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IMPACT OF SALINITY STRESS ON SEED GERMINATION INDICES OF MAIZE (*Zea mays* L.) GENOTYPES

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ABSTRACT. This investigation was done to find germination response of nine maize (*Zea mays* L.) genotypes under three levels of NaCl salinity (0 mM, 100 mM and 200 mM). Seeds were germinated and grown in Petri plates on filter paper, using above mentioned salt solution as treatment with three replications, incubated at 28±1 ^{0}C in a growth chamber following randomized complete block design. Germination percentage (GP), germination speed (GS), germination index (GI), seedling dry weight (SDW), seed vigor index (SVI) and salt tolerance index (STI) 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 levels increased. Interaction between genotypes and salt levels showed varying degree of differences. It is concluded that genotypes showed response variability for seed germination under saline stress. Among the investigated genotypes inbred line CZ-7 expressed as the tolerant genotype and B73 appeared to be more sensitive at germination stage.

INTRODUCTION

Maize, (*Zea mays* L.) belonging to the family Poaceae is the third largest cereal crops after rice and wheat. It is widely cultivated and grown approximately 177 million hectares all over the world in 2012 with a total production of 875 million tons (FAOSTAT, 2013). It has become one of the global food security crops due to its growing ability in various soils and climates.

Global food production will need to increase by 38% by 2025 and by 57% by 2050 (WILD, 2003) if food supply to the growing world population is to be maintained at current levels. The global climate change is feared to promote rapid soil degradations in agricultural lands worldwide. Soil salinization is one of the serious soil degradations, which can arise from natural causes and human-mediated activity such as irrigation in arid and semi-arid regions (HORIE, *et al.*, 2012)**.** Salinization of soil is one of the major factors limiting crop production particularly in arid and semi-arid regions of the world (AHMED, 2009). Most crops are sensitive to salinity caused by high concentration of salts in the soil. It is a global problem and a major threat to irrigated agriculture (VIJAYVARGIYA and KUMAR, 2011). Generally, soils having above

4 dS/m electrical conductivity (EC) of Na^+ ions (US SALINITY LABORATORY STAFF, 1954) corresponding to 40 mM NaCl (IRRI, 2011; MUNNS and TESTER, 2008) are defined as saline soil. Usually, saline soils are found in arid regions, estuaries, and coastal fringes. Saline soil inhibits plant growth by affecting osmosis. It reduces the water uptake ability of plants, and thus by ion-excess, eventually affecting the plant cells (MUNNS, 2002). It also induces nutritional imbalances in plants.

Plants, having the ability to grow under salinity are halophyte (QU *et al.*, 2008), while unable plants are called glycophytes (UNGAR, 1991). Glycophytes covers the majority of agricultural products, and growth and production of this plants group are significantly reduced by salinity stress (HORIE *et al.*, 2012). Salt stress is known to perturb a multitude of physiological processes (NOREEN and ASHRAF, 2008). Osmotic stress (drought problem), ion imbalances, particularly with Ca and K, and the direct toxic effects of ions on the metabolic process are the most important and widely studied physiological impairments caused by salt stress (ZHU, 2001). Like other abiotic stress, it induces oxidative damage to plant cells (AZEVEDO-NETO *et al.*, 2006).

Seed germination is also severely affected by salinity (SHOLI, 2012). Increased salinity caused a significant reduction in germination percentage, germination rate, and root and shoots length and fresh root and shoots weights (JAMIL *et al.*, 2006). Several studies have investigated the effects of the salinity on maize crop, and found that the effect was genotype specific (ZHANG and ZHAO, 2011). Usually, maize is a salt-sensitive crop (MAAS and HOFFMAN, 1977). The response of maize to salinity varies depending on the stage of development (MAAS *et al.*, 1983). Vegetative growth stage appeared to be more sensitive than any other growing stages (CRAMER, 1994).

The development of salt tolerant cultivars has been proposed as the most effective strategy to overcome this problem (EPSTEIN and RAINS, 1987). Selection and breeding of salt tolerant plants is becoming one option to minimize the negative impact of salinity (EPSTEIN *et al.*, 1980). This could be done at different crop growth stages i.e. crop establishment (germination, emergence and seedling growth), vegetative growth and grain filling.

This study was conducted to evaluate the effect of salinity on seed germination of nine maize genotypes under salinity conditions. The primary objective of the present study was to compare germination ability of nine maize genotypes under salinity stress and to select salt tolerant line of maize which can be used for further breeding programme.

MATERIALS AND METHODS

Plant Materials

In this study nine maize genotypes were used to evaluate the degree of salt tolerance at germination stage. Among the nine genotypes eight genotypes were collected from Bangladesh Agricultural Research Institute (BARI), of them three are inbred lines and five are cultivated variety; and other one – a highly popular US inbred line B73 obtained from Chinese Academy of Agricultural Sciences (CAAS), Beijing, China.

Methods

This study was a laboratory-conducted experiment and carried out in Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS) in China, 2011. The seeds were surface sterilized by dipping the seeds in 1.5% (v/v) Sodium Hypochloride solution for 10 minutes and rinsed thoroughly with sterile distilled water. There were three salinity treatments i.e. 0 mM (control), 100 mM, and 200 mM NaCl. These treatments were prepared by dissolving separately calculated amounts of NaCl in deionizer water. All the experiments were conducted in 15 cm diameter sterile Petri plate on filter paper beds in growth chambers. 30 seeds were sown in each Petri plate on filter paper beds, irrigated with 15 ml solution of respective treatment and incubated at 28±1 °C for 10 days. The humidity of the growth chamber was maintained at 65%. Each treatment had three replications. The filter paper beds were irrigated daily with 5 ml solution of the respective treatments. The filter beds were changed with the new one after 48 hrs in order to avoid salt accumulation.

Data Measurement

The emergence of plumule was recorded daily up to ten days as index of germination (CARPICI *et al.,* 2009). The dry weights of the shoots and roots of the seedlings were measured at10 days after the experiment started. The dry weights were measured after drying the shoot and root at 80°C for 48hrs. Based on that information following measurements were calculated.

Germination percentage (GP): GP = (SNG/SN0) x100, SNG = number of germinated seeds, SN0 = number of experimental seeds with viability (LI, 2008).
Germination speed (GS): GS = ΣD×n/Σn, n = number of germinated seeds at each day; D = number of days after the start of the experiment (LI, 2008)
Germination index (GI): GI = ∑(Gt/Tt), Gt = number of seeds germinated on tth day, Tt = number of days up to tth day (LI, 2008).
Mean germination time (MGT): MGT = ΣTiNi/ΣNi, Ni = number of the newly germinated seeds in times of Ti (HU *et al.*, 2005).
Seed vigour index (SVI): SVI = SDW/MGT, MGT = mean germination time, SDW = seedling dry weight (ELLIS and ROBERTS, 1980).
Salt tolerance index (STI): STI = (TDW at Sx/TDW at S1) x100, TDW = total dry weight, S1= control treatment, Sx = salt level treatment (SEYDI, 2003).
Dry weight reduction over control (ROC): (%ROC) = {(value in control – value in saline environment)/value in control} ×100 (ALI *et al.*, 2004).

Statistical Analysis

The experiment was conducted following randomized complete block design with 3 replications. Data were subjected to statistical analysis using ANOVA, a statistical package available from SPSS16. Significant differences between treatments were determined using LSD test at the 0.05 level.

RESULTS

The results of ANOVA manifested that the measured components of maize genotypes were significantly affected by salt concentrations except interaction between genotype and salinity in case of mean germination time (Table 1). In general, salt stress adversely affected germination of maize genotypes.

Table 1. Analysis of variance of the traits determined.

Source of variation	df	Mean Square						
		GP	GS	GI	MGT	SDW	SVI	STI
Genotype (G)	8	2490.742***	178.485***	610.958***	0.146***	0.00023***	5.054E-6***	1493.300***
Salinity (S)	2	6538.273***	698.130***	4520.733***	1.635***	0.005***	0.00015***	11960.616***
G×S	16	614.197***	41.860***	121.299***	0.047^{NS}	0.00034***	8.868E-6***	426.733***
Error	54	61.591	5.418	13.993	0.029	7.392E-6	1.847E-7	11.860
CV (%)		28.95	29.07	35.08	4.58	34.24	72.46	30.34

^{NS} Not significant; *** significant at the 0.001 level of probability; GP: Germination percentage; GS: Germination speed; GI: Germination Index; MGT: Mean germination time; SDW: Seedling dry weight; SVI: Seed vigour index; STI: Salt tolerance index.

According to the results, germination percent was significant in 0.001 percentages for salt levels, genotypes and interaction between salt levels and genotypes. At different salt concentrations, CZ-7 and B73 had the highest and lowest germination percentage as 97.00 and 41.50%, respectively. Inbred line CZ-10 had the second highest germination percentage (91.90%). At different levels of NaCl concentration, CZ-7 demonstrated better tolerance to salt stress than other genotypes for germination percentage (Figure 1).

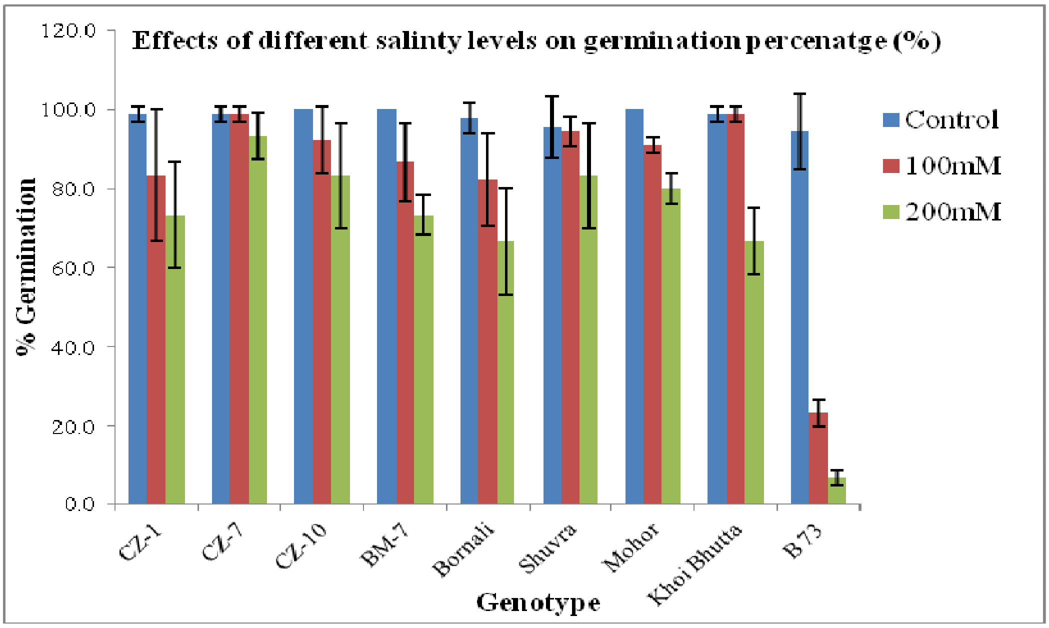

Fig. 1. Effects of different salinity levels on germination percentage (%).

The results showed that germination speed of these maize genotypes were significantly affected by different concentration of salts ($p < 0.001$) (Table 1). Speed of germination was reduced by salinity by 12.95% in CZ-7 and by 95.93% in B73 at highest level (200 mM) of NaCl (Figure 2).

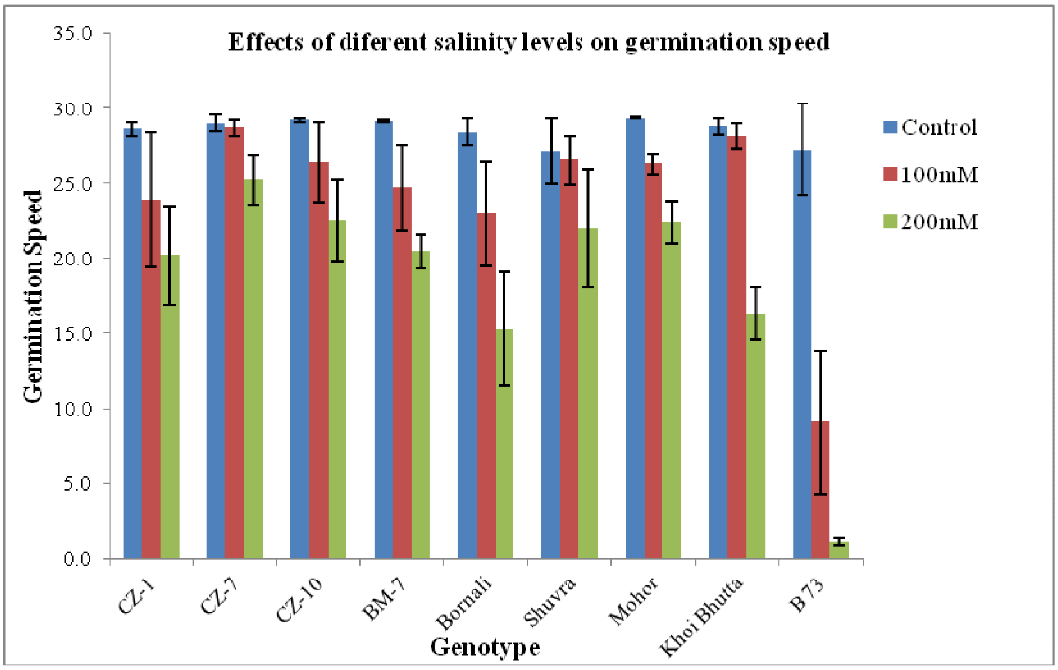

Fig. 2. Effects of different salinity levels on speed of germination.

Among the nine maize genotypes, the highest germination index (GI) value was recorded for CZ-7 (45.07) and lowest for B73 (1.54) recorder at maximum salt stress condition (200 mM), and comparing to the control only minimal decline (18.05%) was recorded for CZ-7 under highest salinity stress and maximum decline (96.94%) was observed in B73. Mean GI values were 53.51in control conditions and 27.69 under salt stress (200 mM). Overall 48.26% decline in GI with salinity was recorded (Figure 3).

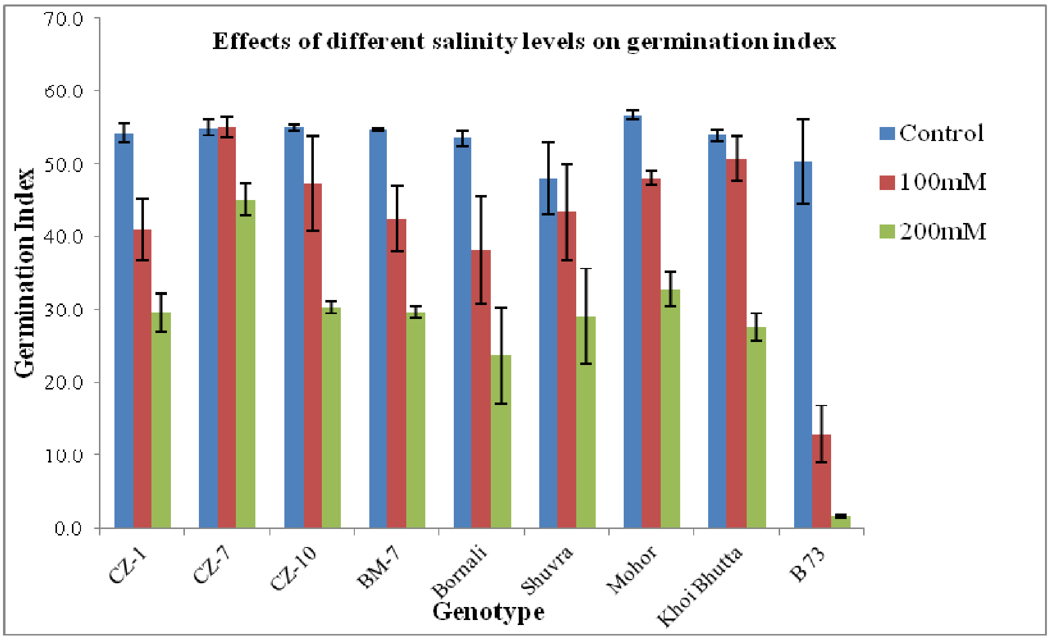

Fig. 3. Effects of different salinity levels on germination index.

Salinity also had considerable effect on germination time of maize seeds, as evident from different germination time delay among the genotypes. CZ-7 had the fastest germinating seeds: 6.21 days (Figure 4). Germination time dramatically increased with salt stress. Mean values of MGT were 6.11 in control conditions and 6.59 under salt stress (200 mM). Overall 7.39% increase in MGT with salinity was recorded (Figure 4).

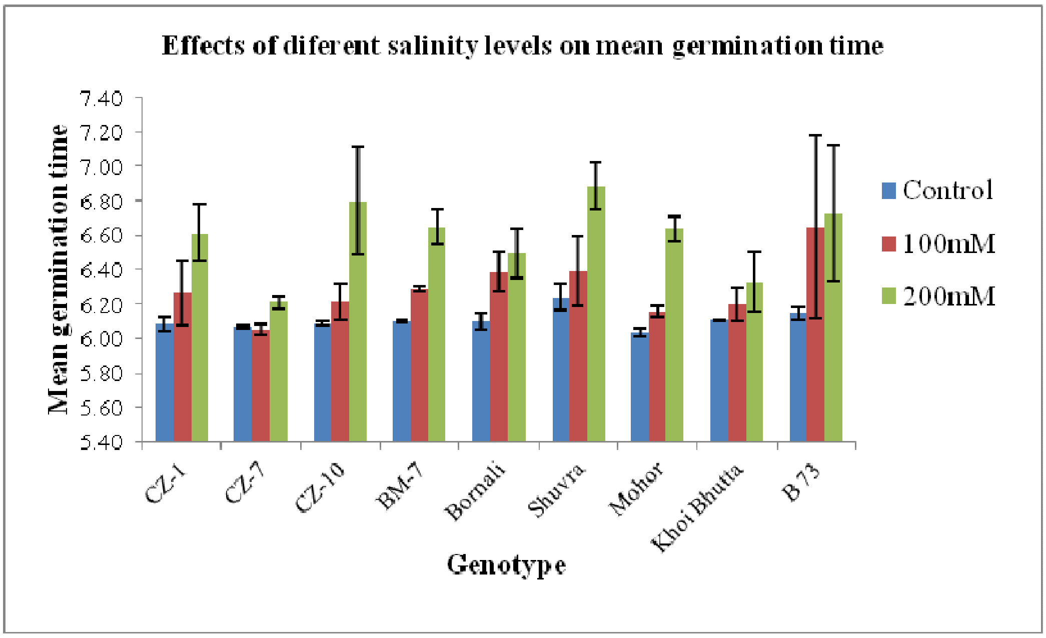

Fig. 4. Effects of different salinity levels on mean germination time.

Seeding dry weight of genotypes decreased significantly as the levels of salinity increased from 0 to 200 mM NaCl. Thus, the highest seedling dry weight was found in the control and the lowest root dry weight at the highest salinity level. Among the genotypes, CZ-7 was affected least by salinity. The rate of reduction in seedling dry weight at 200 mM NaCl in comparison with the control was detected in CZ-1 as 40.43%, CZ-7 as 6.02%, CZ-10 as 38.43%, BM-7 as 41.12%, Bornali as 42.20%, Shuvra as 43.75%, Mohor as 30.83, Khoi Bhutta as 34.67% and B73 as 92.18% (Figure 5).

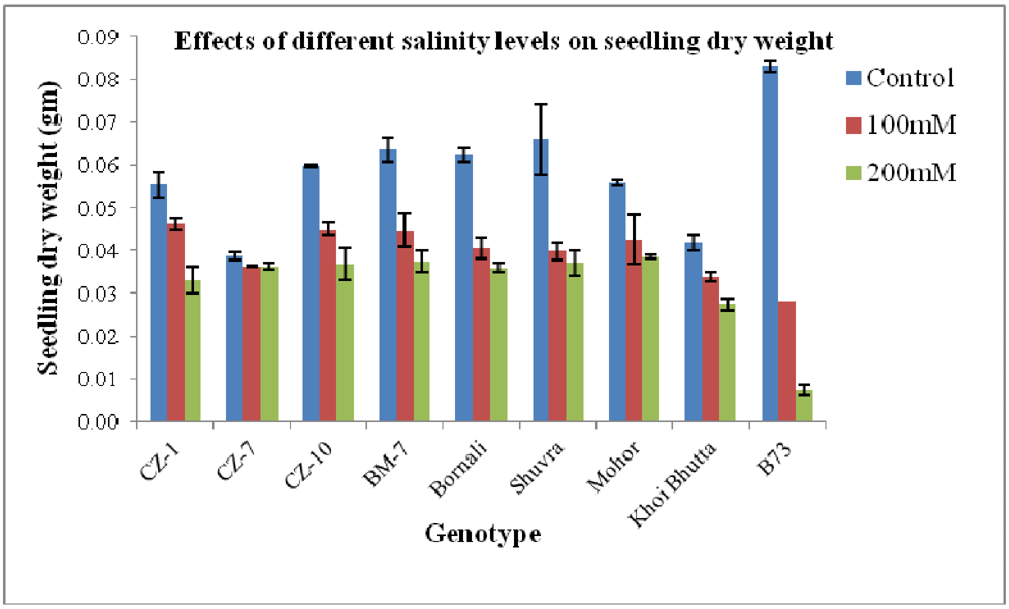

Fig. 5. Effects of different salinity levels on seedling dry weight.

Results of Analysis of Variance for traits seed vigor index showed that there were significant differences between cultivars of these parameters (Table 1). Seed vigor index decreased due to the increased salinity levels from 0mM to 200 mM NaCl. At the highest salinity (200 mM) level the maximum seed vigor index observed in CZ-7 and the minimum vigor index was found in B73 (Figure 6).

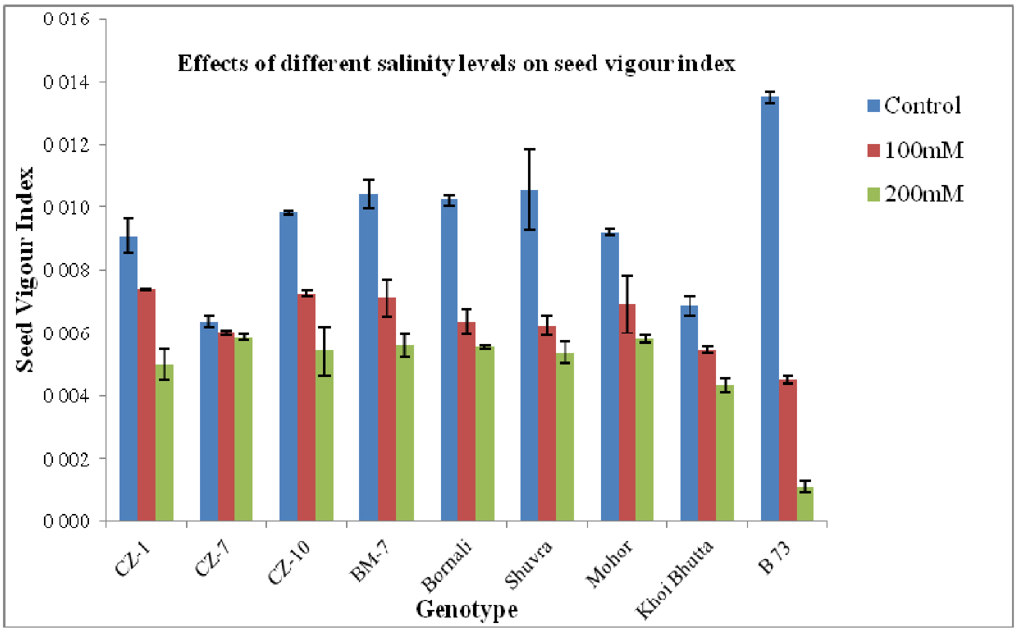

Fig. 6. Effects of different salinity levels on seed vigor index.

From the results it seems that the effects of NaCl on salt tolerance index of different maize genotypes was significant (Table 1). The ranges of mean salt tolerance indices among concentrations were very wide ranging from 71.11 at 100 mM of NaCl to 59.04 at 200 mM of NaCl. The interaction effects of salinity and genotypes were significant for all trials. The highest salt tolerance index was recorded for CZ-7; 94.09 at 100 mM and 93.98 at 200 mM of NaCl. The lowest index was at B73; 33.74 at 100 mM and 8.82 at 200 mM of NaCl (Figure 7).

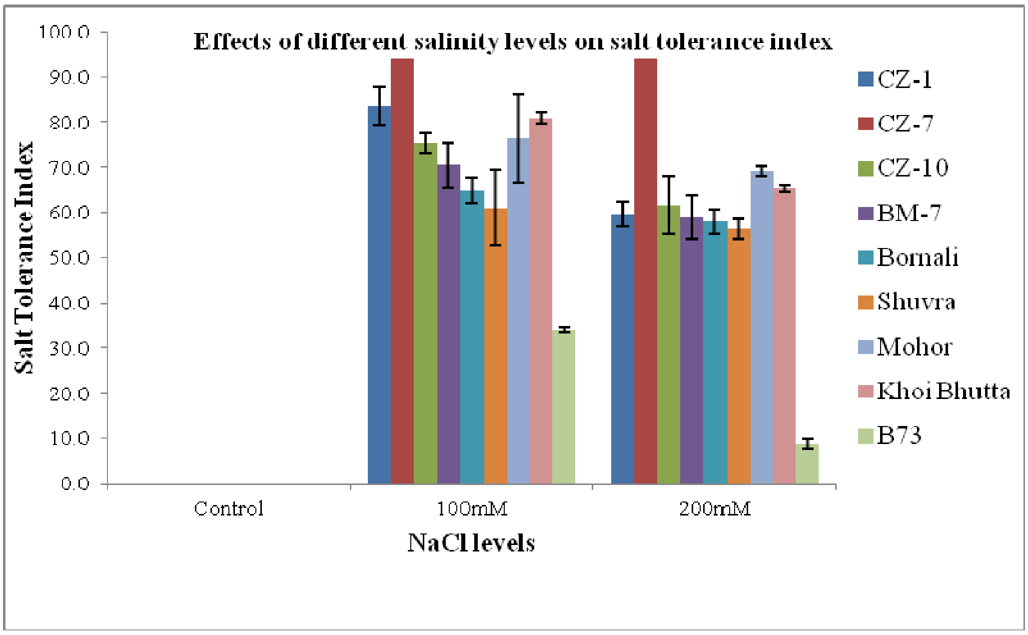

Fig. 7. Effects of different salinity levels on salt tolerance index.

Percent reduction over control results (Table 2) show that as the levels of NaCl increased the reduction in dry weight increased also. The 200mM of NaCl gave the highest reduction (40.96%) followed by the 100mM of NaCl (28.89%). However, different genotypes showed significant differences in percent reduction over control. CZ-7 demonstrated lowest reduction and B73 showed maximum reduction in all salinity levels.

Table 2. Effects of NaCl salinity on percent reduction over control (%) of nine maize genotypes.

Genotype	Levels of NaCl salinity		
	100 mM	200 mM	Mean
CZ-1	16.43	40.43	28.43
CZ-7	5.98	6.02	6.00
CZ-10	24.79	38.43	31.61
BM-7	29.67	41.12	35.40
Bornali	35.06	42.20	38.63
Shuvra	39.01	43.75	41.38
Mohor	23.69	30.83	27.26
Khoi Bhutta	19.09	34.67	26.88
B73	66.26	91.18	78.72
Mean	28.89	40.96	
LSD5% for NaCl levels=7.22; for cultivars=5.31; for interactions=6.08			

DISCUSSION

Germination begins with the imbibition or uptake of water. Salinity prevents water imbibition, thereby hindering the initial process of seed germination (OTHMAN, 2005). Salinity imposes osmotic stress by accumulation of Na and Cl ions. In addition, high absorption of Na and Cl ions during seed germination can be due to cell toxicity that finally inhibits or slows the rate of germination and thus decreases germination percentage (TAIZ and ZEIGER, 2002). RAHMAN *et al.*, (2000) reported that maize cultivars were significantly more tolerant to salt stress at germination than at later stages of growth. Previous studies have shown that increase in salinity delays the initiation of germination leading to reduction in germination percentage (ABBAD *et al.,* 2004). The present results noticeably show that NaCl decreased seed germination significantly with the increment of its concentration. Our results are similar to the findings reported by LI (2008). In maize comparable results were reported by ZHANG and ZHAO (2011).

The speed of germination was decreased significantly in all maize genotypes as the salinity levels increased. The reduction of speed of germination at high salt levels might be mainly due to osmotic stress (HEENAN *et al.,* 1988). The decreasing tendency of germination speed due to increasing salinity was in the conformity with the reports of others (ANBUMALARMATHI and MEHTA, 2013).

Increasing salinity of the seed sown and the highest reduction germination index were significant at higher salinity. NASERI *et al.,* (2012) in barley showed that the germination index decreased with increasing salinity. Similar result was also observed by TANVEER *et al.,* (2013) in field bindweed and CARPICI et al., (2009) and ZHANG and ZHAO (2011) in maize and ABBAS *et al.,* (2013) in rice.

In the present study, seed germination time was delayed by salt. Increase of mean germination time (MGT) indicates that germination is delayed comparatively (HAKIM *et al.,* 2011). The increase in MGT with the increasing NaCl stress was also supported by XU *et al.,* (2011). Similar result was also observed by REDONDO-GOMEZ *et al.,* (2008), where MGT of *Limonium emarginatum* was higher in salt stress condition.

With increasing salinity in all nine maize genotypes seed mass seedling dry weight decreased significantly. This result comes in consistent with previous ones (CARPICI *et al*., 2010). THEERAKULPISUT *et al.,* (2005) stated that the weight of shoots and roots showed high correlation with the level of salinity. ASHRAF (2002) mentioned that the reduction in seedling fresh and dry weight is due to decreasing water uptake by seedlings in salt stress presence. Our results are also similar to the findings reported by RASTEGAR and KANDI (2011) in soybean and by ASHRAF and WAHID (2000) in corn.

In all nine maize genotypes, seed vigor decreased with increasing salinity levels (Figure 6). Generally, seed vigor index is related to special impact of ions and reduction of environmental water potential in the presence of salinity (KESHAVARZI *et al.,* 2011). This is according reports by other researchers. COKKIZGIN (2012); Such KHODARAHMPOUR (2011) and MENSUH *et al.,* (2006) that had been stated vigor reduced by salinity stress.

The salt tolerance indices of genotypes at the germination stage showed a large genotypic variation. The effects of different salinity levels on salt tolerance indices of genotypes were of importance. As the salt concentrations increased the salt tolerance indices of genotypes decreased. Therefore, the lowest value of salt tolerance index was determined at 250 mM NaCl (Figure 7). Finally, salt tolerance index indicated CZ-7 to be relatively salt tolerant as compared to the other genotypes. Similar results were reported by CARPICI *et al.*, (2009) in maize and ZAIF *et al.,* (2009) in hot pepper.

Seed germination is the most important phase for the growth and development of a plant. Salt content is one of the vital features of soil and should be a primary concern when growing any plants. The reason is that salt can dramatically affect plant's growth in all its life stages, from seed to maturity to decline and death. In the current study, the best level of salinity for all investigated traits was control. In general, salt stress adversely affected germination of all maize genotypes. It was noticeable that increasing concentration of NaCl reduced germination percentage, speed of germination, germination index, seedling dry weight and salt tolerance index significantly. In this study, CZ-7 was the most tolerant inbred line while B73 was the least tolerant among the tested genotypes. These genotypes could be used later for any breeding program from improvement of maize for salt tolerance. Nonetheless, criteria such as germination indices and salt tolerance index could be used as physiological markers for assessing salt tolerance in maize.

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