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Evaluation of genetic variation among maize inbred lines for salinity stress at seedling stage through salt-stress-responsive traits

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Abstract: Saline conditions affect plant development and significantly reduce its yield. Maize (*Zea mays*) is the one of main cash crops in Pakistan, and unfavourable saline conditions are among the core reasons for its reduced productivity, especially in arid and semi-arid regions. The identification of potential genotypes is essential for genetic modifications. By considering this situation, the current experiment was conducted to evaluate the inbred maize lines under different salinity levels. We evaluated ten maize inbred maize lines at seedling stage under three salinity levels (0 mM, 75 mM, and 125 mM NaCl). The highly significant ($p \le 0.001$) differences in inbred lines, salinity levels, and in their interaction were revealed by analysis of variance results for most of the traits. The results indicated that inbred lines D-135 and NCIL-20-4 performed better under saline conditions. Our results showed that salinity severely affects seedling growth. Accordingly, a significant decline was observed in root length, shoot length, root weight, and shoot weight, and these traits offered the maximum values for heritability and genetic advance. From the correlation and path coefficient analysis, it has been concluded that root length, shoot length, fresh root weight, and root density are the traits that can be beneficial for the identification of better germplasms under saline conditions and that are helpful for improving tolerance against saline conditions.

Keywords: *Zea mays*, heritability, genetic advance, path coefficient analysis

1. Introduction

Soil salinization is a severe threat to crop productivity [1], and it is expected to be exacerbated in the near future as the consequences of global climatic changes [2]. Salinity affects almost 50% of the world's irrigated land and one-fifth of cultivable land [3], while in Pakistan almost 14 million acres of arable land are affected by salinity [4]. The situation is particularly adverse in arid and semi-arid areas owing to low precipitation and high transpiration, as well as inadequate water and soil management techniques, which disrupt the salt balance in the soil, worsening its impact on plant growth [5]. Maize is a highly valuable agricultural crop that is used for food, animal feed, and bioenergy raw materials all over the world [6], and its production rate has a direct impact on food security [7]. Maize is a salt-sensitive crop [8], and under 1.7 dS m^{-1} of soil electrical conductivity (EC_e) threshold it shows moderate sensitivity, while every 1 dS m^{-1} increase in EC_e results in 12% loss in grain production [9]. Thus, the breeding of salt-tolerant cultivars is one viable approach to rise to this challenge and boost the productivity of crops in salt-affected soils [10]. By 2050, almost half of the arable land is predicted to be damaged by salinity if no major steps will be taken [11].

Soil salinity grossly affects plant growth and development [12]. Plants respond to salinity stress from the molecular to morphological levels [13, 14]. Plant cells respond to salt stress by undergoing major changes – e.g. salt stress induces ion stress that causes ionic imbalance, results in ionic toxicity and osmotic stress, and subsequently produces reactive oxygen species (ROS) in plant cell [15]. Salt stress affects photosynthetic and transpiration rate by decreasing chlorophyll content and stomatal conductance [16]. Soil water potential and leaf water potential decrease due to soil salinity that subsequently disturbs plant–water relations and leads to osmotic stress [17]. High soil salinity caused a reduction in the stem and root length, biomass, and yield [18]. However, different crops respond differently to soil salinity because this is dependent upon their resistance to salinity stress. For example, beans (*Vicia faba*) and rice (*Oryza sativa*) are referred to as salt-sensitive, while barley (*Hordeum vulgare*) and Upland cotton (*Gossypium hirsutum*) are more resilient to salinity stress [19]. Overall, modifications in numerous morpho-anatomical alternations in roots and leaves enable the plant to adjust during salt stress [20].

Salinity is a genetically complicated abiotic stress that is influenced by several physiological and biochemical processes, subsequently affecting yield production [21]. Plant breeders have devised a number of techniques for combating salinity. One of the most important steps is to investigate the genetic variability of the available germplasms in order to find a tolerant genotype that is capable of maintaining a fair production in salt-affected soil [22]. Genetic diversity for salt tolerance has been documented in maize [23]. However, the identification of tolerant and promising genotypes requires a detailed understanding of genetic correlations among many features [24]. For example, correlation is the tool that is used to calculate the link between the traits, and it directly observes the phenotypic and genotypic correlation, which expresses the relationship between traits at the gene level [25, 26]. The path coefficient analysis is also utilized to determine the direct and indirect effects of traits. This method of trait selection is based on the traits' direct and indirect substantial effects on the dependent trait and is useful for determining attributes for selection criteria [27, 28, 29].

In maize, the progress in the determination of genetic architecture of salt tolerance and detecting the underlying genes and genetic factors has slowed down [30]. Therefore, the current study was designed to 1) assess and choose the best-performing maize inbred lines under saline conditions and 2) perform correlation and path coefficient analysis to identify the most contributing traits towards salt tolerance and suggest their role in selection procedure.

2. Material and methods

2.1. Plant material and experimental details

The experiment was conducted in the autumn season of 2018 in the greenhouse of the Department of Plant Breeding and Genetics, University of Agriculture Faisalabad, where the temperature was maintained at 28–30°C. Ten inbred lines used for this evaluation – namely WM-13RA, B-34, W-82-3, A-545, D-135, D-103, NCIL-20-4, OH-54-3A, W-187R, and A-638 (represented as V1, V2, V3, V4, V5, V6, V7, V8, V9, V10 in the figures respectively) – were selected based on the characterization/information provided by the contributor. The inbred lines were sown in polythene bags filled with sand by following completely randomized design having three replications under factorial arrangement. Three salinity levels were applied: control (0 mM), 75 mM, and 125 mM NaCl. The salinity levels were applied on the first day of experiment, and then the normal water was applied throughout the experiment. After 24 days of sowing, chlorophyll content and leaf temperature were examined with the help of chlorophyll content meter CCM200 plus and infrared thermometer, and then seedlings were uprooted and washed. Roots and shoots were detached, and their lengths were measured (cm) using a one-meter ruler. Root density was recorded in grams per millilitre (g/ml) by dipping the roots in a 100 mm test tube. The fresh weight of roots and shoots was measured using a digital balance (g). Then the roots and shoots were oven-dried at 70°C for 72 hrs, and the root dry weight (g) and shoot dry weight (g) were measured. Root/shoot length ratio, fresh root/shoot ratio, and dry root/shoot ratio were also computed.

2.2. Statistical analysis

Analysis of variance of the data for the observed traits was conducted using IBM SPSS statistics 22. The means were compared by employing Tukey's HSD test ($p \leq 0.05$). The variability analysis was performed using R software [31]. Calculations were performed according to Singh and Chaudhary [32].

Heritability was calculated by the following equation:

$$h^2 = \sigma^2_g/\sigma^2_p,$$

where σ^2_g = genotypic variance and σ^2_p = phenotypic variance.

Genetic advance was computed by the following equation:

$$GA = \sigma_p \times h^2 \times I,$$

where σ_p = the phenotypic standard deviation, h^2 = estimate of broad sense heritability, and i = standardized selection differential.

The genetic and phenotypic correlation coefficient ($r_{g,}$ r_p) was calculated by performing the correlation analysis proposed by Kown and Torrie [33].

$r_g = COV_G(X_1\ X_2)/\surd\ V_G\,(X_1).\ V_G\,(X_2)$

$COV_G(X_1\ X_2)$ = genetic covariance between traits X_1 and X_2

$V_G\,(X_1)$ = genetic variance for trait X_1

$V_G\,(X_2)$ = genetic variance for trait X_2

$r_p = COV_p(X_1\ X_2)/\surd\ V_p\,(X_1).\ V_G\,(X_2)$

$COV_p(X_1\ X_2)$ = phenotypic covariance among traits X_1 and X_2

$V_p\,(X_1)$ = phenotypic variance for trait X_1

$V_p\,(X_2)$ = phenotypic variance for trait X_2

The path coefficient analysis was used to split the correlation coefficient into direct and indirect constituents. This method was described by Dewey and Lu [34]. The root/shoot length ratio was considered the dependent variable and checked the direct and indirect effect of other traits upon the dependent variable.

3. Results

3.1. Phenotypic variations of maize inbred lines at seedling stage

The two-way analysis of variance results are presented in *Table 1*. The analysis of variance revealed highly significant variations among the inbred lines for all observed traits. The interaction between inbred lines and salinity levels also revealed highly significant differences for most of the observed traits. Based on the data reported for twelve traits at seedling stage (*Figure 1*), inbred lines V5 (D-135) and V7 (NCIL-

20-4) performed relatively better as compared to other inbred lines under saline conditions. Although the other inbred lines yielded higher values under normal conditions, such as V10 (A-638), they were more susceptible to salinity and had a higher reduction rate (*Table 2*). All observed traits significantly decreased under saline stress, except for leaf temperature, which produced increased values (ranging from 7.03% to 27.95%); thus, the ratio of different traits presented fluctuations in values from decreased to increased *(Figure 1*, *Table 2*). Data regarding the percentage (%) variation of traits under different irrigation conditions as compared to normal conditions are presented in *Table 2*. The chlorophyll content decreased (ranging from 6.74% to 34.48%) in response to salinity stress in all inbred lines. The lowest reduction rate of root length was observed in V5 under both salinity levels (75 mM, 125 mM): 1.04% and 35.86% respectively, while the lowest reduction rate of shoot length was observed in V7, which was 29.64% and 48.83% for both salinity levels [*Figure 1(d)*, *Table 2*]. For fresh and dry root weights, V7 and V5, respectively, performed comparatively better. The reduction rate of fresh root weight was ranging from 8.22% to 22.35%, while the reduction rate for dry root weight was between 18.38% and 56.19% for these two inbred lines under both salinity levels [*Figure 1(e)*, *(h)*, *Table 2*]. The lowest reduction rate in root density was observed in the V7 and V5 inbred lines (ranging from 11.72% to 40.83%). Regarding fresh and dry shoot weights, fresh and dry root/shoot ratios, and root/shoot length ratios, inbred lines presented significant variations among themselves under all applied conditions [*Figure 1(f)*, *(i)*, *(g)*, *(j)*, *(l)*]. The lowest variations in the root/shoot length ratio were observed for V7 and V5, which may indicate their higher survival rate (*Table 2*).

Table 1. Statistical summary of maize traits at seedling stage under different saline conditions

Traits	**Inbred lines (IL)**	**Salinity levels (S)**	**IL × S**
Chlorophyll content	***	***	*
Leaf temperature	***	***	NS
Root length	***	***	***
Shoot length	***	***	**
Fresh root weight	***	***	***
Fresh shoot weight	***	***	**
Fresh root/shoot ratio	***	***	*
Dry root weight	***	***	***
Dry shoot weight	***	***	***
Dry root/shoot ratio	***	***	***
Root density	***	***	***
Root/shoot length ratio	***	***	***

Notes: NS = Non-significant; * = Significant at p ≤ 0.05; ** = Significant at p ≤ 0.01; *** = Significant at p ≤ 0.001

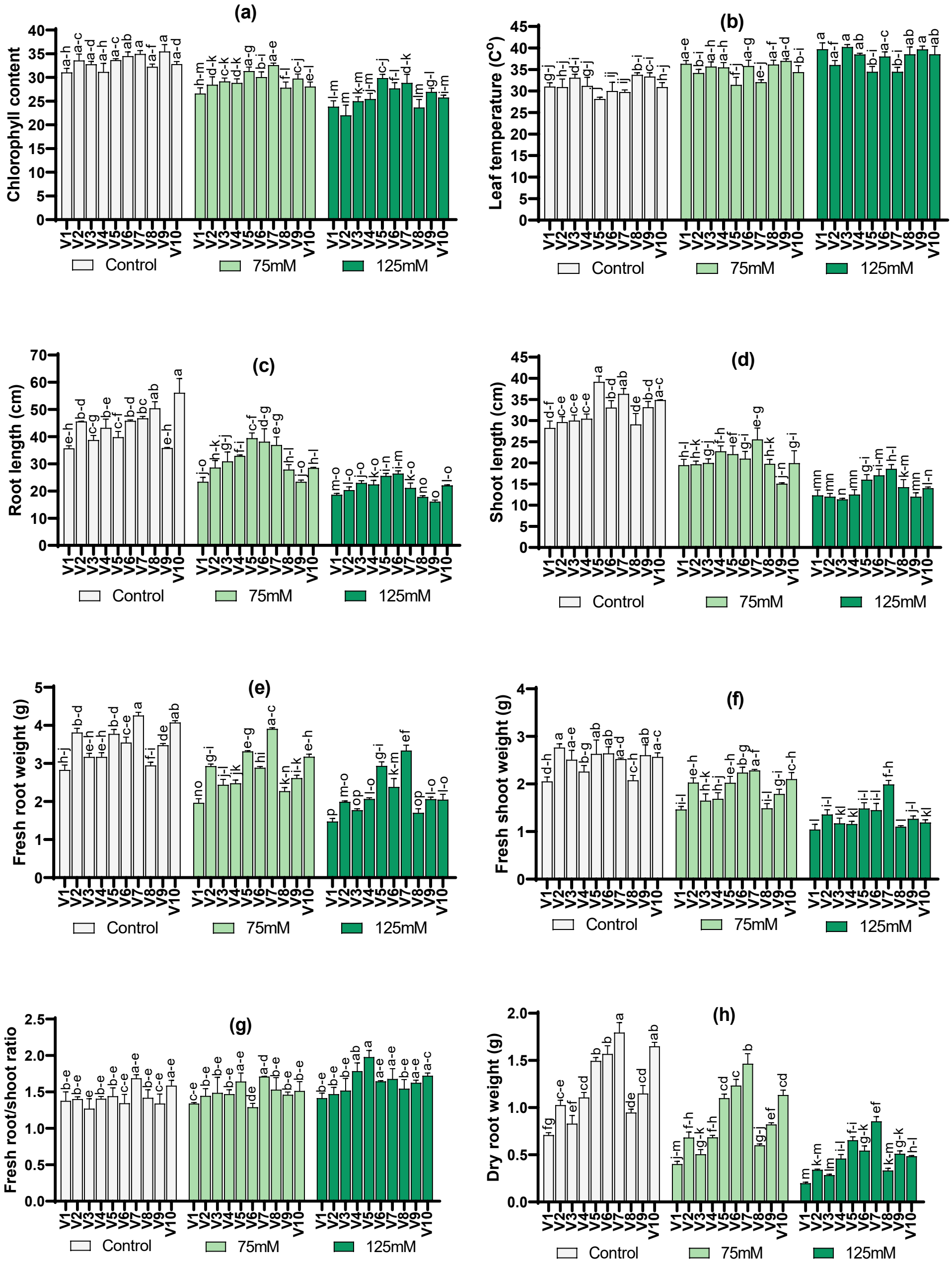

Figure 1a. Comparative morphological characteristics of maize inbred lines at seedling stage under different saline conditions: (a) Chlorophyll content, (b) Leaf temperature, (c) Root length, (d) Shoot length, (e) Fresh root weight, (f) Fresh shoot weight, (g) Fresh root/shoot ratio

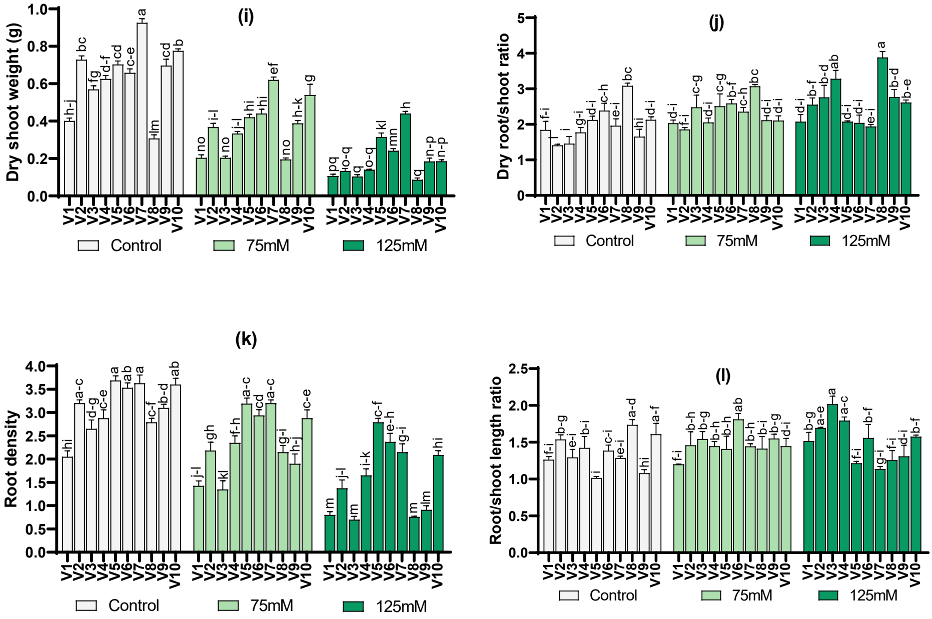

Figure 1b. (h) Dry root weight, (i) Dry shoot weight, (j) Dry root/shoot ratio, (k) Root density, (l) Root/shoot length ratio

Table 2. Percentage (%) variation of the different traits of maize inbred lines as compared to control at seedling stage under different saline conditions

Traits	Levels	Inbred Lines									
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Chlorophyll Content	75 mM	-14.34	-15.25	-10.95	-7.44	-6.86	-12.79	-6.74	-13.8	-16.07	-14.41
	125mM	-23.25	-34.48	-23.75	-18.35	-11.27	-19.68	-18.47	-26.73	-23.99	-21.49
Leaf Temperature	75mM	17.02	10.59	7.85	13.78	11.68	19.32	7.64	7.03	10.89	11.17
	125mM	27.95	16.48	21.55	23.4	22.41	26.73	15.81	14.1	18.89	24.6
Root Length	75mM	-34.5	-37.17	-20.36	-23.92	-1.04	-16.72	-21.02	-44.69	-34.59	-49.15
	125mM	-47.78	-55.37	-40.81	-48.08	-35.86	-42.33	-54.75	-64.67	-54.99	-60.66
Shoot Length	75mM	-31.15	-33.64	-33.46	-25.21	-43.61	-36.42	-29.64	-31.98	-54.46	-39.95
	125mM	-56.35	-59.52	-62.08	-58.87	-46.3	-48.36	-48.83	-51.01	-63.82	-59.82
Fresh Root Weight	75mM	-30.57	-23.2	-23.15	-21.89	-12.17	-18.59	-8.22	-22.88	-24.86	-22.02
	125mM	-47.88	-47.71	-44.09	-34.8	-22.35	-32.82	-21.6	-42.2	-40.66	-49.69
Fresh Shoot Weight	75mM	-28.75	-26.58	-34.26	-25.22	-23.15	-15.31	-9.5	-28.37	-31.29	-18.09
	125mM	-49.17	-50.81	-53.19	-48.67	-43.64	-45.18	-21.09	-46.88	-51.25	-53.7

Traits	Levels	Inbred Lines									
		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10
Fresh Root/ Shoot Ratio	75mM	-2.77	3.12	17.13	4.55	14.05	-3.99	1.41	7.84	8.99	-4.61
	125mM	2.6	4.87	19.51	27.13	37.42	22.3	-0.49	8.68	21.28	8.51
Dry Root Weight	75mM	-43.23	-33.45	-39.1	-38.18	-26.39	-21.34	-18.38	-36.66	-28.55	-31.21
	125mM	-72.01	-66.7	-65.6	-58.48	-56.19	-65.29	-52.37	-64.66	-55.54	-70.61
Dry Shoot Weight	75mM	-49.27	-49.48	-64.13	-46.6	-40.16	-33.08	-32.96	-36.42	-44.21	-30.37
	125mM	-73.55	-81.58	-81.74	-77.63	-55.15	-63.23	-52.4	-71.87	-73.47	-76.08
Dry Root Shoot Ratio	75mM	10.06	31.67	69.84	15.75	18.16	8.47	20.19	-0.41	27.83	-0.92
	125mM	12.6	81.47	89.07	85.2	-2.33	-14.49	-1.18	25.81	67.22	22.92
Root Density	75mM	-30.49	-31.72	-48.96	-18.43	-13.69	-16.74	-11.72	-22.94	-38.61	-20.03
	125mM	-60.98	-57.03	-73.53	-42.61	-24.39	-32.77	-40.83	-72.76	-70.52	-42.01
Root Shoot Length Ratio	75mM	-4.96	-5.18	19.37	1.65	43.9	30.56	12.35	-18.58	43.55	-15.16
	125mM	19.99	10.15	55.87	26.04	19.57	12.46	-11.7	-27.61	24.59	-2.07

Note: Negative (-) values indicate the reduction, and positive (+) values show the increment of the specific traits as compared to the normal.

3.2. Genetic components of the traits of various maize seedling stages

Genotypic variance was slightly lower than phenotypic variance for all the observed traits of maize inbred lines under normal and salinity stress conditions (*Table 3*). The highest values of genetic and phenotypic variance were observed for root length and shoot length traits under all applied conditions, while the other traits yielded lower values, except for chlorophyll content and leaf temperature, which presented slightly higher values under 125 mM salinity level. Other traits displayed marginal changes compared to the normal.

The genotypic coefficient of variance (GCV) was slightly lower than the phenotypic coefficient of variance (PCV). Dry shoot weight, root density, dry root weight, fresh root weight, and shoot length revealed higher values of GCV and PCV under salinity stress. The environmental coefficient of variations (ECV) was higher for shoot length, followed by root density under stressful conditions. These results suggested that shoot length, dry shoot weight, fresh and dry root weight, and root density might be helpful for the selection of germplasm as indicator traits for salinity stress tolerance.

Dry shoot weight, dry root weight, and root density presented the highest values of heritability. The genetic advance was higher for root length and shoot length and moderate for chlorophyll content, leaf temperature, and root density; thus, other traits presented lower values of genetic advance under different salinity levels (*Table 3*).

Table 3. Genetic components for various seedling traits of maize inbred lines under normal and different saline conditions

Traits	Levels	σ^2_g	σ^2_p	GCV	PCV	ECV	h^2	GA
Chlorophyll Content	**Control**	1.691	2.247	3.913	4.510	2.242	0.753	1.5799
	75 mM	2.640	3.111	5.547	6.021	2.342	0.849	2.0955
	125 mM	5.043	5.823	8.677	9.324	3.413	0.866	2.9258
Leaf Temperature	**Control**	2.285	3.092	4.839	5.629	2.876	0.739	1.8191
	75 mM	2.722	3.412	4.731	5.297	2.382	0.798	2.0632
	125 mM	3.674	4.457	5.066	5.579	2.338	0.824	2.4364
Root Length	**Control**	39.763	42.408	14.388	14.858	3.711	0.938	8.5483
	75 mM	29.940	33.046	17.633	18.525	5.679	0.906	7.2916
	125 mM	10.151	10.669	14.906	15.282	3.369	0.951	4.3507
Shoot Length	**Control**	11.533	12.597	10.476	10.948	3.181	0.916	4.5494
	75 mM	10.064	11.350	15.003	15.932	5.362	0.887	4.1823
	125 mM	10.162	10.787	21.943	22.607	5.440	0.942	4.3318
Fresh Root Weight	**Control**	0.224	0.229	13.484	13.635	2.020	0.978	0.6556
	75 mM	0.321	0.325	20.224	20.337	2.147	0.989	0.7889
	125 mM	0.317	0.324	25.808	26.079	3.753	0.979	0.7798
Fresh Shoot Weight	**Control**	0.047	0.061	8.838	9.991	4.660	0.782	0.2698
	75 mM	0.084	0.090	15.423	16.010	4.298	0.928	0.3908
	125 mM	0.072	0.076	20.264	20.839	4.858	0.946	0.3661
Fresh Root/Shoot Ratio	**Control**	0.011	0.015	7.365	8.593	4.426	0.735	0.1263
	75 mM	0.012	0.016	7.241	8.371	4.200	0.748	0.1307
	125 mM	0.023	0.028	9.152	10.177	4.452	0.809	0.1890
Dry Root Weight	**Control**	0.139	0.141	30.359	30.582	3.692	0.985	0.5175
	75 mM	0.122	0.123	40.442	40.682	4.414	0.988	0.4860
	125 mM	0.037	0.037	41.181	41.472	4.899	0.986	0.2668
Dry Shoot Weight	**Control**	0.032	0.032	27.908	28.000	2.259	0.993	0.2487
	75 mM	0.020	0.021	38.564	38.790	4.176	0.988	0.1991
	125 mM	0.013	0.013	58.136	58.316	4.578	0.994	0.1574
Dry Root/Shoot Ratio	**Control**	0.228	0.243	24.060	24.795	5.992	0.942	0.6493
	75 mM	0.112	0.129	14.423	15.474	5.606	0.869	0.4367
	125 mM	0.366	0.388	23.216	23.917	5.750	0.942	0.8217
Root Density	**Control**	0.272	0.280	16.754	16.995	2.851	0.972	0.7197
	75 mM	0.455	0.464	28.606	28.900	4.108	0.980	0.9348
	125 mM	0.574	0.581	48.518	48.828	5.493	0.987	1.0539
Root/Shoot Length Ratio	**Control**	0.047	0.050	15.940	16.461	4.111	0.938	0.2947
	75 mM	0.017	0.024	8.960	10.512	5.497	0.727	0.1573
	125 mM	0.071	0.077	17.635	18.309	4.921	0.928	0.3598

Notes: σ^2_g = Genotypic variance, σ^2_p = Phenotypic Variance, GCV = Genotypic coefficient of variation, PCV = Phenotypic coefficient of variation, ECV = Environmental coefficient of variation, h^2 = Heritability, GA = Genetic advance.

3.3. Genotypic and phenotypic correlation among seedling stage traits

Significant positive genotypic correlation was observed between chlorophyll content and shoot length, fresh root weight, fresh shoot weight, dry root weight, and root density, while highly positive phenotypic correlation was demonstrated for these traits (*Table 4*). Fresh root weight indicated a significant positive correlation with fresh shoot weight, dry root and shoot weight, root density, and shoot length at both the genotypic and phenotypic level. Thus, fresh root weight showed significant negative correlation with leaf temperature for both the genotype and phenotype. Dry root weight and dry shoot weight exhibited significant positive correlation with chlorophyll content, fresh root weight, and fresh shoot weight at both the genotypic and phenotypic level (*Table 4*), while these traits showed significant negative correlation with leaf temperature under all applied conditions. Root density indicated significant positive correlation with all observed traits, except leaf temperature, which showed significant negative correlation (*Table 4*).

3.4. Path coefficient analysis for seedling stage traits

This analysis considered the root/shoot length ratio as dependent trait. The data were divided into direct and indirect effects on the root/shoot length ratio and are presented in *Table 5*. Root/shoot length ratio was dependent upon a variety of connected traits. The highest direct positive effect was presented by the fresh root weight and root length under stressful conditions. Results indicated that the direct effect of the fresh root weight was negative (-0.59311) under normal conditions, but, interestingly, it increased manifold under salinity stress conditions: 0.9279 for 75 mM and 1.94424 for 125 mM salinity stress (*Table 5*). The maximum negative direct effect was presented by fresh shoot weight and dry root and shoot weight under salinity stress conditions. Chlorophyll content and root density indicated the highest positive indirect effect on the dependent trait under salinity stress, via shoot length. Fresh root and shoot weight, dry root and shoot weight, and root density indicated the highest negative effect on the dependent trait under all applied conditions, via shoot length.

Table 4. Genotypic (above diagonal) and phenotypic (below diagonal) correlation coefficient for various seedling traits of maize under control and different saline conditions

Traits	Levels	*Chlorophyll content*	*Leaf temperature*	*Root length*	*Shoot length*	*Fresh root weight*	*Fresh shoot weight*	*Fresh root/shoot ratio*	*Dry root weight*	*Dry shoot weight*	*Dry root/ shoot ratio*	*Root density*	*Root/shoot length ratio*
CC	Control		-0.0260	0.0680	0.7018*	0.7132*	0.8493*	0.2852	0.6701*	0.7340*	-0.0952	0.7631*	-0.3837
	75 mM		-0.7257*	0.8564*	0.7008*	0.9142*	0.7555*	0.9216*	0.8276*	0.6932*	0.2451	0.7504*	0.5711
	125 mM		-0.5164*	0.5703*	0.9022*	0.8881*	0.6960*	0.9519*	0.8924*	0.8649	-0.5261*	0.7570*	-0.5535
LT	Control	-0.1453		-0.1473	-0.8569*	-0.5881*	-0.5456	-0.3840	-0.7080*	-0.6093*	0.0834	-0.6197*	0.3492
	75 mM	-0.6737**		-0.8681*	-0.9869*	-0.9431*	-0.7857*	-0.8932*	-0.6928*	-0.7029*	0.0702	-0.8469*	-0.0639
	125 mM	-0.4559*		-0.4289*	-0.8302*	-0.9435*	-0.9340*	-0.5316	-0.8006*	-0.8459*	0.4797*	-0.8076*	0.5367*
RL	Control	-0.0192	-0.0654		0.1773*	0.4800*	0.1244	0.7281*	0.5252*	0.2049	0.5358*	0.5222*	0.7819*
	75 mM	0.7471**	-0.6908**		0.8797*	0.6982*	0.6834*	0.4877	0.7378*	0.5079*	0.4519*	0.7846*	0.5106*
	125 mM	0.4956*	-0.3663		0.5746*	0.4652*	0.3451	0.6024*	0.3346	0.3797	-0.4704*	0.7845*	0.2247
SL	Control	0.5771**	-0.6713**	0.1438		0.8123*	0.6737*	0.6468*	0.8928*	0.7234*	0.1264	0.8948*	-0.4861*
	75 mM	0.5852**	-0.8714**	0.8331**		0.6761*	0.5334*	0.7227*	0.6026*	0.4888	0.2423	0.7355*	0.0283
	125 mM	0.8001**	-0.7820**	0.5636**		0.8543*	0.7357*	0.7513*	0.8054*	0.8499*	-0.5175*	0.8629*	-0.6589*
FRWT	Control	0.6409	-0.5632**	0.4478*	0.7563**		0.8692*	0.7700	0.8696*	0.9327*	-0.1641	0.8868*	-0.0825
	75 mM	0.8154**	-0.8342**	0.6487**	0.6282**		0.9311*	0.7309	0.9147*	0.9321*	-0.0636	0.8615*	0.2777
	125 mM	0.8040**	-0.8381**	0.4471*	0.8383**		0.9568*	0.7218	0.9846*	0.9805*	-0.5366	0.7936*	-0.5632
FSWT	Control	0.7374**	-0.3762	0.0853	0.5482*	0.7487**		0.3437	0.6097	0.8783*	-0.4796	0.8380*	-0.3220
	75 mM	0.6962**	-0.6284**	0.6479**	0.4544*	0.8987**		0.3864	0.9474*	0.9350*	-0.1251	0.8860*	0.4910
	125 mM	0.6244**	-0.7978	0.3115	0.6995**	0.9359**		0.4874	0.9291*	0.9631*	-0.5736	0.6191*	-0.5307
FRSRT	Control	0.1729	-0.4151	0.6152**	0.5207*	0.6974**	0.0492		0.8127	0.6274	0.2640	0.6043	0.2644
	75 mM	0.6220**	-0.7491**	0.3650	0.6024**	0.6626**	0.2731		0.5099	0.5634	0.1300	0.5425	-0.2114
	125 mM	0.7786**	-0.5078*	0.5399*	0.6870**	0.6548**	0.3501		0.7435	0.6121	-0.1871	0.8931*	-0.3298
DRWT	Control	0.5838**	-0.6181**	0.5095*	0.8433**	0.8578**	0.5339*	0.7023**		0.7868*	0.2044	0.9467*	-0.1004
	75 mM	0.7777**	-0.6317	0.6795**	0.5421*	0.9033**	0.9119**	0.4338		0.9342*	0.1015	0.9327*	0.4674
	125 mM	0.8288**	-0.7200**	0.3398	0.7758**	0.9628**	0.8818**	0.6818**		0.9496*	-0.4440	0.7704*	-0.6392
DSWT	Control	0.6335**	-0.5285*	0.1968	0.6927**	0.9208**	0.7668**	0.5419*	0.7720**		-0.4202	0.7814*	-0.3030
	75 mM	0.6674**	-0.6230**	0.4817*	0.4421	0.9149**	0.9074**	0.4424	0.9293**		-0.2193	0.8475*	0.2066
	125 mM	0.8022**	-0.7801	0.3671	0.8260**	0.9677**	0.9381**	0.5473*	0.9406**		-0.6617	0.7526*	-0.6412
DRSRT	Control	-0.0764	0.0495	0.5071*	0.0932	-0.1484	-0.4139	0.2451	0.2219	-0.4232		0.1661	0.4567
	75 mM	0.1806	-0.0042	0.3338	0.1882	-0.0367	-0.1226	0.2079	0.1145	-0.2189		0.1923	0.4959
	125 mM	0.5005*	0.4291	-0.4266	-0.4892*	-0.5260*	-0.5715**	-0.1336	-0.4159	-0.6525**		-0.5492*	0.1986
RD	Control	0.6679**	-0.5648**	0.4901*	0.8410**	0.8799**	0.7361**	0.5311*	0.9188**	0.7709**	0.1502		-0.0726
	75 mM	0.6654**	-0.7310**	0.7537**	0.7034**	0.8419**	0.8226**	0.4702*	0.9117**	0.8291**	0.1656		0.3228
	125 mM	0.7208**	-0.7234**	0.7461**	0.8297**	0.7844**	0.6117**	0.7808**	0.7524**	0.7450**	-0.5411*		-0.3482
RSLRT	Control	-0.3811	0.3537	0.7898**	-0.4834*	-0.0951	0.2824	0.2102	-0.0911	-0.2977	0.4416	-0.0833	
	75 mM	0.4691*	0.0886	0.5109*	-0.0483	0.2191	0.4691*	-0.2295	0.3975	0.2002	0.3141	0.2831	
	125 mM	-0.4955*	0.5482*	0.2260	-0.6664**	-0.5070*	-0.5070*	-0.3191	-0.6014**	-0.6206**	0.1912	-0.3377	

* = Significant, ** = Highly significant

Table 5. Direct (bold) and indirect effect of various seedling traits of maize inbred lines at root/shoot length ratio under normal and different saline conditions

Traits	**Levels**	Chlorophyll content	Leaf temperature	Root length	Shoot length	Fresh root weight	Fresh shoot weight	Fresh root/ shoot ratio	Dry root weight	Dry shoot weight	Dry root/ shoot ratio	Root density
CC	**Control**	**-0.04766**	0.00112	0.08374	-0.07835	-0.42302	-0.04245	-0.03318	-0.80854	0.99616	-0.05063	0.01916
	75 mM	**0.11195**	-0.04683	1.17629	-1.07834	0.84829	-0.10181	-0.17882	0.56739	-0.83052	-0.01971	0.12320
	125 mM	**0.18169**	0.05971	0.53629	-1.49832	1.72664	-0.82738	-0.32664	-0.94434	0.71682	-0.15250	-0.02541
LT	**Control**	0.00124	**-0.04306**	-0.18151	0.09567	0.34880	0.02727	0.04466	0.85416	-0.82686	0.04436	-0.01556
	75 mM	-0.08124	**0.06453**	-1.19231	1.51856	-0.87510	0.10588	0.17331	-0.47498	0.84213	-0.00564	-0.13903
	125 mM	-0.09382	**-0.11562**	-0.40334	1.37875	-1.83431	1.11038	0.18242	0.84718	-0.70105	0.13905	0.02711
RL	**Control**	-0.00324	0.00634	**1.23234**	-0.01979	-0.28467	-0.00622	-0.08469	-0.63424	0.27808	0.28484	0.01311
	75 mM	0.09588	-0.05601	**1.37348**	-1.35369	0.64788	-0.09210	-0.09463	0.50581	-0.60846	-0.03634	0.12881
	125 mM	0.10362	0.04960	**0.94032**	-0.95429	0.90450	-0.41023	-0.20670	-0.35407	0.31466	-0.13636	-0.02633
SL	**Control**	-0.03345	0.03689	0.21846	**-0.11164**	-0.48177	-0.03367	-0.07523	-1.07716	0.98178	0.06723	0.02247
	75 mM	0.07845	-0.06368	1.20827	**-1.53879**	0.62732	-0.07188	-0.14023	0.41313	-0.58554	-0.01949	0.12075
	125 mM	0.16391	0.09599	0.54031	**-1.66080**	1.66094	-0.87460	-0.25783	-0.85223	0.70437	-0.15002	-0.02896
FRWT	**Control**	-0.03399	0.02532	0.59148	-0.09068	**-0.59311**	-0.04344	-0.08956	-1.04924	1.26574	-0.08725	0.02227
	75 mM	0.10234	-0.06085	0.95899	-1.04033	**0.92790**	-0.12547	-0.14181	0.62709	-1.11666	0.00512	0.14144
	125 mM	0.16135	0.10909	0.43745	-1.41880	**1.94424**	-1.13744	-0.24771	-1.04188	0.81266	-0.15553	-0.02664
FSWT	**Control**	-0.04048	0.02349	0.15333	-0.07521	-0.51552	**-0.04998**	-0.03998	-0.73563	1.19195	-0.25497	0.02104
	75 mM	0.08458	-0.05070	0.93867	-0.82081	0.86397	**-0.13476**	-0.07498	0.64957	-1.12009	0.01006	0.14546
	125 mM	0.12645	0.10800	0.32448	-1.22183	1.86021	**-1.18882**	-0.16725	-0.98311	0.79822	-0.16626	-0.02078

Traits	**Levels**	Chlorophyll content	Leaf temperature	Root length	Shoot length	Fresh root weight	Fresh shoot weight	Fresh root/ shoot ratio	Dry root weight	Dry shoot weight	Dry root/ shoot ratio	Root density
FRSRT	**Control**	-0.01359	0.01653	0.89729	-0.07221	-0.45671	-0.01718	**-0.11631**	-0.98053	0.85152	0.14038	0.01517
	75 mM	0.10317	-0.05763	0.66983	-1.11207	0.67816	-0.05207	**-0.19403**	0.34959	-0.67494	-0.01046	0.08907
	125 mM	0.17294	0.06146	0.56641	-1.24782	1.40345	-0.57941	**-0.34316**	-0.78674	0.50731	-0.05423	-0.02998
DRWT	**Control**	-0.03194	0.03048	0.64782	-0.09967	-0.51580	-0.03047	-0.09453	**-1.20651**	1.06776	0.10868	0.02377
	75 mM	0.09265	-0.04470	1.01332	-0.92725	0.84871	-0.12768	-0.09894	**0.68560**	-1.11923	-0.00817	0.15312
	125 mM	0.16215	0.09257	0.31464	-1.33761	1.91436	-1.10452	-0.25514	**-1.05815**	0.78705	-0.12870	-0.02586
DSWT	**Control**	-0.03498	0.02623	0.25252	-0.08076	-0.55318	-0.04390	-0.07298	-0.94927	**1.35711**	-0.22337	0.01962
	75 mM	0.07761	-0.04536	0.69758	-0.75210	0.86488	-0.12599	-0.10932	0.64051	**-1.19801**	0.01764	0.13914
	125 mM	0.15714	0.09780	0.35700	-1.41145	1.90637	-1.14494	-0.21005	-1.00484	**0.82881**	-0.19181	-0.02526
DRSRT	**Control**	0.00454	-0.00359	0.66025	-0.01412	0.09734	0.02397	-0.03071	-0.24664	-0.57019	**0.53165**	0.00417
	75mM	0.02744	0.00453	0.62068	-0.37285	-0.05902	0.01686	-0.02523	0.06961	0.26277	**-0.08043**	0.03157
	125mM	-0.09559	-0.05547	-0.44236	0.85953	-1.04322	0.68187	0.06419	0.46983	-0.54844	**0.28986**	0.01843
RD	**Control**	-0.03637	0.02668	0.64357	-0.09990	-0.52598	-0.04188	-0.07029	-1.14223	1.06043	0.08829	**0.02511**
	75 mM	0.08401	-0.05464	1.07758	-1.13177	0.79938	-0.11939	-0.10527	0.63944	-1.01529	-0.01546	**0.16418**
	125 mM	0.13754	0.09338	0.73767	-1.43304	1.54301	-0.73603	-0.30648	-0.81519	0.62375	-0.15920	-0.03356

4. Discussion

Salinity affects plant development and is considered as one of the main abiotic stresses damaging crop production [35]. Salinity becomes the mainstream concern for plant scientists in the short as well as long term. Although the breeding techniques have made great progress in the past 15 years, conventional breeding is still the main method to improve the stability and production of crops under stressful conditions – in this method, screening of the potential germplasm is the most fundamental and critical point [36]. The genetic variability for salinity tolerance exists in maize crops due to its highly polymorphic nature [37]. Thus, considering certain agronomical characteristics, screening for salt tolerance in early growth stages is often considered valuable [38, 39]. Here we have screened the maize inbred lines to confirm the most promising salt-tolerant maize germplasm and provide more solid evidence for potentially effective salt-tolerant indicator traits. Our results offer a deeper understanding of maize responses towards salinity stress, and we have revealed the traits that make for salt-tolerant indicators in maize.

Maize is more sensitive for salinity at the vegetative growth stages [40, 41, 42]. In the current study, several morphophysiological traits were assessed in ten maize inbred lines at the seedling stage to investigate their relative tolerance ability to salt stress. The highly significant variations among the genotypes at the control and salt stress levels for almost all traits (*tables 1–2*, *Figure 1*) indicated the genetic difference between the maize genotypes used for salt tolerance. All observed traits, such as root and shoot lengths, fresh root and shoot weights, dry root and shoot weights, root density, and chlorophyll content, exhibited significant decline due to salt stress, whereas leaf temperature showed a rising trend in values. The results show that shoot length is reduced in all genotypes under all salt treatments. The better shoot length value under salt treatments indicated that the specific maize genotypes showed tolerance to salt stress and managed to adjust plant growth and development under stressful environment [43, 44, 45].

In the current investigation, maize inbred line V7 and V5 showed higher shoot length under salt stress as compared with other inbred lines, and therefore these may be used for enhancing the grain productivity of maize in salt-affected soils [46, 47]. Salt stress represses leaf initiation and expansion, including internode growth, which eventually leads to reduction in shoot growth [48, 49, 50]. This repression is mainly due to a reduction in cell elongation [51]. Maize is a salt-sensitive crop, and its shoot growth is highly inhibited at the seedling stage under salt stress [52, 53]. The fresh and dry weight of root and shoot as well as their length as the measure for maintaining growth during salinity stress were considered the driving traits for most variations among the genotypes. These traits have been acknowledged as good predictors of salinity tolerance [54, 55]. Giaveno et al. [56]

proposed that seedling weight should be used to screen for salt-tolerant maize germplasms under salinity stress.

The decrease in chlorophyll content was detected in all maize genotypes under salinity stress, and this decrease is a frequently reported phenomenon, which is used as a vulnerability indicator of cellular metabolic state in several findings [57]. Many plant species reported the decline in chlorophyll content under salinity stress due to membrane deterioration [58, 59]. The reduction of chlorophyll content decreases the photosystem II efficiency and net photosynthetic rate [60]. Salt-induced photosynthesis reductions are associated with both non-stomatal and stomatal limitations and their combination in maize [61]. However, the germplasm with the lowest reduction rate in chlorophyll content exhibited salt-tolerant behaviour [46]. Leaf temperature increment under salinity stress in all the inbred lines was observed to indicate the lower availability of water in leaves because saline conditions hindered plants' water-absorbing capacity [62].

The study of genetic variability and genetic advance offers useful information regarding the extent of variability in available germplasm sources [26, 63]. Based on genetic advance and heritability, selection can be useful [64]. Genetic advances have shown that the additive type of gene action can be used to improve specific traits by fixing them for the next generation [65]. Heritability enabled the entire variation because of genetic variability, and it can play a crucial role in determining the selection criteria [47]. However, heritability alone is not sufficient to determine the selection because high heritability may not always be linked with high genetic advance [66]; thus, heritability and genetic advance may prove to be the most useful for an effective and reliable selection. These selected traits may respond to phenotypic assortment and could be enhanced via heterosis breeding [67]. The highest heritability values were recorded for dry shoot weight, dry root weight, root density, and root length, while genetic advance was higher for root length and shoot length. These results indicated that the salt-tolerant behaviour of maize genotypes depend upon those traits [68], and, consequently, these traits may be used for enhancing the grain productivity of maize in salt-affected soils [46, 69]. Higher heritability in maize seedlings for shoot and root traits under salinity stress was also reported by Tanzeel-ur-Rehman et al. [70], who suggest that root and shoot traits could be taken into consideration for salt tolerance breeding in maize.

For the improvement of traits, the fundamental feature is the assessment of trait variation. It is imperative to describe the relative amount of trait variation components for the development of plant type under any stress condition with further selection parameters by using effective breeding methods [71, 72, 73]. The correlation of seedling traits indicated that the shoot and root length and their weights may be utilized for the selection of better-yielding maize genotypes under salt stress conditions. The higher shoot and root length caused to increase photosynthetic rate in leaves and led to the accumulation of organic matters in the seedling body, due to which the growth and development of maize seedlings improved even under salt stress conditions [69, 74, 75, 76].

For plant breeders, the path coefficient analysis is an effective tool to evaluate the direct and indirect effects of various traits (independent traits) on one dependent trait [64], and it enables a stronger focus on the selection procedure [26, 77]. The traits with the most positive direct effect could be favourable for selection in the breeding cycle [78]. In the current study, the highest direct positive effect was presented by fresh root weight and root length under stressful conditions, while the maximum negative direct effect was exhibited by fresh shoot and dry root and shoot weight under salinity stress conditions, indicating their importance in the selection procedure under stressful conditions [79]. Thus, these traits can be used as reliable screening criteria for the evaluation of salt-tolerant maize genotypes.

5. Conclusions

Comparatively, all inbred lines behaved differently under salinity conditions. Root fresh and dry weights, shoot fresh and dry weights, root and shoot lengths, and root density indicated the highest heritability, which suggested their vital role as selection criteria. Furthermore, root and shoot length and chlorophyll content presented higher genetic advance. The significant correlation among root and shoot lengths and their fresh and dry weights expressed that these traits are essential for assortment against salinity stress conditions. Overall, two inbred lines, namely D-135 and NCIL-20-4, performed comparatively better than other inbred lines. Furthermore, future transcriptomic studies of these tolerant inbred lines could provide an extra glimpse into the gene regulatory components of salt tolerance and investigate their possible use in maize breeding programmes aimed at salinity tolerance.

Authors’ contributions

Farrah Zaidi and Ali Shahzad performed the experiment, measurements, and analyses; Muhammad Ahsan supervised the study; Farrah Zaidi, Ali Shahzad, Hameed Gul, and Shareef Gul wrote the manuscript draft; Muhammad Ahsan and Muhammad Shahzad reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Declarations

Conflict of interest
Authors declare that they have no conflict of interest.

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