

## DETECTION OF SALT TOLERANT HYBRID MAIZE AS GERMINATION INDICES AND SEEDLING GROWTH PERFORMANCE

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### Abstract

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Salinity is a major environmental stress factor for crop production and is an extensive limitation for seed germination. A germination test was carried out with four replications and three levels of salt concentrations (0 dS.m<sup>-1</sup>, 6 dS.m<sup>-1</sup> and 12 dS.m<sup>-1</sup>) in plastic tray under quartz granules. Analysis of variance (ANOVA) indicated that all traits were significance ( $P < 0.01$ ) for genotypes, treatment and their interaction. Germination percentage (GP), germination speed (GS), germination index (GI), maximum root length (MRL), maximum shoot length (MSL), total dry matter (TDM), and salt tolerance index (STI), seed vigor index (SVI) were all decreased as the level of NaCl was increased. Mean germination time (MGT) and Percent reduction in dry weight over control (ROC%) was increased as the NaCl concentration increased. From the result, it is concluded that 962, Super gold, Kaveri-50, PAC-999, 9120, 900M Gold and C-6485 hybrids would be tolerant while prince articulated as salt sensitive.

*Key words:* maize; salinity; stress; germination; tolerant

*List of abbreviation:* GP – germination percentage; GS – germination speed; GI – germination index; MGT – mean germination time; MRL – maximum root length, cm; MSL – maximum shoot length, cm; TDM – total dry matter, g; STI – salt tolerance index; SVI – seed vigor index; ROC% – percent reduction in dry weight over control

### Introduction

Soil salinity is one of the most principal challenges for salt sensitive crop production in salt affected area of the world (Billah et al., 2017). As a common abiotic stress factor, salinity is extremely affecting on crop production in different regions of the world, mostly in arid and semi-arid region (Khodarahmpour et al., 2012). Therefore, salinity stresses affecting more than 800 million hectares of land throughout the world (Munns, 2005). In Bangladesh, about 1.05 million hectares of arable lands are affected by varying levels of salinity out of 2.85 million hectares of the coastal and off-shore lands (Ahmed, 2011). In irrigated lands, salinization is spreading more rapidly due to wrong supervision of irrigation and drainage. Furthermore, rain, cyclones and

wind enrich NaCl to coastal agricultural lands (FAO, 2008). World food production will prerequisite to increase by 38% by 2025 and by 57% by 2050 (WILD, 2003) to meet the food security of growing population. Nevertheless, the claim for new salt tolerant crop genotypes is increased for affected 20% of the global area of highly productive irrigated land and 2% of the world's rain-fed zones which represent over 800 million ha worldwide (Mohammad et al., 2014).

Plant growth is suppressed by salinity through affecting osmotic potential. Seed germination of many crops are severely affected by salinity through raising an osmotic potential outside the seed and obstructing of water absorption, or by Na<sup>+</sup> and Cl<sup>-</sup> toxicity effects (Khajeh-Hosseini et al., 2003). The principal perilous and most sensitive stage of plant life cycle is seed sowing where seeds are often ex-

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posed to critical environmental states that may conciliate the establishment of seedling (Misra et al., 2004). Capability of seeds germination at in high salt concentrations soil is a crucial significance for the persistence of many plant species (Sali et al., 2015). With increasing of salinity, significantly reduces germination percentage, germination rate, root and shoots length and fresh root and shoots weights. Several experiments have been inspected the effects of the salinity on maize crop, and found that the consequence was variety specific (Zhang et al., 2011). However, maize (*Zea mays* L.) is considered as one of the most global food safety crops after wheat and rice. It cultivates wide range of agro-ecological environment of the world but it is salt sensitive crops.

Thus, considering the above discussion, the present study was performed to find the best performer hybrids at germination stage, which would be the highest salt tolerant genotypes.

## Materials and Methods

### Plant Materials

In this experiment, 33 commercial hybrids of maize were used to investigate the better performer genotypes.

### Methods

The germination test was carried out in the greenhouse of Plant Breeding Division, Bangladesh Agricultural Research Institute (BARI), Gazipur-1701. The temperate was maintained at 28°C – 30°C for 14 hours under light and at 22°C for 10 hours under dark conditions. The light intensity of the greenhouse room is 657  $\mu\text{mol m}^{-2} \text{s}^{-1}$ , and the relative humidity was 50%. The experiment was conducted with completely randomize block design (RCBD) with four replications and three treatments (control, 6 dS.m<sup>-1</sup>, and 12 dS.m<sup>-1</sup> NaCl) were set. Each replication contains 25 seeds. After surface sterilization with 0.05% HgCl<sub>2</sub> for 10 minutes and washed with deionized water three times then the seeds were sown under plastic tray in quartz granule then treatment were applied and covered with black polythen for 4 days in growth room for germination and kept under light for another 4 days. Data of germination was recorded regularly up to eight days. After 10 days seedlings were noted for root length, shoot length and number of seminal roots. Total dry matter was measured after oven drying at 80°C for five days.

### Data measurement

➤ *Germination percentage (GP)*:  $GP = (SNG/SN0) \times 100$ , SNG = number of germinated seeds, SN0 = number of experimental seeds with viability

➤ *Germination speed (GS)*:  $GS = \Sigma D \times n / \Sigma n$ , n = number of germinated seeds at each day; D = number of days after the start of the experiment

➤ *Germination index (GI)*:  $GI = \Sigma(Gt/Tt)$ , Gt = number of seeds germinated on t<sup>th</sup> day, Tt = number of days up to t<sup>th</sup> day

➤ *Mean germination time (MGT)*:  $MGT = \Sigma TiNi / \Sigma Ni$ , Ni = number of the newly germinated seeds in times of Ti

➤ *Seed vigour index (SVI)*:  $SVI = SDW/MGT$ , MGT = mean germination time, SDW = seedling dry weight

➤ *Salt tolerance index (STI)*:  $STI = (TDW \text{ at } Sx / TDW \text{ at } S1) \times 100$ , TDW = total dry weight, S1 = control treatment, Sx = salt level treatment

➤ *Maximum root length (cm) (MRL)*: Maximum root length measured from the coleoptile node to last tip of the primary root.

➤ *Maximum shoot length (cm) (MSL)*: Maximum shoot length measured from coleoptile node to highest tip of the leaf.

➤ *Total dry matter (g)*: Dry weight was determined after placing plant samples in oven at 60°C for seven days.

➤ *Dry weight reduction over control (ROC)*:  $(\%ROC) = (\text{value in control} - \text{value in saline environment}) / \text{value in control} \times 100$

### Statistical analysis

For individual and combined analysis of variance of completely randomize design (RCD) with four replications was used and, the statistical analyses were performed using R-statistics software Version 3.1.2 for window (R Development Core Team, 2013).

## Results

Analysis of variance (ANOVA) indicated that the measured parameters of maize hybrids were significantly affected by salt treatment. Box plots showed that differences under the treatments among maize genotypes for measured traits (Figure 1). In the box plot, box edges show upper and lower quintile and the median as shown in the middle of the box. At least 25% of all values are below the lower quartile and 25% of all values are above the upper quartile. 50% of all values are containing middle of the box. Lower trail and upper trail indicate maximum and minimum ranges. Individuals falling outside of the rank of whisker are revealed as circle. All the traits fitted with normal distribution in all treatment conditions except some trait skewed left and some are right. The highest variations were observed in GP and GI at 12 dSm<sup>-1</sup> salinity level.

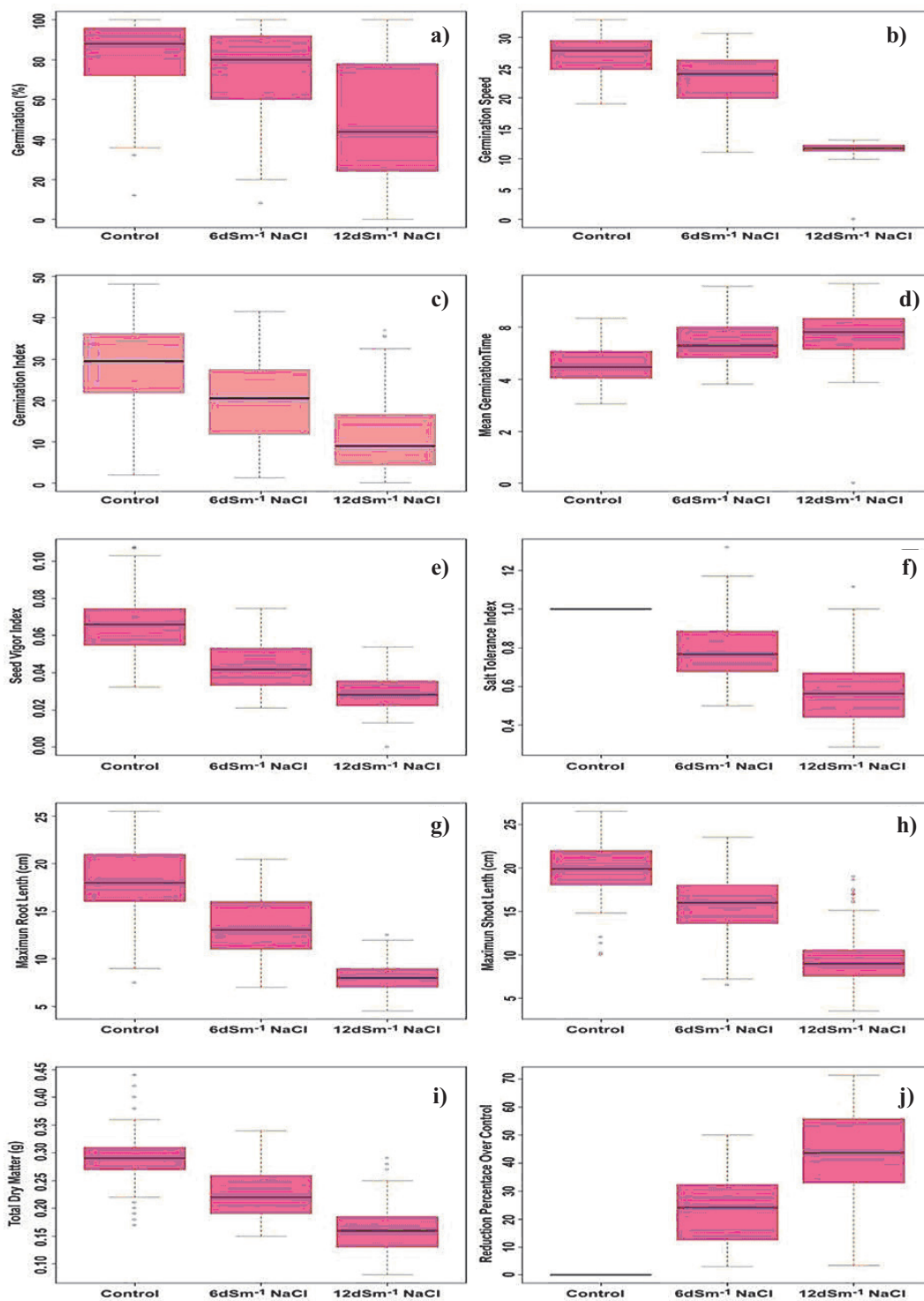
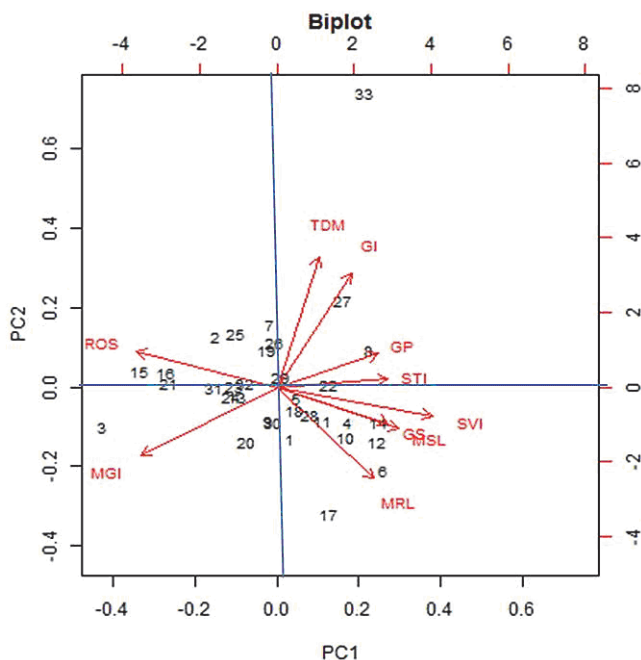


Fig.1. Box plots showing differences under control and two levels of salinity

### Genotype by trait interactions

A genotype  $\times$  traits biplot was created from a two-way matrix of ten traits and 33 genotypes using the relative value of the trait (Figure 2). The plot summarizes the information from this matrix into principle components, where the cosine of the angle between vectors connecting traits to the origin is proportional to the correlation coefficient between those traits. Again, traits on opposite sides of the origin are negatively correlated and traits near each other are positively correlated. Also, traits at 90° to each other are not correlated, with respect to the origin. This biplot expressed superior genotypes with relatively greater expression of combinations of favorable traits. Total outcome suggest that TDM, GI, GP, STI, SVI, GS, MSL and MRL might be helpful to identify superior genotypes in elite germplasm.



**Fig. 2. Genotype  $\times$  trait biplot based on seedling traits of maize**

### Cluster analysis for classification of maize hybrids

Cluster analysis was performed at 12 dSm<sup>-1</sup> level with relative mean values of each trait. Euclidian distance coefficients were calculated for all maize hybrids based on seedling traits. Dendrogram from UPGMA clustering indicated grouping of 33 genotypes of maize into five clusters (Figure 3). Cluster I, II, III, IV and V comprised with 17, 7, 1, 7 and 1 hybrids, respectively (Table 1). Among the five cluster, cluster number IV holding most tolerant genotypes for the

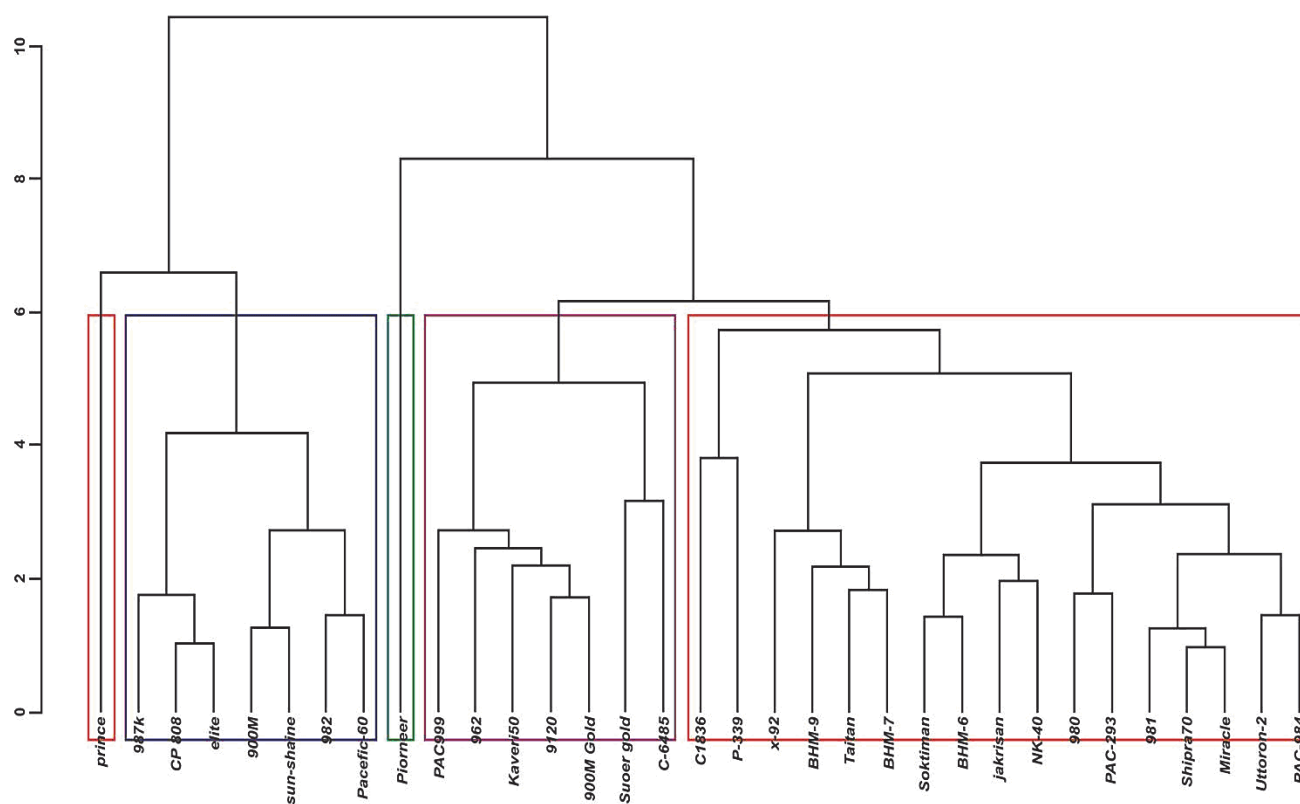
highest number of relative mean (Table 2) GP, GI, SVI, STI, MRL, MSL, TDM and ROS. The lowest relative mean value exhibited in the cluster number III, this group displayed lowest relative mean value for GP, GS, GI, SVI, STI, MRL, MSL, TDM and ROS and the highest for MGT, followed by cluster II it indicated that genotypes belonging to cluster number III and II are susceptible.

### Discussion

According to the result, the germination percent was significance in 1% level for genotypes, salinity and interaction between genotypes and salt level. High levels of soil salinity can significantly inhibit seed germination and seedling growth, due to the combined effects of high osmotic potential and specific ion toxicity. Salt stress had adverse effects on the functioning and metabolism of plants considerably hinders the productivity. However, during seed germination cell toxicity arises due to high absorption of Na<sup>+</sup> and Cl<sup>-</sup> ions that finally hinders or reduces the rate of germination and consequently declines germination percentage (Figure 1A). High concentration of salinity increases the osmotic potential as a result plant become unable to absorbed water or nutrient. Great number of scientists investigated similar results in maize (Mohammad et al., 2014; Sali et al., 2015; Zhang et al., 2011).

The speed of germination of genotypes was significantly affected ( $p < 0.01$ ) by salt treatment (Figure 1B). Osmotic stress is the main fact for diminishing germination speed. The tendency of decreasing of germination speed with the increasing of salt level was confirmed by others investigation (Roychoudhury et al., 2011). Furthermore, all indices of germination for all cultivars declined with increasing salt concentration (Carpócy et al., 2009). With the increasing of salt concentration germination index significantly reduced (Figure 1C). In rice, germination index decreases with the increasing of salt concentration (Abbas et al., 2012). Similar finding was observed in barley (Naseri et al., 2012) and in maize (Zhang et al., 2011). The mean germination time was late by NaCl stress treatment (Figure 1D). The same result was investigated in safflower (Khodadad, 2011). Seed vigor index reduced significantly with increasing salt concentration level (Figure 1E). Seed vigor index is depends on special ions impact and decrease of environmental water potential due to salt stress (Theerakulpisut et al., 2005). From the result, salt tolerant index declined significantly with increasing salt treatment (Figure 1F). The higher salt tolerant index indicated that the genotypes response less salt sensitivity. Comparable effects were described in maize (Ziaf et al., 2009).

**Dendrogram using Agglomerative Clustering Method**



**Fig. 3. Dendrogram from UPGMA clustering for 33 maize hybrids using Euclidean genetic distance based on all seedling traits measured**

**Table 1**  
**Cluster groups and their containing varieties name**

Cluster	Size	Name of the varieties
I	17	Shipra70, 980, C1836, 981, Uttoron-2, P-339, Miracle, Jai-kisan, X-92, Saktiman, PAC-984, Taitan, PAC-293, BHM-7, NK-40, BHM-6, BHM-9
II	7	982, 900M, 987k, CP 808, Sun-shaine, Elite, Pacific-60
III	1	Prince
IV	7	962, Super gold, Kaveri-50, PAC-999, 9120, 900M Gold, C-6485
V	1	Pioneer

**Table 2**  
**Mean values for five clusters based on ten quantitative traits of maize hybrids**

Cluster	GP	GS	GI	MGI	SVI	STI	MRL	MSL	TDM	ROS
I	0.45	0.45	0.48	1.32	0.49	0.62	0.48	0.47	0.57	43.24
II	0.55	0.41	0.60	1.44	0.31	0.44	0.31	0.37	0.42	58.11
III	0.13	0.19	0.17	1.98	0.15	0.37	0.51	0.33	0.36	64.00
IV	0.88	0.44	0.76	1.19	0.56	0.63	0.51	0.73	0.79	20.83
V	0.54	0.41	1.22	0.45	0.48	0.72	0.39	0.55	45.16	45.16

As plant organ, root activities provide the beneficial facts regarding the salt tolerant for potential of the plants (Akram et al., 2010). Impact salinity decreased the root length of all maize hybrids significantly (Figure 1G). Salinity inhabits the cell enlargement and cell division as a result plant growth decreased. Shoot length also drastically reduced at high salt ( $12\text{dSm}^{-1}$  NaCl) treatment (Figure 1H). Most probably, extreme gathering of salts in the cell wall elasticity decreases the shoot length. The salt stresses affect badly to the plant morphology, functioning and homeostasis, and decrease the plant biomass (Parvaiz, 2014). In the present study total dry matter declined significantly with increasing salt treatment (Figure 1). Therefore, shoots and roots exhibited high correlation with the level of salinity (Theerakulpisut et al., 2005). Salinity reduced the plant growth by diminishing biomass production. Similarly, our investigation was supported by several scientists in maize (Carpýcý et al., 2009; Mohammad et al., 2014). Nevertheless, this observation has permitted a better knowledge of germination indices and seedling growth at different salinity levels in maize seed.

## Conclusions

At seedling stage, it is required to find out the less salt sensitive hybrids for cultivation saline area. The present study directed that the application of high level of salt, adversely affected the germination indices and seedling growth of all maize hybrids. A significant difference was investigated among 33 hybrids maize in salt stress. However, among the all hybrids, 962, Super gold, Kaveri-50, PAC-999, 9120, 900M Gold and C-6485 were demonstrated less salt impact and 982, 900M, 987k, CP 808, Sun-shaine, Elite, Pacific-60 were exhibited high salt effect. Finally, these hybrids would be used for salt tolerant breeding program as decent genetic materials.

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