

## Characterizing Maize Genotypes for Salt Tolerance Using Morphological and Ionic Traits at Seedling Stage

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**Keywords:** Hydroponics, salinity, growth, categorization

**Abstract:** Maize crop is the third most important cereal crop, mostly grown for food, feed and fodder purpose. In spite of the fact the crop is susceptible to salt stress but exhibits a considerable genotypic variability for salt tolerance. The present study was carried out to determine to which extent the maize genotypes with contrasting morphological traits respond to salt stress. Seven days old seedlings of thirty maize genotypes were exposed to NaCl stress of EC less than 4 (control), equal to 8 and 12 dS m<sup>-1</sup> for further 28 days in a temperature controlled greenhouse. The salt stress imposition was completed in two increments every other day from the date of planting. At the age of 35 days, the seedlings were evaluated for contrasting morphological traits. The genotypes exhibited considerable variations for each of the 10 measured morphological and ionic traits. PCA was employed to identify the most suitable morphological trait to be used as selection criterion for salt tolerance. Based on the PCA results, dry shoot weight (DSW) was used to classify thirty maize genotypes into salt-tolerant-T, moderately tolerant-MT and salt sensitive-S groups. Two (2) out of thirty genotypes i.e. SB-9617 and FH-949 had the highest average of percent dry shoot weight (PDSW) values > 70% were classified as salt tolerant (T). The fifteen (15) genotypes showed average of PDSW values in the range of 55-69.9 % were classified as moderately tolerant (MT) by indicating the sequence order of salt tolerance as YH-1898 > MMRI-yellow > S-2002 > FH-988 > FH-1292 > HC-12 > MS-2018 > NCEV-1270-7 > Pahari > Pearl White > FH-1114 > NCEV-1270-3 > Iqbal > NCEV-1297 > DK-6724. However, thirteen (13) out of total thirty (30) genotypes attained the average PDSW values <55% were classified as salt sensitive (S) following the sequence order as NCEV-1530-9 > Composite > FRI-22 > Azam > MS-2015 > P-1543 > Neelum > Afgoi > Malka-2016 > MS-1501 > HNG > NCEV-1270-5 > NCEV-7004. It was also noticed that the declared salt tolerance was positively correlated with dry shoot weight (DSW), shoot length (SL), stem diameter (SD), fresh shoot weight (FSW), fresh root weight (FRW), dry roots weight (DRW), root length (RL), leaf area per plant (LA), number of leaves per plant (NL) and K<sup>+</sup>/Na<sup>+</sup> ratio. The highest positive Pearson correlation coefficients were determined in LA vs SD (r=0.900), DSW vs SD (r=0.899), SL vs DSW (r=0.891), SL vs FSW (r=0.890) and DSW vs FSW (r=0.887). Additionally, the salt tolerant (T) group of genotypes maintained higher K<sup>+</sup>/Na<sup>+</sup> ratios compared to moderately tolerant (MT) and salt sensitive (S) groups. The results clearly showed that dry shoot weight (DSW) could be viable option to classify maize genotypes into different salt tolerance groups and to identify the most suited and best adapted salt tolerant genotype for cultivation in saline soils. Furthermore, this scientific information could help the plant scientists to improve and develop the new salt tolerant cultivar.

### 1. Introduction

Maize (*Zea mays* L.) crop is on rank 3<sup>rd</sup> after wheat and rice. In Pakistan it is grown on area of 1,418 thousand hectares with the total production of 8.47 million tons in 2020-21 [18] contributed 0.6 percent to GDP. The crop is moderately sensitive to salt stress and mostly grown for multiple purposes such as food, feed and fodder.

Salinity impacts crop growth and development through its osmotic and ionic effects via restricting the water and nutrients supply to plants [54, 34, 59, 47]. Moreover, the influence of salt stress on plant growth occurs in two phases: limits water supply to plants in the first phase i.e. osmotic phase [48,49,31] and disrupts nutrients supply through higher accumulation of toxic ions specifically  $\text{Na}^+$  in the second phase i.e. ionic phase [49]. Furthermore, the reactive oxygen species (ROS) when produced in mitochondria; chloroplast and peroxisomes in response to saline stress, are more reactive than molecular  $\text{O}_2$  and have the greater tendency to react with cellular membrane causing more leakiness of membranes [44,2,24]. Additionally, the ROS also cause significant damage to plant genomics and disrupts various physiological, biochemical, and metabolic processes, more specifically the bio-synthesis of proteins and chlorophylls [46, 43, 41, 66, 64, 36, 25, 12].

However, different breeding procedures such as introduction, selection and hybridization are reported in the literature for the development of improved varieties. Thus, the existence of sufficient genetic variability among the genotypes for various attributes is prerequisite for a successful breeding programme. Among the traits, morphological and ionic attributes can effectively be used to determine genotypic variability for salt tolerance among the genotypes [23]. The genetic variability based on these traits determines salt tolerance among the genotypes [6].

As maize crop is categorized as moderately sensitive to salt stress but exhibits considerable genetic variability for morphological and ionic traits among genotypes [40, 15, 26, 33, 7]. The morphological and ionic traits which experience negative impacts of salt stress greater are shoot length (SL), root length (RL), stem diameter (SD), number of leaves (NL) & leaf area (LA), fresh & dry root weights (FRW & DRW), fresh & dry shoot weight (FSW & DSW) and  $\text{K}^+/\text{Na}^+$  ratios [13,20]. Such type of variations may serve as mile stone for developing new/advance breeding lines and varieties with improved characters. Therefore, the acquisition of knowledge about the genotypic variability in the available germplasm resources, especially regarding the morphological and ionic attributes, is the foremost step before starting any breeding programme (3, 55). It enables the plant breeder to select the most suitable/appropriate genetic material for developing salt tolerant cultivars by considering the morphological traits as selection criteria [57,69]. To ease the selection of desirable maize genotypes, the maize breeders must have information regarding the nature and extent of relationships among the morphological and ionic attributes [37]. Some powerful methods and strategies to analyze the degree of functional genetic divergence in the available genotypes include multivariate analysis [27, 68]. Various statistical techniques such as correlation, PCA, regression, cluster analysis, and factor analysis are used to assess the genotypic variability [6,37, 68].

In view of the above perspective, the present study was conducted with the objectives (i) to find out the strength of relationships among studied attributes of the experimental genotypes (ii) to explore the existence of genetic variability among the tested genotypes (iii) to identify the most suitable trait to use as selection criterion among the studied morphological and ionic attributes. PCA and then Murillo et al. [5, 4] classification were employed on the data to categorize maize genotypes into different salt tolerance groups i.e. tolerant (T), moderately tolerant (MT) and sensitive (S) genotypic groups.

## 2. Materials and Methods

The study was planned to categorize promising maize genotypes into different salt tolerance groups based on genotypic variability using morphological and bio-chemical traits as a selection criterion. For this purpose thirty maize genotypes viz., FH-1114, FH-988, FH-1292, HC-12, YH-1898, DK-6724, P-1543, HNG, SB-9617, FH-949, MMRI-yellow, Pearl white, Composite, Malka-2016, Afgoi, Azam, Iqbal, Pahari, S-2002, Neelum, MS-2018, NCEV-1270-3, NCEV-1297, NCEV-1530-9, FRI-22, MS-2015, NCEV-1530-10, MS-1501, NCEV-1270-5 and NCEV-7004 were collected from National Agricultural Research Centre Islamabad, Ayub Agricultural Research Institute Faisalabad, Maize and Millet Research Institute Sahiwal and Fodder Research Institute Sargodha. The seed of each genotype was surface sterilized with hypochlorite (1%) solution. After

sterilization the seed was washed with distil water 2-3 times and allowed to dry under the shade. Thereafter, sand was purchased from the local market and sieved properly to remove all the inert material and gravels. The sieved sand was then washed with water three time and spread on the polythene sheet for sun drying. After that, the sand was filled in seedling trays. One tray was maintained for each genotype by planting one healthy seed per hole of the seedling tray. These seed planted trays were placed in the rain protected glass house with regulated temperature (30°C) at Institute of Soil Science, PMAS-Arid Agriculture University Rawalpindi (33.6518°N and 73.0806°E). The trays were moistened daily to avoid drying. On the day 7<sup>th</sup> from planting of seed, five healthy and uniform seedlings from each genotype were uprooted carefully and wrapped separately with foam at the root shoot junction. After wrapping, each seedling was fixed into the holes of polystyrene thermo pole sheet floating on the water in a plastic tub (60×40×30 cm<sup>3</sup>). Moreover, five seedlings per replica were maintained. Furthermore, a weighed quantity of sodium chloride (NaCl) of analytical grade was added into each tub to develop the requisite salt stress levels viz. <4 (control), 8 and 12 dS m<sup>-1</sup>. It was noteworthy that salinity stress was imposed in two equal splits on each alternate day after the shifting of seedlings. Half-strength of the Hoagland's [14] nutrient solution was also added in each tub on the very next day to meet the nutrients requirement of the seedlings. Additionally, the pH of the solution was maintained at 6.5±0.5 with 1M NaOH or HCl when required. Nutrient solution was changed accordingly after one week interval. An aeration pump (ACO-208, Guangdong Hailea Group Co., Ltd, China) was installed in each tub with all its piping arrangements to ensure the continuous supply of air.

At the age of 35 days, the seedlings of each genotype from each salt stress treatment were taken and separated into root and shoot parts with the help of a sharp paper cutter blade. Accordingly, the morphological and bio-chemical traits were recorded by employing the standard procedures as under: Fresh weights of the shoots and roots were recorded with digital balance (Model: AND-GF 3000). Shoot and root lengths of the seedlings were measured with the help of an ordinary foot scale. Stem diameter of the seedlings measured using vernier calliper. Leaves of the five seedlings from each genotype were counted and then averaged to compute number of leaves per seedling. Leaf area was measured by multiplying the maximum length and width of the leaves with 0.75. Dry weights of the shoots and roots were recorded after oven drying at 70 °C for 72 h. However, the Na<sup>+</sup> and K<sup>+</sup> contents were determined in the dried shoots were determined by digesting the plant material with perchloric acid and concentrated H<sub>2</sub>SO<sub>4</sub>. The digested samples were diluted and run at flame photometer (Digiflame, DV-710) for the quantification of K<sup>+</sup> and Na<sup>+</sup>.

After the quantification of all the morphological and bio-chemical attributes, PCA was performed to identify the most suitable trait to be used as selection criterion. By relying on the PCA results, dry shoot weight (DSW) and fresh root weight (FRW) were appeared as the major contributing traits to be used as selection criterion for salt tolerance. Moreover, both the variables contributed maximally and equally to explain the overall variations. Furthermore, it has been proven previously that shoots experiences more salts stress than roots. In this study, dry shoot weight (DSW) was therefore used as a sole criterion to categorize maize genotypes for salt tolerance using Murillo et al., [5, 4] classification. Accordingly, dry shoot weights (DSW) were used to compute PDSW8 (A) and PDSW12 (B) and finally to work out average of PDSW (C) for each genotype by using the formulae given as under:

$$PDSW8 = \frac{DSW (g) \text{ in EC } 8}{DSW (g) \text{ in control}} \times 100 \quad (A)$$

$$PDSW12 = \frac{DSW (g) \text{ in EC } 12}{DSW (g) \text{ in control}} \times 100 \quad (B)$$

$$\text{Average of PDSW} = \frac{A + B}{2} \times 100 \quad (C)$$

Where PDSW8 and PDSW12 are the percent dry shoot weights at EC 8 and 12dSm<sup>-1</sup>, respectively.

According to this classification, the genotypes which showed average of PDSW  $\geq 70$  % were designated as tolerant (T). However the genotypes whose average of PDSW values falls between 55-69.9 % were categorized as moderately tolerant (MT). Moreover, the genotypes that exhibited the average of PDSW  $< 55$  were placed under sensitive (S) category.

**Statistical Analysis:** Analysis of variance (ANOVA) was performed by using a statistical package, SPSS version 16.0 and the means were compared by standard error.

### 3. Results

#### *Shoot length (cm):*

The thirty (30) maize genotypes responded differently in response to salt stress and considerable variations between the control and salt treatments were observed in almost all traits. Data presented in the Table-1 indicated the decreased shoot length (SL) with the increasing salt stress levels. Results of the current study showed that SB-9617 performed better among the genotypes with respect to yielding maximum shoot length i.e.  $33.50 \pm 0.47$ ,  $30.77 \pm 0.80$  and  $26.50 \pm 0.70$  cm at EC =  $< 4, 8$  and  $12 \text{ dS m}^{-1}$ . However, the minimum shoot lengths i.e.  $22.30 \pm 0.50$  cm ( $< 4 \text{ dS m}^{-1}$ ),  $19.80 \pm 0.90$  ( $8 \text{ dS m}^{-1}$ ) and  $14.60 \pm 1.04$  cm ( $12 \text{ dS m}^{-1}$ ) were recorded in NCEV-1530-9. Additionally, SB-9617 had seedlings with 60 % higher average shoots length compared to NCEV-1530-9 among the other studied genotypes.

**Table 1.** Effect of different levels of salt stress on the shoot length (cm) of different maize genotypes in 35 days old seedlings under solution culture

Shoot length (cm) per plant				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	26.37±0.72	20.50±0.93	17.33±1.02	21.40±1.40
Azam	27.57±0.64	23.00±1.27	18.13±0.86	22.90±1.44
Iqbal	28.30±0.64	23.83±0.69	19.57±0.41	23.90±1.29
S-2002	30.30±0.65	26.60±0.81	22.63±0.56	26.51±1.16
FH-988	30.07±0.55	27.20±0.49	21.70±0.87	26.32±1.27
FH-1292	30.10±0.32	24.83±0.71	21.10±1.00	25.34±1.36
FH-1114	28.90±1.31	25.53±1.05	18.43±0.82	24.29±1.63
Composite	30.23±0.41	23.40±0.64	16.23±0.60	23.29±2.04
MS-2015	25.97±0.93	23.20±0.55	18.87±0.55	22.68±1.09
NCEV-1270-3	28.30±0.57	24.30±0.64	19.90±0.68	24.17±1.25
MS-2018	28.67±1.61	24.80±0.82	20.53±0.69	24.67±1.30
HC-12	28.90±0.64	24.57±1.39	21.57±1.04	25.01±1.19
SB-9617	33.50±0.47	30.77±0.80	26.50±0.70	30.26±1.07
YH-1898	31.57±0.50	28.00±0.93	23.50±0.85	27.69±1.23
Malka-2016	25.57±1.48	22.77±0.78	17.07±1.53	21.80±1.41
P-1543	26.07±0.71	23.00±1.32	17.90±0.50	22.32±1.28
Neelum	25.73±0.90	23.30±0.76	17.07±0.84	22.03±1.36
FRI-22	29.70±0.92	24.97±1.28	14.07±0.22	22.91±2.35
MMRI-Yellow	31.23±1.02	27.97±0.43	22.77±0.74	27.32±1.29
Pahari	29.63±0.88	24.57±0.79	19.77±0.73	24.66±1.48
DK-6724	26.07±0.73	23.67±0.49	21.00±0.60	23.58±0.80
FH-949	31.33±1.24	29.50±1.07	23.47±1.48	28.10±1.34
Pearl White	29.03±0.96	25.70±1.10	19.00±0.93	24.58±1.56
NCEV-1270-5	23.53±1.19	20.50±0.81	15.20±0.93	19.74±1.31
Afgoi	24.87±0.90	20.97±0.81	19.93±1.02	21.92±0.88
NCEV-1270-7	29.97±1.30	23.83±0.91	20.17±1.17	24.66±1.54

NCEV-1530-9	28.77±0.57	22.47±0.54	18.83±1.11	23.36±1.50
NCEV-1297	26.83±0.95	24.50±0.40	19.97±0.80	23.77±1.08
NCEV-7004	22.30±0.50	19.80±0.90	14.60±1.04	18.90±1.21
HNG	26.40±0.70	22.40±0.42	14.63±0.56	21.14±1.75
Mean	28.19±0.16	24.35±0.30	19.38±0.33	23.97±0.28

Mean ± Standard error (Mean ± S.E)

### *Fresh shoot weight (g) per plant:*

Table 2 indicated the deleterious effects of incremented salt stress levels on fresh shoot weight (FSW) of thirty maize genotypes. The maximum fresh shoot weights viz., 5.78±0.09 3.82±0.16 2.33±0.07 g per plant were obtained in SB-9617 at EC <4, 8 and 12 dS m<sup>-1</sup>, respectively followed by FH-949 produced fresh shoot weights of 5.56±0.24 3.81±0.14 2.02±0.07 g per plant at the above salt stress levels respectively. The minimum fresh shoot weights viz., 2.74±0.06 1.35±0.03 0.54±0.06 g per plant were attained with NCEV-7004 with the increasing levels of salt stress. Furthermore, SB-9617 showed the lowest reductions of fresh shoot weight 33.9 & 59.7 % at 8 and 12dSm<sup>-1</sup> with respect to control treatment, respectively.

**Table 2.** Effect of different levels of salt stress on the fresh shoot weight of maize genotypes in 35 days old seedlings under solution culture

Fresh shoot weight [g per plant]				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	3.08±0.05	1.46±0.03	0.66±0.03	1.74±0.36
Azam	3.33±0.07	1.71±0.06	0.81±0.05	1.95±0.37
Iqbal	4.40±0.09	2.34±0.11	1.23±0.07	2.68±0.47
S-2002	5.23±0.12	3.62±0.08	1.18±0.08	3.34±0.59
FH-988	5.35±0.13	3.53±0.07	1.27±0.03	3.38±0.59
FH-1292	3.95±0.11	2.49±0.07	1.03±0.05	2.49±0.42
FH-1114	3.81±0.12	2.15±0.04	1.02±0.06	2.33±0.41
Composite	4.35±0.15	2.44±0.06	0.90±0.03	2.57±0.50
MS-2015	4.17±0.24	2.37±0.15	0.77±0.02	2.44±0.50
NCEV-1270-3	3.26±0.17	2.11±0.21	0.54±0.04	1.97±0.40
MS-2018	3.89±0.16	2.32±0.03	1.01±0.05	2.41±0.42
HC-12	4.17±0.09	2.59±0.14	1.02±0.03	2.59±0.46
SB-9617	5.78±0.09	3.82±0.16	2.33±0.07	3.98±0.50
YH-1898	5.33±0.15	3.30±0.09	1.98±0.05	3.54±0.49
Malka-2016	3.60±0.08	1.62±0.08	0.89±0.07	2.03±0.41
P-1543	3.74±0.09	2.27±0.19	0.54±0.05	2.19±0.47
Neelum	3.31±0.09	2.01±0.07	0.47±0.06	1.93±0.41
FRI-22	3.93±0.15	2.58±0.09	0.45±0.02	2.32±0.51
MMRI-Yellow	4.86±0.15	2.91±0.31	1.64±0.04	3.14±0.48
Pahari	3.78±0.17	2.42±0.08	0.80±0.03	2.33±0.43
DK-6724	3.81±0.05	2.37±0.11	0.67±0.05	2.29±0.46
FH-949	5.56±0.24	3.81±0.14	2.02±0.07	3.80±0.52
Pearl White	4.74±0.12	2.82±0.06	1.16±0.05	2.91±0.52
NCEV-1270-5	2.63±0.11	1.32±0.05	0.44±0.04	1.46±0.32
Afgoi	2.69±0.10	1.48±0.04	0.50±0.05	1.56±0.32
NCEV-1270-7	4.32±0.12	2.46±0.07	1.22±0.07	2.67±0.45
NCEV-1530-9	3.41±0.26	1.74±0.08	0.92±0.06	2.02±0.38
NCEV-1297	3.20±0.12	1.81±0.05	0.77±0.02	1.93±0.35
NCEV-7004	2.74±0.06	1.35±0.03	0.54±0.06	1.54±0.32
HNG	3.08±0.03	1.61±0.05	0.42±0.03	1.70±0.39
Mean	3.98±0.09	2.36±0.08	0.98 ±0.05	2.44±0.09

Mean ± Standard error (Mean ± S.E)

**Dry shoot weight (g) per plant:**

The increasing salt stress also caused drastic effects on the dry shoot weight (DSW) in all the maize genotypes as indicated in the Table-3. The maximum dry shoot weights viz.,  $1.45 \pm 0.09$ ,  $1.19 \pm 0.05$  and  $0.90 \pm 0.03$  were determined in SB-9617 where EC less than 4 and equal to 8 and  $12 \text{dSm}^{-1}$  were maintained in the nutrients medium solution. The minimum dry shoot weights viz.,  $0.68 \pm 0.10$ ,  $0.37 \pm 0.03$  and  $0.11 \pm 0.01$  were recorded in NCEV-7004 at same levels of salt stress respectively. The other tested maize genotypes were noticed between the two genotypes with respect to dry shoot weight.

**Table 3.** Effect of different levels of salt stress on the dry shoot weight of maize genotypes in 35 days old seedlings under solution culture

Dry shoot weight [g per plant]				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	0.80±0.04	0.51±0.03	0.23±0.03	0.51±0.08
Azam	0.90±0.06	0.72±0.03	0.18±0.03	0.60±0.11
Iqbal	0.99±0.12	0.77±0.08	0.33±0.01	0.70±0.11
S-2002	1.21±0.07	0.94±0.07	0.64±0.03	0.93±0.09
FH-988	1.21±0.11	0.84±0.05	0.72±0.02	0.92±0.08
FH-1292	1.19±0.05	0.81±0.08	0.70±0.04	0.90±0.08
FH-1114	0.99±0.06	0.78±0.09	0.40±0.04	0.72±0.09
Composite	0.92±0.07	0.74±0.08	0.21±0.01	0.63±0.11
MS-2015	0.90±0.02	0.67±0.05	0.23±0.04	0.60±0.10
NCEV-1270-3	0.99±0.12	0.77±0.17	0.39±0.08	0.72±0.11
MS-2018	1.07±0.09	0.80±0.04	0.55±0.08	0.81±0.08
HC-12	1.14±0.02	0.85±0.03	0.60±0.03	0.86±0.08
SB-9617	1.45±0.09	1.19±0.05	0.90±0.03	1.18±0.09
YH-1898	1.29±0.11	1.08±0.03	0.71±0.07	1.03±0.09
Malka-2016	0.81±0.05	0.53±0.08	0.23±0.03	0.52±0.09
P-1543	0.89±0.08	0.62±0.08	0.25±0.03	0.59±0.10
Neelum	0.87±0.03	0.62±0.07	0.22±0.02	0.57±0.10
FRI-22	0.90±0.03	0.72±0.10	0.19±0.03	0.60±0.11
MMRI-Yellow	1.29±0.05	1.103±0.05	0.63±0.06	1.01±0.10
Pahari	1.00±0.07	0.79±0.03	0.42±0.02	0.74±0.09
DK-6724	0.96±0.08	0.76±0.04	0.30±0.02	0.67±0.10
FH-949	1.34±0.09	1.15±0.07	0.77±0.03	1.09±0.09
Pearl White	1.00±0.07	0.78±0.11	0.41±0.05	0.73±0.09
NCEV-1270-5	0.76±0.03	0.48±0.01	0.17±0.02	0.47±0.10
Afgoi	0.84±0.08	0.62±0.06	0.19±0.03	0.55±0.09
NCEV-1270-7	1.03±0.03	0.80±0.04	0.51±0.03	0.78±0.10
NCEV-1530-9	0.94±0.06	0.75±0.14	0.25±0.03	0.65±0.08
NCEV-1297	0.96±0.05	0.76±0.06	0.31±0.03	0.67±0.11
NCEV-7004	0.68±0.10	0.37±0.03	0.11±0.01	0.39±0.10
HNG	0.76±0.04	0.49±0.07	0.19±0.01	0.48±0.09
Mean	1.01±0.02	0.76±0.03	0.39±0.02	0.71±0.02

Mean± Standard error (Mean ± S.E)

***Number of leaves and Leaf area (cm<sup>2</sup>) per plant:***

The reductions of numbers of leaves (NL) and leaf area (LA) per plant in all the genotypes were observed with incrementing the salt concentrations in the nutrient medium (Table-4 & 5). In the present study the highest numbers of leaves ( $6.00 \pm 0.00$ ,  $5.67 \pm 0.33$  &  $5.33 \pm 0.33$ ) and leaf area ( $204.48 \pm 1.16$ ,  $161.12 \pm 2.02$  &  $120.65 \pm 3.57$  cm<sup>2</sup>) per plant were recorded in SB-9617 at salinity stress levels <4, 8 & 12dSm<sup>-1</sup> respectively. Whereas, the lowest number of leaves ( $4.33 \pm 0.67$ ,  $3.33 \pm 0.33$  &  $2.67 \pm 0.33$ ) and leaf area ( $115.90 \pm 7.79$ ,  $87.23 \pm 7.12$  &  $70.74 \pm 4.13$  cm<sup>2</sup>) per plant were observed in NCEV-7004 with the same levels of salt stress. Furthermore, SB-9617 produced 64.8 and 229.2 % higher number of leaves (NL) and leaf area (LA) per plant than NCEV-7004.

**Table 4.** Effect of different levels of salt on effect of the number of leaves of maize genotypes counted in 35 days old seedlings under solution culture

Number of leaves per plant				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	4.67±0.333	4.33±0.33	3.33±0.33	4.11±0.26
Azam	5.00±0.00	4.67±0.33	4.00±0.58	4.56±0.24
Iqbal	5.00±0.00	4.67±0.33	4.33±0.33	4.67±0.17
S-2002	5.33±0.67	5.00±0.00	4.67±0.33	5.00±0.24
FH-988	5.33±0.33	5.00±0.00	4.67±0.33	5.00±0.17
FH-1292	5.33±0.00	5.00±0.00	4.67±0.33	5.00±0.17
FH-1114	5.00±0.00	5.00±0.00	4.33±0.33	4.78±0.15
Composite	5.00±0.00	4.67±0.33	4.33±0.33	4.67±0.17
MS-2015	5.00±0.00	4.33±0.67	4.00±0.58	4.44±0.29
NCEV-1270-3	5.00±0.00	4.67±0.33	4.33±0.33	4.67±0.17
MS-2018	5.33±0.33	5.00±0.00	4.33±0.33	4.89±0.20
HC-12	5.33±0.33	5.00±0.58	4.67±0.33	5.00±0.24
SB-9617	6.00±0.00	5.67±0.33	5.33±0.33	5.67±0.17
YH-1898	6.00±0.00	5.33±0.67	5.00±0.00	5.44±0.24
Malka-2016	5.00±0.00	4.33±0.33	3.33±0.33	4.22±0.28
P-1543	5.00±0.00	4.33±0.33	4.00±0.00	4.44±0.18
Neelum	5.00±0.00	4.33±0.67	3.67±0.33	4.33±0.29
FRI-22	5.00±0.00	4.67±0.33	4.00±0.58	4.56±0.24
MMRI-Yellow	5.67±0.33	5.33±0.33	4.67±0.33	5.22±0.22
Pahari	5.33±0.33	5.00±0.00	4.33±0.33	4.89±0.20
DK-6724	5.00±0.00	4.67±0.33	4.33±0.67	4.67±0.24
FH-949	6.00±0.00	5.33±0.33	5.00±0.00	5.44±0.18
Pearl White	5.00±0.00	5.00±0.00	4.33±0.33	4.78±0.15
NCEV-1270-5	4.33±0.33	4.00±0.58	3.00±0.58	3.78±0.32
Afgoi	5.00±0.00	4.33±0.33	3.67±0.33	4.33±0.24
NCEV-1270-7	5.33±0.67	5.00±0.58	4.33±0.33	4.89±0.31
NCEV-1530-9	5.00±0.00	4.67±0.33	4.33±0.33	4.67±0.17
NCEV-1297	5.00±0.00	4.67±0.33	4.33±0.33	4.67±0.17
NCEV-7004	4.33±0.67	3.33±0.33	2.67±0.33	3.44±0.29
HNG	4.68±0.00	4.00±0.58	3.33±0.33	4.00±0.29
Mean	5.13±0.06	4.71±0.08	4.18±0.08	4.67±0.05

Mean± Standard error (Mean ± S.E)

**Table 5.** Effect of different levels of salt stress on the leaf area of maize genotypes measured in 35 days old seedlings under solution culture

	Leaf area [cm <sup>2</sup> per plant]			
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	87.10±4.66	69.29±3.36	41.71±2.58	66.03±6.85
Azam	105.06±5.40	83.74±3.39	61.92±3.66	83.57±6.58
Iqbal	116.83±3.24	87.42±7.50	72.07±6.53	92.11±7.39
S-2002	144.70±2.93	107.23±2.33	92.30±5.44	114.75±8.02
FH-988	129.88±10.18	104.76±1.90	90.08±4.85	108.24±6.68
FH-1292	124.86±4.22	103.67±2.70	84.28±7.41	104.27±6.40
FH-1114	118.80±15.71	89.62±3.11	73.04±7.76	93.82±8.43
Composite	109.14±3.06	84.67±6.36	67.34±2.49	87.05±6.44
MS-2015	102.65±4.00	81.00±5.32	61.87±10.73	81.84±6.92
NCEV-1270-3	118.55±3.23	88.97±3.94	72.35±2.20	93.29±6.94
MS-2018	122.65±8.48	99.54±2.01	83.09±1.58	101.76±6.28
HC-12	124.85±2.55	102.08±4.16	83.59±5.48	103.51±6.33
SB-9617	204.48±1.16	161.12±2.02	120.65±3.57	162.08±12.16
YH-1898	154.37±8.09	116.11±1.30	102.55±2.47	124.34±8.14
Malka-2016	88.06±3.34	69.34±2.24	44.95±3.65	67.45±6.43
P-1543	101.99±4.46	80.78±3.23	55.27±2.10	79.35±6.96
Neelum	93.84±4.48	74.27±3.60	53.17±3.16	73.76±6.17
FRI-22	107.45±4.02	84.50±6.81	62.83±2.22	84.93±6.86
MMRI-Yellow	152.49±2.72	107.95±3.33	93.38±3.74	117.94±9.04
Pahari	120.58±12.16	98.56±5.63	77.42±4.85	98.86±7.47
DK-6724	115.88±1.64	85.74±6.44	70.12±6.38	90.58±7.22
FH-949	186.08±2.73	119.85±3.05	103.94±4.75	136.62±12.70
Pearl White	120.00±4.89	94.11±5.84	75.04±5.96	96.38±7.09
NCEV-1270-5	79.03±1.77	63.35±5.46	40.66±3.71	61.02±5.91
Afgoi	88.19±4.23	69.86±2.12	51.78±2.93	69.94±5.49
NCEV-1270-7	120.89±7.93	99.38±4.58	82.73±4.79	101.00±6.28
NCEV-1530-9	113.80±3.90	85.42±4.90	68.92±2.92	89.38±6.83
NCEV-1297	115.90±7.79	87.23±7.43	70.74±4.12	91.29±7.39
NCEV-7004	70.58±5.26	47.94±1.84	28.95±2.19	49.15±6.26
HNG	83.95±10.82	65.44±2.33	41.25±2.26	63.55±6.98
Mean	117.42±3.19	90.43±2.30	70.93±2.30	92.93±1.91

Mean± Standard error (Mean ± S

***Stem diameter (cm) per plant:***

All the maize genotypes respond differently to varying salt stress levels for stem diameter (Table-6). However, SB-9617 outperformed among the other maize genotypes and yielded maximum stem diameter (SD) viz.,  $0.57 \pm 0.01$ ,  $0.50 \pm 0.01$  &  $0.47 \pm 0.02$  cm at EC less than 4 and equal to 8 and 12dSm<sup>-1</sup>, respectively. Moreover, NCEV-7004 was found the least performer which yielded the lowest stem diameter ( $0.367 \pm 0.01$ ,  $0.26 \pm 0.01$ , &  $0.24 \pm 0.02$  cm) with the increasing levels of salt. On overall basis, SB-9617 attained the maximum stem diameter ( $0.51 \pm 0.02$ ) than attained ( $0.29 \pm 0.01$  cm) by NCEV-7004, which was 75.9% higher in SB-9617 compared to NCEV-7004.



**Table 6.** Effect of different levels of salt effect on the stem diameter of maize genotypes measured in 35 days old seedlings under solution culture

Stem diameter [cm per plant]				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	0.443±0.012	0.363±0.015	0.323±0.009	0.377±0.019
Azam	0.457±0.015	0.403±0.009	0.343±0.009	0.401±0.017
Iqbal	0.472±0.003	0.427±0.009	0.370±0.006	0.423±0.015
S-2002	0.527±0.012	0.453±0.009	0.423±0.009	0.468±0.016
FH-988	0.527±0.009	0.447±0.015	0.413±0.009	0.462±0.018
FH-1292	0.520±0.006	0.440±0.021	0.407±0.009	0.456±0.018
FH-1114	0.477±0.009	0.427±0.009	0.373±0.009	0.426±0.016
Composite	0.463±0.009	0.417±0.009	0.350±0.017	0.410±0.018
MS-2015	0.450±0.011	0.403±0.009	0.340±0.015	0.398±0.017
NCEV-1270-3	0.473±0.009	0.427±0.009	0.373±0.009	0.424±0.015
MS-2018	0.507±0.018	0.437±0.009	0.390±0.006	0.444±0.018
HC-12	0.520±0.006	0.437±0.007	0.403±0.012	0.453±0.018
SB-9617	0.573±0.012	0.500±0.006	0.467±0.015	0.513±0.017
YH-1898	0.540±0.015	0.473±0.009	0.427±0.009	0.480±0.017
Malka-2016	0.447±0.009	0.367±0.009	0.327±0.012	0.380±0.018
P-1543	0.450±0.021	0.377±0.009	0.337±0.009	0.388±0.018
Neelum	0.447±0.012	0.377±0.009	0.333±0.015	0.386±0.018
FRI-22	0.463±0.015	0.413±0.012	0.343±0.017	0.407±0.019
MMRI-Yellow	0.530±0.006	0.470±0.012	0.423±0.009	0.474±0.016
Pahari	0.493±0.009	0.433±0.009	0.380±0.006	0.436±0.017
DK-6724	0.463±0.018	0.420±0.021	0.360±0.012	0.414±0.017
FH-949	0.563±0.012	0.487±0.015	0.430±0.012	0.493±0.020
Pearl White	0.480±0.006	0.433±0.009	0.377±0.009	0.430±0.015
NCEV-1270-5	0.423±0.009	0.357±0.009	0.280±0.006	0.353±0.021
Afgoi	0.447±0.015	0.373±0.009	0.330±0.006	0.383±0.018
NCEV-1270-7	0.493±0.009	0.433±0.009	0.390±0.012	0.439±0.016
NCEV-1530-9	0.463±0.018	0.420±0.006	0.360±0.015	0.414±0.017
NCEV-1297	0.467±0.009	0.423±0.009	0.367±0.009	0.419±0.015
NCEV-7004	0.367±0.012	0.263±0.009	0.240±0.017	0.290±0.021
HNG	0.430±0.006	0.360±0.012	0.307±0.015	0.366±0.019
Mean	0.479±0.005	0.415±0.005	0.366±0.005	0.420±0.004

Mean± Standard error (Mean ± S.E)

**Root length (cm) per plant:**

Root length (RL) was also seriously affected by higher levels of salt stress in all the studied genotypes (Table-7). In the present study, root lengths in all the genotypes were drastically influenced by incrementing the salt stress levels. However, the maximum reductions in the root lengths ( $10.93 \pm 0.35$ ,  $8.20 \pm 0.42$  &  $7.03 \pm 0.37$  cm) were recorded in NCEV-7004 at <4, 8 and  $12\text{dSm}^{-1}$  respectively. Additionally, these reductions were recorded 33.1 % greater than control at  $8\text{dSm}^{-1}$  and 55.4 % higher than control at  $12\text{dSm}^{-1}$  in the same maize genotype. Among the maize genotypes, SB-9617 proved the best performing genotype having more root lengths ( $17.60 \pm 0.91$ ,  $15.10 \pm 1.10$  &  $13.57 \pm 0.62$  cm) at all the salt stress levels. On overall basis, SB-9617 attained 20.3 % higher more root length than NCEV-7004.

**Table 7.** Effect of different levels of salt stress on the root length of different maize genotypes in 35 days old seedlings under solution culture

Root length [cm per plant]				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	13.83±0.43	11.27±0.48	7.17±0.24	10.76±0.99
Azam	13.77±0.94	11.77±0.18	9.40±0.60	11.64±0.71
Iqbal	15.00±0.28	13.20±0.92	8.80±0.32	12.33±0.97
S-2002	16.60±1.00	12.93±0.65	11.43±0.49	13.66±0.85
FH-988	17.17±0.77	12.47±0.50	10.57±0.58	13.40±1.03
FH-1292	15.93±0.22	12.83±1.02	11.07±0.32	13.28±0.78
FH-1114	14.20±0.57	12.47±0.55	11.20±0.31	12.62±0.50
Composite	14.73±0.62	12.00±0.25	8.50±0.55	11.74±0.94
MS-2015	14.57±0.38	11.67±1.01	8.63±0.33	11.62±0.92
NCEV-1270-3	15.83±1.02	11.83±0.58	10.00±0.25	12.56±0.93
MS-2018	15.43±0.49	13.10±0.17	10.97±0.15	13.17±0.66
HC-12	16.00±0.78	14.33±0.58	9.33±0.23	13.22±1.04
SB-9617	17.60±0.91	15.10±1.10	13.57±0.62	15.42±0.74
YH-1898	17.97±0.84	13.33±0.61	11.23±0.18	14.18±1.04
Malka-2016	12.67±0.73	11.13±0.29	8.50±0.31	10.77±0.66
P-1543	12.83±0.76	11.93±0.62	9.53±0.44	11.43±0.58
Neelum	13.03±1.13	11.13±0.22	9.77±0.52	11.31±0.60
FRI-22	14.03±0.91	11.73±0.48	9.37±0.70	11.71±0.76
MMRI-Yellow	16.97±0.52	13.53±0.72	10.73±0.37	13.74±0.94
Pahari	14.83±0.38	12.70±0.25	11.20±0.42	12.91±0.56
DK-6724	14.03±0.66	12.03±0.43	10.20±0.17	12.09±0.60
FH-949	17.17±1.15	14.43±0.46	12.40±0.41	14.67±1.41
Pearl White	15.13±0.38	12.90±0.21	10.47±0.52	12.83±0.70
NCEV-1270-5	11.70±0.76	8.57±0.93	6.60±0.74	8.96±0.85
Afgoi	14.60±0.55	9.67±0.50	8.17±0.44	10.81±1.00
NCEV-1270-7	15.03±1.04	13.20±0.78	11.17±0.27	13.13±0.68
NCEV-1530-9	14.53±1.20	11.67±0.44	9.47±0.45	11.89±0.83
NCEV-1297	14.47±0.66	12.33±0.33	9.63±0.09	12.14±0.73
NCEV-7004	10.93±0.35	8.20±0.42	7.03±0.37	8.72±0.61
HNG	13.13±0.59	10.83±0.73	7.93±0.48	10.63±0.81
Mean	14.79±0.21	12.14±0.18	9.80±0.21	12.24±0.17

Mean± Standard error (Mean ± S.E)

***Fresh root weight (g) per plant:***

Fresh root weights (FRW) in the tested genotypes were also affected by the negative impacts of increasing salinity (Table-8). In comparison, almost all the maize genotypes experienced the reduction of fresh root weight with the higher levels of salt stress but SB-9617 was found least affected by the increasing salt concentrations i.e. at EC=8 dSm<sup>-1</sup> the reduction of fresh shoot weight was 12.8 % and in the similar way the reduction in the FRW was 45 % at EC equal to 12dSm<sup>-1</sup> compared to control. The highest reductions of FRWs i.e. 36.4 & 74.4 % were recorded in NCEV-7004 at 8 and 12dSm<sup>-1</sup> respectively. On an average, SB-9617 produced 212.3% higher fresh root weight (FRW) than NCEV-7004 under the influence of salt stress. The remaining genotypes were present in between them with respect to FRW (Table-8).

**Table 8.** Effect of different levels of salt stress on the fresh root weight of maize genotypes in 35 days old seedlings under solution culture

Fresh root weight [g per plant]				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	1.44±0.105	1.03±0.044	0.42±0.036	0.96±0.15
Azam	1.72±0.031	1.15±0.076	0.69±0.020	1.19±0.15
Iqbal	2.12±0.095	1.45±0.129	0.97±0.101	1.51±0.18
S-2002	2.65±0.046	2.13±0.175	1.35±0.087	2.04±0.20
FH-988	2.72±0.081	2.17±0.035	1.43±0.035	2.11±0.19
FH-1292	2.55±0.061	2.09±0.136	1.31±0.146	1.98±0.19
FH-1114	2.29±0.106	1.68±0.072	1.07±0.153	1.68±0.19
Composite	1.72±0.112	1.17±0.091	0.65±0.056	1.18±0.16
MS-2015	1.68±0.122	1.13±0.046	0.62±0.131	1.14±0.16
NCEV-1270-3	2.27±0.208	1.58±0.291	1.10±0.087	1.65±0.20
MS-2018	2.57±1.246	1.79±0.046	1.20±0.058	1.85±0.41
HC-12	2.94±1.386	2.23±0.131	1.15±0.104	2.11±0.48
SB-9617	3.13±0.148	2.73±0.060	1.72±0.046	2.53±0.22
YH-1898	2.92±0.076	2.47±0.259	1.54±0.087	2.31±0.22
Malka-2016	1.49±0.026	1.05±0.128	0.49±0.069	1.01±0.15
P-1543	1.67±0.058	1.14±0.106	0.57±0.091	1.13±0.17
Neelum	1.59±0.051	1.13±0.095	0.57±0.095	1.10±0.15
FRI-22	1.73±0.064	1.15±0.031	0.63±0.182	1.17±0.17
MMRI-Yellow	2.78±0.215	2.23±0.157	1.50±0.058	2.17±0.20
Pahari	2.52±0.184	1.87±0.116	1.23±0.065	1.87±0.20
DK-6724	1.75±0.024	1.27±0.085	0.67±0.078	1.23±0.16
FH-949	3.07±0.051	2.65±0.110	1.68±0.050	2.47±0.21
Pearl White	2.31±0.153	1.78±0.104	1.01±0.059	1.70±0.20
NCEV-1270-5	1.37±0.150	0.92±0.032	0.29±0.044	0.86±0.16
Afgoi	1.57±0.060	1.09±0.125	0.55±0.042	1.07±0.15
NCEV-1270-7	2.46±0.061	1.93±0.106	1.17±0.140	1.85±0.20
NCEV-1530-9	2.13±0.148	1.27±0.148	1.05±0.098	1.48±0.18
NCEV-1297	1.98±0.104	1.37±0.089	0.85±0.035	1.39±0.17
NCEV-7004	1.29±0.161	0.82±0.074	0.33±0.042	0.81±0.15
HNG	1.37±0.228	0.98±0.092	0.38±0.015	0.91±0.16
Mean	2.13±0.080	1.58±0.061	0.94±0.046	1.55±0.05

Mean± Standard error (Mean ± S.E)

**Dry root weight (g) per plant:**

Dry root weight (DRW) presented in the Table-9 showed the deleterious effects of salt stress in thirty (30) maize genotypes. The results depicted that SB-9617 performed better among the other tested genotypes with respect to DRW i.e.  $0.39 \pm 0.01$ ,  $0.35 \pm 0.01$  and  $0.30 \pm 0.01$  g per plant at EC less than 4 (non-saline), equal to 8 and  $12 \text{dSm}^{-1}$  respectively. It is furthermore added that SB-9617 produced 10.3 and 23.1 % higher DRW at 8 and  $12 \text{dSm}^{-1}$  respectively, compared to control treatment. However, NCEV-7004 was noticed as the least performer yielding DRWs of  $0.17 \pm 0.01$ ,  $0.11 \pm 0.01$  and  $0.08 \pm 0.01$  g per plant where EC was less than 4, equal to 8 &  $12 \text{dSm}^{-1}$  respectively. On overall basis, SB-9617 (showing highest mean DRW) produced 192 % higher dry root weight than NCEV-7004 (showing least mean DRW).

**Table 9.** Effect of different levels of salt stress on dry root weight of maize genotypes in 35 days old seedlings under solution culture

Dry root weight [g per plant]				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	0.16±0.012	0.14±0.009	0.11±0.012	0.14±0.009
Azam	0.18±0.012	0.16±0.006	0.13±0.010	0.16±0.009
Iqbal	0.21±0.010	0.19±0.012	0.16±0.006	0.19±0.009
S-2002	0.28±0.018	0.26±0.015	0.22±0.015	0.25±0.012
FH-988	0.27±0.010	0.24±0.010	0.21±0.012	0.24±0.010
FH-1292	0.28±0.007	0.23±0.018	0.22±0.015	0.24±0.011
FH-1114	0.25±0.012	0.23±0.006	0.19±0.012	0.22±0.010
Composite	0.21±0.010	0.19±0.012	0.10±0.010	0.17±0.018
MS-2015	0.19±0.012	0.16±0.006	0.13±0.010	0.16±0.010
NCEV-1270-3	0.26±0.010	0.23±0.010	0.18±0.015	0.22±0.013
MS-2018	0.25±0.015	0.23±0.015	0.20±0.015	0.23±0.011
HC-12	0.27±0.013	0.25±0.012	0.21±0.012	0.24±0.010
SB-9617	0.39±0.012	0.35±0.010	0.30±0.007	0.35±0.014
YH-1898	0.28±0.003	0.26±0.015	0.24±0.017	0.26±0.009
Malka-2016	0.18±0.015	0.14±0.012	0.11±0.010	0.14±0.012
P-1543	0.19±0.012	0.16±0.015	0.12±0.006	0.16±0.012
Neelum	0.20±0.015	0.15±0.003	0.09±0.017	0.15±0.017
FRI-22	0.20±0.006	0.16±0.015	0.11±0.006	0.16±0.014
MMRI-Yellow	0.29±0.012	0.26±0.015	0.22±0.006	0.26±0.012
Pahari	0.25±0.010	0.23±0.010	0.20±0.010	0.23±0.009
DK-6724	0.19±0.006	0.20±0.012	0.16±0.012	0.18±0.008
FH-949	0.33±0.010	0.29±0.012	0.24±0.006	0.29±0.014
Pearl White	0.25±0.015	0.22±0.010	0.19±0.015	0.22±0.011
NCEV-1270-5	0.16±0.012	0.12±0.006	0.09±0.006	0.12±0.011
Afgoi	0.17±0.012	0.15±0.012	0.12±0.006	0.15±0.009
NCEV-1270-7	0.25±0.010	0.23±0.012	0.20±0.006	0.23±0.009
NCEV-1530-9	0.19±0.006	0.18±0.007	0.14±0.009	0.17±0.009
NCEV-1297	0.23±0.015	0.18±0.012	0.12±0.006	0.18±0.013
NCEV-7004	0.17±0.012	0.11±0.006	0.08±0.007	0.12±0.017
HNG	0.17±0.015	0.12±0.006	0.09±0.006	0.13±0.013
Mean	0.23±0.006	0.20±0.006	0.16±0.006	0.20±0.004

Mean ± Standard error (Mean ± S.E)

***K<sup>+</sup>/Na<sup>+</sup> ratios***

All the 30 maize genotypes were also evaluated for K<sup>+</sup>/Na<sup>+</sup> ratios against the graded levels of salt stress. Data presented in the Table-10 clearly indicated that all the genotypes responded differently to incrementing salt concentrations. The maize genotype SB-9617 comparatively exhibited the highest K<sup>+</sup>/Na<sup>+</sup> ratios i.e.  $7.24 \pm 0.836$ ,  $2.76 \pm 0.112$  and  $1.13 \pm 0.019$  at EC less than 4, equal to 8 & 12dSm<sup>-1</sup> respectively. However, the maize genotype NCEV-7004 attained the lowest K<sup>+</sup>/Na<sup>+</sup> ratios i.e.  $0.64 \pm 0.101$ ,  $0.35 \pm 0.032$  and  $0.09 \pm 0.003$  at the above levels of salt stress. The other maize genotypes were positioned between these two genotypes with respect to K<sup>+</sup>/Na<sup>+</sup> ratios on overall basis.

**Table 10.** Effects of different levels of salt stress on  $K^+$ :  $Na^+$  ratio of maize genotypes in 35 days old seedlings under solution culture

K <sup>+</sup> /Na <sup>+</sup> ratio				
Maize genotypes	Salinity levels			Mean
	<4 (control)	8	12	
	[dSm <sup>-1</sup> ]			
MS-1501	0.78±0.012	0.45±0.035	0.15±0.015	0.46±0.092
Azam	1.27±0.0285	0.63±0.028	0.25±0.007	0.72±0.149
Iqbal	1.77±0.0361	0.91±0.113	0.32±0.047	1.00±0.214
S-2002	5.14±0.841	1.83±0.172	0.64±0.082	2.54±0.718
FH-988	4.71±0.632	1.57±0.047	0.60±0.067	2.30±0.647
FH-1292	3.94±0.788	1.51±0.062	0.56±0.040	2.00±0.552
FH-1114	2.07±0.091	1.03±0.069	0.34±0.021	1.15±0.254
Composite	1.51±0.163	0.69±0.066	0.27±0.030	0.82±0.190
MS-2015	1.26±0.118	0.60±0.050	0.24±0.018	0.70±0.154
NCEV-1270-3	1.99±0.310	0.99±0.045	0.32±0.012	1.10±0.259
MS-2018	3.14±0.301	1.45±0.144	0.42±0.034	1.67±0.408
HC-12	3.33±0.378	1.48±0.012	0.50±0.054	1.77±0.428
SB-9617	7.24±0.836	2.76±0.112	1.13±0.019	3.71±0.945
YH-1898	5.91±0.417	1.98±0.217	0.92±0.063	2.93±0.772
Malka-2016	0.80±0.020	0.47±0.048	0.16±0.015	0.47±0.093
P-1543	1.09±0.075	0.58±0.020	0.23±0.029	0.64±0.128
Neelum	1.06±0.118	0.55±0.013	0.21±0.009	0.61±0.128
FRI-22	1.30±0.136	0.63±0.012	0.25±0.024	0.73±0.158
MMRI-Yellow	5.01±0.314	1.92±0.246	0.71±0.087	2.55±0.651
Pahari	2.49±0.224	1.27±0.083	0.40±0.043	1.39±0.312
DK-6724	1.60±0.091	0.80±0.045	0.29±0.026	0.90±0.193
FH-949	6.39±0.656	2.17±0.132	1.05±0.067	3.20±0.836
Pearl White	2.43±0.229	1.04±0.067	0.37±0.006	1.28±0.311
NCEV-1270-5	0.65±0.045	0.40±0.035	0.11±0.007	0.39±0.079
Afgoi	0.89±0.102	0.52±0.020	0.19±0.015	0.53±0.106
NCEV-1270-7	2.98±0.281	1.40±0.129	0.41±0.032	1.60±0.384
NCEV-1530-9	1.57±0.180	0.75±0.032	0.37±0.020	0.89±0.185
NCEV-1297	1.76±0.170	0.83±0.038	0.29±0.024	0.96±0.220
NCEV-7004	0.64±0.101	0.35±0.032	0.09±0.003	0.36±0.085
HNG	0.70±0.070	0.44±0.020	0.14±0.015	0.43±0.083
Mean	2.51±0.203	1.07±0.066	0.40±0.028	1.33±0.089

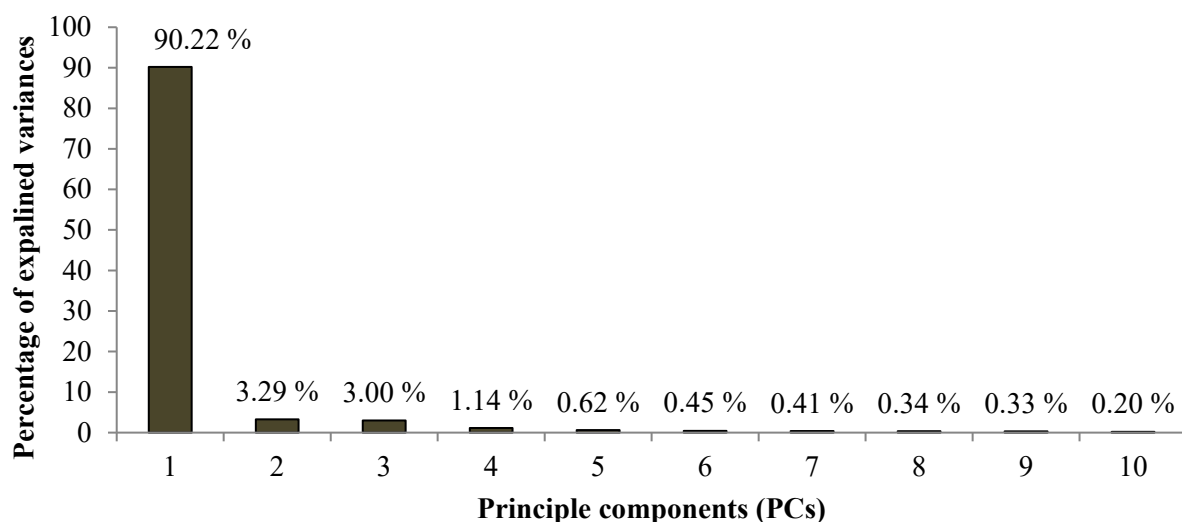
Mean± Standard error (Mean ± S.E)

### ***Pearson's Correlation and Principle Component Analysis (PCA)***

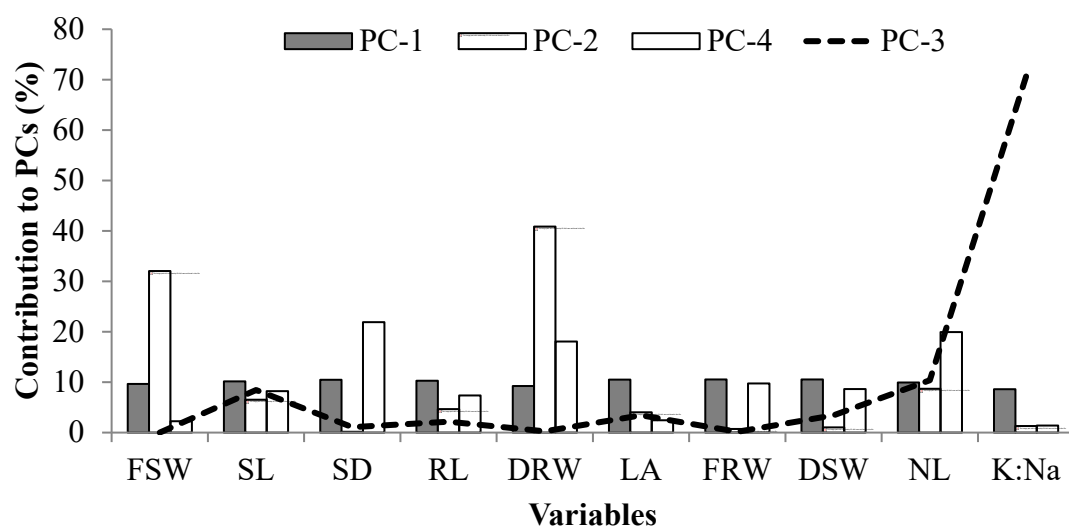
Table-12 indicated the highly significant ( $P<0.01$ ) positive and negative correlation among the different morphological and bio-chemical attributes of 30 different maize genotypes as influenced by graded levels of salt stress. The highest positive correlations viz.,  $r=0.900$ ,  $0.899$ ,  $0.891$ ,  $0.890$  and  $0.0887$  were noticed in LA vs SD, SD vs DSW, SL vs DSW, SL vs FSW and DSW vs FSW, respectively.

PCA was carried out to show the dispersion of different maize genotypes in different co-ordinated on the basis of their individual response to the graded levels of salt stress. PC1 and PC2 explained the highest variability 90.22 % and 3.29 % respectively, towards the total variability (Figure-1). In comparison, dry shoot weight (DSW) and fresh root weight (FRW) both contributed the maximum and equally (10.55%) to PC1. Furthermore, the dry root weight (DRW) (40.86%) and

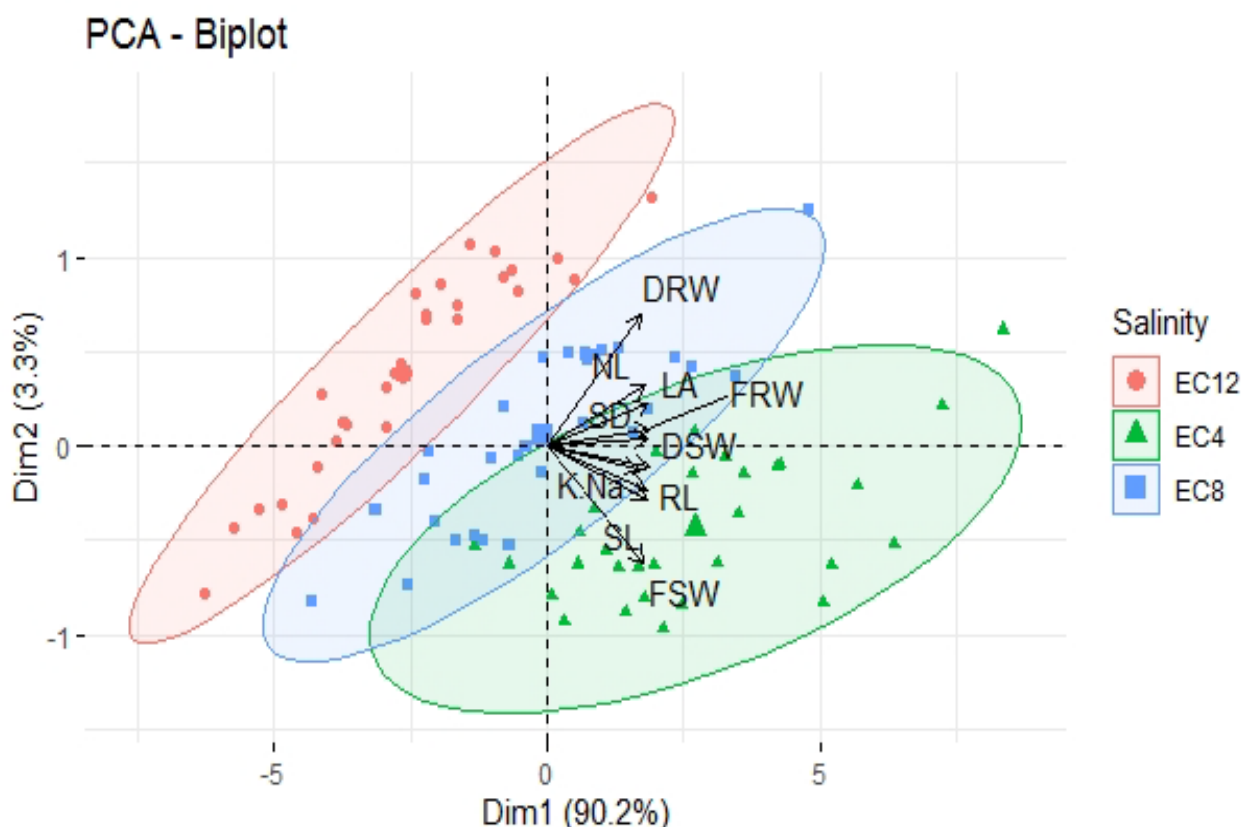
fresh shoot weight (32.04%) contributed maximally to PC2 (Figure-2). However the ion accumulation traits such as  $K^+$ :  $Na^+$  ratio showed their maximum influence on PC3 i.e. 70.63 % (Figure-2).



**Figure 1:** Percent contribution of each principle component (PC) to the total explained variations



**Figure 2:** Contribution of different variables to PC-1 (Gray bars), PC-2 (Brown bars), PC-3 (Black dotted line) and PC-4 (White bars)



**Figure 3:** PCA Bi-plot ellipsis showed the distribution dimension of different maize genotypes based on the various morphological, growth and bio-chemical traits influenced by graded levels of salt stress

### ***Salt Tolerance Groups of Maize Genotypes:***

#### ***i. Tolerant group (T)***

The highest average of PSDW values viz., 72.07 & 71.64 % obtained from SB-9617 and FH-949 respectively (Table-11).

#### ***ii. Moderately tolerant group (MT)***

Based on the average of PSDW values (Table-11), the sequence order of the moderately tolerant genotypes with respect to salt tolerance was as YH-1898>MMRI-yellow>S-2002 > FH-988 > FH-1292 > HC-12 > MS-2018 > NCEV-1270-7 > Pahari > Pearl White > FH-1114 > NCEV-1270-3 > Iqbal > NCEV-1297 > DK-6724.

#### ***iii. Sensitive group (S)***

The order sequence of maize genotypes for sensitive group on the basis of average of PDSW (Tabl-11) was observed as under: NCEV-1530-9 > Composite > FRI-22 > Azam > MS-2015 > P-1543 > Neelum > Afgoi > Malka-2016 > MS-1501 > HNG > NCEV-1270-5 > NCEV-7004.

**Table 11.** Characterization of maize genotypes for salt tolerance on DSW basis (values derived from Table-3)

Maize genotypes	*PDSW8	**PDSW12	Average of PDSW	Tolerance group
MS-1501	63.75	28.75	46.25	S
Azam	80.00	20.00	50.00	S
Iqbal	77.78	33.33	55.56	MT
S-2002	77.68	52.89	65.29	MT
FH-988	69.42	59.50	64.46	MT
FH-1292	68.07	58.82	63.45	MT
FH-1114	78.79	40.40	59.56	MT
Composite	80.43	22.83	51.63	S
MS-2015	74.44	25.56	50.00	S
NCEV-1270-3	77.78	39.39	58.59	MT
MS-2018	74.77	51.40	63.09	MT
HC-12	74.56	52.63	63.60	MT
SB-9617	82.07	62.07	72.07	T
YH-1898	83.72	55.04	69.38	MT
Malka-2016	65.43	28.39	46.91	S
P-1543	69.66	28.08	48.87	S
Neelum	71.26	25.29	48.28	S
FRI-22	80.00	21.11	50.56	S
MMRI-Yellow	85.27	48.84	67.06	MT
Pahari	79.00	42.00	60.50	MT
DK-6724	79.17	31.25	55.21	MT
FH-949	85.82	57.46	71.64	T
Pearl White	78.00	41.00	59.50	MT
NCEV-1270-5	63.16	22.37	42.76	S
Afgoi	73.81	22.61	48.21	S
NCEV-1270-7	77.67	49.51	63.59	MT
NCEV-1530-9	79.79	26.60	53.20	S
NCEV-1297	79.17	32.29	55.73	MT
NCEV-7004	54.41	16.17	35.29	S
HNG	64.47	25.00	44.74	S

\*Percentage of dry shoot weight at  $EC=8dSm^{-1}$ ; \*\* Percentage of dry shoot weight at  $EC=12dSm^{-1}$ .  
T= tolerant; MT=moderately tolerant and S= sensitive



**Table-12:** Pearson correlation coefficients of different morphological and bio-chemical traits for 30 maize genotypes as influenced by graded levels of salt stress at seedling stage

	FSW	DSW	SL	SD	LA	FRW	DRW	RL	NL	K <sup>+</sup> /Na <sup>+</sup> Ratio
FSW	1	0.887**	0.890**	0.860**	0.845**	0.807**	0.717**	0.850**	0.650**	0.814**
DSW	0.887**	1	0.891**	0.899**	0.871**	0.846**	0.821**	0.852**	0.713**	0.775**
SL	0.890**	0.891**	1	0.880**	0.842**	0.823**	0.776**	0.830**	0.695**	0.739**
SD	0.860**	0.899**	0.880**	1	0.900**	0.828**	0.832**	0.854**	0.748**	0.794**
LA	0.845**	0.871**	0.842**	0.900**	1	0.837**	0.871**	0.833**	0.745**	0.850**
FRW	0.807**	0.846**	0.823**	0.828**	0.837**	1	0.814**	0.791**	0.645**	0.756**
DRW	0.717**	0.821**	0.776**	0.832**	0.871**	0.814**	1	0.746**	0.684**	0.739**
RL	0.850**	0.852**	0.830**	0.854**	0.833**	0.791**	0.746**	1	0.703**	0.734**
NL	0.650**	0.713**	0.695**	0.748**	0.745**	0.645**	0.684**	0.703**	1	0.594**
K <sup>+</sup> /Na <sup>+</sup> Ratio	0.814**	0.775**	0.739**	0.794**	0.850**	0.756**	0.739**	0.734**	0.594**	1

FSW=Fresh shoot weight, DSW=Dry shoot weight, SL=Shoot length, LA=Leaf area, FRW=Fresh root weight, DRW=Dry root weight, RL=Root length, NL=Number of leaves per plant, K<sup>+</sup>/Na<sup>+</sup>=Potassium to Sodium ratio

\*\*Correlation is significant at 0.01 levels of significance

## Discussion

Salt stress adversely affects almost all the developmental stage in maize but causes severe damage to early seedling establishment [49]. In spite of the fact that maize crop is sensitive to salt stress but contains excellent genotypic variability for salt tolerance [10]. To cope with salinity, the crop undergoes some necessary alterations in the shoot and roots morphology [53, 67]. Therefore, quantification of the morphological traits associated with shoot and roots at seedling stage are of great significance to be used as selection criterion for salt tolerance in maize. In the present study, different morphological traits such as lengths of shoot and root, fresh and dry weights of shoot and roots, stem diameter, number of leaves per plant and leaf area per plant were studied.

Among all the morphological traits, shoot growth characters in maize exhibits higher vulnerability to salt stress [22]. In the current study, shoot morphological traits such as shoot length (SL), fresh shoot weight (FSW) and dry shoot weight (DSW) were severely affected by incrementing the salt stress level. The decreased shoot length with increasing salt stress levels might be attributed to the stunted growth with reduced intermodal development. The results of the present study were in accordance with the findings as reported in the previous studies held by Akram et al. [29]; Qu et al. [11]; Batool et al. [32]; Chen et al. [63] and Shafique et al. [16] wherein it was investigated that leaf abscission and /or senescence disrupts the production of photosynthates process and their translocation to growing tissues, thereby leading to the production of seedlings with stunted growth and shorter length. As far as the fresh and dry shoot weights are concerned, a significant number of research reports are available which clearly indicate the dual aspect of salt stress either positive or negative. Furthermore, the response of crop to salt stress changes with salt type, its concentration and the plant type [35, 9, 61, 56]. In our findings, the gradual reductions of fresh and dry shoot weights were also noticed in all the maize genotypes with higher levels of salt stress. However, the reduced SFW and DSW were recorded in SB-9817, FH-949 and YH-1898 might be due to having more inherited potential to prevent the accumulation of lethal concentration of salts in the biological tissues. Similar findings were also reported by Maqbool et al. [38]; Huqe et al. [37]; Shafique et al. [16]; Zahra et al. [42] that salt lenient maize genotypes attained higher shoot biomass than sensitive genotypes under salt stressed conditions.

Stem diameter (SD), number of leaves (NL) and leaf area (LA) are the other morphological stress indicators whose estimation also determines the intensity of salt damage to crop. In the present results, it was observed that the increasing levels of salt stress caused significant reduction of the stem diameter (SD). Tas and Basar [8]; Farhana et al. [53]; Gao et al. [62] and Nassar et al. [51] also concluded the similar findings that salinity stress alters the stem anatomy which eventually reduces the stem thickness, thereby affects the stem diameter (SD).

Salt stress through its ionic and osmotic effects accelerates the leaf senescence which resultantly cause wilting and then death of the leaves [49, 42, 60]. These effects significantly reduced the number of leaves and leaf area per plant in our current study. The results of the presents study are further supported by the findings of Szalai and Janda [17], Qu et al. [11] and Agami et al. [50] where it was investigated that salts when applied in higher concentrations suppress the leaf growth and leaf initiation by reducing the number as well as the rate of elongating cells. Similar reports were also presented in various studies that increasing concentration of salt stress deleteriously impacts the leaf area by reducing the number of leaves per plant [21, 28, 42, 16].

Saltiness also had drastic effects on the root morphological traits such as length of the roots and fresh and dry weights. The reduction of these parameters might be associated with inhibited roots growth due to excessive accumulation of  $\text{Na}^+$  in the roots (Munns and Tester, 2008). Similar reports were also published by Farhana et al. [53]; Hasan and Miyake [45] and Shafique et al. [16] wherein the excessive accumulation  $\text{Na}^+$  in the roots tissues was held responsible for altered morphology and anatomy of the roots. Furthermore, the sodium ions displace the calcium ions from the cellular membranes causing them more perforated, thereby leading to the loss of vital electrolytes [19], which resultantly reduced the cell division and cell elongation [17]. The results of the present study were further supported by the findings as reported by Maqbool et al. [38], Dikobe et al. [58] and Sandhu et al. [13] that salt stress causes significant reduction of roots length and fresh & dry weights of roots.

Based on the morphological traits, all the genotypes were found superior at least in one character and inferior in other characters. Therefore, describing maize genotypes for each character separately was very difficult. Hence, PCA-Biplot multivariate analysis was employed using nine morphological traits across 30 maize genotypes against the graded levels of salt stress ( $\text{EC} < 4, 8 \text{ \& } 12 \text{ dSm}^{-1}$ ) to identify the most suitable characters to be used as selection criterion for salt tolerance. PCA is a type of that combines characters and objects in two dimensions together and minimizes overlapping variations, facilitating the determination of main characters for selection [1]. The first and second principle components (PC1 & PC2) were used which contributed maximally (90.7 & 3.3 %, respectively) to explain the overall variation (94.0%) as shown in the Figure-1. Two (DSW & FRW) out of nine studied morphological characters were found of great significance which exhibited the highest and equal contribution (10.55%) to explain the variations in PC1. Thus, they are the fair estimates of performance of the maize genotypes under salinity stress.

After determining the most suitable morphological character i.e. DSW (Based on PCA results) was used as selection criterion for salt tolerance in maize genotypes using Murrillo et al. [5,4] classification to categorize genotypes into tolerant (T), moderately tolerant (MT) and salt sensitive (S) groups.

Based on morphological features, the tolerant (T) group was least affected by the adverse effects of salinity stress than moderately tolerant (MT) and sensitive (S) genotypic groups. Varsha et al., [23] and Huqe et al. [37] also used the similar statistical procedure of multivariate analysis i.e. PCA to identify the salt tolerant maize genotypes on the basis of morphological traits. The differences among the genotypes for morphological traits as observed in the current study might be associated with the differential strategic behaviour of genotypes to cope with harmful effects salinity. The sensitivity of maize genotypes to increased levels of salt stress depends on their inability to retain higher  $\text{K}^+$  contents in the plant tissues that eventually reduced the  $\text{K}^+/\text{Na}^+$  ratio. Under the influence of salt stress, plants tend to accumulate more  $\text{Na}^+$  than  $\text{K}^+$  in the shoot and roots

tissues. Whereas, the salt tolerant genotypes retain lower  $\text{Na}^+$  and maintained higher levels of  $\text{K}^+$  in the plant body thereby leading to higher  $\text{K}^+/\text{Na}^+$  ratio (65,37, 30), which is very much consistent with our findings (Table-10). In short summary, the current study provided scientific information to the breeders to boost the development process of new, more productive and better adapted maize cultivars.

## Conclusions

Based on the results of the PCA employed on morphological traits, it was concluded that dry shoot weight (DSW) could effectively be used as selection criterion to categorize maize genotypes into different salt tolerance groups. Accordingly, two maize genotype SB-9617 and FH-949 were categorized as salt tolerant (T), fifteen were moderately tolerant genotypes with their sequence order relative to salt tolerance as YH-1898 > MMRI-yellow > S-2002 > FH-988 > FH-1292 > HC-12 > MS-2018 > NCEV-1270-7 > Pahari > Pearl White > FH-1114 > NCEV-1270-3 > Iqbal > NCEV-1297 > DK-6724. Moreover, the sequence order of thirteen sensitive genotypes was as NCEV-1530-9 > Composite > FRI-22 > Azam > MS-2015 > P-1543 > Neelum > Afgoi > Malka-2016 > MS-1501 > HNG > NCEV-1270-5 > NCEV-7004. Moreover, it was also concluded that salt tolerant maize genotypes with higher  $\text{K}^+/\text{Na}^+$  ratios performed better under the influence of salt stress. The study also provides the understanding for the breeders to utilize the existing plant genetic resources for the development of new salt-tolerant maize cultivars.

## Conflicts of Interest

Author declares not any conflict of interest regarding the publication of this manuscript.

## Acknowledgements

This scientific information is the part of Ph. D research work of the author. Laboratory facilities provided by Soil Salinity Research Institute (SSRI), Pindi Bhattian, Pakistan, are highly appreciated. Authors would like to thank anonymous reviewers for their valuable comments to improve the manuscript.

## Authors' contributions

Syed Saqlain Hussain and Dr. Muhammad Rasheed conceived the idea, designed and carried out the experiment and collected data; Prof. Dr. Ghulam Jilani supervised the experimental work and improved the manuscript. Prof. Dr. Zammurad Iqbal Ahmed improved the manuscript and technically guided in the application of statistical analysis software. All authors contributed equally in the write up, and approval of the manuscript final version.

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