The characterization of wheat genotypes for salinity tolerance using morpho-physiological indices under hydroponic conditions

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Abstract

Salinity affects plant growth, development, yield, and is a big challenge for wheat growth across the globe. Possible feasible solution is creation of salt-tolerant material, genetic variation is a criterion to developing genetically superior individuals. To assess the genetic variation for salt tolerance, nationally and internationally-derived 81 wheat genotypes were selected and evaluated in 0- and 150-mM salt in nutritional culture at seedling stage. Results indicate that salinity levels reveal significant (p < 0.01) differences for fresh root weight (RW), shoot length (SL), fresh shoot weight (SW), total plant length (TL), total fresh weight (TW), root/shoot weight ratio (RSWR), root/shoot length ratio (RSLR), and relative growth rate for weight (RGR-Wt). While, there was no difference for root length (RL). Hierarchical Clustering and Pairwise correlation analysis showed TW, RGR-Wt, SL, SW, and RW were positively correlated among themselves, whereas RL had poor correlations with all the traits except TL and RSLR. Hence, selection of SL can improve the performance of other parameters. Based on PCA analysis, SW and RGR-Wt were the major discriminative components for wheat genotypes. Present study explained that shoot related parameters could be used as a selection criterion to categorize salt-tolerant genotypes. Outperforming genotypes 1104 and 1106 in saline conditions could be used as parents in creation of salt-tolerant wheat genotypes, and parameters such as SL, SW, TW, and RGR-Wt for early screening will be important for creating salt-tolerant and high yielding wheat genotypes.

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1. Introduction

Globally wheat (Triticum aestivum L.) is cultivated to meet the consumable food demand of the humanity (Sehgal et al., 2012; Uzair et al., 2016). Being a primary source of nutrition for millions of individuals, wheat contributes 30% of the total grain production of the world. However, annual yield gain in crop productivity is prolonged to meet the future demands for plant-based products for the projected global population in 2050 (Priyamvada et al., 2011; Tilman et al., 2011). Globally, different kinds of stresses are the main threats for production of crops (Hussain et al., 2011; Iqbal et al., 2018; Naveed et al., 2020; Uzair et al., 2021). Plants are immobile, for their growth and for survival they need nutrients and water from the soil. Previously it has been reported
that around 20% of the world cultivated area is being pretentious by salinity (Oproi and Madosa, 2014). Soil salinity adversely affects crop production up to 60%, and its drastic effect is more pronounced in the perspective of climate change scenario (Xie et al., 2016). Deposition of more salts in the upper layer of soil poses highly stressful environment for plant development, which ultimately causes reduction in yield or plant death (Naveed et al., 2020; Akbarimoghaddam et al., 2011; Munns, 2002; Niamat et al., 2019; Ahmad et al., 2020). In Pakistan, salinity affected the 6.3 × 106 ha of irrigated land (Qureshi and Barrett-Lennard, 1998). This makes the circumstance disturbing and it represents a danger of deficiency of food. Recently, much consideration is given to wheat improvement by joining both new and traditional crop breeding approaches along with advances in management practices to cope with salinity problem (Dodig et al., 2010; Gosal et al., 2009). Improvement through conventional breeding requires the exploitation of the existing genetic variation in the wheat crop for salt tolerance. Past examinations (Salam et al., 1999; Ali et al., 2007; Marvi et al., 2011; Ali et al., 2012) have uncovered large variation for salt resilience in wheat. Genetic diversity is also high in local and/or exotic material, which might be valuable for the improvement of salt-tolerance in future breeding plans. For this purpose, different methods were used which includes solution, sand, and pot cultures, saline raised beds, and field screening (Munns et al., 2006; Shahzad et al., 2019). Field evaluation may be sometime misleading or inaccurate as salinity intermingles with drought. In solution culture, supplements are promptly accessible to plant in differentiating with soil, where filtering or chelation of supplements to soil ruins their accessibility to plants. This strategy is reasonable due to the lower climatic variation (Munns et al., 2006), and it has been used by several researchers for genotype screening, especially under salinity (Salam et al., 1999; Ali et al., 2007; Hussain et al., 2015; Radi et al., 2013).

Within the field evaluation, different elements (soil fertility, water availability, insect pests, plant productivity, and climatic conditions etc.) create problems inside the assurance of salt-tolerant genotypes which are lenient in one climate may not be lenient in another climate (Raza et al., 2019). Additionally, soil features like pH, sodicity and harmful components i.e., boron change from zone to region indeed from land to land (Rengasamy, 2002). Changing field and climates, genotype × climate associations should be analyzed cautiously for prevalent and trustworthy outcomes in salt affected regions. In wheat crop, root, shoot, and their biomass have moreover been detailed as principal characteristics which bestow salt resistance. Wheat researchers concluded that shoot biomass and plant biomass might be utilized for choice of salt-tolerant genotypes (Munns and James, 2003; Oyiga et al., 2016; El-Hendawy et al., 2009; Genc et al., 2019; Meneguzzo et al., 2000). Salt stress created a huge decrease in wheat plant biomass, and this decline was more clear at high levels of salt stress as compared to low levels (Radi et al., 2013). Since salt-tolerant genotypes had the option to hold their growth and had more biomass than the salt-susceptible ones. Increased biomass of resistant genotypes might be connected with their cap-potential to keep a preferable photosynthesis rate over the susceptible genotypes (Ashraf and Ashraf, 2012). In light of association and heritability, it transformed into presumed that lengths of root and shoot, their fresh and dry weights, have positive associations and high heritability, so these might be exceptionally gainful norms for settling on salt tolerant genotypes (Shahzad et al., 2012).

Identification of hereditary variation is an essential of any breeding plan pointed toward creating salt-tolerant genotypes. This study was directed to portray the relationship of considered seedling parameters and to choose reasonable determination standards under both control and saline climates. The present study will provide novel breeding material to develop a salt tolerant genotype suitable for saline conditions.

2. Materials and methods

2.1. Plant material and developing conditions

The current research was directed in the experimental area of University of Agriculture, Faisalabad (UAIF), 31°26’N, 73°06’E, and 184.4 m above sea level (ASL). Total 81 genotypes; including 29 genotypes from Pakistan, 44 genotypes from the International Maize and Wheat Improvement Center (CIMMYT), Mexico, and 8 genotypes from Australia (Supplemental Table S1) and salinity check genotype LU26s was collected to measure the effects of normal and salt stress at the seedling stage. Seeds of each genotype were planted in polythene bags (27 cm depth and 12.7 cm diameter) containing soil, under natural conditions. Bags were kept at 24/12 ± 2 °C Day/Night temperature with 10 h photoperiod. Irrigation was applied as per requirement.

At two leaf stage, seedlings were shifted in iron tubes (L118 × W88 × H30 cm) containing 200 litters 1/2 strength Hoagland supplements solution (Hoagland and Arnon, 1950). Nutrient’s solution was prepared with ddH2O and air pump was used for aeration. At the time of shifting, initial RL and SL (cm), and RW and SW (g) of single plants were recorded. Following two days of seedling shifted to hydroponic conditions, commercial salt was added to build up two NaCl levels of 0 (control) and 150 mM (15 dsm−1) in isolated tubs. The pH of the solutions 6.0 to 6.5 was balanced daily by adding 1 M HCl or NaOH. The experiment followed completely randomized two factors factorial design. Four plants of every genotype in every replicate were used to record the data of RL, SL, and their weights after 15 days of seedlings growth under NaCl stress. Data were collected for the following characters at two different stages one at the time of salt application and second after 15 days of exposure to salinity.

RL (cm) from the root-shoot joint to the end of the root tip and SL (cm) from the root-shoot joint to the upper tip of leaf of the same plants were measured with the help of a graduated ruler. TL (cm) was obtained by summing both RL and SL. RSLR was computed through dividing of RL by SL.

Root – Shoot Ratio (RL : SL) = RL/SL

Each seedling was cut down at the junction of root and shoot to separate each part. RW and SW (g) were weighted using digital electronic balance (Compax, RS 232C). RW and SW were summed to get TW (g). RW was divided by SW to get weight ratio (RSWR). RGR-Wt was calculated by the following formula as mentioned previously (Hoffmann and Poorter, 2002).

\[ RGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \]

where \( ln W_1 \) and \( ln W_2 \) are the means of plant weights at times \( t_1 \) and \( t_2 \).

2.2. Data analysis

QTL lciMapping V4.2 (freely available from https://www.is-breeding.net/) (Lei et al., 2015) was used for analysis of variance (ANOVA). Genotypic and phenotypic variances were estimated with the help of following formulas:

Phenotypic variance \( \sigma^2_p = \sigma^2_g + \sigma^2_{err} \)

Genetic variance \( \sigma^2_g = (MS_g - MS_{ge}) \times (rt) \)
Genotype × treatment interaction variance \( \sigma^2_{gt} \)  

\[ (MS_{gt} - MS_e) \times (r) \]

where \( r \) and \( t \) representing the number of replications and the number of treatments; \( MS_e \), genotypic mean square; \( MS_{gt} \), genotypic × treatment interaction mean square; \( \sigma^2_{gt} \), Error mean square.

Agglomerative Hierarchical Cluster (AHC) analysis was performed using XLSTAT. Dissimilarities were calculated by calculating Euclidean distance and applying Ward’s method. Heatmaps were constructed using R package pheatmap (version 1.7) (Kolde and Kolde, 2015; Rasool et al., 2021). For the estimation of broad sense heritability (H), data was subjected to ANOVA and then the total variances were subdivided into its parts. The differences among genotypes and within genotypes were utilized to workout broad sense heritability with help of following equation:

\[
\text{Broad sense heritability (H)} = \sigma^2_g
\]

\[
[(\sigma^2 + \sigma^2_g + \sigma^2_{gt}) \times (r)] \times (rt)
\]

where \( \sigma^2_g \) is the genetic variance; \( \sigma^2_g \), genotype × treatment interaction; \( r \), replication; \( t \), treatments and \( \sigma^2_{gt} \), phenotypic variance. Association and PCA were performed to explore the associations among parameters and genotypes under salt stress using R 3.4.5.

3. Results

Mean squares for RL, SL, RW, SW, RSLR, RSWR, TL, TW and RGR-Wt under control and 150 mM salt stress (Table 1). Genotypes varied significantly \((p < 0.01)\) for all parameters analyzed indicating presence of genotypic variation variability to be exploited. Apart from RL, all the traits differ significantly \((p < 0.01)\) in both conditions. Under control and salt stress conditions, it was shown that all the genotypes behaved differently because the interaction among the genotypes and treatments was highly significant \((p < 0.01)\) for all traits. Under saline conditions some genotypes that showing variation in performance for nine seedling traits as compared to those which are studied; among them best performing genotypes were considered salt tolerant (Table 2 and Fig. 1).

3.1. Mean variability

Under control conditions mean values of RL of 81 wheat genotypes altered significantly, ranging from 21.26 to 43.63 cm, however, under saline conditions RL ranges from 20.06 to 47.6 cm (Fig. 1 and Table 2). Genotypes 1073 (47.6 cm), 1064 (47.16 cm) and 1237 (46.1 cm) showed maximum RL however minimum RL was observed in genotypes 1202 (20.06 cm) and 1217 (21.86 cm) followed by AAS11 (22.03 cm). When these genotypes compared with other already tested genotypes (AAS-11 and AARI-11) then these genotypes considered as salt-tolerant as well as susceptible. Similarly, SL of 81 genotypes were recorded in saline conditions differed from each other, highest SL was observed in 1240 (21.66 cm) and 1197 (21.63 cm) closely followed by 1104 (21.46 cm). While, lowest SL was observed in 1202 (13.1 cm) and 1091 (14 cm) followed by 1141 (14.16 cm).

Significant variation in RW of 81 wheat genotypes arose under 150 mM level of NaCl and ranges from 0.666 to 7.566 g (Fig. 1 and Table 2). Maximum RW was noted in genotypes 1104 (7.56 g) and 1099 (6.66 g) followed by 1133 (6.16 g). While, lowest RW was noted for the genotypes 1124 (0.66 g) and 1490 (0.76 g) followed by 1202 (1.13 g). From 81 wheat genotypes, 7 and 4 genotypes had more RW than AARI-11 and AAS-11 respectively. Discrete variation in the wheat genotypes for SW was observed under 150 mM NaCl salinity level. It ranges from 0.5 to 8.76 g (Fig. 1 and Table 2). Maximum SW production per plant was detected in the genotypes 1104 (8.76 g) and 1230 (7.73 g) followed by 1106 (7.53 g) and 1145 (7.23 g). While, minimum SW was observed for the genotypes 1124 (0.5 g) and 1055 (0.96 g) (Table 2 and Fig. 1). TL range from 33.16 to 65.53 cm under saline condition (Fig. 1 and Table 2). Maximum and minimum TL was recorded for the genotypes 1237 (65.53 cm) and 1202 (33.16 cm), respectively (Fig. 1 and Table 2). Under saline conditions, total plant weight (TW) range from 1.16 to 16.33 g (Fig. 1 and Table 2). Maximum TW was gain by genotypes 1104 (16.33 g) and 1145 (12.7 g), whereas the genotypes 1124 (11.66 g) followed by 1202 (2.2 g) and 1055 (2.23 g) gain minimum TW (Table 2 and Fig. 1).

RSLR for genotypes under studied changed significantly, it ranges from 0.1 to 3.14 cm under control conditions, and from 1.19 to 3.23 cm under saline conditions, as presented in Fig. 1 and Table 2. As the salt stress increases, a decline was detected among all the genotypes. RSWR ranging from 0.43 to 1.83 g under control conditions while under saline conditions it ranges from 0.35 to 1.38 g. For RSLR and RSWR the genotypes 1073 (3.23 g) and 1481 (1.38 g) classified as salt tolerant (Table 2 and Fig. 1). RGR-Wt express the growth behavior of genotypes in a precise period. Data measured for RGR-Wt of wheat seedlings changes significantly from –0.06 to 0.13 under control conditions and from –0.03 to 0.097 under salt stress conditions (Fig. 1 and Table 2). The genotypes 1104 (0.095), 1063 (0.088) trailed by 1064 (0.087) with higher RGR-Wt under salt-stress were tolerant. For each trait, top five best and worst performing genotypes are enlisted in Table 3.

| Table 1 | Mean square from Analysis of variance (ANOVA) for nine morpho-physiological parameters at early seedling stage of 81 wheat genotypes evaluated in hydroponic culture under control and saline (150 mM) conditions. |
|---------|-------------------------------------------------|
|            | Genotype | Treatment | G × T | Error | Total | Heritability |
| DF       | 80       | 1        | 80    | 324   | 485   | 0.75        |
| RL       | 147.26** | 0.37**   | 22.82* | 0.5262|       | 0.75        |
| SL       | 41.35**  | 768.9**  | 17.78* | 0.7131|       | 0.51        |
| RW       | 8.79**   | 9.34**   | 2.88** | 0.0212|       | 0.59        |
| SW       | 21.53**  | 17.6**   | 3.06** | 0.0305|       | 0.77        |
| TL       | 209.42** | 735.35*  | 43.92* | 1.2067|       | 0.69        |
| TW       | 55.53*   | 52.6*    | 9.79*  | 0.0987|       | 0.73        |
| RSLR     | 0.75**   | 5.1**    | 0.19*  | 0.0084|       | 0.64        |
| RSWR     | 0.22**   | 0.06**   | 0.11*  | 0.0054|       | 0.46        |
| RGR-Wt   | 0.005    | 0.0005** | 0.0007**| 0.0001|       | 0.78        |

** Highly significant \((P < 0.01)\); * significant \((P < 0.05)\); n.s = non-significant.

SOV = source of variation; G × T = genotype by treatment interaction; DF = degree of freedom; RL = root length (cm); SL = shoot length (cm); RW = root weight (g); SW = shoot weight (g); TL = Total Plant length (cm); TW = Total plant weight (g); RSLR = Root/shoot length ratio; RSWR = Root/shoot weight ratio; RGR-Wt = Relative growth rate for weight (g/day).
Table 2
Summary statistics of nine morpho-physiological parameters at early seedling stage of 81 wheat genotypes under control and saline conditions.

| Trait | Treatment | Maximum | Minimum | Mean | Variance | SD |
|-------|-----------|---------|---------|------|----------|----|
| RL    | Control   | 43.64   | 21.27   | 32.10| 24.78    | 4.98|
|       | Salinity  | 47.6    | 20.07   | 32.15| 31.93    | 5.65|
| SL    | Control   | 29.5    | 12.64   | 19.98| 16.29    | 4.04|
|       | Salinity  | 21.66   | 13.10   | 17.47| 3.43     | 1.86|
| RW    | Control   | 7.24    | 0.67    | 3.60 | 2.14     | 1.47|
|       | Salinity  | 7.57    | 0.67    | 3.33 | 1.76     | 1.33|
| SW    | Control   | 11.50   | 0.50    | 4.44 | 5.17     | 2.28|
|       | Salinity  | 8.77    | 0.50    | 4.06 | 3.04     | 1.75|
| TL    | Control   | 71.57   | 36.30   | 52.08| 50.46    | 7.11|
|       | Salinity  | 65.54   | 33.17   | 49.62| 33.99    | 5.83|
| TW    | Control   | 17.97   | 1.37    | 8.04 | 13.32    | 3.65|
|       | Salinity  | 16.34   | 1.17    | 7.38 | 8.47     | 2.91|
| RSLR  | Control   | 3.15    | 1.07    | 1.67 | 0.15     | 0.39|
|       | Salinity  | 3.24    | 1.19    | 1.89 | 0.17     | 0.41|
| RSWR  | Control   | 1.84    | 0.44    | 0.89 | 0.06     | 0.24|
|       | Salinity  | 1.39    | 0.36    | 0.87 | 0.05     | 0.23|
| RGR-Wt| Control   | 0.13    | -0.06   | 0.04 | 0.01     | 0.04|
|       | Salinity  | 0.10    | -0.04   | 0.05 | 0.08     | 0.03|

SD = standard deviation; RL = root length (cm); SL = shoot length (cm); RW = root weight (g); SW = shoot weight (g); TL = Total length (cm); TW = Total plant weight (g); RSLR = Root/shoot length ratio; RSWR = Root/shoot weight ratio; RGR-Wt = Relative growth rate for weight (g/g/day).

Fig. 1. Variability for nine morpho-physiological parameters at early seedling stage of 81 wheat genotypes. Boxplots contains average, median, minimum, maximum values, and circles showing outliers. Significant at (*P < 0.05, **P < 0.01, ***P < 0.001).
Performance of nine morpho-physiological parameters at early seedling stage of 81 wheat genotypes under salt stress condition.

Heritability is thought to be an essential sign for the growth of improved population. Selection of single plant at the seedling stage can be proved more operational for a trait that is exceptionally heritable when contrasted with less heritable attributes. Heritability assessments under 150 mM salt (Table 1) were above 70% for RGR-Wt (78%), SW (77%), RL (75%), TW (73%) and followed by TL (69%). This shows that over 70% hereditary differences communicated to offspring was additive in nature. Thus, determination for these attributes might be valuable during early generations.

3.2. Estimation of heritability (H) for salinity tolerance

Heritability is estimated as a measure of the degree to which genetic differences among individuals in a population play a role in variation of traits. Heritability is often expressed as the proportion of variation due to genetics in relation to the total variation. It is calculated using the formula: Heritability (H) = [(Variation due to genotype) / Total variation] × 100.

Table 3

| Trait                             | Best-Performing Genotypes: Names and Mean Values (Salt-Tolerant) | Worst-Performing Genotypes: Names and Mean Values (Salt-Susceptible) |
|-----------------------------------|----------------------------------------------------------------|------------------------------------------------------------------|
| RL                               | 1073 (47.6) and 1064 (47.16) followed by 1237 (46.1)          | 1202 (20.06) and 1217 (21.86) followed by AAS11 (22.03)          |
| SL                               | 1240 (21.66) and 1197 (21.63) followed by 1104 (21.46)        | 1202 (13.1) and 1091 (14.14) followed by 1141 (14.16)           |
| RW                               | 1104 (7.56) and 1099 (6.66) followed by 1113 (6.16)           | 1124 (0.66) and 1490 (0.76) followed by 1202 (1.13) and 1055 (1.26) |
| SW                               | 1104 (8.76) and 1230 (7.73) followed by 1106 (7.53) and 1145 (7.23) | 1124 (0.5) and 1055 (0.96) followed by 1202 (1.06) and 1076 (1.5) |
| TL                               | 1237 (65.53), 1064 (64.06), 1106 (63.03) followed by 1073 (62.3) | 1202 (33.16) and AAB311 (38.06) followed by 1217 (39.5)         |
| TW                               | 1104 (16.33), 1145 (12.7) followed by 1099 (12.46) and 1220 (12) | 1124 (1.16) followed by 1220 (22) and 1055 (2.23)               |
| RSLR                             | 1073 (3.23) and 1091 (3.00) followed by 1141 (2.92) and 1064 (2.79) | AAS11 (1.19) and 1217 (1.240) followed by 1197 (1.248)          |
| RSWR                             | 1481 (1.38), 1124 (1.355) followed by 1073 (1.35) and 1055 (1.31) | 1042 (0.359) and 1232 (0.423) followed by 1490 (0.446)          |
| RGR-Wt                           | 1106 (0.097), 1104 (0.095), 1063 (0.088) followed by 1064 (0.087) | 1073 (~0.036) and 1124 (~0.030) followed by 1055 (~0.009)       |

3.3. Correlation analysis

The proportion of connection among at least two autonomous factor is known as correlation. Pearson’s correlation results indicated that RL showed low correlations with all the traits except TL and RSLR in salt stress conditions (Fig. 2), so during assortment of salt tolerant genotypes these traits cannot be used. Under the salt stress, TW showed highly significant (p-value < 0.001) association with SL (r = 0.48***), SW (r = 0.96***), and RW (r = 0.93***). Similarly, RGR-Wt also have positive correlation with SL (r = 0.40***), RW (r = 0.66***), SW (r = 0.78**), and TW (r = 0.77***). Significant at (P < 0.05, **P < 0.01, ***P < 0.001).

3.4. Cluster analysis

To find the genotypes which are similar or homogenous among each other. So, cluster analysis was performed by using the data recorded under both control (Fig. 3a) and saline conditions (Fig. 3b). It showed the two-way clustering. Based on the correlation among the parameters, they were divided into two subgroups under both environments. In controlled conditions RSLR and RSWR were separated from rest of the parameters and make a separated cluster. Similarly, under saline conditions SL, RGR-Wt, RW, SW, and TW makes a separated group (Fig. 3b). The hierarchical clustering under saline conditions was also performed and on the bases of performance of the wheat genotypes under salinity, they were divided into three groups susceptible, intermediate, and tolerant (Fig. 4). Out of 81 wheat genotypes, first group (susceptible) contains 36 genotypes, second group (intermediate) contains 11 genotypes, while the third (tolerant) group contains 34 genotypes.

3.5. Principal component analysis (PCA)

To assess the scope of the germplasm and the connection among seedling parameters under control and salt stress, patterns of variation have been contemplated in 81 wheat genotypes utilizing PCA in view of the relationship network. In the studied germplasm, under control conditions first two PCs covered 78.1% of the total variability (Fig. 5a) while, in saline conditions first two PCs covered 75.3% of the total variability (Fig. 5b). PC1 accounted for 56.8% of the variance and PC2 accounted for 21.3% under control conditions. Similarly, in saline conditions PC1 contributed 46.2% of the total variance, and PC2 contributed 29.1%. Upon projection of parameters on PC1 and PC2 in normal and saline conditions shown that RW, SL, SW, and TW were extremely and positively related to RGR-Wt. These results are further confirmation of the hierarchical cluster analysis (Fig. 3b). The genotypes 1104, 1106, 1042, 1237, 1489 and 1064 were opposite to 1202, 1124, 1088 and AAS-11 under the control conditions. A clear difference was existed among salt-tolerant and salt-susceptible genotypes. The genotypes 1104, 1106, 1237, 1159, 1073, and 1064 were opposite to genotypes 1202, 1124, 1088, 1161, 1088, and 1223 in saline conditions.

4. Discussion

In the present research work solution culture technique was used. To assess the pattern of variability for salt tolerance, four-week-old seedlings of 81 diverse wheat genotypes were used. It also provides very valuable data about growth of plant at seedling stage. Through this technique it is convenient to recover tolerant seedlings for seed production. To study salt tolerance in rice (Bado et al., 2016; Aslam et al., 1993) and wheat (Hussain et al., 2015), this technique is very effective. The role of seedling stage in the growth of plant towards abiotic stresses is very important as described in previous studies (Shah et al., 2021; Khan et al., 2020; Ali et al., 2014). Pre-selection of populations before field assessments can also be possible (Ali et al., 2012; Munns and James, 2003; Shahzad et al., 2012). Huge differences are observed among species and even genotypes within the species towards response to salinity. For screening, selection of salt concentration is another main and essential factor. According to the results for identification of salt tolerant genotypes, 150 mM or higher amount of NaCl can be used (Wahid et al., 2007; Yao et al., 2021). For screening of wheat for salt tolerance preference should be controlled conditions since it has minimum variations in ecological conditions (Munns and James, 2003) as field has changing soil pH, and harmful components from one region to another and even land to land.

Previous studies stated that RL seemed a vital parameter for salt stress tolerance (Ali et al., 2007; Shahzad et al., 2021). Salinity affected genotypes showed reduced growth rate and it led towards minimum root length. Same results were described by (Ali et al., 2007; Ali et al., 2012; Raddi et al., 2013; El-Hendawy et al., 2009). Role of plant roots is very important and vital because it absorbs water as well mineral nutrients through direct interaction with soil. Therefore, under salt stress situations, substantial symptom is also provided by roots. During experiment, no significant incre-
At 150 mM NaCl stress, RL was reduced when compared with controlled conditions. So, it was recommended that it is a salinity sensitive trait (Ashraf and Ashraf, 2012). It was also stated that under salinity stress RL can be used as a selection criterion. When RL enhances the growth rate under stressed circumstances then it helps the plant in many ways, as it absorbs water from deep soil and tolerance mechanism also enhanced (Wasaya et al., 2018). The reason may be that there was reduction in it due to death of older roots and stop of formation of new roots. Therefore, to enhance the salt tolerance in crop plants, it was suggested that longer roots selection can become one of the reasons (Azhar et al., 1998). Salinity effected genotypes show a wide range of response in plants in the form of minimum SL as compared to tolerant genotypes and it also decreased the growth rate of sensitive genotypes. The relations between environment and genotype are called phenotypic response for any trait. Our results showed that through salinity stress the most effected trait was SL. In present studies it was noticed that decrease happened in SL and SW, since shoots were withered away, and breakage happened in them, so these harms lead to weight and height misfortune. Same consequences were stated about shoot growth that under stress condition it effect most as compare to root growth (Soomro et al., 2008).

In the form of RW production, salinity stress gives a lot of variation of response in genotypes. Salt susceptible genotypes showed minimum RW when it compared with salt tolerant genotypes. RW production can be enhanced through lateral roots produced by salt tolerant genotypes. Sensitive genotypes show decline in SW as compared to tolerant ones while damaged leaves also lead toward reduction in growth rate. Among these genotypes some performed well against salinity and give maximum shoot biomass as compared to sensitive genotypes. Susceptible genotypes show decline in plant biomass as well as plant weight in salt stress condition. On the other hand, tolerant genotypes performed well and shows great enhancement in normal growth rate which ultimately increase plant biomass production. This decrease in morphological parameters is because of lessening in photosynthetic pigments (Ashraf and Ashraf, 2012), rate of transpiration, synthesis of carbohydrates, and proteins in plants (Radi et al., 2013). But salt tolerant genotypes performed well even under saline conditions and provide normal growth of plants and are less affected by salinity as compared to sensitive genotypes (Munns, 2005). The plants which had the option to support high development rate indicated tolerance. Relative development rate for weight of genotypes uncovered addition or misfortune in biomass of the genotype with respect to as g/g/day and this development rate is the immediate pointer of a genotype possibly it had its generally expected development, or its development was hindered under salt stress.

In plant breeding correlation analysis is used to expose the relative importance of different plant traits (Ghafoor et al., 2013; Ali et al., 2013).
et al., 2008). According to previous studies at seedling stage fresh shoot weight and plant biomass can be used as a selection criterion for salt tolerance (Radi et al., 2013; Iqbal, 2005; Moud and Maghsoudi, 2008; Mahmood, 2009; Akhtar et al., 2003). Under saline conditions those genotypes which had maximum shoot or plant biomass can increase their normal growth rate by maintaining normal photosynthetic process, which lead towards increase growth rate of plant (Ashraf and Ashraf, 2012). Overall, it was observed that shoots related traits have strong correlations as compared to root related traits, as well as due to higher correlation it can also be used as a good selection criterion for salt tolerance.

PCA is a multivariate measurable assessment for inspecting and improving convoluted and enormous datasets. These investigations (e.g., PCA and PLS-DA) changes the wide variety of correlated factors into smaller ones (Azam et al., 2020; Ali et al., 2015; Sisodia

![Fig. 3. Two-way hierarchical clustering of nine morpho-physiological parameters at early seedling stage of 81 wheat genotypes under control (a) and 150 mM salt stress (b) conditions. Rows represents number of genotypes while the columns represent the morphological parameters. Color scheme in which dark blue and white represents high and low values, respectively. RL = root length (cm); SL = shoot length (cm); RW = root weight (g); SW = shoot weight (g); TL = Total plant length (cm); TW = Total plant weight (g); RSLR = Root/shoot length ratio; RSWR = Root/shoot weight ratio; RGR-Wt = Relative growth rate for weight (g/g/day).]
and Rai, 2017; Wang et al., 2021). Essentially, for parental choice in breeding programs, a biplot analysis may be used in determination of variables that may be categorized into fundamental groups and subgroups primarily based on homogeneity and uniqueness (Ali et al., 2012; Hussain et al., 2015). Previously PCA was used by many researchers to determine diversity and grouping in wheat at seedling stage (Sisodia and Rai, 2017) as well as in field (Saima et al., 2012; Baranwal et al., 2013; Hussain et al., 2013). The diverse response of wheat genotypes under salt conditions distinguished in this work might mirror the effect of genetic components and the impact of conditions. Regardless, there is a basic need to examine hereditary variation inside the present used germplasm to save a positive level of hereditary variation for future wheat rearing.

Fig. 4. Grouping of wheat genotypes on the bases of their performance under saline conditions. Three main groups green (susceptible), red (intermediate), and dark blue (tolerant) were created.
5. Conclusion

In total 81 wheat genotypes were assessed against salt stress and appeared significant inhibitory impact on all the parameters. However, the genotypes contrasted in their behavior to salt stress. This was due to the genetics of the plant types, environment, and genotype × environment interactions. Correlation, Hierarchical Cluster Analysis, and PCA revealed that SL, RW, SW, and TW were correlated to each other and had a positive association with RGR-Wt. This study proposed that presence of genetic variability and shoot related indices due to their genetic bases, can be utilized as a selection criterion to recognize salt tolerant wheat genotypes.
The genotypes 1104, 1106, 1199, 1073, 1240, 1136, 1237, 1489, 1022, 1124, 1042, 1088 and 1079 appeared to be sensitive. The genotypes 1063, and 1042 appeared to be most tolerant whilst the genotypes 1104, 1106, 1199, 1073, 1240, 1136, 1237, 1489, 1022, 1124, 1042, 1088 and 1079 appeared to be sensitive. The salinity stress displayed drastic impacts on plant development.

The present investigations well explained to recognize the best salinity prone regions under wheat cultivation.

6. Institutional review board statement

Not applicable.

7. Compliance with ethical standards

This manuscript does not contain any studies with human/animals performed by any of the authors.

8. Data availability statement

All the relevant data are within the paper and its supporting information file.

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Author contributions

All authors contributed to the study conception and design, Material preparation, data collection and analysis were performed by Muhammad Uzair, Zulfiqar Ali, Mohsin Ali, Sajid Fiaz, Korb Attia, Naem Khan, Abdullah A. Al-Doss, and Muhammad Ramzan Khan. Muhammad Uzair wrote the original draft, and Zulfiqar Ali edited the manuscript. Zulfiqar Ali supervised the whole study. All authors have read and approved the submitted manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary Table S1: 81 wheat genotypes with their pedigrees/parentages tested at normal and salt stress conditions. Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103299.

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