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# Uncovering the Iranian wheat landraces for salinity stress tolerance at early stages of plant growth

Sukhjit Kaur[1] · Ankita Suhalia[1] · R. S. Sarlach[2] · Mohd Shamshad[2] · Pritpal Singh[2] · Gomti Grover[2] · Anureet Brar[2] · Achla Sharma[2]

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## Abstract

Soil salinity is a major global environmental factor limiting plant growth and productivity. Wheat seeds need to be able to germinate and establish seedlings in saline soils for sustained productivity. In this study, we investigated seed germination-related traits under salt stress conditions in 239 diverse Iranian wheat landraces for evaluation of salt stress tolerance. Seed of the landraces along with relevant checks was germinated in salt and control solutions until 14 days. Initially, 10 randomly selected accessions were subjected to six different (25 mM, 50 mM, 75 mM, 100 mM, 125 mM, 150 mM) salinity levels for standardization. The salinity level 125 mM NaCl was found more effective concentration for the discrimination of genotypes for various physiological indices, viz. germination percentage, coleoptile length, root and shoot length, fresh root and shoot weight, dry root and shoot weight, and vigor index. After 14 days, germination percentage and all seedling traits were found to be affected due to salinity. Salt tolerance index maintained a significant positive correlation with seedling traits which indicates that these parameters could be used as selection criteria for screening wheat genotypes against salt stress. Significant differences were observed for coleoptile length, root–shoot length, fresh root–a shoot weight, dry shoot weight, and vigor index among the wheat landraces. From the overall observation of germination percentage and early seedling growth, it was concluded that the wheat landraces accessions including IWA 8600278, IWA 8600291, IWA 8611786, IWA 8600179, IWA 8600303, and IWA 8610487 showed better salt tolerance than Kharchia 65, the universal salt-tolerant variety used so far in wheat-breeding programs.

**Keywords** Iranian wheat landraces · Salinity stress · Seedling growth · Physiological indices · Stress tolerance index

## Introduction

Salinity is one of the most important environmental factors limiting crop production of marginal agricultural soils in many parts of the world. It has been reported that more than 6 million ha land in India is salt affected (Chatrath et al. 2007). Soil salinization is increasing due to excessive irrigation and industrial pollution and is emerging as the main threats facing modern agriculture sustainability (Hamam and Negim 2014; Klay et al. 2014; Ben-Romdhane et al. 2018). Saline soils are defined as having electric conductivity (ECe) > 4dS$m^{-1}$, and alkali soils are also referred as sodic soils with pH greater than 8.2, exchangeable sodium percentage (ESP > 15) and soluble salts mostly carbonate and bicarbonate of sodium, capable of alkaline hydrolysis (Abrol et al. 1980). Salt accumulation in soil affects plant growth to different degrees at different stages (Bernstein 1975), and different plant species exhibit different growth response (Glenn et al. 1999). In India, 6.7 M ha land under wheat cultivation is affected by salt including 3 M ha by salinity and 3.7 M ha by sodicity/alkalinity, distributed across 15 of the 29 states. Out of these 15 states, eight contribute ~ 97% of national wheat production and have ~ 5.6 M ha affected by salt. Soil acidity affects 25 M ha of Indian soils, including ~ 30% of current areas under cultivation (Khokhar et al. 2017). Indian Punjab, usually known as the wheat bowl of India, has around 3.52 million hectare area under wheat crop annually. In view of changing climate, many new soil stresses have emerged in different niches in Indian Punjab, the

✉ Sukhjit Kaur
sukhjitdhaliwal789@gmail.com

1 Department of Botany, Punjab Agricultural University, Ludhiana 141004, India

2 Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141004, India

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soil salinity being much pronounced in South Western districts of the state.

Among the abiotic stresses, salinity is one of the most important stresses that adversely effect the rate of germination, seedling establishment, plant growth, and development (Mehmet et al. 2006; Saleh and Madany 2015). Most of the cereals are sensitive to salinity stress at the germination and early seedling phase of development (Ghoulam and Fares 2001). Salinity also inhibits plant reproduction that decreases spikelet number, thousand grain weight, and finally grain yield (Ghogdi et al. 2012). Additionally, salinity effects the plant growth by inhibiting photosynthesis, water and ion transport, through reducing stomatal conductivity and increased intracellular carbon dioxide concentration. High NaCl levels alter the nutrient uptake from the soil; decrease P, K, and Ca ions and increase $Na^+$ concentration; and also inhibit protein synthesis, enzyme activities, and membrane permeability (Radhakrishnan and Lee 2015). Germination percentage, root length, callus size, coleoptile length, and growth are also known to be reduced by the increasing salinity levels. Similarly, it has been reported that the limited $Na^+$ transport to shoot in the salt-tolerant wheat genotype was due the regulation of xylem loading transporters (Hussain et al. 2015). Landraces, synthetic hexaploid wheat, wild species, and other germplasm sources have been promoted as providing a potential source of novel genetic variation useful for bread wheat improvement (Mujeeb-Kazi 2003). A number of such materials have been introduced as breeding parents for programs aiming at salinity tolerance (Zavala-Fonseca et al. 1998; Pritchard et al. 2001, 2002; Mujeeb-Kazi and Diaz De Leon 2002; Colmer et al. 2006). Many efforts have been diverted by the major wheat-breeding programs of the region to breed salt-tolerant varieties. The availability of the donors for any trait is the prerequisite for initiating any targeted breeding program. In India, the universal salinity donor namely 'Kharchia' has been extensively used for this purpose (Chatrath et al. 2007). There is an urgent need to scout for new sources of tolerance to have diversity in donors. The present study reports the variation in response to salt stress in a set of Iranian landraces at seedling stage with the aim to identify salt-tolerant genotype(s). The identified genotypes would be valuable resources for breeding programs and scientific research toward better understanding of salt tolerance mechanisms of crop with the goal of creating new local wheat varieties of higher adaptation abilities to soil salinization in agricultural lands.

## Materials and methods

### Plant material

A set of 239 Iranian wheat landraces procured from CIMMYT, a universal salt-tolerant wheat cultivar, 'Kharchia 65,' KRL 213, a salt-tolerant genotype developed by Central Soil Salinity Institute, Karnal, Haryana, India, and two popular commercial wheat varieties of the region (HD 2967 & PBW 725) constituted the plant material. PBW 725 (PBW 621//GLUPRO/3*PBW 568/3/PBW621) is the variety developed and released by Punjab Agricultural University, Ludhiana, for cultivation under timely sown irrigated (TSI) conditions and HD2967 (ALD/COC//URES/3/HD2160M/HD 2278) is developed and released by IARI, New Delhi, India, for cultivation under TSI conditions. Kharchia 65 (EG-593/Kharchia local) and KRL 213 (Cndo/R143//Ente/Mexi_2/3/*Ae.sq.*/4/Weaver/5/2*Kauz) are the stress-tolerant cultivars which have been specifically bred for cultivation under salt stressed lands. Kharchia 65 was developed and released in 1971, whereas KRL 213 was released in 2010. To ensure that pure seeds were used and to minimize heterogeneity and contamination, multiplication using single ear method was carried out and the harvested seeds were then used for the evaluation at germination and seedling stage under controlled conditions.

### Screening of germplasm against NaCl stress

Seeds were surface sterilized using 0.1% mercuric chloride solution for about 2 min, then rinsed with water, and were air-dried. Ten random seeds with no visible damage from each landrace were placed on germination paper, moistened with NaCl solution with 3 replications, and subsequently rolled up in cigar roll method as described by Zhu et al. (2005). These were placed in growth chamber in darkness at 25 °C for 3 days and then grown under a photoperiod of 16/8 h (light/dark). Cigar roll method was used with some local modifications. After 2 weeks of seedling growth in commercial-grade NaCl solution (at dose as per the specific experiment), the observations on germination percentage, root Length (RL), shoot length SL (SL), fresh root weight (FRW), fresh shoot weight (FSW), and vigor index VI [(Average SL + Average RL) × germination percentage] were measured. Later, dry root weight (DRW) and dry shoot weight (DSW) were calculated after drying the shoot and root for 72 h in oven at 60–62 °C.

For coleoptile length (CL) measurement, 20 uniform-sized seeds of each genotype with no physical damage were placed in the middle of a moist heavy grade germination paper with NaCl solution and distilled water, about one centimetre apart with germ end down. The germination paper was then folded vertically in half with the seed placed in the crease; the folded half was again folded horizontally four times and placed in a plastic box with holes at the base to drain excess water. The plastic boxes were then placed inside a growth chamber at a constant temperature of 25 °C in complete darkness. After 10 days, the average CL of 20 randomly selected seedlings were recorded to the nearest millimeter measuring from the base of the seed to the coleoptile tip.

Stress tolerance index (STI) for various traits was recorded according to the following formula:

Germination stress tolerance index (GRSI):

$$\text{GRSI} = \frac{\text{Germination percentage of stressed plant}}{\text{Germination percentage of control plant}} \times 100 \tag{1}$$

Coleoptile length stress tolerance index (CLSI):

$$\text{CLSI} = \frac{\text{Coleoptile length of stressed plant}}{\text{Coleoptile length of control plant}} \times 100 \tag{2}$$

Root length stress tolerance index (RLSI):

$$\text{RLSI} = \frac{\text{Root length of stressed plant}}{\text{Root length of control plant}} \times 100 \tag{3}$$

Shoot length stress tolerance index (SLSI):

$$\text{SLSI} = \frac{\text{Shoot length of stressed plant}}{\text{Shoot length of control plant}} \times 100 \tag{4}$$

Root fresh weight (RFW) stress tolerance index (REWSI):

$$\text{RFWSI} = \frac{\text{Root fresh weight of stressed plant}}{\text{Root fresh weight of control plant}} \times 100 \tag{5}$$

Shoot fresh weight (SFW) stress tolerance index (SFWSI):

$$\text{SFWSI} = \frac{\text{Shoot fresh weight of stressed plant}}{\text{Shoot fresh weight of control plant}} \times 100 \tag{6}$$

Root dry weight (RDW) stress tolerance index (RDWSI):

$$\text{RDWSI} = \frac{\text{Root dry weight of stressed plant}}{\text{Root dry weight of control plant}} \times 100 \tag{7}$$

Shoot dry weight (SDW) stress tolerance index (SDWSI):

$$\text{SDWSI} = \frac{\text{Shoot dry weight of stressed plant}}{\text{Shoot dry weight of control plant}} \times 100 \tag{8}$$

Vigor index stress tolerance index (VISI):

$$\text{VISI} = \frac{\text{Vigour index of stressed plant}}{\text{Vigour index of control plant}} \times 100 \tag{9}$$

### Standardization of the optimum dose of NaCl for inducing salt stress to evaluate the germplasm set

Any level of stress which is able to finally elucidate the physiological differences among landraces in response to salt stress would be considered the optimum stress level. For this, an experiment was set up using Kharchia 65, KRL 213, PBW 725, HD 2967 and ten randomly selected Iranian landraces with aim to standardize the dose of stress creating agent (NaCl) at seedling stage using the cigar roll method. The set was subjected to different doses of NaCl (0, 25, 50, 75, 100, 125, and 150 mM) for creating stress environment. This was critical as much knowledge about response of Iranian landraces to salinity stress is not available. The experiment was carried out in completely randomized design (CRD) design with three replications under laboratory conditions. The concentrations of NaCl [T1, control (distilled water), T2 (25 mM), T3 (50 mM), T4 (75 mM), T5 (100 mM), T6 (125 mM), and T7 (150 mM)] were prepared by dissolving calculated amount of scientific-grade NaCl in distilled water.

### Statistical analysis

The data were analyzed as standard procedure PROC GLM in SAS (Version 9.3, SAS Institute) for analysis of variance (ANOVA), and means were used for construction of different graphs. The description of the recoded observation was depicted as the bar graphs, and hierarchical cluster was performed followed by K-mean matrix for various traits using Minitab 18. The correlation between different traits was calculated using the Pearson correlation coefficient using the IBM SPSS statistics 25.

## Results

### Optimization of NaCl concentration for evaluation

The experiment clearly revealed that response of seedling parameters in wheat cultivars used as control and Iranian landraces up to 75 mM NaCl could not differentiate the genetic differences for to salt stress tolerance. There was a significant reduction in germination percentage and other seedling traits at dose of 100 mM concentration of NaCl in wheat varieties PBW 725 and HD2967 (Supplementary Table S1). Stress induced using 100 mM NaCl did not differentiate much among landraces, whereas stress induced using 150 mM NaCl concentration showed differences in landraces, but at the same time, inhibited total germination in few accessions (IWA 8607746 and IWA 8614325). Maximum differences were observed at 125 mM NaCl concentration. Based upon these observations, the NaCl concentration of 125 mM was chosen to be the best dose to differentiate the Iranian landraces effectively with the aim to identify tolerant landrace accessions.

## Screening of germplasm

The complete germplasm set consisting of 239 Iranian landraces and four wheat varieties was screened for seedling traits at 125 mM NaCl and distilled water as control (0 mM). There was significant variation for response to salt stress tolerance in Iranian landraces (Table 1). ANOVA revealed significant differences among landraces and variables response to 125 mM NaCl used for creating salinity stress. Interaction between the landraces and salt concentration was also observed to be significant for all the traits except germination and RDW. The analysis showed variation in CL, germination percentage, and other seedling traits (Fig. 1A). There were significant differences in germination percentage, CL, RL, SL, RFW, RDW, SFW, SDW, and VI score between the genotypes ($P < 0.05$). The reduction under saline conditions compared to control conditions for germination percentage and CL was 17.53% and 18.48%, respectively. The average root and SL decreased by 26.27% from 17.76 cm under control to 13.09 cm under salt stress conditions and by 47.55% from 14.72 cm under control conditions to 7.72 cm under saline conditions, respectively. The average RFW reduced by 41.20% from 0.134 g under control to 0.079 g under stress conditions. The average root and SDW also decreased but to a lesser extent, from 0.030 g in control conditions to 0.0179 g under salt stress and by 42.41% from 0.055 g to 0.032 g, respectively. The average SFW decreased by 42.30% from 0.337 g under control conditions to 0.194 g under salt stress conditions. The average VI decreased by 42.37% from 3350.95 to 1930.86 under control and salt stress conditions, respectively.

Stress tolerance index for all the traits of whole set is given as supplementary data, (Supplementary Table S2). Landraces IWA 8600338, IWA 8600031, IWA 8600084, IWA 8600841, IWA 8602728, IWA 8606739, IWA 8611786, IWA 8613166, and IWA 8611583 had minimum effect of stress on germination under stress conditions and had better germination percentage than Kharchia 65 and KRL 213. IWA 8600291, IWA 8600338, IWA 8607803, IWA 8604640, IWA 8613426, IWA 8611786, IWA 8611326, IWA 8610487, IWA 8600527, IWA 8600278, IWA 8600303, and IWA 8600179 landraces had superior performance based upon VI as compared to Kharchia 65 and KRL 213, whereas Kharchia 65 genotype was more vigorous than PBW 725 and HD 2967. RLSI, SFSI, RFSI, VI VISISDSI, and RDSI clearly pointed toward the influence of stress conditions on the genotypes.

From the data on physiological stress tolerance indices like GRSI, CLSI, RLSI, SLSI, VISI, etc., it is demonstrated that five landrace accessions (IWA 8600278, IWA 8600291, IWA 8611786, IWA 8600179, and IWA 8600303) (Table S2) were ranked better than the best salt-tolerant check and these indices can be used to screen the wheat germplasm for salt tolerance and can be the quick reflective characters for salt tolerance when selecting a large set of germplasm for tolerance. Table S2 also shows the landraces categorized as potential highly tolerant (HT), moderately tolerant (MT), and non-tolerant (NT) genotypes against salinity stress. These can be further characterized for subcomponents of tolerance and can be efficiently used by wheat breeders to pyramid different components of salt stress tolerance in elite wheat germplasm. In the era of accelerated plant breeding, very efficient techniques like double haploid production (Chahal and Gosal 2002), speed breeding or accelerated breeding (Watson et al. 2017), etc. are available for quick mobilization of traits of interest into agronomical desirable backgrounds.

The mean value for all individual traits is given in supplementary material (Table S3). CL is the important seedling trait and is adversely affected by osmotic stress. There was significant ($P < 0.05$) reduction in CL under stress conditions. CL ranged between 2.37 cm and 6.57 cm with an average value of 4.58 cm under controlled condition and 1.13 cm to 6.00 cm with an average value of 3.78 cm under saline conditions. In saline conditions, maximum value of CL (6.00 cm) was observed in landrace IWA 8602728 followed by IWA 8600397 (5.86 cm) and

**Table 1** Analysis of variance (ANOVA) for coleoptile length, germination percentage, and seedling traits in Iranian landraces and wheat cultivars (included as controls) under salt stress conditions

| Mean square of the characters | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Source of variation | DF | GERM% | RL | SL | CL | RFW | RDW | SFW | SDW | VI |
| Treatment | 1 | 12,688.59* | 7568.01* | 25,739.35* | 159.74* | 0.506* | 0.010* | 7.715* | 0.0377* | 658,346,528.7* |
| Genotypes | 243 | 200.32* | 46.03012* | 46.1848* | 2.677* | 0.0156* | 0.00017* | 0.023* | 0.00048* | 1,610,080.3* |
| Trt*geno | 243 | 107.79 | 15.42111* | 25.2915* | 1.109* | 0.00496* | 0.00012 | 0.011* | 0.00033* | 632,186.9* |
| Error | 976 | 80.25 | 7.32014 | 7.34863 | 0.652425 | 0.001346 | 0.00011 | 0.005 | 0.00015 | 411,770 |

*DF* degree of freedom, *Germ %* germination percentage, *RL* root length, *SL* shoot length, *CL* coleoptile length, *RFW* root fresh weight, *RDW* root dry weight, *SFW* shoot fresh weight, *SDW* shoot dry weight, *VI* vigor index

*Significant differences at 5% level

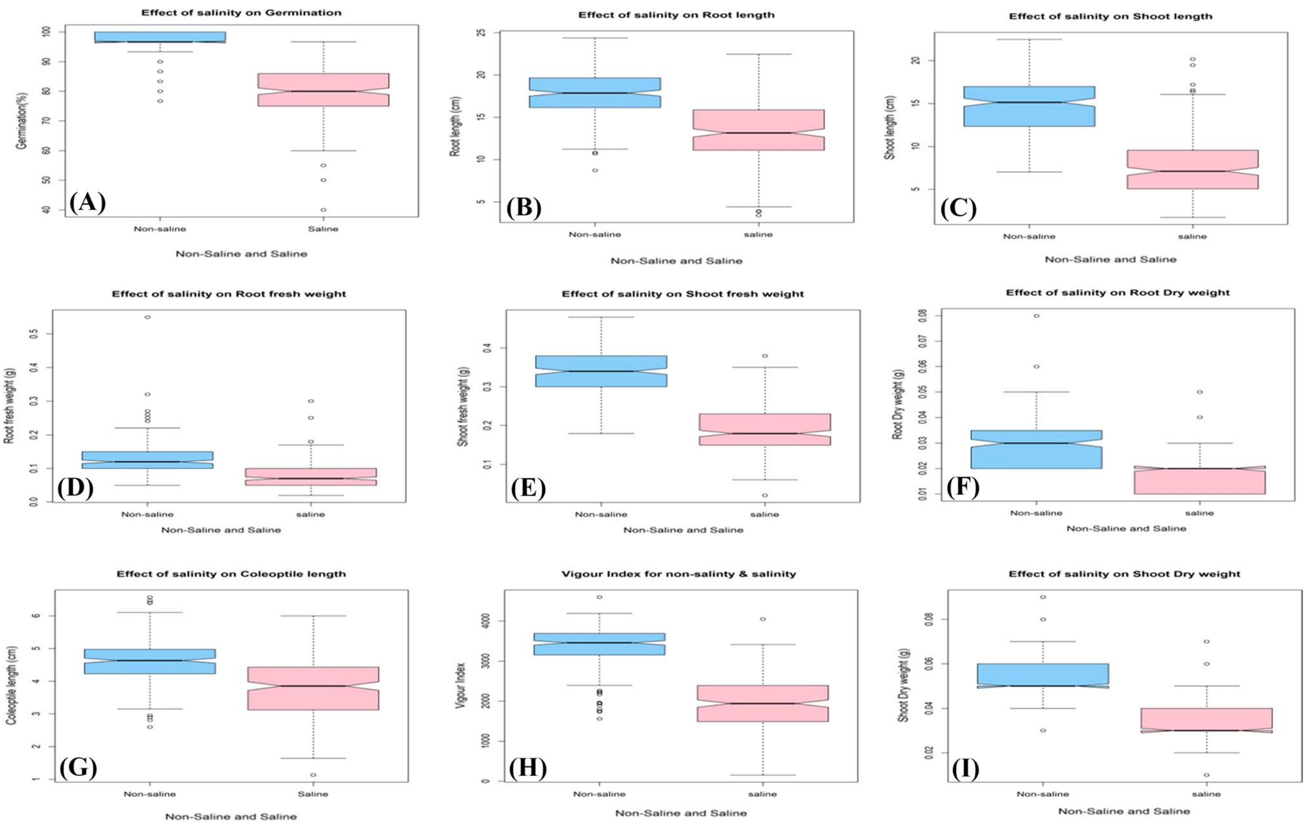


**Fig. 1** Box plots of growth trait data of *Triticum aestivum* under 0 mM NaCl (C) and 125 mM NaCl (S). Germination percentages (**A**), root length (**B**), shoot length (**C**), root fresh weight (**D**), shoot fresh weight (**E**), root dry weight (**F**), coleoptile length (**G**), vigor index (**H**), and shoot dry weight (**I**). Box edges show upper and lower quartile, and the median is shown in the middle of the box. Mild outliers are shown as dots

Kharchia 65 (5.80 cm) and minimum was 1.13 cm in IWA 8603011 followed by IWA WILSON VE 141 (1.64 cm) (Supplementary Table S3).

Significant reduction in RL was found under higher salt concentration. RL ranged between 3.39 and 20.00 cm with an average value of 13.08 cm under saline conditions. Maximum RL (20.00 cm) was found in landrace IWA 8607803 followed by IWA 8604640 (19.73 cm) under saline conditions. One landrace (IWA 8600527) had equal reduction in RL as in Kharchia 65, whereas 21 landraces had even less reduction in RL. Root and SFW are the measure of root and shoot development and hence their biomass. Salinity inhibited SL of all genotypes and hence influenced the SLSI. Dry weight, measured after drying the root and shoot, gives the absolute weight of the tissue without water. These are the traits which can be used as preliminarily criteria to evaluate growth of a specific genotype under stress conditions. The minimum reduction in dry root and shoot weight among control cultivars was in Kharchia 65 and KRL 213 genotypes, respectively. IWA 8607910, IWA 8600461, PI 225290 landraces had less reduction in RDW, whereas 50 landraces had less reduction in SDW than the best check (Supplementary Table S3).

Dendrogram from cluster analysis (Fig. 2) showed that all genotypes were divided into six clusters. The cluster analysis based on complete linkage correlation coefficient distance was performed in the present study which split the 243 wheat genotypes into six clusters (Table 2, Fig. 2). The cluster-1 comprised of twenty-six genotypes, among which one was ranked highly tolerant and rest 25 were grouped as non-tolerant landraces. Cluster 2 comprised of maximum number of tolerant genotypes (188) which could be categorized into 2 highly tolerant, 8 moderately tolerant, and rest 178 as non-tolerant landraces. Cluster-3 comprised of twenty-two genotypes consisting 9 highly tolerant, 6 moderately tolerant, and 7 non-tolerant genotypes. Clusters 4, 5, and 6 had two, four, and one genotypes, respectively. Cluster three represented the maximum landrace accessions with salt stress tolerance. The best check Kharchia 65 was included in cluster 5, whereas KRL 213 and PBW 725 were in cluster 2. HD 2967 was grouped in cluster 1. This shows diversity in the landraces set and

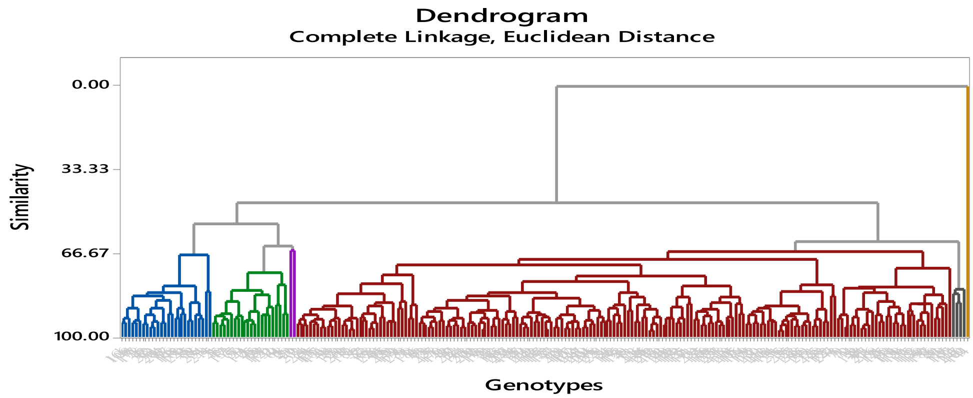


**Fig. 2** Dendrogram from cluster analysis for salt tolerance in different genotypes

**Table 2** Distribution of Iranian landraces and check varieties in clusters

| Cluster number | Number | Salinity tolerance status | Cluster number | Number | Salinity tolerance status |
|---|---|---|---|---|---|
| 1 | 26 | 1 highly tolerant<br>25 non-tolerant | 5 | 4 | 2 highly tolerant<br>2 non-tolerant |
| 2 | 188 | 2 highly tolerant<br>8 moderately tolerant<br>178 non-tolerant | 6 | 1 | Non-tolerant |
| 3 | 22 | 9 highly tolerant<br>6 moderately tolerant<br>7 non-tolerant | | | |
| 4 | 2 | 1 moderately tolerant<br>1 non-tolerant | | | |

hence can be used as parents to build up populations for selection of transgressive segregants against salt stress in subsequent generations.

The correlation analysis was also performed to understand the architecture of sub components of salt stress tolerance at seedling stage for screening the germplasm. The correlation analysis (Fig. 3) indicated some important associations among the germination, CL, root and SL, root and shoot biomass and VI. Significant and positive correlation was observed between CLSI and RDWSI, RFW stress tolerance index (RFWSI), RL stress tolerance index (RLSI), SFW stress tolerance index (SFWSI), SLSI, SDWSI, and VISI, similarly for RDWSI and RFWSI, RLSI, SFWSI, SLSI, SDWSI, and VISI. Significant and positive correlations were also obtained between RFWSI and RLSI, SFWSI, SLSI, and SDWSI, and relationship between RFWSI and RLSI, SFWSI, SLSI, and SDWSI was also positive. Also, significantly positive correlation was recorded between CLSI and RDWSI, RFWSI, RLSI, SFWSI, SLSI, SDWSI, and VISI which clearly depict that these physiological indices can be utilized to screen the genotypes for salinity tolerance. These physiological indices could be a reliable and efficient method for assessing salt tolerance in wheat genotypes.

## Discussion

Present results indicated that stress tolerance indices could explain some of the mechanisms indicating tolerance to salinity. Landrace accession IWA 8600278, IWA 8600291, IWA 8611786, IWA 8600179, and IWA 8600303 was most tolerant for salt stress. The genotypes could be efficiently categorized into highly tolerant, moderately tolerant, and non-tolerant classes based on the seedling indices so as to facilitate their further use in breeding programs. The data in the present study indicated that the genotypes with high GRSI, CLSI, RLSI, SLSI, RFWSI, SFWSI, and VISI were tolerant to salt stress. Stress Tolerance Index (STI) and Geometric Mean Productivity index (GMP) are efficient for identification of genotypes with good performance to non-stress and stress conditions (Khalili et al. 2004; Golbashy et al. 2010). Furthermore, Moghaddam and Hadizadeh (2012) found that stress tolerance index (STI) was also useful in selection of maize genotypes significantly differing in a response under stress and non-stress conditions. Generally, indices having high correlations with plant response in stress and non-stress conditions are introduced as the best ones (Ashraf et al. 2015). Different

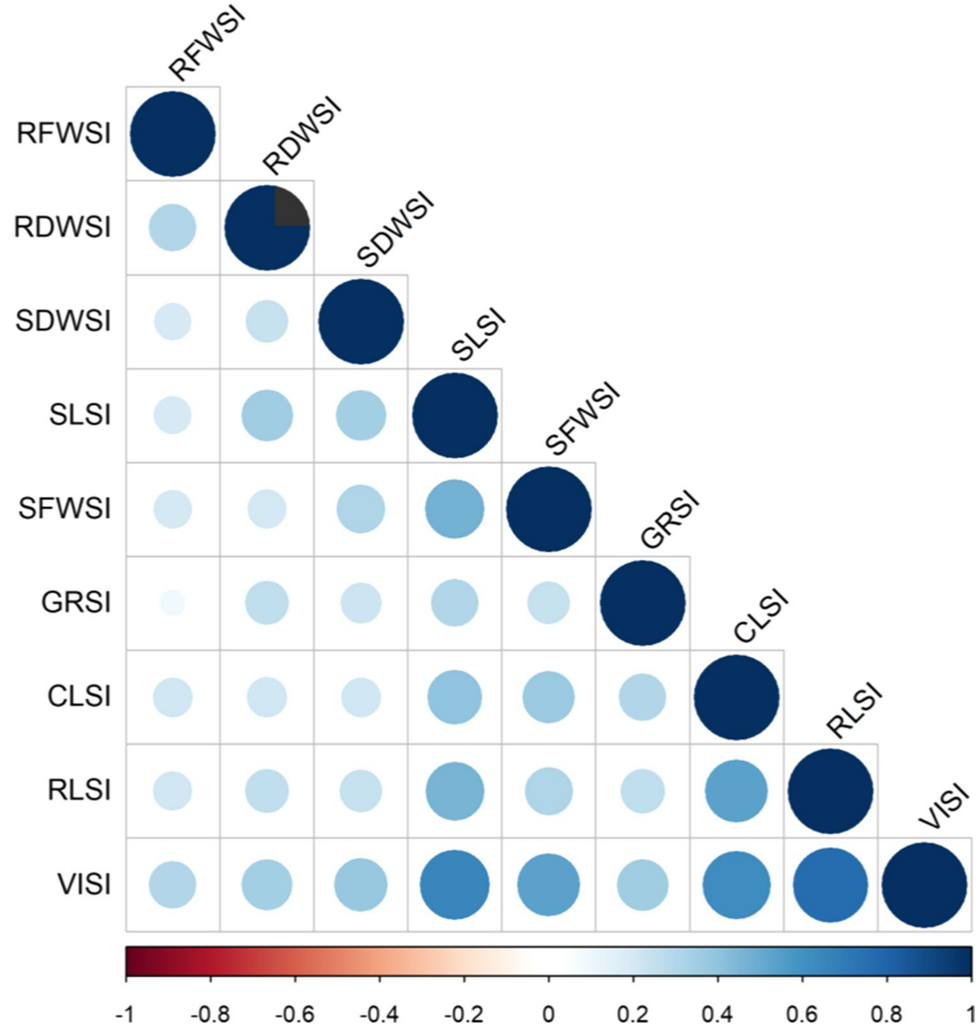


**Fig. 3** Correlation among different seedling traits

statistical approaches like absolute salt tolerance (Dewey 1962), relative salt tolerance (Maas 1986), susceptibility index (Fischer and Maurer 1978), and GGE biplot (Yan 2001; Ali et al. 2012) have been used to determine the salt tolerance response of crop plants. The tolerance to various stresses in field at adult plant stage is usually reflected by the tolerance at seedling stage of plant. This fact has been exploited with success in maize (Khan et al. 2003a), sorghum (Kausar et al. 2012), wheat (Ali et al. 2002; Khan et al. 2003b), soybean (Kamal et al. 2003), and cotton (Azhar and Ahmad 2000).

Iranian wheat landraces provide a rich source of genetic diversity and carry resistance for many biotic stresses such as bunt diseases (Bonman et al. 2015), Russian wheat aphid (Ehdaie and Baker 1999; Valdez et al. 2012; Bonman et al. 2015), leaf and stripe rusts (Kertho et al. 2015), stem rust (Rouse et al. 2011; Newcomb et al. 2013), and abiotic stresses such as salinity (Jafari-Shabestari et al. 1995), drought and heat (Ehdaie et al.1988). To date, most of the Iranian germplasm lines have not been characterized and efficiently used in modern plant breeding (Hoisington et al. 1999; Akbarpour et al. 2015). These germplasm lines not only provide new source of resistance to biotic and abiotic stresses, but also can enhance the diversity of breeding materials (Huang et al. 2010). These findings are in accordance with the results of Kausar et al. (2012).

Seed germination and early seedling growth determine stand establishment and yield potential. Germination is a crucial stage for plant establishment (Song et al. 2008) as poor germination may lead to poor stand establishment, resulting in lower grain yields and large number of studies on salt tolerance in different crop species is mostly based on the germination percentage (Song et al. 2008; Tlig et al. 2008; Badridze et al. 2009). Despite the importance of germination to production on salt-affected soils, more is known of the mechanism of salt tolerance in vegetative and reproductive stages than during germination (Zhang et al. 2010). In addition, the mechanisms for salt tolerance that have been identified during vegetative compartmentalization (Qiu et al. 2007) or $Na^+$ bound in starch granules (Kanai et al. 2007) are energy intensive and reduce carbohydrate reserves available to the seedling. Thus, although excess sodium and chloride ions imbalance has a deleterious effect on many cellular systems at all growth stages (Zhu et al. 1997), metabolic tolerance to these ions is more important during germination than at later life stages, due to the limited carbohydrates reserves available in the seed (Zhang et al. 2010). Saboora et al. (2006) reported nine wheat cultivars at germination and early seedling growth under six salt treatments and found that different salt treatments had significant negative effect on germination percentage, rate of germination, total dry weight, and dry weight of root and shoot. RL as a good selection criterion under salt stress conditions has also been reported by Ashraf et al. (1986). Previous studies also reported reduction in RL (Adcock et al. 2007), SL (Munns and James 2003), fresh root weight (Radi et al. 2013), and fresh shoot weight (Rahman et al. 2008). The genotypes with a higher VI under stress conditions were considered as salt-tolerant genotypes. Different researchers have used cluster analysis to group different wheat genotypes based on various characteristics and found similarities of wheat genotype within a group (Nookr and Khaliq 2007). The literature emphasizes on the use of cluster analysis to screen the crop germplasm for stress tolerance (Noorifarjam et al. 2013). Selected landraces could be used in further breeding programs for salt tolerance.

Soil salinization is a major factor contributing to the loss of productivity of cultivated soils, and a large salt-affected area and economical losses due to low productivity are a matter of concern for agriculturists. Moreover, population growth, urbanization, industrialization, and climate change including salinity stress create an alarming situation that poses a threat to national and international food security (Hussain et al. 2015). Developing wheat varieties that give a good yield on saline soils seems to be the long-term possible solution. In India, almost all salt-tolerant wheat germplasm is derived from Kharchia 65, a line developed from selections from farmers' fields in the sodic-saline soils of Kharchi–Pali area of Rajasthan (Rana 1986). KRL 1–4, as cross of Kharchia 65 with WL 711, has done well on the saline soils of northern India which was developed by pedigree method of selection and released through CVRC in 1990, but not in Pakistan, possibly because of the heavier soils and greater problems of waterlogging (Hollington 2000). In addition, two more varieties KRL 210 and KRL 213 have been released for cultivation under salt affected soils. The Pakistan selection Lu26s showed improved yields on saline soils in Pakistan (Qureshi et al. 1980), but it is susceptible to rust and not adapted to dense saline-sodic soils where there is the possibility of waterlogging. Lu26s was crossed with Kharchia, and two salt-tolerant genotypes, S24 and S36, were selected from $F_3$ seed at maturity levels of 24 and 36dS/m, respectively (Ashraf and O' Leary 1996). Cultivar S24 was derived from the cross of Lu26s and Kharchia (Ashraf and O' Leary 1996). S24 had high salt tolerance, as high as Kharchia and SARC-1 possibly due to its low leaf $Na^+$ accumulation (Ashraf 2002). Yield can be increased significantly by developing salt-tolerant crop plants (Clark and Duncan 1993) by exploiting genetic diversity for salt tolerance in species and developing reliable screening techniques.

Usually the leads obtained by screening wide germplasm are not utilized for cultivar development (Munns et al. 2006). Though the identified landraces could not be directly cultivated on salt-affected soils, it can be efficiently used for breeding germplasm tolerant to salt stress. The information regarding significant correlations among the characters is important for initiation of any breeding program because it provides a chance for selection of desirable genotypes with desirable traits simultaneously (Ali et al. 2009). Since the area under salinity stress is increasing at an alarming rate, the identification of tolerant sources and quick mobilization of components of tolerance in elite backgrounds is very urgent; these identified donors can be of immense use. The tolerant landraces identified in this study are under further investigation to determine the genetic basis of tolerance and mobilization of tolerance into adapted elite cultivars.

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s42976-022-00245-6.

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