

## EFFECTS OF SALINITY ON MORPHO-PHYSIOLOGICAL TRAITS IN SIX MAIZE HYBRIDS AT SEEDLING STAGE

Ali Raza<sup>1\*</sup>, Ali Hassan<sup>1</sup>, Shaheen Abbas<sup>1</sup>, Waqas Razzaq<sup>2</sup>, Shahzaib Hazoor<sup>1</sup>,  
Muhammad Noshewan<sup>1</sup>, Abdul Rehman<sup>1</sup> and Iftikhar Ali<sup>1\*</sup>

<sup>1</sup>Department of Plant Breeding and Genetics (PGB), The Islamia University of Bahawalpur (IUB), Punjab, Pakistan

<sup>2</sup>University of Avignon, France

\*Corresponding author: [iftikhar.ali@iub.edu.pk](mailto:iftikhar.ali@iub.edu.pk) (IA); [alizraza005@gmail.com](mailto:alizraza005@gmail.com) (AR)

### ABSTRACT

Increased soil salinity due to climate change and poor irrigation practices has detrimental effects on the maize seedling stage than lateral growth phases. Therefore, this experiment evaluated maize's morphological and physiological response to salinity by screening tolerant genotypes. Six hybrids were grown in plastic pots in a warehouse using a completely randomized design (CRD) with three replications. Ten-day-old seedlings were subjected to controlled salinity stress (control, 4, 7, and 10dS/m NaCl). Significant differences ( $p < 0.05$ ) were revealed in all genotypes, treatments, and their interaction by analysis of variance. The mean comparison of parameters exhibited the maximum reduction in chlorophyll content (80.4%), shoot length (55.4%), and shoot fresh weight (54%) and the least reduction in root fresh weight (30.73%) at 10dS/m salt application. Under the highest salinity stress, the strong association among shoot fresh weight, shoot length, root dry weight, shoot dry weight, stomatal conductance, and root length shows these parameters have a common genetic base; improvement in one trait can boost another, resulting in improved salinity stress. While PCA analysis demonstrated that fresh root-to-shoot weight, chlorophyll content, root fresh weight, stomatal conductance, leaf area, and relative water content is effective parameters for evaluating and identifying resilient germplasm under salinity stress. Among investigated hybrids, Pak Afghoi SG-2002 and S-2266 were tolerant, while Sohni Dharti-626 was susceptible, demonstrating the spectrum of adaptability and susceptibility within genotypes. Biochemical and genetic analysis of tolerant genotypes can be utilized to get an expanded outlook of gene regulatory mechanisms underlying salinity tolerance.

**Keywords:** Salinity; *Zea mays L.*; Seedling stage; Growth factors; Chlorophyll content

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### 1. INTRODUCTION

Maize (*Zea mays L.*) is the third most important cereal crop being grown in all regions of the world, excluding Antarctica (Orhun et al. 2013) and is the staple food for people living in Central America, Southern and Eastern Africa, and Mexico (Ranum et al. 2014). Maize, a versatile crop, is largely used for feed (56%), human food consumption (13%), a fifth for non-food including maize grain processing by-products, maize stover, and forage or silage that provide important feed sources for animals (Erenstein et al. 2022). Maize grain endosperm contains variety of nutrients including starch (85%), protein (8.5%), a small quantity of fats, antioxidants, and bioactive compounds like carotenoids, anthocyanin, and phenolic compounds that have many health-promoting role for human beings (Singh et al. 2014). The introduction of mutant allele ( $\alpha 2$ ) has led to the development of quality protein maize (QPM) with high lysine, and tryptophan contents (Hossain et al. 2019).

Thirty crop species, including maize that provide 90% of our food, often experience high yield losses under moderate salinity. Therefore, creating a need to breed the salinity tolerant crop cultivars to ensure the food security with increasing population (Zörb et al. 2019). Climate change has led to increased root-zone soil salinity in agricultural areas, especially shallow water tables, coastal areas with seawater intrusion, and water-scarce areas relying on degraded groundwater for irrigation (Corwin 2021). Salinity negatively effects the different morphological, physiological, and biochemical parameters of maize at all stages during its growth and development (Yildiz et al. 2020) but germination and seedling stages are more sensitive than the lateral growth phases (Farooq et al. 2015). Morphological traits that are effected by salinity includes reduced germination and seedling growth (Khodarahmpour et al. 2012), decreased root and shoot length, lessened biomass, diminished leaf area with altered

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leaf morphology, plant height (Hu et al. 2022) and root-to-shoot ratio (Song et al. 2023).

Under high saline conditions, adversely affected physiological parameters are reduced relative water content (RWC) (Cicek and Çakirlar 2002), impaired stomatal conductance (Gs) and transpiration (Azevedo Neto et al. 2004), deceased chlorophyll content leading to reduction in net photosynthetic rate (Pn) (Cha-Um and Kirdmanee 2009) and increased respiration rate (Dikobe et al. 2021). Biochemical effects include increased electrolyte leakage (EL), enhanced hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), malondialdehyde and ionic toxicity by Na<sup>+</sup> and Cl<sup>-</sup> ions accumulation in maize seedling (AbdElgawad et al. 2016; Iqbal et al. 2020).

Maize exhibits various morpho-physiological tolerance mechanisms to survive at the intense salinity levels. Morphological adaptations include higher biomass production especially fresh shoot weight (Hoque et al. 2015; Huqe et al. 2021), alteration in root anatomy, reduced leaf area and thicker leaves to reduce the rate of transpiration, and deeper roots to have access water and minerals in deeper soil layers (Acosta-Motos et al. 2017). Other important tolerance mechanisms to cope with high saline conditions include lipid peroxidation, increased activities of antioxidant enzymes including peroxidase, catalase, superoxide dismutase and ascorbate peroxidase (Azooz et al. 2009), accumulation of potassium and calcium ions, and osmolytes (Kholova et al. 2010), stress protein synthesis (Dubey and Stress 1999), enhanced water use efficiency by reducing stomatal conductance (Liao et al. 2022), accumulation of proline for osmotic adjustment (Carpici et al. 2010) and regulated phytohormones activity (Kaya et al. 2009).

Although a lot of investigations have been carried out to understand the effects of salinity and dynamics of its tolerance in different crop species but still there is limited comprehension creating a hurdle in developing salt tolerant genotypes. This study was carried out to investigate the diverse effects of NaCl on different morpho-physiological parameters and identify the traits which can be used for evaluating the maize under salinity stress at seedling stage. Additionally, evaluated tolerant genotypes can be utilized as valuable germplasm in developing salinity tolerant genotypes, increasing overall agricultural productivity.

## 2. MATERIALS AND METHODS

### 2.1. Study Area and Planting Material

The experiment was conducted from March 2 to April 4, 2024, in the warehouse at The Islamia University of Bahawalpur, Punjab, Pakistan to investigate the effects of salinity on morpho-physiological parameters of maize at the seedling stage. The experimental site is located at an altitude of 115.85 m above sea level with latitude 29° 23' 53" N and longitude 71° 41' 28" E. Seeds of maize hybrids were obtained from the certified seed supplier of Pak-Agro Chemicals and Seeds store located in the Bahawalpur. Names of these hybrids

are given in Table 1.

**Table 1:** Six Maize Hybrids used in the study to check the effects of NaCl treatments

Genotypes	Names
G1	S-2266
G2	S-7720
G3	Pak Afghoi SG-2002
G4	KS-17
G5	CM-1079
G6	Sohni Dharti 626

### 2.2. Experimental Design and Treatment

The study was carried out using a completely randomized design (CRD), and treatments were comprised of control, 4dS/m (low), 7dS/m (moderate), and 10dS/m (highest) sodium chloride (NaCl) solutions. Each treatment was replicated three times. Seeds of equal size and weight were selected, surface sterilized with 5% sodium hypochlorite (NaClO) solution for 5min, and washed three times with double distilled water. Maize was grown in plastic pots (height 25cm, top diameter 7.9cm, bottom diameter 5.3cm with two holes) filled with 0.91kg of air-dried sandy loam soil obtained from a local farm with EC of 0.41dS/m. Five seeds of each hybrid are sown in separate pots, with each pot containing seeds planted at a depth of 1.5cm. Seeds were germinated in approximately seven days in all pots and thinned to three plants per pot after emergence. The total salt solution for each treatment was divided into two equal splits. The first split was applied ten days after emergence. The second half was administered three days later. After applying the saline solutions, seedlings remained in the pots for the next 11 days and data were collected for analysis.

### 2.3. Data Collection

Data were recorded on different morpho-physiological traits including root length (cm), shoot length (cm), root fresh weight (g), shoot fresh weight (g), root dry weight (g), shoot dry weight (g), chlorophyll content (SPAD), stomatal conductance (mmol/m<sup>2</sup>s), leaf area (cm<sup>2</sup>), and relative water content (%). Chlorophyll content (CC) and stomatal conductance (SC) were measured by using a chlorophyll content meter and leaf perimeter, respectively, at the end of the experiment. Root length (RL) and shoot length (SL) were recorded with the wooden

meter rod after uprooting the plants from the soil. Uprooted plants were cut to separate the roots, shoots, and leaves, washed with distilled water, and dried under the fan for 1 hour to measure the root fresh weight (RFW) and shoot fresh weight (SFW) by using an electric balance. Roots and shoots were dried in an oven at 85°C for 60h to get the root dry weight (RDW) and shoot dry weight (SDW). Separated leaves were placed in fresh water for 5h, air dried, and turgid weight (TW) was recorded and then dried in the oven. Relative water content (RWC) was measured by using the formula:

$$RWC = \frac{(TW - DW)}{(FW - DW)} \times 100$$

Where TW is turgid weight, DW is dry weight, and FW is fresh weight. (Turner and Soil 1981)

The leaf area (LA) of the second leaf from the bottom was measured by using the formula:

$$LA = L \times W \times A$$

Where L is leaf length, W is leaf maximum width and A is constant (A = 0.75) (Derviş and Modeling 2013).

### 2.4. Statistical Analysis

Collected data were analyzed by two-way ANOVA (analysis of variance) under factorial design, followed by the least significance difference (LSD) test application to identify the differences between the means of genotypes and treatments at the significance level of <0.05 by using Statistics 8.1 software. Using Origin Pro software, Pearson correlation (association test) was performed to find how traits influence each other at controlled and treatment conditions. Principle component Analysis (PCA) was performed, and PCA biplots were used to study the genotypes and traits simultaneously, giving the visual observation of their relationship and identifying the key traits associated with salinity tolerance at the highest treatment level (10dS/m).

## 3. RESULTS

Results of analyzed data revealed the significant values for genotypes (G), NaCl treatment (T), and their interactions (G×T) except DRSTW (genotype, treatment and their interactions) and RTSL (treatment value) as shown in Table 2. Mean square (MS) values were considered highly significant (\*\*) and significant (\*) when p-values were less than 0.1 and 0.5, respectively. LSD test also identified the significant differences between G×T means. Therefore, reduction percentages were calculated from these mean values for each trait in all genotypes by comparing them with the controlled conditions.

**Table 2:** Analysis of Variance (ANOVA)

SOV	Df	SL	RL	SFW	RFW	SDW	RDW	CC	SC	RWC	LA	RTSL	FRTSW	DRTSW
G	5	253.547**	242.298**	3.53821**	5.24**	0.0918**	0.0734**	23.92**	296.54**	470.2**	744.26**	0.24415**	0.85736**	0.05903ns
T	3	280.077**	198.98**	6.84079**	1.66**	0.1258**	0.09938**	292.45**	312.49**	5454.31**	732.31**	0.04987ns	1.10688**	0.02092ns
G×T	15	4.359*	19.79**	0.06785*	0.35**	0.004*	0.00616**	3.42**	5.31*	24.8*	25.63**	0.12164**	0.16012**	0.06798ns

Two-way ANOVA for 13 morpho-physiological traits, demonstrating the effects of NaCl treatments on genotypes (G), treatments (T), and their interaction (G×T). The significance of mean square (MS) values is indicated by \* and \*\* for P<0.05 (significant) and P<0.01 (highly significant), and ns for non-significant results.

### 3.1. Shoot Length

This study revealed that the shoot length in all genotypes ranging from (25.3 to 11.6cm) at controlled conditions was decreased to (17.66 to 5.28cm) at 10dS/m NaCl stress. Shoot length reduction persisted with the increasing salinity, although some genotypes were more susceptible than others. SOHNI DHARTI 626 exhibited maximum reduction (60.11%) closely followed by cm-1079 (58.1%), S-7720(54.69%), and KS-17 (54.58%) as shown in Fig. 1(a). PAK AFGHOI SG-2002 was the most tolerant, showing the least reduction in shoot length by (30.2%) at 10dS/m. The average reduction in all genotypes at 4dS/m was 22.26%, which increased to 51.44% at the highest NaCl treatment.

### 3.2. Root Length

Overall, salinity stress negatively affected this parameter, but one genotype exhibited the increase in root length while the others showed the resistance in reduction with increasing salinity. Results revealed that an increase in the salinity enhanced the root length in Pak Afghoi SG-2002 by (8.68%) and (1.56%) at 4dS/m and 7dS/m, respectively, in comparison to controlled conditions but with ultimate reduction at 10dS/m. Sohni Dharti 626 and S-2266 were also showing less reduction in root length at the highest salt concentration in comparison to moderate stress application. cm-1079 revealed the lesser drop at 7dS/m in comparison to 4dS/m but was also showing the maximum reduction percentage in root length by (68.78%) among all genotypes followed by S-7720 (64.23%), KS-17 (50.46%), and S-2266(48.28%) at 10dS/m as shown in Fig. 1(b).

### 3.3. Shoot Fresh Weight

Statistical analysis results disclosed that fresh shoot weight was consistently dropped with increasing NaCl concentration in all six genotypes as given in Fig. 1(c). SOHNI DHARTI 626, being susceptible to salinity stress, lessened the fresh shoot weight by 60%, closely succeeded by S-7720(57%),cm-1079(57%), KS-17 (55%), and S-2266 (54%) at 10dS/m. Meanwhile, Pak Afghoi SG-2002 showed the lowest reduction of 40%. The decrease in fresh shoot weight in all genotypes was substantial at 4dS/m and 7dS/m (24.5% and 43.7%, respectively), and at 10dS/m, reduction was enhanced to (54%), indicating an increase in reduction from 7dS/m to 10dS/m was not remarkable (only 10.3%).

### 3.4. Root Fresh Weight

Reduction in root fresh weight was significantly lower than shoot fresh weight across all the genotypes. However, this reduction showed the non-linear behavior at different salinity conditions in almost all hybrids. At first level of treatment, PAK AFGHOI SG-2002 revealed only 3.1% reduction in shoot fresh weight. In fact, S-2266, with increasing salt stress, increased its RFW up to (23.45%) and (13.93%) at 7dS/m and 10dS/m, respectively.cm-1079 showed a maximum reduction of 49.23% at the highest level of treatment. Nearly all genotypes had less percentage reduction at the second level of treatment in comparison to the first treatment, as shown in Fig. 1(d).

### 3.5. Shoot Dry Weight (SDW)

Overall, comparative analysis revealed that shoot dry weight had a higher reduction than shoot fresh weight, showing a negative correlation between increased salinity and SDW.PAK AFGHOI SG-2002 and cm-1079 being tolerant and sensitive to NaCl stress, exhibited maximum reduction by (27.2%) and (69.7%), respectively, at 10dS/m. Although two genotypes showed non-linear responses to varied salt treatments. In Pak Afghoi SG-2002 and SOHNI DHARTI 626, there was more reduction in SDW at 4dS/m (29.7% and 47.7%) in comparison to 7dS/m treatment application (22.8% and 39.6%), respectively, indicating the possible tolerance mechanism of these genotypes against salinity. Shoot dry weight in S-2266, S-7720, KS-17, and cm-1079 was linearly decreased with increasing salinity as shown in Fig. 1(e).

### 3.6. Root Dry Weight (RDW)

Overall, root dry weight showed more depletion than root fresh weight, as plants absorb more water under salinity stress to mitigate the negative effects of salinity. The reduction proportion ranged from 66.15 to 23.9% in all genotypes at 10dS/m salt application. PAK AFGHOI SG-2002 and KS-17, having the root dry weight of 0.41g and 0.16g at controlled conditions, reduced to 0.31g and 0.12g, revealing the least reduction ratio of 23.9 and 25.9%, respectively, at the highest salt application. SOHNI DHARTI 626 was more sensitive to dry weight lessening than other genotypes. S-7720, cm-1079, and S-2266 then succeeded with a minimization percentage of 56.8, 48.3, and 45.7%, respectively, as shown in Fig. 1(f).

### 3.7. Leaf Area (LA)

Current investigation proved that, like other morphological characters, NaCl adversely affected leaf area in maize seedlings. Average leaf area at controlled conditions for all genotypes was 29.67cm<sup>2</sup> which reduced to 22.21cm<sup>2</sup>, 19.71cm<sup>2</sup>, and 12.36cm<sup>2</sup> at first, second, and third levels of treatments, indicating the most severe reduction was at 10dS/m.cm-1079 had highest reduction percentage with 67.06%, followed by SOHNI DHARTI 626 (47.19%), S-7720 (45.44%), KS-17 (44%), and S-2266 (39.46%) as shown in Fig. 2(a). PAK AFGHOI SG-2002 was tolerant to leaf reduction, showing the least minimization percentage of (27.56%) when compared to normal.

### 3.8. Stomatal Conductance (SC)

Among investigated physiological traits, stomatal conductance was least affected, showing a net percentage reduction of (38.93%) in all genotypes at elevated salinity levels. PAK AFGHOI SG-2002, showing better performance for other morpho-physiological traits, disclosed its same potential tolerance with a percentage reduction of (22.7%) at the highest stress application. The most sensitive genotype was cm-1079 with the lessened proportion of (52.4%) diligently tracked by SOHNI DHARTI 626 (47%), KS-17 (43.4%), and S-7720 (40.2%) at 10dS/m as shown in Fig. 2(b).

### 3.9. Relative Water Content (RWC)

All genotypes showed a consistent reduction in RWC with increasing salinity stress. The average RWC for all genotypes was 85.07% under the controlled conditions, which decreased to 43.17% at 10dS/m NaCl application, showing a net reduction of 49.25%. SOHNI DHARTI 626 had a maximum reduction percentage of (56%) closely

followed by-1079 (55%), KS-17 (52%), and S-7720 (49%), demonstrating their sensitivity to salt stress, as shown in Fig. 2(c). PAK AFGHOI SG-2002 and S-2266 exposed the least diminution of 40.19 and 44.79%, respectively, highlighting their potential resilience.

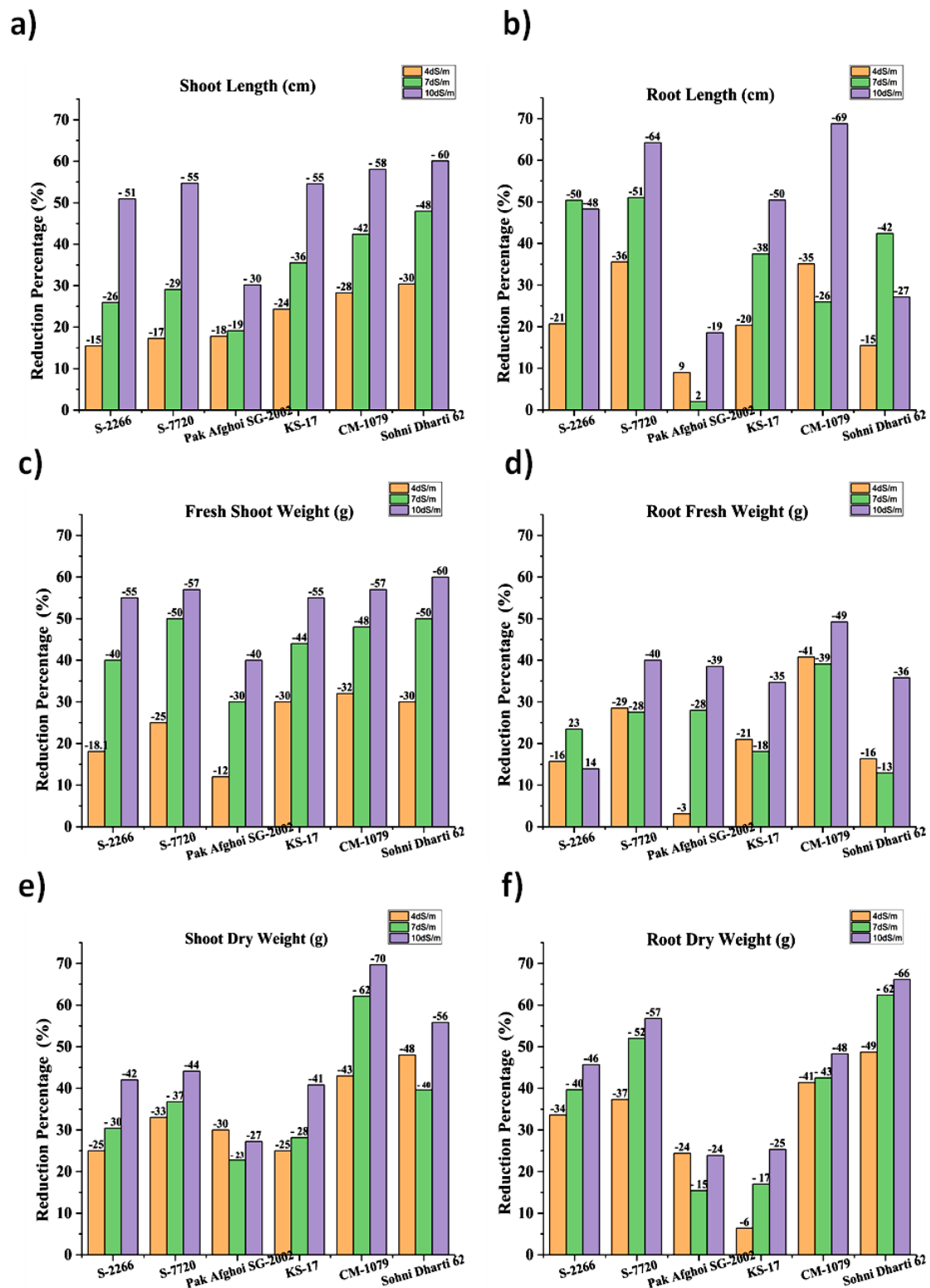


Fig. 1: Percentage Change (%) in investigated traits in 6 maize hybrids at three Salinity Levels. Each bar indicates percentage variation value, reductions with minus sign (-) and increments indicated as positive.

### 3.10. Chlorophyll Content (CC)

Chlorophyll content holding the mean value of 11.10 SPAD units for all genotypes under control conditions consistently decreased to 5.52, 3.96, and 1.63 at 4dS/m, 7dS/m, and 10dS/m, respectively. It showed the maximum reduction (85.31%) among all morpho-physiological traits. Interestingly, PAK AFGHOI SG-2002 revealed the least reduction for almost all traits, exposed the maximum short of (89.09%) for this parameter while least minimization was shown by cm-1079 (81.67%) as shown in Fig. 2(d).

### 3.11. Root to Shoot Length (RTSL)

Root to shoot length was intensified in most of the genotypes with the varied percentages under increased salinity levels, indicating more root growth as compared shoot growth in comparison to standardized conditions. SOHNI DHARTI 626 and cm-1079 demonstrated the maximum augmentation in root to shoot length by (92%) and (49%) under third and second levels of salinity as shown in Fig. 2(e). PAK AFGHOI SG-2002, being the tolerant genotype exhibited an increase in this ratio at all levels of treatment when compared to monitored conditions. Only S-7720 reflected the reduction in root-to-shoot length at all three levels.

### 3.12. Fresh Root to Shoot Weight (FRTSW)

Studied maize genotypes disclosed an increase in fresh root-shoot weight ratio, indicating higher fresh root weight than shoot weight in all treatment applications except SOHNI DHARTI 626, showing the reduction with increasing salinity as shown in Fig. 2(f). This ratio ranged from 1.71 to 0.62 at controlled conditions and increased to 2.19 to 0.89 at the highest dose of treatment in all genotypes. S-2266 had a maximum increment percentage of 154.1%, followed by KS-17 (48%), cm-1079 (44%), and S-7720 (43.5%) at 10dS/m. PAK AFGHOI SG-2002 demonstrated the least increase in this ratio by (5%) at the first level of treatment (10dS/m).

### 3.13. Dry Root to Shoot Weight (DRTSW)

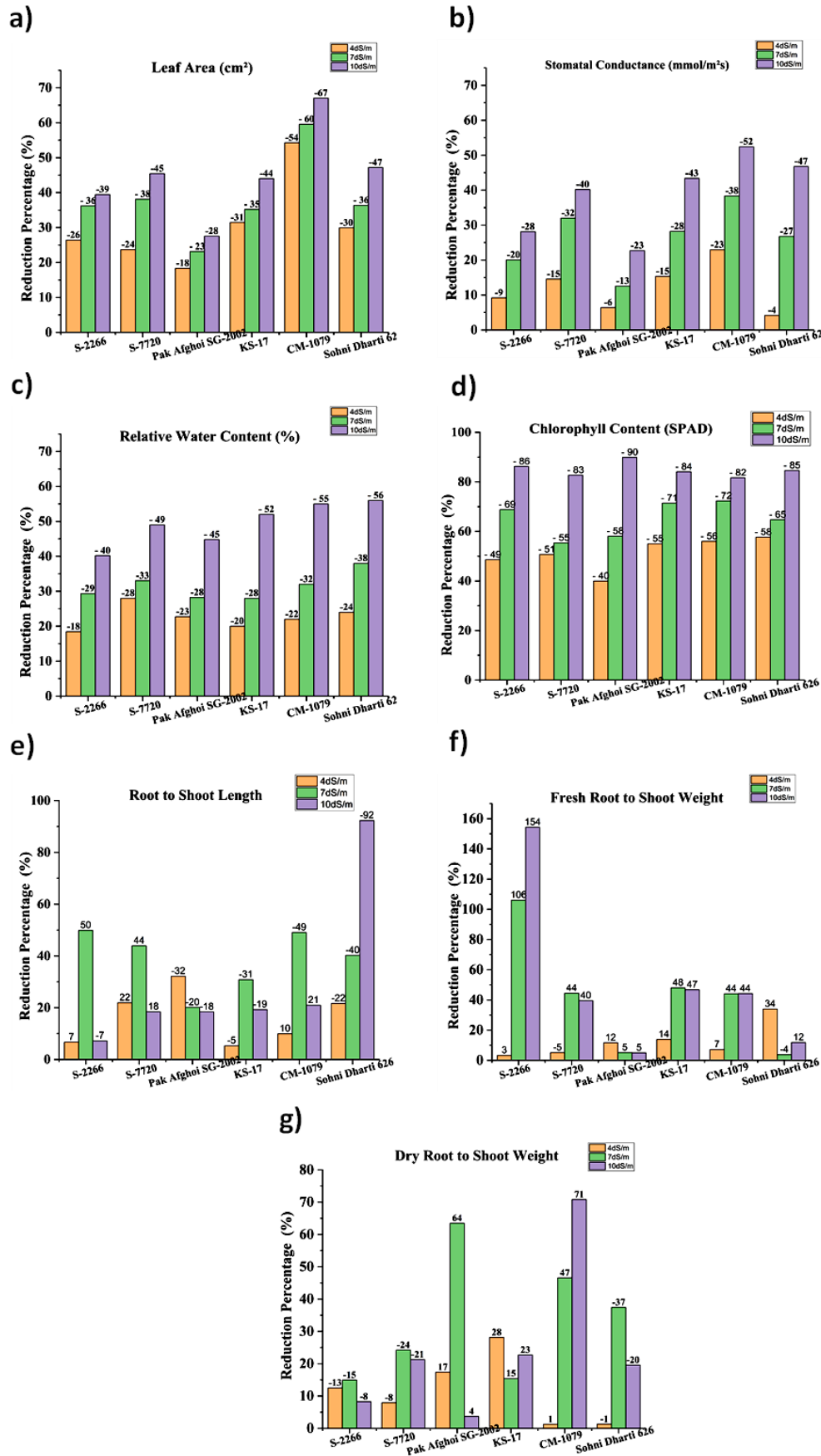
Although mean square (MS) values for dry root-shoot ratio for genotypes, treatments, and G×T interaction were non-significant ( $P>0.05$ ) percentage comparison analysis to controlled conditions revealed an increase in ratio for cm-1079 by 70.81% at 10dS/m followed by PAK AFGHOI SG-2002 (63.52%) and cm-1079 (46.56%) at 7dS/m, as shown in Fig. 2(g).

### 3.14. Correlation Analysis

Pearson's correlation analysis was performed to investigate the impact of salinity on association among all traits studied in the experiment. Under controlled conditions, correlation coefficient values ranged between (-0.15 and 0.99) for all traits. For morphological traits, the computed magnitude of correlation among SL, SFW, SDW, RL, RFW, RDW, and LA was significantly strong and above 0.81, except that the association of RDW with SFW and RDW was non-significant. For these parameters, RFW had the strongest and most significant association with RL ( $r=0.99$ ) and SFW ( $r=0.93$ ). The measured association of SL with RL, SFW, SDW, RFW, and RDW was spotted between (0.83 to 0.88).

Comparison among morpho-physiological traits under normal conditions revealed that the association magnitude of SC with SDW, SFW, and SL was ( $r=0.98$ , 0.94, and 0.93) and that of RWC with SFW, SDW, SL, and RDW was ( $r=0.97$ , 0.96, 0.93, and 0.90), respectively. RTSL, FRTSW, and DRTSW displayed a non-significant and very weak association with almost all studied traits. Only non-significant negative correlation was between FRTSW and SFW ( $r=-0.15$ ) as shown in Fig. 3(a).

Correlation coefficient values at highest stress application were fluctuating between (-0.51 to 0.99) for all studied parameters. For morphological traits, correlation computed magnitude among SL, SFW, SDW, RL, RFW, RDW, and LA was spotted between (0.44 to 0.98) clearly showing the effects of treatment on association among corresponding parameters in comparison to control. At 10dS/m NaCl application, RDW showed the highest positive correlation with SFW ( $r=0.99$ ), SDW( $r=0.98$ ), and SL( $r=0.97$ ). The correlation of SL with SFW and SDW was strong and positive, and RL had a significant association with RDW ( $r=0.80$ ) and a non-significant weak association with RFW ( $r=0.44$ ). At intense salt application, the correlation among physiological traits was changed compared to those in controlled conditions. The association of SC with RWC ( $r=0.92$ ) was still highly significant but weak and non-significant with CC ( $r=0.14$ ). CC revealed a non-significant and very weak correlation with RWC. It was the only physiological trait showing a moderate to very weak correlation with all investigated traits in the study, as shown in Fig. 3(b). Among morpho-physiological traits, the correlation of RWC with SDW (0.91), RDW (0.89), LA (0.88), RFW (0.86), and SFW (0.83) was significant but non-significant with SL and RL. SC also exhibited a strong positive association with SDW (0.99), RDW (0.98), SFW (0.95), and SL (0.95) and a non-significant association with RFW.



**Fig. 2:** Percentage Change (%) in investigated traits in 6 maize hybrids at three Salinity Levels. Each bar indicates percentage variation value, reductions with minus sign (-) and increments indicated as positive.

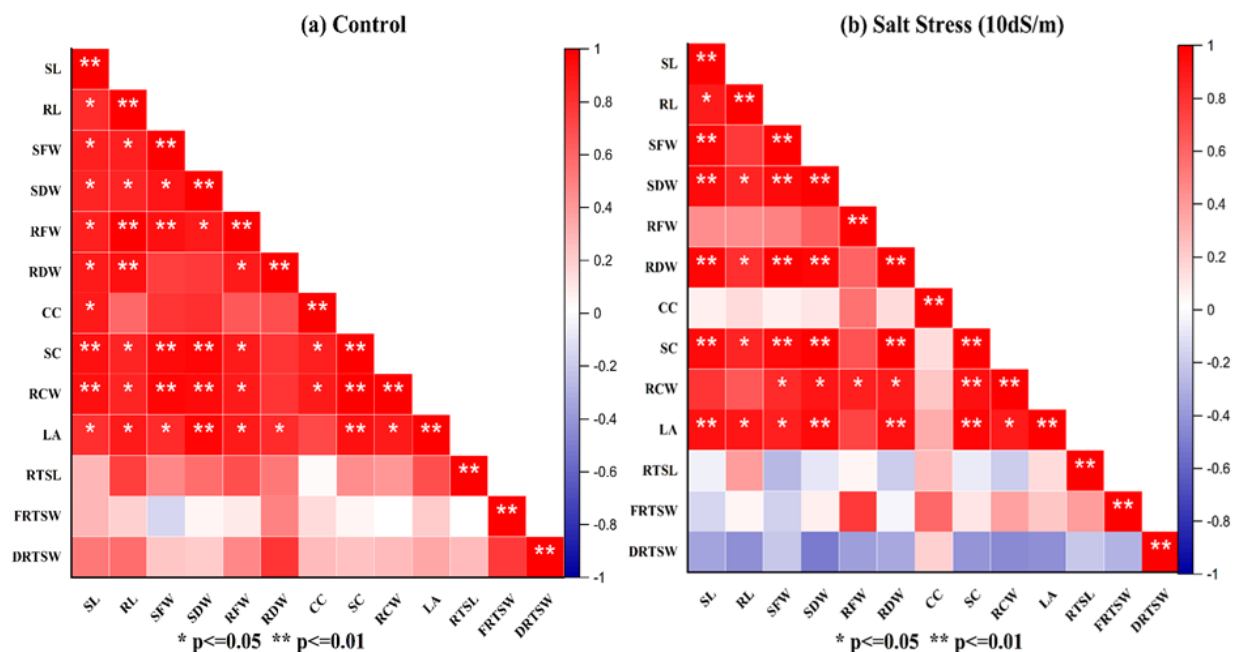


Fig. 3: Pearson's Correlation matrix of 13 traits under normal and highest stress application.

At normal conditions, the association of RTSL, FRTSW, and DRTSW with all traits was positive, but at the highest level of treatment, these parameters showed a non-significant and weak to moderate negative association with all morpho-physiological traits. The highest negative correlation coefficient in this study was displayed between DRTSW and SFW ( $r = -0.51$ ).

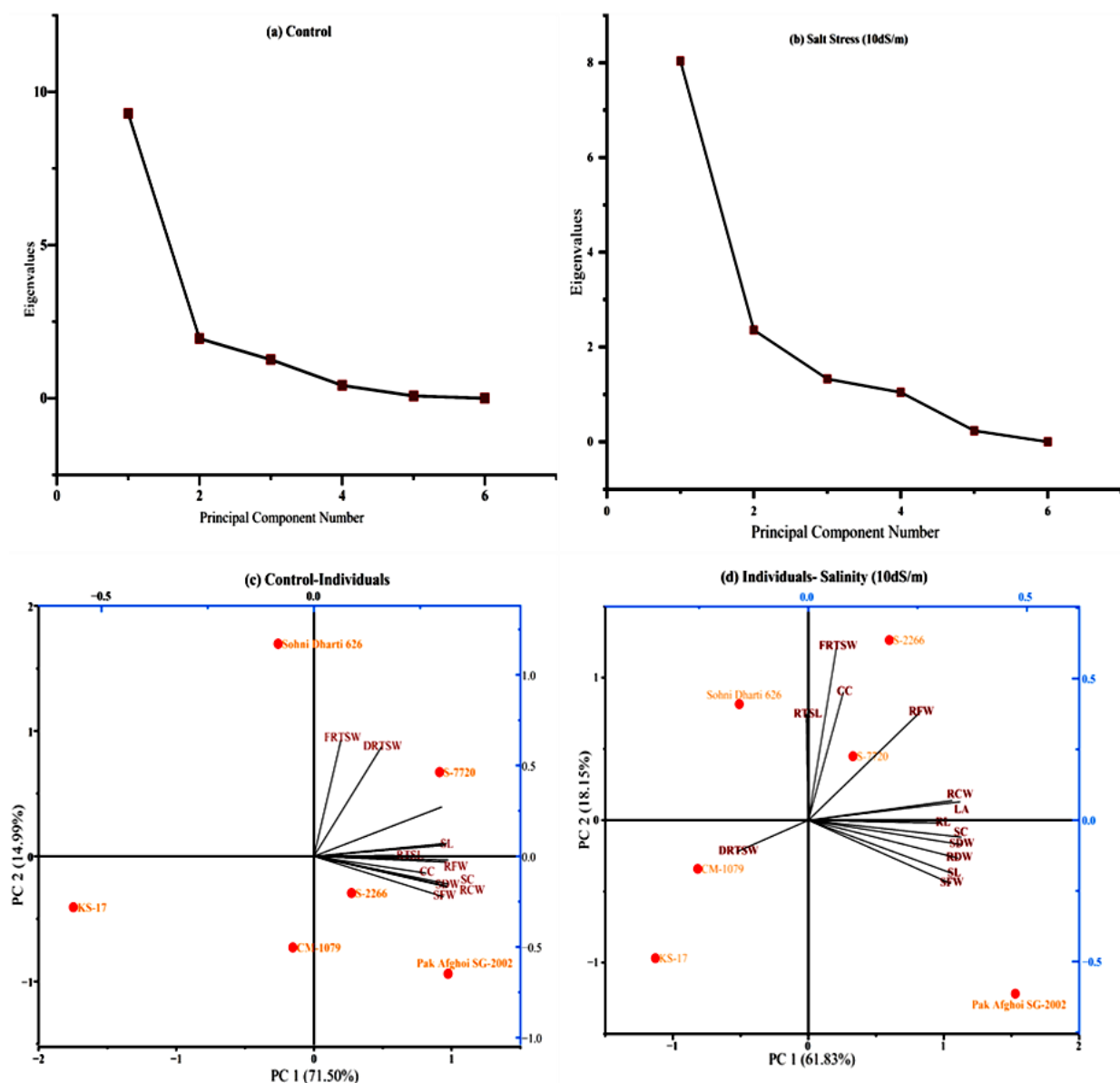
### 3.15. Principle Component Analysis (PCA)

As all genotypes had different performances for morpho-physiological traits under normal and highest stress conditions, PCA analysis was performed to reduce the dimensionality of data, discriminate among traits, and visualize the salinity-tolerant genotypes. Eigenvalues of the first three components under the controlled conditions and the first four values at 10dS/m were greater than one, but only the first two PCs were counted as they were capturing the maximum variability. Under normal conditions, eigenvalues for the first and second components were 9.29 and 1.95, respectively and captured (86.48%) of the total variance. These values were changed to 8.03 and 2.35 for PC1 and PC2 at the highest level of treatment (10dS/m), contributing the share of (79.99%) for total variance as shown in Fig. 4(a) and (b) scree plots.

Under controlled conditions, PC1 was predominantly interpreted by RFW, SFW, SL, RL, SC, RWC, and LA, and eigenvectors of these traits were strongly associated with each other, showing a very strong correlation among them, while FRTSW and DRTSW explained PC2. PAK AFGHOI SG-2002 and S-7720 were performing optimally in all traits associated with PC1, while the performance of KS-17 and cm-1079 was largely not good in these traits associated with PC1 and PC2. SOHNI DHARTI 626 was performing well for traits that were associated with PC2. While S-2266 had a moderate performance for major growth traits associated with PC1, FRTSW, and DRTSW did not enhance their effectiveness, as shown in Fig. 4(c).

But under 10dS/m stress, there was a change in the length and angle of each trait, as PC1 was largely explained by SC, LA, SDW, RDW, SL, RWC, and SFW, and these traits were positively associated with each other as shown in Fig. 4(d). While FRTSW, CC, and RFW largely explained PC2. PAK AFGHOI SG-2002 had a well-rounded performance for traits associated with PC1 and inadequate performance for parameters associated with PC2 (FRTSW, CC, RFW). Under salinity conditions, there was a shift in the position of S-7720 as it positively impacted both PCs but moderately performed well for all studied traits underlying the investigation. The performance of S-2266 was significantly superior under salinity stress, as it was remarkably influencing both PCs, showing its higher tolerance against saline conditions among all studied genotypes. SOHNI DHARTI 626, in comparison to control conditions, displayed deteriorating performance for almost all traits associated with PC1 and PC2. But cm-1079 and KS-17 were the most susceptible genotypes because of their amplified negative impact on both PCs in contrast to a regulated environment, indicating their poor performance and susceptibility to high salinity stress.





**Fig. 4:** Principal Component analysis (PCA) of 6 maize hybrids based on 13 morpho-physiological traits under control and highest stress application (10dS/m). (a) and (b) are the Scree Plots, each indicating the first six principal components. (c) and (d) are PCA-Biplots each with the red dots indicating 6 hybrids, eigenvectors represent investigated variables under control and highest salt application.

Overall investigation with the PCA biplots, it was concluded that Pak Afghoi SG-2002 and S-2266 are the most tolerant hybrids while KS-17 is the most sensitive under salinity stress. While FRTSW, RFW, CC, SC, LA, and RWC explained the major proportion of variability in data under saline.

#### 4. DISCUSSION

Salinity is a complex abiotic factor that is responsible for growth retardation in crops during all stages of their development (Gao et al. 2015), resulting in massive yield losses; therefore, plant breeders have to develop salinity-tolerant genotypes to ensure food security. Due to the polymorphic nature of maize, diverse alleles bearing optimal traits are present within the species. Hence different breeding methods can be used to incorporate desired traits in a single genotype with improved salinity tolerance (Zaidi et al. 2022). Results of studied traits expressed that salinity negatively affected all morphological and physiological traits in all genotypes at the seedling stage. Two-way ANOVA and, subsequently, LSD mean comparison revealed significant variations among almost all genotypes, treatments, and their combination.

Among morphological traits, SL, SFW, SDW, RL, RFW, LA, and RDW were decreased. This reduction can be attributed to osmotic stress, hormonal imbalances, and deterioration of maize physiological processes, causing the above and below-ground parts of the plant to show stunted growth, resulting in overall biomass reduction. The results of our study are similar to the investigations carried out by many workers (Akram et al. 2010; Wang et al. 2020; Ali et al. 2021). An adaptive mechanism in plants under salinity stress is the accumulation of different ions from varied organelles and organs (especially from roots to shoot), resulting in increased ionic toxicity and ultimately causing SL reduction (Arif et al. 2020). This reduction in SL with increasing salinity has been reported in other members of the grass family, like ryegrass (Pessarakli and Kopec 2009).

In our investigation, there was a net reduction in RL in all genotypes. This decrease can be attributed to the menacing of cellular processes due to increased accumulation of  $\text{Na}^+$  ions, diminished  $\text{K}^+$  content, and soluble sugars, resulting in reduced osmotic adjustment and osmotic potential responsible for root length reduction (Hajlaoui et al. 2010). But an increase in RL at a higher salinity level in Pak Afghoi SG-2002 at 4dS/m and 7dS/m and less reduction in Sohni Dharti 626 at 10dS/m in comparison to 7dS/m is a possible important salt tolerance mechanism enabling the plant to absorb more water and nutrients under high osmotic stress. In other abiotic stress conditions like drought, maize exaggerated the root length in deeper soil zones to absorb the maximum water and nutrients as a tolerance mechanism to cope with the stress conditions (Zhan et al. 2015). An investigation carried out in cotton seedlings displayed no effects of moderate salinity on primary root length and weight but a reduction at higher stress levels above 75mM (Shelden and Munns 2023).

According to our findings, there was more reduction in dry biomass than fresh root and shoot biomass. More reduction in SDW and RDW as compared to SFW and RFW indicates more water retention capacity and osmotic adjustments in maize plants under salinity stress conditions (Fatima et al. 2021). As for other characters, leaf area was reduced in all hybrids but cm-1079 displayed the maximum reduction in this parameter. This significant reduction in leaf area is due to a decrease in the activity of the hydrogen pump, changes in enzymes, and enhanced pH of the apoplastic pathway (Pitann et al. 2009).

An increase in RTSL (more root growth as compared to shoot growth) indicates more resource allocation to roots under salinity stress than shoots to help the maize plant survive under severe salinity stress. Other researchers in corn and wheat have confirmed this increase in ratio (Zaidi et al. 2022) (Khatun et al. 2013). An increase in FRTSW indicates shoot biomass has a higher reduction percentage than root biomass under salinity stress, a common accommodative mechanism under salinity stress in maize (Zia et al. 2022). DRTSW exposed non-significant results showing that it was least affected by NaCl treatments due to strong genetic control, limiting the effect of the environment.

Maximum RWC reduction in SOHNI DHARTI 626 and least reduction in PAK AFGHOI SG-2002 is highlighting their different response mechanism under stress conditions. Similar studies in maize and water dropwort (vegetable), revealed that RWC reduction is possibly due to their varied mechanism for ion intake (Maqbool et al. 2020), (Kumar et al. 2021). Among physiological traits, SC was least affected as compared to CC and RWC but revealed consistent reduction with increasing salinity, especially in cm-1079. SC, an important plant physiological mechanism, is massively deteriorated by salinity and drought because plants are unable to obtain enough moisture content, resulting in stomatal closure, responsible for reduced SC and transpiration (Liao et al. 2024).

Of all investigated traits, CC exhibited maximum drop-down with increasing NaCl concentrations, revealing this character is more susceptible to salinity than others. CC showed maximum reduction in PAK AFGHOI SG-2002 and KS-17. Higher sodium and chloride ions accumulation in chloroplast reduces its functionality, nutrients impairment, more reactive oxygen species (ROS) accumulation, inhibition of enzymes activation controlling Calvin cycle, and hormonal changes (Das and Roychoudhury 2014; Alamer et al. 2022; Chattha et al. 2022).

It is essential to understand the correlation among morpho-physiological traits because they are essential indicators of maize adaptability under stressful conditions like salinity. Under normal conditions all morpho-physiological traits were positively and strongly correlated to each other, showing that these parameters are genetically and physiologically linked (Hall and Richards 2013), contributing to excellent maize performance under normal conditions. Many other researchers have also reported significant and positive correlations among these traits in maize (Ali et al. 2014; Zhu et al. 2024).

At 10dS/m NaCl application, a very strong correlation of SC with RWC, SL, SFW, SDW, and LA, and SL with SFW, SDW, RL, and RDW was observed, indicating that these traits have co-adaptive and common genetic base helping the plant to survive under salinity stress. Traits with positive and significant associations can be used in breeding programs striving to develop the salinity tolerant genotypes (Shannon 1997). On the other hand, shoot parameters (SL, SFW, SDW) showed a significant correlation with root parameters (RL, RDW) under salinity stress. These findings are in harmony with other investigations (Masuda et al. 2021) but the association among some root parameters and that of shoot traits with RFW was non-significant and moderate, representing that these traits were negatively affected by salinity.

Under salty conditions, CC was the only physiological parameter showing a weak and non-significant correlation with morphological traits. So, CC had a differential response and more sensitivity to salinity stress, resulting in co-regulation disturbance and weak association with other traits in comparison to control (Turan et al. 2009). The association of RTSL, FRTSW, and DRTSW among them and with other morpho-physiological traits was non-significant but positive under normal conditions that interestingly changed to negative under salty conditions. This change in correlation under salinity stress indicates the trade-off between allocation of resources and disruption in plant physiology due to the accumulation of Na<sup>+</sup> and Cl<sup>-</sup> ions in the soil (Hasana and Miyake 2017).

PCA analysis proved that Pak Afghoi SG 2002 and S 2266 are resilient genotypes while Sohni Dharti 626 is susceptible at 10dS/m stress application, possibly due to the differences in their genetic makeup responsible for the varied responses for osmotic adjustment and ions regulation. So, these resilient genotypes can be selected as parents in breeding programs to develop salinity-tolerant genotypes, and their further biochemical and genetic analysis should be carried out to understand the ion homeostasis and osmotic adjustment mechanisms. FRTSW, CC, RFW, SC, LA, and RWC are morpho-physiological traits that can be used to evaluate the maize under salinity stress because of their maximum contribution to overall variability. In wheat and other cereal crops, several morphological and physiological adaptations are required to tolerate the high osmotic stress to conserve the maximum water and maintain the growth and developmental parameters (Munns et al. 2006).

Our current investigation yielded a profound comprehension of the detrimental impacts of salinity on morpho-physiological traits and specific parameters that can be used to evaluate the maize under salinity stress at the seedling stage. However, it is crucial to incorporate large genetic diversity and biochemical and genetic markers to grasp the comprehensive perception and illustration of complex salinity tolerance mechanisms. An exhaustive understanding of molecular processes like sodium and chloride ions' compartmentalization in vacuoles, explicitly stated signaling pathways and a combination of genomics and phenomics can assist the plant breeders in mitigating the negative impacts of salinity on growth and developmental stages and developing salinity tolerant genotypes for maize and other crop species, resulting in magnified agricultural productivity (Munns and Tester 2008).

## 5. CONCLUSION

Comprehensive research, understanding, and interpretation of the relationship of traits with genotypes experiencing salinity challenges are crucial to developing salt-tolerant hybrids. Therefore, current research that set out to investigate the effects of NaCl treatments on different structural and physiological parameters in six maize hybrids demonstrated the adverse effects of salinity on all examined variables. However, a comparative analysis of means revealed the maximum reduction in CC followed by SL and SFW in all genotypes, exhibiting their increased vulnerability compared to other traits. At 10dS/m stress application, the strong association among SFW, SL, SDW, RDW, SC, and RL showed that these parameters have a common genetic base; optimizing one trait can positively impact others, leading to improved salinity tolerance. Furthermore, FRTSW, CC, RFW, SC, LA, and RWC can be used to assess maize under salinity stress, aiding in the development of more tolerant genotypes. Overall, Pak Afghoi SG 2002 and S2266 were the genotypes that were more tolerant than others, so in the future, their molecular analysis can be engaged in reviewing the osmotic adjustment mechanism under salinity stress. Despite this, it is crucial to incorporate large genetic diversity with biochemical and genetic analysis for broader applicability of results to obtain a better understanding of the mechanism underlying salinity tolerance.

**Author's Contribution:** IA, A.Raza, and WR conceived and designed the experiment. IA, A.Raza, AH, SH, MN, and MS performed the study, collected the data, and conducted lab analyses. IA supervised and coordinated the experiments, and A.Rehman provided salt solution preparation formula. IA, A.Raza, WR, and A.Rehman performed statistical analyses of experimental data and prepared the manuscript format. IA, A.Raza, AH, and WR prepared the manuscript draft. All authors critically revised the manuscript and approved the definitive version.

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