tolerant cultivars show higher value of membrane stability as compared to sensitive ones.

Salt tolerance mechanism in plants depends upon ion accumulation pattern, ion transport, distribution with in plant cell and organ (Ahmad *et al*., 2013; Manzoor *et al*., 2015). Plant tolerance for high salt is defined on the basis of plant ability to exclude and accumulate salt or to maintain high K/Na ratio (Gurmani *et al*., 2014). Accumulation of sodium and chloride in leaves interrupts photosynthetic machinery and physiological processes. Chakwal-97 and Frontana show higher sodium concentration under salt stress compared to control. Minimum Sodium content was recorded for Local White and Pasban-90 under salt stress compared to their controls. Salt sensitive wheat genotypes grown in saline environment absorb more sodium ion compared to potassium. Potassium ions are required to maintain cell membrane integrity and processes but in saline condition sodium compete with potassium absorption (Kader & Lindberg, 2010). Pasban-90 and Local White were recorded with higher level of chloride content under salt stress compared to control. K/Na ratio is an important selection criterion to identify salt tolerant wheat genotypes (Gurmani *et al*., 2014). Local White and Pasban-90 maintained higher K/Na ratio compared to Chakwal-97 and Frontana.

Munns & James (2003) findings depict that plant show another tolerance mechanism to accumulate metabolites such as protein, proline and sugar under salt stress (Gurmani *et al*., 2014). Wheat genotypes having higher K/Na ratio and chlorophyll content were able to accumulate higher concentration of metabolites. Salt tolerant genotypes (Local White & Pasban-90) accumulate higher concentration of osmolytes compared to salt sensitive genotypes (Chakwal-97 and Frontana) under salt stress. Protein production and accumulation is genotype dependant under salt stress (Gurmani *et al*., 2014). In current study, Local White and Pasban-90 accumulated higher protein concentration under salt stress conditions and also accumulated higher concentration of proline and sugarsas compared to sensitive wheat genotypes. Salt stress promotes free radical production which leads to oxidative stress. Wheat plant produces enzymatic and non-enzymatic anti-oxidant responses such as superoxide dismutase (SOD), peroxide dismutase (POD) and catalase (Cat) to overcome oxidative stress and SOD helped in detoxification of free radicals and SOD level increased with increasing salt levels. Wheat tolerant genotypes (Pasban-90 and Local White) were recorded with high activity of SOD compared to sensitive genotypes under salt stress (Gurmani *et al*., 2014). POD's main role is in scavenging of free radical mainly hydrogen peroxide. POD activity showed indistinctive trends among wheat genotypes but Pasban-90 showed higher level of POD under salt stress. Similar observation was recorded by Gurmani *et al*., (2014) in wheat genotypes with variying degree of salt tolerance. Catalase (Cat) play an important role against free radicals (Gurmani *et al*., 2014) and Cat activity was increased with increasing salt levels. In present investigation, Cat activity was higher in tolerant genotypes under salt stress.

## Conclusion

The present study demonstrates that salt stress significantly affects germination, seedling growth, physiological, biochemical, and ionic attributes of wheat cultivars, with clear differences among genotypes. Local White and Pasban-90 exhibited higher germination percentages (37.5% and 33.5% at 150 mM NaCl, respectively), greater seedling vigor, and maintained higher

$K^+/Na^+$ ratios under salt stress, indicating their relative tolerance. In contrast, Chakwal-97 and Frontana showed greater reductions in germination, seedling growth, and $K^+/Na^+$ ratios, reflecting higher susceptibility. Biochemical analyses revealed that tolerant genotypes maintained higher total chlorophyll content and antioxidant activity under salinity. Genetic diversity analysis using RAPD and SSR markers further supported these findings, clustering tolerant genotypes together with 73.3% similarity, and separating them from susceptible genotypes (83.4% similarity within the susceptible cluster; 54% intercluster similarity). These results provide a comprehensive screening framework for salt tolerance in wheat and identify Local White and Pasban-90 as promising genotypes for breeding programs. It is recommended that these tolerant cultivars be used in crosses with other wheat lines to enhance salinity tolerance in Pakistan, addressing the critical challenge of soil salinization on national wheat production.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Author's contribution:** A.S. conceived and designed the study; N.B. conducted the experiments; S.H.S. prepared the initial manuscript draft; K.A. contributed in physiological and biochemical analyses; R.I. and S.B. participated in laboratory work, data collection, and statistical analysis; S.A. provided overall supervision, technical guidance, and finalized the manuscript for submission. All authors read and approved the final manuscript.

## References

Abbas, Q. and A. Shafique. 2019. Integrated use of organic and inorganic fertilizers improves soil health, growth and yield of wheat (*Triticum aestivum* L.). *Adv. Agric. Biol.,* 2: 48-54. https://doi.org/10.63072/aab.19007

Abdullah, Z., M.A. Zia, S. Shoukat, S. Aziz, I.U. Khan, A. Khan, H. Maab and S. Zaib. 2025. Co-expression of *ZmVPP1*, *ZmNAC111*, and *ZmTIP1* confers enhanced drought tolerance in maize (*Zea mays*). *J. Plant Prod. Sustain.,* 1: 12-19.

Ahmad, P., M. Sarwat and S. Sharma. 2013. Reactive oxygen species, antioxidants, and signaling in plants under salt stress. In: (Eds.): M.A. Hossain, L.W.P. Tran & S. Sharma. *Salinity stress in plants: Signaling, genes, and products* (pp. 61–81). Springer, New York, NY.

Ahmed, A. (2024, December 22). *Pakistan loses 30pc farm land to salinity: FAO*. Dawn.

Akca, Y. and E. Samsunlu. 2012. The effect of salt stress on growth, chlorophyll content, proline and nutrient accumulation, and K/Na ratio in walnut. *Pak. J. Bot.,* 44: 1513-1520.

Alamgeer, M., L.A. Chaudhry, W. Anum, A. Ali, D. Zafar, A. Hamid and R. Abbas. 2022. Effect of different sowing dates, planting methods and irrigation intervals on performance of wheat varieties under agro-climatic conditions of Bahawalpur region. *Adv. Agric. Biol.,* 5: 35–45. https://doi.org/10.63072/aab.22006

Ali, B., A.Hussain, M.Alamgeer, W.Anum and A.Ali. 2025. Exploring stage-specific responses of chickpea (*Cicer arietinum* L.) to supplementary irrigation under agro-climatic conditions of Bahawalpur, Pakistan. *J. Plant Prod. Sustain.,* 1: 1-15.

Ali, T., J. Akhtar, M.Anwar-ul-Haq and M.Maqsood. 2024. Effects of $ZnSO_4$ on growth, some key physiological processes and ionic homeostasis of different bread wheat (*Triticum aestivum* L.) genotypes under salinity stress. *Pak. J. Bot.,* 56: 815-826.

Anonymous. 2005. International rules for seed testing. International seed testing association. Zurich, Switzerland.

Arnon, D.I. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.,* 24: 1.

Ashraf, M., H.R. Athar, P.J.C. Harris and T.R. Kwon. 2008. Some prospective strategies for improving crop salt tolerance. *Adv. Agron.,* 97: 45-110.

Atta, K., S. Mondal, S. Gorai, A.P. Singh, A. Kumari, T. Ghosh, A. Roy, S. Hembram, D.J. Gaikwad, S. Mondal, S. Bhattacharya, U.C. Jha and D. Jespersen. 2023. Impacts of salinity stress on crop plants: Improving salt tolerance through genetic and molecular dissection. *Front. Plant Sci.,* 14: 1241736.

Batool, S.G., A. Wahid, A. Kiran, Z.A. Malik and Z. Hassan. 2025. Evaluating the effect of exogenous application of salicylic acid on heat stress tolerance in wheat (*Triticum aestivum* L.). *J. Plant Prod. Sustain.,* 1: 16-37.

Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.,* 72: 248-254.

Cakmak, I., D. Strbac and H. Marschner. 1993. Activities of hydrogen peroxide-scavenging enzymes in germinating wheat seeds. *J. Exp. Bot.,* 44: 127-132.

Capell, B. and K. Doerffling. 1993. Genotype-specific differences in chilling tolerance of maize in relation to chilling-induced changes in water status and abscisic acid accumulation. *Physiol. Plant.,* 88: 638-646.

Dinsa, T. and U. Balcha. 2024. Evaluation of agronomic performance and grain yield stability of bread wheat (*Triticum aestivum*) genotypes in East Shewa zone, Oromia. *Adv. Agric. Biol.,* 7: 58-67. https://doi.org/10.63072/aab.24007

Egea, I., Y. Estrada, C. Faura, J.M. Egea-Fernández, M.C. Bolarin and F.B. Flores. 2023. Salt-tolerant alternative crops as sources of quality food to mitigate the negative impact of salinity on agricultural production. *Front. Plant Sci.,* 14: 1092885.

Evans, S., W.H. Ryan and M.A. Schneegurt. 2013. Isolation and characterization of halotolerant soil fungi from the great salt plains of Oklahoma. *Cryptogam. Mycol.,* 34: 329-341.

Giannopolitis, C.N. and S.K. Ries. 1997. Superoxide dismutase I. Occurrence in higher plants. *J. Plant Physiol.,* 59: 309-314.

Gurmani, A.R., S.U. Khan, F. Mabood, Z. Ahmed, S.J. Butt, J. Din, A. Mujeeb-Kazi and D. Smith. 2014. Screening and selection of synthetic hexaploid wheat germplasm for salinity tolerance based on physiological and biochemical characters. *Int. J. Agric. Biol.,* 16: 681-690.

Hafeez, M.B., A. Raza, N. Zahra, K. Shaukat, M.Z. Akram, S. Iqbal and S.M.A. Basra. 2021. Gene regulation in halophytes in conferring salt tolerance. In: *Handbook of Bioremediation*; Elsevier: Amsterdam, The Netherlands; Academic Press: Cambridge, MA, USA; pp. 341-370.

Hamilton, P.B. and D.D. Van Slyke. 1943. Amino acid determination with ninhydrin. *J. Biol. Chem.,* 150: 231-233.

Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. California Agriculture Experiment Station Circular, 347.

Hussain, S., A. Khaliq, A. Matloob, M.A. Wahid and I. Afzal. 2013. Germination and growth response of three wheat cultivars to NaCl salinity. *Soil Environ.,* 32: 36-43.

Iqbal, J., S.F.H. Shah and I. Ahmad. 2018. Effect of sowing time, advanced genotype, and potassium application on seed cotton yield in relay cropping with wheat. *Adv. Agric. Biol.,* 1: 15-22. https://doi.org/10.63072/aab.18003

Iqbal, M. and A.A. Qureshi. 2021. Biostimulants and salinity: Crosstalk in improving growth and salt tolerance mechanism in Fennel (*Foeniculum vulgare*). *Adv. Agric. Biol.,* 4: 8-13. https://doi.org/10.63072/aab.21002

Jamali, S.S., A. Borzouei and M. Aghamirzaei. 2015. Cell membrane stability and biochemical response of seven wheat cultivars under salinity stress. *Brazil. J. Bot.,* 38: 63.

Kader, M.A. and S. Lindberg. 2010. Cytosolic calcium and pH signaling in plants under salinity stress. *Plant Signal. Behav.,* 5: 233-238.

Khayatnezhad, M., R. Gholamin, S.H. Jamaatie-Somarin and R. Zabihi-Mahmoodabad. 2010. Effects of PEG stress on corn cultivars (*Zea mays* L.) at germination stage. *World J. Appl. Sci.,* 11: 504-506.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin Phenol reagent. *J. Biol. Chem.,* 193: 265-275.

Manzoor, K., N. Ilyas, N. Batool, B. Ahmad and M. Arshad. 2015. Effect of salicylic acid on the growth and physiological characteristics of maize under stress conditions. *J. Chem. Soc. Pak.,* 37: 588-592.

Mehmood, K., M. Arshad, G.M. Ali, A. Razzaq and R. Qureshi. 2020. Optimization of factors affecting callus-based gene transformation by Agrobacterium tumefaciens in wheat (*Triticum aestivum* L.). *Adv. Agric. Biol.,* 3: 23-31. https://doi.org/10.63072/aab.20004

Melino, V. and M. Tester. 2024. Salt-tolerant crops: Time to deliver. *Annu. Rev. Plant Biol.,* 74: 671-696.

Miri, Y. and S.A. Mirjalili. 2013. Effects of salinity stress on seed germination and some physiological traits in primary stages of growth in purple coneflower (*Echinacea purpurea*). *Int. J. Agron. Plant Prod.,* 4: 142-146.

Munns, R. and R.A. James. 2003. Screening methods for salt tolerance: A case study with tetraploid wheat. *Plant Soil,* 253: 201-218.

Rafique, A., A. Iqbal, M. Riaz and H.G. Abbas. 2025. Assessment of growth and yield traits under irrigated and rainfed environments for genotypes selection in barley (*Hordeum vulgare* L.). *J. Plant Prod. Sustain.,* 1: 1-11.

Rahman, M., U.A. Soomro, M.Z. Haq and S. Gul. 2008. Effects of NaCl salinity on wheat (*Triticum aestivum* L.) cultivars. *World J. Agric. Sci.,* 4: 398-403.

Rani, J. and C.R. Merlin. 2012. Salt stress tolerance and stress proteins in wheat (*Triticum aestivum* L.). *Int. Res. J. Pharm.,* 3: 143-146.

Rehman, S.U., J. Yang, J. Zhang, L. Zhang, X. Hao, R. Song, S. Chen, G. Wang and L. Hua. 2025. Salt stress in wheat: A physiological and genetic perspective. *Plant Stress,* 16: 100832.

Rohlf, F.J. 2005. NTSYS pc (Numerical Taxonomy and Multivariate Analysis System). Version 2.2. Exeter Software Appl. Biostat Inc., New York.

Saeed, B., H. Gul, P. Shah, A. Khan, S. Anwar, A. Azra and S. Ali. 2012. Yield of wheat varieties under solid and skip row geometries. *ARPN J. Agric. Biol. Sci.,* 7: 591-594.

Safder, M., M. Ali and M.U. Haq. 2025. Influence of socio-economic characteristics and agronomic practices on wheat production in Shujaabad, Multan, Pakistan. *J. Plant Prod. Sustain.,* 1: 20-31.

Scholander, P., H. Hammel, E. Bradsheet and E. Hemminogsen. 1965. Sap pressure in vascular plants. *Plant Sci.,* 148: 339-345.

Shafqat, N., H. Ahmed, Z. Khan, A. Shehzad, S.H. Shah, M. Islam and R. Masood. 2023. Characterization of wheat-thinopyrum bessarabicum genetic stock for stripe rust and karnal bunt resistance. *Brazil. J. Biol.,* 83: e246440. https://doi.org/10.1590/1519-6984.246440

Shaheen, A., A. Shahzad, S.H. Shah, S.H. Khattak, S. Idrees and S.U. Khan. 2023. Molecular and morphological evaluation of salt tolerance genes in advanced wheat lines. *Pak. J. Bot.,* 55: 813-823.

Shahzad, A., M. Ahmad, M. Iqbal, I. Ahmed and G.M. Ali. 2012. Evaluation of wheat landrace genotypes for salinity tolerance at vegetative stage by using morphological and molecular markers. *Genet. Mol. Res.,* 11: 679-692.

Shehzad, R.A., G. Sarwar, S.H. Shah, M.A. Tahir, N.U. Sabah, S. Muhammad, M. Aftab, M.Z. Manzoor and I. Shehzad. 2022. Efficacy of P enriched organic manures to improve soil health and nutrient acquisition of wheat. *Pak. J. Agric. Res.,* 35: 266-273.

Shehzad, R.A., G. Sarwar, S.H. Shah, M.A. Tahir, N.-u.-S. Sabah, S. Muhammad, M. Aftab, M.Z. Manzoor, I. Shehzad and U. Saleem. 2023. Growth and yield response of wheat to organic manures (farm yard manure, phospho-compost (PROM), and press mud) alone and in combination with mineral fertilizer. *Pak. J. Agric. Res.,* 36: 1-8.

Singh, P., N. Singh, K.D. Sharma and M.S. Kuhad. 2010. Plant water relations osmotic adjustment in Brassica species under salinity stress. *J. Am. Sci.,* 6: 30-35.

Sourour, A., N. Rassaa, Z. Chamekh, S. Beji, F. Karoui, T. Bouzaien, M. Mrabit and B.Y. Mongi. 2014. Effect of salt stress (sodium chloride) on germination and seedling growth of durum wheat (*Triticum durum* Desf.) genotypes. *Int. J. Biodiv. Conserv.,* 6: 320-325.

Syed, A., G. Sarwar, S.H. Shah and S. Muhammad. 2020. Soil salinity research in 21st century in Pakistan: Its impact on availability of plant nutrients, growth and yield of crops. *Commun. Soil Sci. Plant Anal.,* 51: 183-200.

Weatherly, P.E. 1950. Studies in the water relations of cotton. The field measurement of water deficits in leaves. *New Phytol.,* 49: 81-97.

Zaman, M.S. and A.A. Qureshi. 2018. Deciphering physiological, biochemical, and molecular responses of potato under salinity stress: A comprehensive review. *Adv. Agric. Biol.,* 1: 54-60. https://doi.org/10.63072/aab.18008