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Research article

Assessment of the salt tolerance of diverse bread wheat (*Triticum aestivum* L.) genotypes during the early growth stage under hydroponic culture conditions

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ABSTRACT

Objectives: Soil salinity affects the growth of crop plants, leading to reduced productivity, and is a major challenge for wheat production worldwide. Various adaptations and mitigation approaches in combination with tolerant wheat genotypes can be useful for the sustainability of crop production in saline environments. However, the development of salt-tolerant wheat genotypes is one of the best and most efficient solutions for obtaining desirable yields. Considering these issues, an investigation was carried out under hydroponic nutrient culture conditions to assess the genetic variability and selection of salt-tolerant wheat genotypes by categorizing inequitable morphophysiological and genetic variability as well as multivariate analysis.

Methods: To meet the objectives of this study, 100 wheat genotypes were tested hydroponically in 0 (control) and 15 dS m^{-1} salt solutions.

Conclusion: For all the wheat genotypes grown under saline conditions, the shoot length (SL), root length (RL), shoot fresh weight (SFW), root fresh weight (RFW), total fresh weight (TFW), shoot dry weight (SDW), root dry weight (RDW), and total dry weight (TDW) decreased significantly. Furthermore, significant variation was observed among the genotypes in terms of their characteristics only under saline conditions. In the case of genetic diversity analysis, a high genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), genetic advance in the percentage of the mean (GAM) and high heritability (h^2b) were recorded for all tested wheat genotypes based on the SDW, RDW and TDW. Correlation analysis for both genotypic and phenotypic relationships revealed strong positive correlations for TDW, SDW, TFW and SFW. Principal component analysis (PCA) revealed that TDW, TFW, SDW, and SFW were the most

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discriminative variables for the wheat genotypes, which was confirmed by discriminant function analysis (DFA). PCA-biplot analysis also revealed significant positive correlations between SDW and SFW and between TDW and TFW. Hierarchical cluster analysis was performed for ten clusters based on the relative performance of the genotypes, where the genotypes were characterized into salt-tolerant, medium-salt-tolerant, medium-salt-susceptible and salt-susceptible groups. Among the genotypes, G11, G25 and G29 under cluster VII were categorized as salt tolerant based on their outstanding performance in terms of characteristics only under saline conditions. D^2 analysis proved that the wheat genotypes of this cluster were highly divergent from the other cluster genotypes; as a result, these genotypes might be utilized as parents in the development of salt-tolerant wheat genotypes. The current study concluded that SDW and TDW could be employed as criteria for selecting and defining salt-tolerant genotypes during the early growth stage of wheat.

Table 1
List of wheat genotypes used as experimental materials.

Genotype code	Genotype name	Genotype code	Genotype name
G1	Shatabdi	G51	BAW 1410
G2	BARI Gom 25	G52	BAW 1411
G3	BARI Gom 33	G53	BAW 1412
G4	KRL- 19	G54	BAW 1413
G5	KRL-210	G55	BAW 1414
G6	BioW-6	G56	BAW 1415
G7	BioW-12	G57	BAW 1416
G8	BioW-13	G58	BAW 1417
G9	BioW-23	G59	BAW 1418
G10	BioW-27	G60	BAW 1419
G11	BioW-31	G61	BAW 1420
G12	BioW-41	G62	BAW 1421
G13	BioW-46	G63	BAW 1422
G14	BioW-50	G64	BAW 1423
G15	BioW-65	G65	BAW 1424
G16	BioW-67	G66	BAW 1425
G17	BioW-68	G67	G67
G18	Nax1	G68	G68
G19	Nax2	G69	G69
G20	BAW 1147	G70	G70
G21	BAW 1290	G71	G71
G22	BAW 1243	G72	G72
G23	BAW 1286	G73	G73
G24	BAW 1290	G74	G74
G25	BAW 1318	G75	G75
G26	BAW 1322	G76	G76
G27	BAW 1340	G77	G77
G28	BAW 1344	G78	G78
G29	BAW 1349	G79	G79
G30	BAW 1354	G80	G80
G31	BAW 1359	G81	G81
G32	BAW 1373	G82	G82
G33	BAW 1374	G83	G83
G34	BAW 1378	G84	G84
G35	BAW 1385	G85	G85
G36	BAW 1390	G86	G86
G37	BAW 1391	G87	G87
G38	BAW 1393	G88	G88
G39	BAW 1394	G89	G89
G40	BAW 1397	G90	G90
G41	BAW 1399	G91	G91
G42	BAW 1401	G92	G92
G43	BAW 1402	G93	G93
G44	BAW 1403	G94	G94
G45	BAW 1404	G95	G95
G46	BAW 1405	G96	G96
G47	BAW 1406	G97	G97
G48	BAW 1407	G98	G98
G49	BAW 1408	G99	G99
G50	BAW 1409	G100	G100

1. Introduction

Wheat (*Triticum aestivum* L.) is the number one crop grown to meet human food needs worldwide [1–3]. Wheat is considered the staple food of more than 36% of the world's population, and it provides 20% of the calories and 55% of the carbohydrates globally [4,

Fig. 1. Pictorial view of hydroponically grown wheat genotypes under different levels of salinity.

5]. It dominates most arable land (38.85%) with relatively high grain protein (12–15%). Previously, it was reported that salinity affects approximately 20% of cultivated land worldwide [6]. Soil salinity decreases the production of crops by up to 60%, and its impact is more noticeable in the context of climate change [7]. The accumulation of additional salts in the soil top layer provides a highly stressful environment for plant growth, resulting in yield loss or plant death [8–12]. In Bangladesh, 1.056 million hectares of cultivable land in the southern region of the country are affected by various degrees of soil salinity and fallowing during the dry season [13]. As a consequence, there is an opportunity to enhance the cultivation of wheat in this area by utilizing salt-tolerant genotypes and narrowing the substantial gap between production (1.085 million metric tons) and needs (approximately 8.0 million metric tons) [14]. To address this problem, much attention has recently been given to developing salt-tolerant wheat plants by integrating new and old approaches to crop breeding with advances in management. For salt tolerance, conventional breeding needs to exploit existing genetic variation in the wheat crop. Research has not been performed in Bangladesh to identify significant variations in salt resistance in wheat. Local and/or exotic genetic diversity is also considerable and might help improve salt tolerance in future breeding strategies. Various approaches have been utilized for this purpose, including hydroponic systems, sand and pot cultures, saline-raised beds, and field screening [15,16].

Screening procedures for wheat genotypes at early stages of growth involving solution culture in hydroponic systems are helpful methods for distinguishing tolerant wheat genotypes because examining a large number of genotypes in the field for salt tolerance is challenging because of soil variability in salinity concentration and organic carbon, as well as because of the emission of carbon dioxide (CO_2) from the soil [17]. In contrast to soil, supplements are readily available to plants in solution culture, where the leaching or binding of supplements to soil reduces their availability to plants. Due to the low climatic variation, this technique is plausible [16] and has been employed by several investigators for genotype evaluation, particularly under saline conditions [18–21].

Furthermore, roots, shoots, and biomass have been identified as key features contributing to salt tolerance in wheat plants. Wheat researchers eventually reached the conclusion that shoot biomass and plant biomass might be used to select salt-tolerant genotypes [22–25]. Salt stress caused a significant decrease in wheat plant biomass, which was more pronounced at high levels of salt stress than at low levels [19]. Given that salt-tolerant genotypes could have more relative characteristics than salt-susceptible genotypes, the greater biomass of tolerant genotypes could be linked to their ability to maintain a greater photosynthetic rate than that of susceptible genotypes [26]. Based on association and inheritance, it was assumed that root and shoot lengths and their fresh and dry weights exhibit positive relationships and high heritability, implying that these factors could be beneficial standards for selecting salt-tolerant genotypes [15]. Detecting genetic variation is a critical component of any breeding strategy for generating salt-tolerant genotypes. The current study aimed to investigate the genetic variability and selection of salt-tolerant wheat genotypes by categorizing inequitable morphophysiological and genetic variability and performing multivariate analysis.

2. Materials and methods

2.1. Plant materials and location

In this experiment, one hundred wheat genotypes were used, as shown in Table 1. These genotypes were obtained from the Regional Station of the Bangladesh Wheat and Maize Research Institute (BWMRI), Gazipur, Bangladesh. The current study was conducted in a hydroponic system (Fig. 1) at the Regional Station of the Bangladesh Wheat and Maize Research Institute (BWMRI), Gazipur, which is located 23099/N, 90041/E, 14 m above sea level. The genotypes were randomized following a split-plot completely randomized design (CRD) with four replications, where salinity was the main plot treatment and genotype was the subplot treatment.

2.2. Hydroponic system and application of salinity

Seedlings were grown in rectangular fibre-reinforced plastic trays (1.22 × 0.61 × 0.11 m), in which a lid (1.22 × 0.61 m) with 50 holes (diameter of 10 cm) was placed on each tray. A perforated fibre-reinforced plastic Petri dish was placed in each well (Fig. 1). Thus, fifty Petri dishes were placed in a tray. The diameter of every spot in the Petri dishes was 3 mm, with four holes of 4 mm at equal distances from the periphery of the Petri dish. A mesh-like bandage cloth with four wicks was placed over the Petri dishes so that the wicks could fit through the 4 mm holes to absorb the salt solution inside the tray. Each tray contained approximately 80 L of solution and was painted on the outside to hinder algae growth by creating a gloomy environment inside the tray. The trays were connected to four water tanks through supply pipes, and a regulator could control the solution supply from the container to every tray (Fig. 1).

Twenty-five seeds of each genotype were placed on January 11, 2021 on a bandage cloth in the hole of a Petri dish so that the roots could easily reach the salt solution in the tray. Seedlings were grown with normal water supplied from the container to trays for ten days. Ten days after the seeds were placed in the Petri dish, the water in half of the trays was replaced with a salt solution (15 dS $m^{-1)}$ mixed with nutrient solution from the container, and half of the trays were replaced with regular water mixed with nutrient solution. Ten plants were kept in each tray before the salt solution was added, and the remaining plants were removed. The salt solution was made by diluting the seawater with tap water. Seawater was collected from the Bay of Bengal with a 32 dS m^{-1} EC salinity level. Hoagland solution [27] was used for fertilization, and 10 ml/L solution was mixed with the solution in the water tanks. First, a nutrient solution was prepared with distilled water. The trays were aerated using an air pump. The pH of the solution was maintained between 6.0 and 6.5 by adding NaOH as needed. The complete hydroponic system was covered with a net to protect the plants from bird damage.

2.3. Data collection

Thirty-five-day-old plants of each genotype were collected from all replications to record shoot length (SL) and root length (RL) in cm and shoot fresh weight (SFW) and root fresh weight (RFW) in g. A graduated ruler was used to measure RL from the root–shoot joint to the end of the root tip and SL from the root–shoot joint to the upper tip of the leaf of the same plant. The seedlings were then trimmed at the root–shoot junction to divide each portion. The weights of the RWs and SWWs were calculated using a digital electronic balance (High Precision Electric Balance, FGH series, A&D Company Ltd., Korea). The total fresh weight (TFW) in g was calculated by adding RFW and SFW. The shoots and roots were dried at 70 °C for 72 h. After drying, the shoot (SDW) and root (RDW) were weighed, and the corresponding data were recorded in mg. The SDW and RDW per plant were computed by dividing by the number of plants sampled. The total dry weight (TDW) per plant was computed by summing the SDW and RDW. The relative traits were calculated using the following formula:

$$\text{Relative variable} = \frac{\text{Value at control}}{\text{Value at salinity}}$$

2.4. Statistical analysis

The Statistical Tool for Agricultural Research (STAR) software, version 2.0.1, designed by the IRRI [28], was used for analysis of variance (ANOVA), principal component analysis (PCA) and cluster analysis for variables evaluated under salinity stress. SPSS software version 29.0.10 (171) was used to perform discriminant function analysis (DFA) and Mahalanobis (D^2) distance analysis [29]. The genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability (h^2b), genetic advance (GA), genetic advance in the percentage of the mean (GAM), and genotypic and phenotypic correlation coefficients among traits were calculated using the OPSTAT Statistical Software Package for Agricultural Research Workers [30]. Genotypic and phenotypic variance were calculated via ANOVA using Microsoft Excel and are presented in Table 2.

3. Results

3.1. Analysis of variance (ANOVA) and descriptive evaluation

The ANOVA findings for traits are displayed in Table 3. For all the variables investigated, ANOVA revealed highly significant variation among treatments, genotypes, and interactions between genotype and salinity. Because of the highly significant interaction between genotype and salinity, genotype and trait selection were performed using variable data under salinity conditions. ANOVA for attributes only under salinity conditions revealed highly substantial differences across the genotypes.

The descriptive statistics are shown in Table 4. Except for SL and RL, the eight quantitative characteristics exhibited a wide range of variability. Salinity inhibited the growth of all the plant components, resulting in lower TFW and TDW.

Salinity considerably reduced the values of all control variables. The SL in the control treatment varied from 24.0 to 46.5 cm, with a mean of 35.1 cm, while the salinity decreased to 11.1–35.0 cm, with a mean of 21.7 cm (Fig. 2 (a)). The range of RL measurements varied from 27.0 to 56.0 cm in the control, with an average of 39.9 cm (Fig. (b)). This range decreased to 7.0–38.0 cm under saline conditions, with an average of 23.8 cm (Fig. 2 (b)). The SFW ranged from 0.67 to 1.76 g in the control, with an average of 1.19 g plant^{-1}. When exposed to saline conditions, the SFW decreased from 0.25 to 0.86 g plant^{-1}, with an average of 0.51 g plant^{-1} (Fig. 2 (c)). The RFW varied between 0.52 and 1.76 g plant^{-1} under control conditions, with an average of 0.80 g plant^{-1}. However, under saline conditions, it decreased from 0.26 to 0.65 g plant^{-1}, with an average of 0.42 g plant^{-1} (Fig. 2(d)). In the control, the TFW ranged from 1.33 to 3.30 g plant^{-1}, with a mean of 1.98 g plant^{-1} (Fig. 2(e)).

Table 2
A list of formulas for calculating genetic parameters.

Genetic parameter	Abbreviation	Formula
Genotypic variance	δ^2g	$\delta^2\,g = \frac{GMS - EMS}{r}$
Phenotypic variance	δ^2p	$\delta^2 p = \delta^2 g + EMS$
Genotypic coefficient of variation	GCV	$GCV\,(\%) = \frac{\sqrt{\delta^2\,g}}{\bar{x}} \times 100$
Phenotypic coefficient of variation	PCV	$PCV\,(\%) = \frac{\sqrt{\delta^2\,p}}{\bar{x}} \times 100$
Heritability in the broad sense	h^2b	$h^2 b = \frac{\delta^2\,g}{\delta^2\,p} \times 100$
Genetic Advance	GA	$GA = h^2 b.i.\delta p$
Genetic Advance in Percent of Mean	GAM	$GAM = \frac{GA}{\bar{x}}$

GMS = genotypic mean square, EMS = error mean square, r = number of replications, $\bar{x}$ = population mean, i = selection differential, the value of which is 1.76 at the 10% level of selection intensity, δp = phenotypic standard deviation.

Table 3
Combined analysis of variance of traits of evaluated wheat genotypes.

Source of variation	df	Mean square							
		SL	RL	SFW	RFW	TFW	SDW	RDW	TDW
Salinity (S)	1	35687**	51912**	92.01**	28.45**	222.70**	2354016**	174138**	3808800**
Error I	6	67.26	167.71	0.112	0.0069	0.1717	560.83	557.11	2207.04
Genotype (G)	99	60.45**	53.18**	0.127**	0.0597**	0.2763**	4790**	321.46**	5799**
S x G	99	14.92**	35.02**	0.061**	0.037**	0.1384**	2138**	234.69**	2704**
Error II	594	6.42	13.80	0.0042	0.0009	0.0056	28.37	1.37	29.97
The mean square of values for only salinity									
Genotype	99	40.49**	43.76**	0.0332**	0.0154**	0.0699**	1793.63**	40.42**	2071.28**
Error	300	6.30	14.86	0.0022	0.0008	0.0036	56.23	5.58	71.65

df indicates degrees of freedom, p*<0.05, p**<0.01, SL = shoot length (cm), RL = root length (cm), SFW = shoot fresh weight (g), RFW = root fresh weight (g), TFW = total fresh weight (g), SDW = shoot dry weight (mg), RDW = root dry weight (mg), and TDW = total dry weight (mg).

Table 4
Descriptive statistics for wheat genotype traits.

Traits	Control		Salinity (15 dS m^{-1})	
	Range	Mean ± SD	Range	Mean ± SD
SL (cm)	24.0–46.5	35.1 ± 3.8	11.1–35.0	21.7 ± 3.9 (0.62 ± 0.07)
RL (cm)	27.0–56.0	39.9 ± 4.8	7.0–38	23.8 ± 4.7 (0.60 ± 0.09)
SFW (g $plant^{-1}$)	0.67–1.76	1.19 ± 0.21	0.25–0.86	0.51 ± 0.10 (0.43 ± 0.08)
RFW (g $plant^{-1}$)	0.52–1.55	0.80 ± 0.15	0.26–0.65	0.42 ± 0.07 (0.54 ± 0.09)
TFW (g $plant^{-1}$)	1.33–3.30	1.98 ± 0.31	0.54–1.46	0.93 ± 0.14 (0.47 ± 0.07)
SDW (mg $plant^{-1}$)	124–300	197 ± 35.8	40–196	90.1 ± 22.1 (0.46 ± 0.10)
RDW (mg $plant^{-1}$)	23–98	43 ± 11.6	6–26	13.8 ± 3.7 (0.33 ± 0.09)
TDW (mg $plant^{-1}$)	160–366	242 ± 40.3	48–205	103.9 ± 23.8 (0.43 ± 0.09)

SL = shoot length, RL = root length, SFW = shoot fresh weight, RFW = root fresh weight, TFW = total fresh weight, SDW = shoot dry weight, RDW = root dry weight, TDW = total dry weight, Min = minimum, Max = maximum, SD = standard deviation. The figure in parentheses shows the average relative values as a ratio of control and salinity-affected plants.

The salinity decreased from 0.54 to 1.46 g $plant^{-1}$, with a mean of 0.51 g plant-1. The SDW ranged from 124 to 300 mg plant-1 in the control, with a mean value of 197 mg $plant^{-1}$ (Fig. 2 (f)). However, under saline conditions, the trait decreased from 40 to 196 mg $plant^{-1}$, with a mean value of 0.51 g $plant^{-1}$. The RDW ranged from 23 to 98 mg $plant^{-1}$ in the control, with a mean of 43 mg $plant^{-1}$, but decreased from 6 to 26 mg $plant^{-1}$ under saline conditions, with a mean of 13.8 mg $plant^{-1}$ (Fig. 2(g)). The crucial characteristic TDW varied between 160 and 366 mg $plant^{-1}$ in the control group, with a mean of 242 mg $plant^{-1}$; in terms of salinity, it decreased from 48 to 205 mg $plant^{-1}$, with a mean of 103.9 mg $plant^{-1}$(Fig. 2(h)). The vast variance in all the plant attributes provided a selection opportunity for diverse quantitative wheat characteristics. Box plots revealed differences in the measured plant attributes among the wheat genotypes under saline and nonsaline conditions (Fig. 2(a–h).

3.2. Genetic diversity analysis

The genotypic and phenotypic variances (σ^2g and σ^2p), phenotypic and genotypic coefficients of variation (GCV and PCV), broad-sense heritability (h^2b), genetic advance (GA) and genetic advance in the percentage of the mean (GAM) derived using trait values grown under salinity are shown in Table 5.

The PCV was greater than the GCV for all the relative characteristics, indicating that there was some interaction with the environment. The characteristics examined in this study demonstrated intermediate to high PCV and GCV (15.0–24.5 and 11.4–23.2, respectively), with high levels of PCV and GCV in the SDW (24.5 and 23.2%, respectively), RDW (24.2 and 22.7%, respectively) and TDW (22.7 and 21.6%, respectively). Moderate PCV and GCV were found for all the remaining traits. A trait of heritability is deemed extraordinarily high or high when the figure is 80% or higher, moderate when it varies from 40 to 80%, and low when it is less than 40%. In the present study, except for SL and RL, all the traits exhibited high heritability (>80%), whereas SL had medium heritability (58.4%), and RL had low heritability (33.8%).

3.3. Correlation analysis

The genotypic and phenotypic correlation coefficients for all pairs of the eight characteristics are shown in Table 6. All the traits showed significant positive correlations with each other in terms of both genotypic and phenotypic correlation, and all the genotypic correlation coefficients were greater than the phenotypic correlation coefficients, with the same correlation coefficients between SDW and TDW (0.992) in both cases.

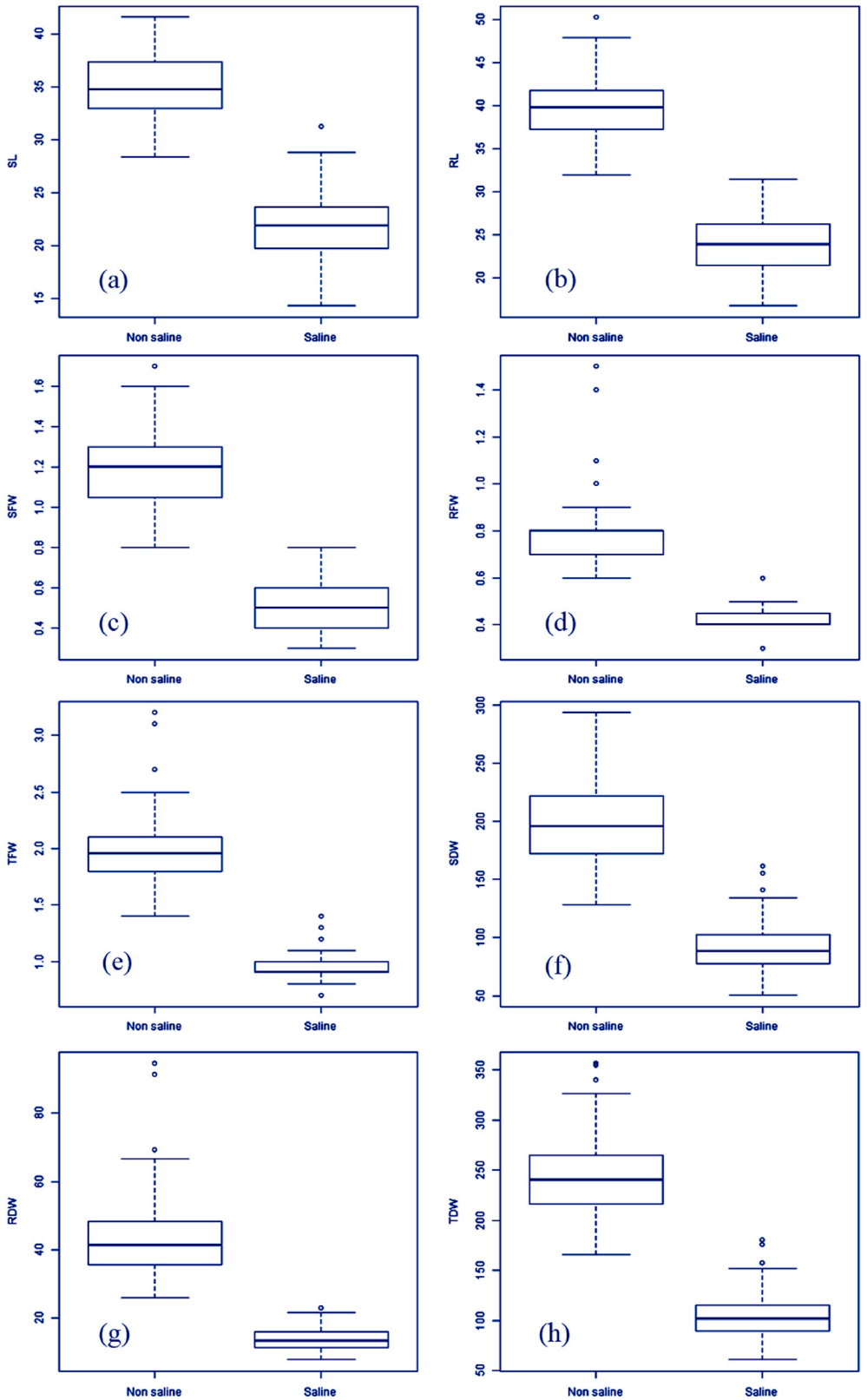

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Fig. 2. Box plots illustrating genotypic differences based on (a) shoot length (SL), (b) root length (RL), (c) shoot fresh weight (SFW), (d) root fresh weight (RFW), (e) total fresh weight (TFW), (f) shoot dry weight (SDW), (g) root dry weight (RDW) and (h) total dry weight (TDW) both in non-saline and saline environments.

Table 5
Genetic variability in wheat genotypes based on trait values under salinity.

Relative traits	σ^2g	σ^2p	GCV (%)	PCV (%)	h^2_b (%)	GA	GAM
SL	8.5475	14.8475	13.4	17.6	58.4	4.60	21.16
RL	7.225	22.085	11.4	19.5	33.8	3.23	13.59
SFW	0.00775	0.00995	17.5	19.2	82.9	0.17	32.74
RFW	0.00365	0.00445	14.3	15.8	81.9	0.11	26.75
TFW	0.016575	0.020175	13.9	15.0	86.5	0.25	26.73
SDW	434.35	490.58	23.2	24.5	89.5	40.64	45.13
RDW	8.71	14.29	22.7	24.2	88.3	6.1	43.97
TDW	499.9075	571.5575	21.6	22.7	90.6	44.0	42.38

SL= Shoot length, RL = Root length, SFW= Shoot fresh weight, RFW= Root fresh weight, TFW = Total fresh weight, SDW= Shoot dry weight, RDW= Root dry weight, TDW = Total dry weight, σ^2g: Genotypic variance, σ^2p: Phenotypic variance, GCV: Genotypic coefficient of variation, PCV: Phenotypic coefficient of variation, h^2_b: Heritability (broad sense), GA: Genetic advance, GAM: Genetic advance in percentage of mean.

Table 6
Genotypic (upper diagonal) and phenotypic (lower diagonal) correlations among the trait relative values of the evaluated wheat genotypes.

	SL	RL	SFW	RFW	TFW	SDW	RDW	TDW
SL		0.720**	0.628**	0.169**	0.509**	0.780**	0.307*	0.767**
RL	0.321**		0.440*	0.198**	0.394**	0.510**	0.341**	0.521**
SFW	0.485**	0.242**		0.491**	0.914**	0.793**	0.517**	0.809**
RFW	0.121*	0.106*	0.417**		0.802**	0.420**	0.642**	0.480**
TFW	0.398**	0.220**	0.900**	0.771**		0.739**	0.653**	0.778**
SDW	0.589**	0.283**	0.690**	0.369**	0.660**		0.456**	0.992**
RDW	0.218**	0.214**	0.437**	0.545**	0.567*	0.406**		0.562**
TDW	0.581**	0.295**	0.707**	0.422**	0.691**	0.992**	0.521**	

p*≤0.05, p**≤0.01, SL = shoot length, RL = root length, SFW = shoot fresh weight, RFW = root fresh weight, TFW = total fresh weight, SDW = shoot dry weight, RDW = root dry weight, TDW = total dry weight.

3.4. Principal component analysis (PCA)

3.4.1. Eigenvalues and latent vectors connected with principal component analysis (PCA)

Eigenvalues and latent vectors connected with PCA for wheat genotypes based on trait values are shown in Table 7. It is a data reduction approach that has been used to investigate interdependence and seeks to reduce complicated and diverse relationships through a set of discernible variables by recognizing standard dimensions or components that are linked with apparently unconnected variables. PCA was performed on all the plant characteristics under salinity. PCA clearly and concisely explained the genotypic variance in the degree of salt tolerance in the wheat genotypes. The authors combined the first three PCs with eigenvalues greater than one, explaining 87.13% of the genotypic diversity in salt tolerance. The first PC accounted for 61.83% of the genotypic variation in tolerance to salinity, to which all the traits contributed positively. Among the traits, TDW contributed significantly, followed by TFW, SDW, and SFW, with PC values of 0.4182, 0.4102, and 0.4038, respectively (Table 7). The second PC explained 16.10% of the

Table 7
Eigenvalues and latent vectors connected with principal components in wheat genotypes based on trait values.

Relative traits	Latent vectors		
	1	2	3
SL	0.3209	0.4894	−0.0221
RL	0.235	0.4168	0.7764
SFW	0.3974	−0.0137	−0.2522
RFW	0.2909	−0.5629	0.1877
TFW	0.4102	−0.2733	−0.0864
SDW	0.4038	0.202	−0.2978
RDW	0.305	−0.3682	0.3902
TDW	0.4182	0.1364	−0.2225
Eigenvalues	4.9461	1.2877	0.7361
Proportion of variance (%)	61.83	16.10	9.20
Cumulative variance (%)	61.83	77.93	87.13

variation, where the major contributor was SL, followed by RL and PC, with values of 0.4894 and 0.4168, respectively. The third PC contributed 9.20% of the variance, in which the main contributor was RL (PC score of 0.7764), followed by RDW (PC score of 0.3902).

A biplot of the genotype × trait based on comparable characteristics of the wheat genotypes is presented in Fig. 3. PC1 and PC2 created a PC biplot based on the principal component analysis. By constructing acute angles, the trait vectors demonstrated positive relationships.

3.5. Cluster analysis

One hundred genotypes (Table 1) with high genetic variability (Table 5) were grouped into ten clusters using agglomerative Ward's linkage cluster analysis (Fig. 4). The salinity traits were used to group the genotypes. Cluster I consisted of nineteen genotypes (G1, G2, G7, G10, G15, G16, G17, G19, G36, G41, G44, G55, G59, G61, G62, G63, G74, G77 and G86); cluster II comprised fourteen genotypes (G3, G31, G32, G37, G39, G42, G50, G53, G56, G66, G87, G89, G92 and G97); cluster III included twelve genotypes (G4, G13, G28, G33, G40, G52, G60, G65, G76, G78, G80 and G83); cluster IV had three genotypes (G5, G14 and G18); cluster V was composed of eight genotypes (G6, G27, G30, G47, G64, G84, G91 and G100); and cluster VI was composed of sixteen genotypes (G8, G9, G20, G21, G24, G38, G48, G51, G54, G57, G58, G73, G79, G81, G88.

The mean values of the clusters are presented in Table 8. Cluster VII had the greatest mean trait values for all characteristics except relative SL and RL, where RL (26.50 cm) was the second largest and SL was also good (23.62 cm); thus, the genotypes in Cluster VII might be considered salt tolerant.

Cluster X was characterized by the genotypes with the highest SL (27.38 cm) and the second most important characteristics, TDW (147.72 g plant^{-1}), SDW (133.13 g plant^{-1}), SFW (0.60 g plant^{-1}) and a high amount of TFW (1.01 g plant^{-1}). Cluster V included the genotypes with the second highest TFW (1.05 g plant^{-1}) and SFW (0.60 g plant^{-1}) and with good amounts of TDW, SDW, RDW and RFW per plant. Consequently, clusters V and X were moderately salt-tolerant groups. The poorest and closest values of traits to each other were found for the genotypes in clusters I, II, VI, VIII and IX; these were considered moderately salinity-susceptible genotypes. The lowest values for all the characteristics were observed in cluster III, except for SDW, followed by cluster IV for most of the characteristics; thus, the genotypes in these two clusters were identified as salt susceptible.

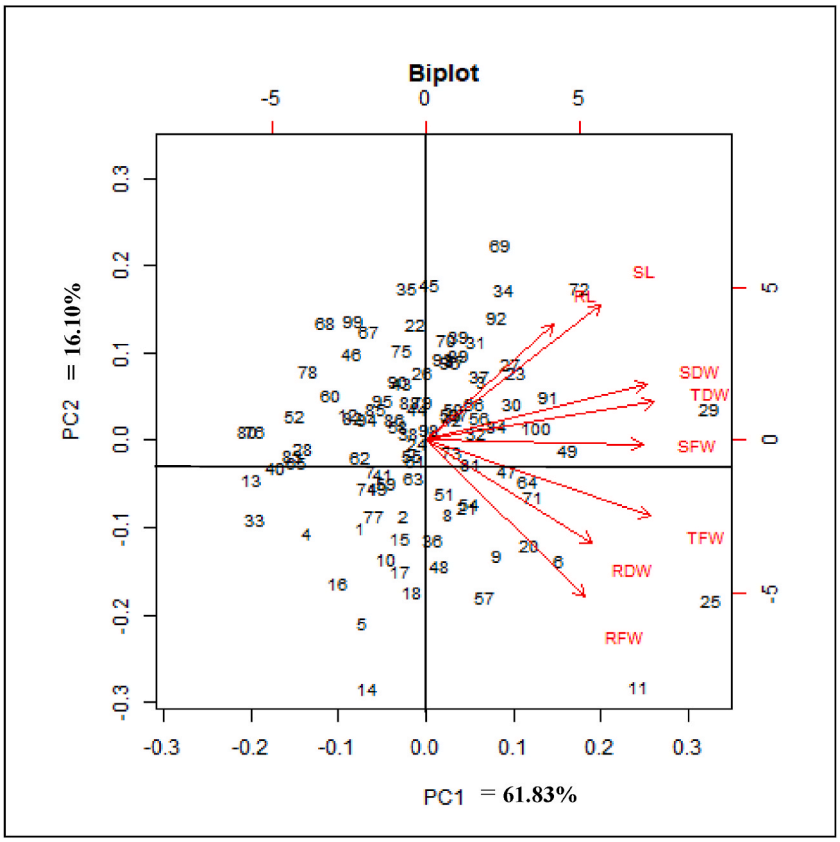

Fig. 3. Genotype × trait biplot based on comparable characteristics of wheat genotypes.

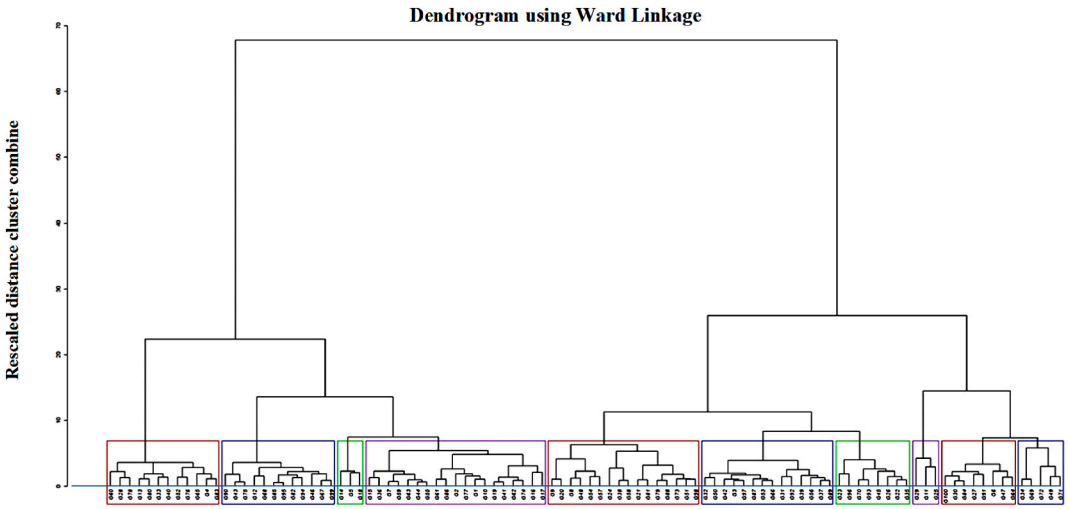

Fig. 4. Dendrogram of 100 wheat genotypes generated by using the agglomerative clustering method with Ward's linkage and Euclidean distance based on traits under salinity. Cophenetic Correlation Coefficient = 0.43.

Table 8
Comparison among ten clusters of wheat genotypes clustered by the agglomerative Ward's linkage method based on mean trait values under salinity.

Traits	Clusters									
	I	II	III	IV	V	VI	VII	VIII	IX	X
Genotypes (no.)	19	14	12	3	8	16	3	12	8	5
SL (cm)	20.01	23.52	17.20	17.52	24.22	21.84	23.62	21.43	24.52	27.38
RL (cm)	20.91	27.81	19.25	21.83	25.78	24.78	26.50	24.62	25.41	22.55
SFW (g plant^{-1})	0.49	0.53	0.39	0.44	0.60	0.52	0.78	0.43	0.58	0.60
RFW (g plant^{-1})	0.42	0.42	0.34	0.49	0.45	0.48	0.58	0.38	0.37	0.42
TFW (g plant^{-1})	0.91	0.95	0.73	0.93	1.05	0.99	1.36	0.82	0.95	1.01
SDW (mg plant^{-1})	79.89	98.53	65.6	59.5	109.83	89.05	147.25	82.45	93.47	133.13
RDW (mg plant^{-1})	13.43	15.1	10.51	18.58	17.88	14.18	20.92	10.82	11.49	14.72
TDW (mg plant^{-1})	93.32	113.63	76.11	78.08	127.7	103.23	168.17	93.27	104.95	147.85

SL = shoot length, RL = root length, SFW = shoot fresh weight, RFW = root fresh weight, TFW = total fresh weight, SDW = shoot dry weight, RDW = root dry weight, TDW = total dry weight.

3.6. Discriminant function analysis (DFA)

DFA was performed to determine whether a specific set of characteristics from the eight previously described plant characteristics was used to separate the ten clusters. DFA is especially beneficial for discovering genotype groupings according to previous clustering criteria. It also provides a graphical display indicating the presence of clusters. Table 9 shows the association coefficients between the eight discriminatory factors and DFA-derived discriminant functions.

The variables were sorted according to the magnitude of their correlation with functions. The relative TDW was found to be at the

Table 9
Correlation matrix between discriminating variables and standardized canonical discriminant functions of 100 wheat genotypes as a classification criterion.

Relative traits	Canonical discriminant coefficients		
	1	2	3
TDW	0.589*	0.229	−0.451
TFW	0.586*	−0.542	−0.316
SDW	0.529*	−0.625*	−0.228
SFW	0.523	−0.218	−0.304
RDW	0.323	0.376	−0.461
RL	0.322	0.045	0.879*
RFW	0.300	0.611	−0.344
SL	0.203	−0.251*	0.061

[a] Variables are ordered by the absolute extent of correlation within the function: [the] largest correlation between each variable and any discriminant function.

top of the list of discriminatory variables, followed by TFW and SDW, with correlation coefficients of 0.589, 0.586, and 0.529, respectively, under function 1. TDW, followed by TFW and SDW, was found to play the most dominant role in explaining the tremendous variance in 100 wheat genotypes according to stepwise DFA. RFW, followed by RDW, had the highest correlation (0.611 and 0.376, respectively) between each variable and any discriminant function in function 2. The RFW and RDW were the most significant variables explaining the maximum variance under function 2. Fig. 5 shows how the wheat genotypes were classified into ten groups based on the first two discriminating functions. Therefore, better groups would rely principally on the researcher's (breeders') objectives in the breeding program. However, the graphical representation of the discriminating analysis provides information about genetic discrimination among clusters.

With some exceptions for RL and SL, the genotypes dispersed on the right side of the graph had greater values of traits, and those spread on the left side had lower values of the characters analysed. This was anticipated because the attributes contributed more to identifying genetic variability in function 1. The positive and significant contribution of the identical characteristics indicated in DFA to PC1 distinguished Cluster VII from the other clusters. The Cluster VII genotype could be salt tolerant due to its exceptional values, except for RL and Sl (Table 8). However, the characteristics of the genotypes in clusters V and X were intermediate. Because of the lower values of plant traits, the genotypes scattered on the left side of the graph (clusters III and IV) were susceptible to salinity. DFA used the test of equality of group means to determine the overall effects of the eight variables evaluated in the analysis under each cluster. The group means of the eight variables presented in Table 8 were significantly different from each other at a probability level of 0.001 according to the test of equality for DFA.

3.7. Mahalanobis (D^2) and intracluster distances

The Mahalanobis distance (D^2) of intercluster distances among the clusters was obtained via DFA, and intracluster distances were calculated via MS Excel via Euclidean distance matrices of the genotypes. Table 10 displays the inter- and intracluster (bold) distances. Genotypes in the same cluster (intracluster) are assumed to be more genetically correlated than the genotypes in another cluster (intercluster). All of the intercluster distances were greater than the intracluster distances, suggesting that there was more divergence among genotypes of different clusters than among those from the same cluster, suggesting that the clustering method grouped homogenous genotypes. The intracluster distances were small and close to each other, ranging from 0.139 to 0.246. Cluster VII had the shortest intracluster distance, whereas Cluster I had the greatest intracluster distance.

Clusters III and VII were the farthest apart, with 171.73 units, followed by Clusters III and X, which were 160.70 units apart. Except for RL, Cluster VII contained the genotypes that performed best in all of the characteristics. The Cluster III genotype, on the other hand, performed the worst. Clusters I and VIII (0.52 units) were most closely related, indicating that they were most similar, followed by Clusters III and IV (0.96 units). The most significant intercluster difference indicated that the genotypes in Cluster VII were very different from the genotypes in the other clusters.

3.8. Analysing the prediction efficacy of discriminating functions

DFA is also used to identify misclassified genotypes that have been reassigned to the proper cluster. The classification matrix of 10

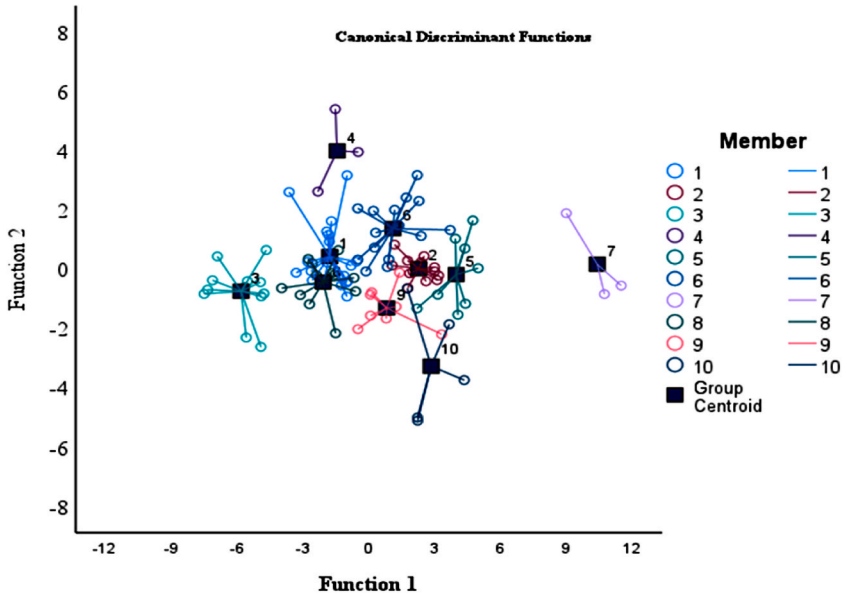

Fig. 5. Graphical representation of discriminant function analysis (DFA) of ten clusters of wheat genotypes treated with different levels of salinity.

Table 10
Intercluster (Mahalanobis distances- D^2) and intracluster (bold) distances of wheat genotypes based on trait values under salinity.

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X
I	0.246	30.05[a]	16.12[a]	11.56[a]	54.15[a]	7.81[a]	126.18[a]	0.52	11.14[a]	120.44[a]
II		0.221	75.18 [a]	40.39 [a]	6.98 [a]	7.21 [a]	62.95 [a]	17.92 [a]	1.40	47.36[a]
III			0.195	0.96	100.77 [a]	40.45 [a]	171.73 [a]	18.28 [a]	40.01 [a]	160.70 [a]
IV				0.172	59.32[a]	23.67[a]	123.98[a]	13.57[a]	27.02[a]	109.12[a]
V					0.177	24.73[a]	32.79[a]	38.61[a]	11.49[a]	17.94[a]
VI						0.215	91.86[a]	3.20	1.12	79.48[a]
VII							0.139	108.17[a]	67.74[a]	4.01[b]
VIII								0.207	6.25[a]	97.31[a]
IX									0.163	51.96[a]
X										0.208

[a] Distances differed from zero at a 99% confidence interval.
[b] Distances differed from zero at a 95% confidence interval.

wheat genotype clusters described the prediction performance of discriminatory functions during classification into distinct genotype clusters. Based on discriminant functions, each genotype was assigned. Clusters III, IV, V, VII, IX, and X were 100% accurate according to the DFA data. In the remaining clusters, more than 80% of the genotypes were appropriately assigned to clusters, with an average of 94% of the genotypes assigned to the original clusters (Table 11).

4. Discussion

To generate salinity-tolerant wheat genotypes, a selection procedure and genotypic variation are required [31,32]. However, the genetic basis of salt tolerance in wheat breeding is limited [22,33,34]. The shortage of genetic diversity restricts progress in improving wheat salt tolerance [35]. In the present study, wheat plants were grown for up to 30 days in a hydroponic system to evaluate the variability in tolerance to salt stress among 100 genotypes. This method offered extremely significant data regarding plant growth at the seedling stage, and it is easier to restore tolerant seedlings for seed production using this strategy [36–38]. This technique is exceptionally effective for studying salt tolerance in rice [39,40] and wheat [18,37,38]. Previous research has demonstrated the importance of the seedling stage in plant growth in response to abiotic stressors [41–43]. It is also possible to preselect populations before performing field evaluations [44,45].

All the investigated traits showed genotypic variation and responded to salinity stress. Another important and necessary component in screening is choosing the salt concentration during the study. In the present study, we used the maximum 150 mM salt solution based on the earlier findings of Uzair et al. [38] to identify wheat genotypes that are tolerant to salinity stress. To determine the best wheat genotype for a saline environment, a controlled environment is also important for determining phenotypic and genotypic variability [46,47].

In our study, salinity stress significantly reduced the SL, RL, RFW, SFW, TFW, SDW, and TDW (Table 4; Fig. 2). However, a wide range of genotypic and phenotypic variations for these tested traits were recorded among the genotypes. Some of these genotypes performed well against salinity by producing more biomass and longer roots than did the control, and some were highly susceptible to salinity. This is due to an overabundance of Na^+ in the solution around the roots, which leads to an imbalance in nutrient absorption, resulting in diminished plant development, the death of older roots, and the halting of root formation [48,49]. Due to a decrease in photosynthesis and an increase in the respiration rate in growing plants, salt stress is associated with considerable decreases in shoot and root length as well as shoot and root biomass [50,51]. According to Uzair et al. [38], under 150 mM NaCl stress, RL, SFW, RFW, and TFW were lower than those under control conditions but not significantly lower than those under control conditions.

The decline in morphological parameters is due to a decrease in photosynthetic pigments [26,52], transpiration rate, and carbohydrate and protein synthesis in plants [19,53,54]. Previous research has shown that saline stress reduces the fresh weight of rice and wheat roots and shoots [37,55–59]. The relationships between the environment and genotype are called phenotypic responses for any trait. When the average relative values of the traits studied were compared, the average relative values of RL (0.60) and SL (0.60) were comparatively greater, which suggested that these two traits are less useful for adapting to a high-salt environment. However, the relative RDW was the lowest (0.33), which was the most comprehensive range among the traits (Table 4), confirming that most of the genotypes performed poorly in terms of RL under salt stress conditions. In this case, RFW or RDW might increase in response to lateral roots produced by salt-tolerant plant genotypes. As a result, it can be concluded that RL was an important determinant of salt tolerance in our study. Previous research has indicated that RL is a vital characteristic of salt stress tolerance [15,20,60]. Ashraf and Ashraf [26] suggested that the RL is sensitive to salinity. It was also mentioned that the RL can be employed as a selection criterion under salinity stress. When RL increases the growth rate under stressed conditions, it benefits the plant in various ways, including through the absorption of water from deep soil and the enhancement of tolerance [61]. Prolonged root selection has thus been proposed to be one of the causes of improved salt tolerance in crop plants [62,63]. In this study, the SFW (0.43), SDW (0.46), TFW (0.47), and TDW (0.43) were important relative characteristics for salt tolerance in wheat genotypes. According to previous research, shoot growth is more affected by salt stress than is root growth [38,64].

According to Dashora et al. [65], Singh et al. [66] and Deshmukh et al. [67], wheat genotypes demonstrated genetic diversity concerning PCV and GCV for the traits investigated in the present study. High PCVs were detected for RL, SDW, RDW, and TDW, as was

Table 11
Classification matrix of ten clusters of 100 wheat genotypes (rows are observed categories, and columns are predicted categories).

Clusters	% correct	I	II	III	IV	V	VI	VII	VIII	IX	X	Total observed number
I	89.5	17	0	0	0	0	1	0	1	0	0	19
II	92.9	0	13	0	0	0	1	0	0	0	0	14
III	100.0	0	0	12	0	0	0	0	0	0	0	12
IV	100.0	0	0	0	3	0	0	0	0	0	0	3
V	100.0	0	0	0	0	8	0	0	0	0	0	8
VI	87.5	0	0	0	0	0	14	0	1	1	0	16
VII	100.0	0	0	0	0	0	0	3	0	0	0	3
VIII	91.7	1	0	0	0	0	0	0	11	0	0	12
IX	100.0	0	0	0	0	0	0	0	0	8	0	8
X	100.0	0	0	0	0	0	0	0	0	0	5	5
Total predicted number	94.0	18	13	12	3	8	16	3	13	9	5	100

high GCV for SDW, RDW, and TDW, whereas all the other characteristics had medium PCV and GCV (Table 5). Except for RL, PCV was relatively larger than GCV, with a small difference in magnitude. This revealed that the significant influence of environmental parameters on phenotypic expression was limited and that there was a greater chance of improving these traits through selection based on phenotypic performance under salinity scenarios [65,66]. However, the difference in RL between PCV and GCV was relatively large. This means that environmental factors have a greater influence on the phenotypic expression of this trait, making it difficult or nearly impossible to exercise selection based on phenotypic performance to improve the characteristics of plants under salt stress conditions [63,65,66]. Haque et al. [37] reported high PCVs for SFW, RFW, and RDW; medium PCVs for SDW and RL; and low PCVs for SL in bread wheat plants grown under salt stress conditions under hydroponic conditions. They also discovered that GCV was high in RFW and RDW, medium in SFW and SDW, and low in SL and RL. In the present study, except for SL and RL, a high degree of heritability was found for all of the studied characteristics, with high genetic advance in terms of the percentage of the mean (GAM), implying that these characteristics are highly heritable and that these high-performing genotypes can be selected for saline environmental conditions [68,69]. The heritability of these attributes is most likely due to additive gene effects, and selection for these traits may be successful in early generations [20,70]. Like in our work, Haque et al. [37] reported high heritability solely in RFW and low heritability in RL. In all these cases, there is an urgent need to investigate the genetic variation within the currently utilized genotypes to maintain a positive level of genetic variation for future wheat breeding.

The traits in this study showed a highly significant positive relationship with each other in terms of genotypic and phenotypic correlations, and the genotypic correlation coefficients were greater than the phenotypic correlation coefficients. These findings indicate that the association was due to genetic factors [71–73] and that these are important characteristics that could be utilized as criteria for selecting for wheat plants under saline conditions. Among these relationships, TDW, SDW, TFW, and SFW demonstrated stronger relationships with each other in terms of both genotypic and phenotypic relationships (Table 6), confirming that shoot biomass contributes more to total biomass. Correlation analysis is used in plant breeding to determine the relative significance of many plant characteristics [74,75]. Previous research has shown that fresh shoot weight and plant biomass can be applied as selection criteria for salt tolerance at the seedling stage [19,76–79]. Uzair et al. [38] discovered that shoot-related attributes have stronger correlations than root-related traits. Such higher correlations can be utilized as an adequate selection criterion for salt tolerance, which corroborated our findings.

Principal component analysis (PCA) helps determine which axis of differentiation contributes the most to the total variation in each axis of differentiation [80,81]. The first three PC axes explained most of the variation (87.13%) in the current study (Table 7). The first PC axis explained 61.83% of the genotype variation, and all the traits contributed positively to this variation. However, the most significant contributors were TDW, TFW, and SDW, whereas the second PC axis explained 16.10% of the variation, with RL and SL as major contributors. In the third PC axis, which exhibited 9.20% variation, the highest contributor was the RL, followed by the RDW. Taken together, these findings suggested that almost all the characteristics contributed to the maximum variation. However, the PCA of the PC biplot constructed from PC1 and PC2 showed that SDW, SFW, TDW, and TFW were significantly and positively correlated. Under salinity, Uzair et al. [38] reported associations between shoot length, fresh weight, fresh weight, and total fresh weight in wheat plants, similar to our findings. PCA reduces a large number of linked components to a small number [82–85]. Biplot analysis can be used to determine factors that can be categorized and visualized into fundamental groups and subgroups based on homogeneity and uniqueness in breeding programs [18,44]. Many researchers have previously used PCA to determine major variables for determining the diversity and grouping of wheat plants at the seedling stage [37,38,84] and in the field [86–89]. According to Iqbal et al. [90], quantitative characteristics that contribute positively to principal component analysis could be very important for the genetic materials under testing.

DFA also revealed that the TDW, TFW, SDW, RFW and RDW were the most critical variables responsible for genotype variation (Table 9). However, both techniques revealed that TDW was the most discriminatory variable, followed by TFW and SDW, and RL was the least discriminating variable in distinguishing the genotypes. RL, SL, RFW, and RDW were not the same for the two techniques; DFA characterized RFW and RDW, while PCA described SL and RL as secondary key variables for grouping genotypes. SL and SFW contributed roughly equally to describing the first components in both approaches.

Cluster analysis is a more useful measure for estimating divergence. The genotypes that are close to each other are classified into a group from diverged genotypes through cluster analysis. In this study, ten clusters were generated using the studied relative traits

through hierarchical cluster analysis (Fig. 4), and the intercluster (Mahalanobis distance-D^2) among the clusters and intracluster with the cluster were calculated. Four types of genotype responses to salinity were identified among the ten clusters. DFA was used to perform group mean equality tests with significance levels for each variable, revealing that the group means of eight variables were significantly different from each other at a probability of 0.01. All intercluster distances were greater than the intracluster distances, indicating that there was more divergence among genotypes within different clusters than among those from the same cluster, implying that the clustering method grouped homogenous genotypes. DFA is also used to identify misclassified genotypes that have been reassigned to the correct cluster. On average, 94% of the genotypes were accurately assigned to their respective clusters. However, based on the relative effectiveness of the variables evaluated, four categories of genotypic response to salinity were identified in our test. Among the clusters, the cluster VII genotype was selected for salt tolerance because of its excellent performance under salinity, which was more than 50% of that of the control. The moderately salt-tolerant group included Clusters V and X because of their intermediate performance. Clusters I, II, VI, VIII, and IX were classified as somewhat salt susceptible due to their poor overall performance. Moreover, Cluster III was categorized as salt susceptible due to its poorest relative performance, followed by Cluster IV. Cluster VII, with three genotypes, was the most distant from the most inferior Cluster III, indicating that the genotypes in Cluster VII were widely separated from the genotypes in the other clusters. Many scientists have used cluster analysis to categorize different wheat genotypes according to salt tolerance status based on various attributes and found similarities between wheat genotypes within a group [7,31,38,86]. Islam et al. [91] used DFA to improve mungbean flooding tolerance.

The results of the current study revealed that the interaction between genotype and salt stress treatment is significant; therefore, selection under salt stress may be recommended. This criterion could be implemented as a selection index to select high-yielding and salt stress-tolerant wheat genotypes as parents or varieties for sustainable wheat production, particularly in salinity-prone coastal regions of South Asia, including Bangladesh.

5. Conclusions

The current study aimed to identify wheat genotypes that are tolerant of soil salinity. In this context, 100 wheat genotypes were tested hydroponically under control and saline conditions. For all the wheat genotypes grown under saline conditions, the shoot and root length; shoot, root and total fresh weight; and dry weight decreased significantly. Considering the genetic diversity analysis, a high genotypic coefficient of variation, phenotypic coefficient of variation, and genetic advance in the percentage of the mean and high heritability were recorded for all tested wheat genotypes based on their shoot, root and total dry weight values. Correlation analysis for both genotypic and phenotypic relationships of all the genotypes revealed strong positive correlations for shoot, root and total fresh weight and dry weight. Principal component analysis (PCA) revealed that shoot fresh weight and dry weight and total fresh weight and dry weight were the most discriminative variables for the wheat genotypes, which was confirmed by discriminant function analysis. The biplot analysis also revealed significant positive correlations between shoot fresh weight and dry weight and between total fresh weight and dry weight. Furthermore, significant variation was observed among the genotypes in terms of variables only under salinity. The hierarchical cluster analysis grouped 100 genotypes into ten clusters based on their salinity, where the genotypes were characterized as salt-tolerant, medium-salt-tolerant, medium-salt-susceptible, or salt-susceptible. Among the genotypes, G11, G25 and G29 under cluster VII were categorized as salt tolerant based on their outstanding performance in terms of variables under salinity. Intercluster (Mahalanobis distance-D^2) analysis proved that the wheat genotypes of this cluster were strongly divergent from the other cluster genotypes; as a result, these genotypes might be utilized as parents in the development of salt-tolerant wheat genotypes. The present study revealed that shoot fresh weight and dry weight and total fresh weight and dry weight (shoot-related characteristics) could be employed as criteria for selecting and defining salt-tolerant genotypes during the early growth stage of wheat. Since genotyping by salt stress-treatment interactions is significant, selection under salt stress is recommended, and this criterion could be implemented as a selection index to select high-yielding and salt stress-tolerant genotypes as parents or varieties, particularly for salinity-prone coastal regions of South Asia, including Bangladesh.

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Ethics declaration statement

Not applicable.

Availability of data

The data will be made available upon request to the corresponding authors.

CRediT authorship contribution statement

Md Mustafa Khan: Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology,

Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Md Mahbubur Rahman:** Writing – original draft, Visualization, Validation, Methodology, Data curation, Conceptualization. **Md Mahamudul Hasan:** Writing – original draft, Visualization, Validation, Methodology, Investigation, Conceptualization. **Mohammad Forhad Amin:** Writing – original draft, Visualization, Validation, Formal analysis, Data curation, Conceptualization. **Mohammad Quamrul Islam Matin:** Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Golam Faruq:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Lamya Ahmed Alkeridis:** Writing – review & editing, Software, Project administration, Funding acquisition, Formal analysis, Data curation. **Ahmed Gaber:** Writing – review & editing, Software, Resources, Project administration, Funding acquisition, Formal analysis, Data curation. **Akbar Hossain:** Writing – review & editing, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization.

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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