African Journal of Biotechnology Vol. 11(2), pp. 298-304, 5 January, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.2624
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Effects of NaCl salinity on maize (*Zea mays* L.) at germination and early seedling stage

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Accepted 9 December, 2011

The response of eight maize hybrids against five different salinity levels namely 0, 60, 120, 180 and 240 mM) were studied at germination and early seedling stage. This investigation was performed as factorial experiment under completely randomized design (CRD) with three replications for each salinity level. Analysis of variance (ANOVA) showed that there were significant differences (P < 0.05) between salinity stress levels, hybrids and interaction effects for all investigated traits. Supplementary analysis showed that there were significant differences (P < 0.05) between hybrids for germination percentage, germination rate, mean germination time and seed vigor in all salinity levels. But, there were no significant differences found between studied hybrids at salinity level of 240 mM for the length of radicle, the length of plumule and the length of whole seedling. Results also indicated that maximum reduction in germination percentage (77.4%), germination rate (32.4%), length of radicle (79.5%) and plumule (78%), seedling length (78.1%) and seed vigor (95%) were obtained in highest level of salinity (240 mM). Results further depicted that hybrid K166B×K47/2-2-21-2-1-1-1 was the most tolerant hybrid than other hybrids under salinity stress.

Key words: Germination indices, maize hybrid, salinity stress.

INTRODUCTION

Salinity is a common abiotic stress factor seriously affecting crop production in different regions, particularly in arid and semi-arid regions of the world. It is estimated that over 800 million hectares of land in the world are affected both by salinity and sodicity (Munns, 2005).

Among the cereal species, maize (*Zea mays* L.) seems to be sensitive to salt stress (Maas et al., 1986). Maize is being increasingly cultivated in Iran. Its cultivation area is expanding to areas having high potential for accumulation of salts in the soil profile, such as Khuzestan. It is, therefore, important to develop new maize varieties with high genetic capacity to tolerate salt stress. The first important step in breeding new varieties with high salt tolerance is to have a useful and substantial genetic variation in tolerance to salinity stress. Breeders seek to develop and identify cultivars that are more tolerant toward salinity and water stress (Janmohammadi et al., 2008).

Seed sowing generally considered the first critical and most sensitive stage in the life cycle of plants and seeds are frequently exposed to unfavorable environmental conditions that may compromise the establishment of seedling (Figueiredo-e-Albuquerque and Carvalho 2003; Misra and Dwivedi, 2004). Germination and seedling characteristics are the most viable criteria used for selecting salt tolerance in plants. Germination percentage, germination rate and seedling growth are most suitable criteria for selection of cultivars. One of the commonest experiments in germination of the seeds is the application of NaCl. Seed response to salinity can be simulated by NaCl induced ionic stress in the germination experiments. Ionic stress is caused by a toxic accumulation of NaCl in plant tissues. Germination rates decrease with an increase in NaCl concentration (Murillo-Amador et al., 2002).

The present study was therefore mainly conducted to evaluate the effect of salinity on seed germination and seedling growth of eight maize hybrids under salinity conditions. The primary objective of the present study was to compare eight maize hybrids toward salinity stress and

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Table 1. Pedigree/origin of studied inbred lines of maize.

Inbred lines	Pedigree sources/origin
Lancaster Sure Crop (LSC)	
K19	Derived from MO17 changes in Iran
Reid Yellow Dent (RYD)	
A B73 back-cross derived line [(A662 × B73)(3)]	A679
Extracted from late synthetic (Created in Iran)	
SYN-Late(Iran)	K3651/1
SYN-Late(Iran)	K3640/5
Lines extracted from CIMMYT originated materials in Iran	
K47/2-2-21-2-1-1-1	K166A

to select the most tolerant hybrid of maize.

MATERIALS AND METHODS

Effect of salt stress induced by different levels namely 0, 60, 120, 180 and 240 mM of NaCl on germination and early seedling development of maize were studied. To achieve the purpose, eight hybrids the combination of selected inbred lines (Table 1) of maize were used. This investigation was performed as factorial experiment under completely randomized design (CRD) with three replications (for each hybrid and salinity level) at Seed Laboratory, Islamic Azad University - Shoushtar Branch, Iran in year 2011. This study was performed in Petri-dishes (11 cm) containing 2 layered filter paper (90 mm). The selected seeds of each hybrid were first sterilized in sodium hypochlorite (1%) solution and then washed and the washed twice in deionized distilled water. Then Petri-dishes containing double layer filter paper were moistened by respective prepared salinity solutions. Thereafter a selected number of seeds of each hybrid were soaked in these Petri dishes and then kept in an incubator (40% relative humidity) at 25°C. Daily germination rate was measured and filter papers were replaced when needed. Similarly respective salinity solutions were added when required. Seeds were considered germinated when the emergent radicle reached to 2 mm length. After 7 days, germination percentage was measured by ISTA (International Seed Testing Association) standard method. By the end of the 7th day, the germination percentage, mean germination time (MGT) (Ellis and Robert, 1981), germination rate, the length of radicle and plumule of seeds and length of seedling and seed vigor were also measured.

Formula 1: $$GP = \frac{SNG}{SN0} \times 100$$

Where, GP is the germination percentage; SNG is the number of germinated seeds and SN0 is the number of experimental seeds with viability (Scott et al., 1984).

Formula 2: $$GR = \frac{\sum N}{\sum (n \times g)}$$

Where, GR is the germination rate; n is the number of germinated seed on gth day and g is the number of total germinated seeds (Ellis and Robert, 1981).

Formula 3: Seed vigor = germination percentage × seedling length
For statistical analysis the data of germinating percentage were transformed to arcsin $\sqrt{\frac{X}{100}}$.

Analyses were done using the SPSS var. 16 software. Differences between means were determined by Duncan's Multiple Range Tests (DMRT) at probability level 5%. Draw plots using software EXCEL carried out.

RESULTS AND DISCUSSION

Analysis of variance showed that there were significant differences between salinity stress levels. The results of this study reveal that various concentrations of NaCl had a significant effect on the all measured traits. For hybrids, there were significant differences for all traits. Also, analysis of variance showed that interaction effects were significant for all investigated traits (Table 2).

Germination percentage of all hybrids was adversely affected due to the application of different levels (0, 60, 120, 180 and 240 mM) of applied NaCl salt. The differences between the means (hybrids and salinity stress levels) were compared by Duncan multiple range test and are shown in Figures 1 and 2. It was observed that in all of the hybrids there was a linear decrease in germination percentage because of gradual salinity stress increment. While in this experiment, different hybrids showed various responses toward salinity stress. Among the maize hybrids, K166B×K47/2-2-21-2-1-1-1 had the highest germination percentage and the hybrids K166B×K19 and A679×K19 had the lowest germination percentage (Figure 2). However, maximum reduction in germination

Table 2. Analysis of variance (ANOVA) for various measured traits of maize (*Zea mays* L.) hybrids subjected to salinity stress

Source of variance	Df	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Length of radicle (cm)
Salinity levels	4	3049.2**	5.93**	0.04**	45.1**
Hybrid	7	306.1**	1.6**	0.009**	3.49**
Salinity levels × Hybrid	28	85.2*	0.45*	0.092*	0.85*
Error	80	47.6	0.37	0.002	0.57

Source of variance	Df	Length of plumule (cm)	Length of seedling (cm)	Seed vigour
Salinity levels	4	31.05**	150.6**	438113**
Hybrid	7	1.5**	6.97**	19051.9**
Salinity levels× Hybrid	28	0.43*	1.14*	8337.1**
Error	80	0.24	1.03	3402.4

* and **: significant at 5% and 1% probability levels, respectively.

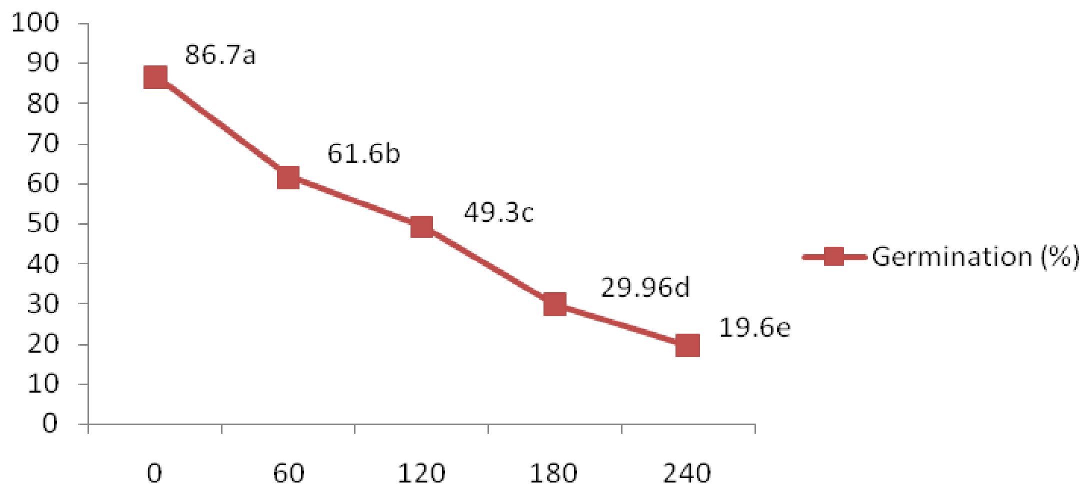

Figure 1. Germination percentage under different salinity stress levels (mM).

percentage was observed at the highest level of applied salts that is, 240 mM of NaCl (Figure 1). Hybrids K166B×K47/2-2-21-2-1-1-1, K3651/1×K166B and K3651/1×K3640/5 had the highest germination rate and seems hybrid K166B×K47/2-2-21-2-1-1-1 relativelytolerant to salinity stress (Table 3). There were found significant differences among hybrids for their germination percentage and rate of germi-nation in all levels of salinity stress (Table 4).

Results of means comparison values deciphered that germination percentage and germination rate

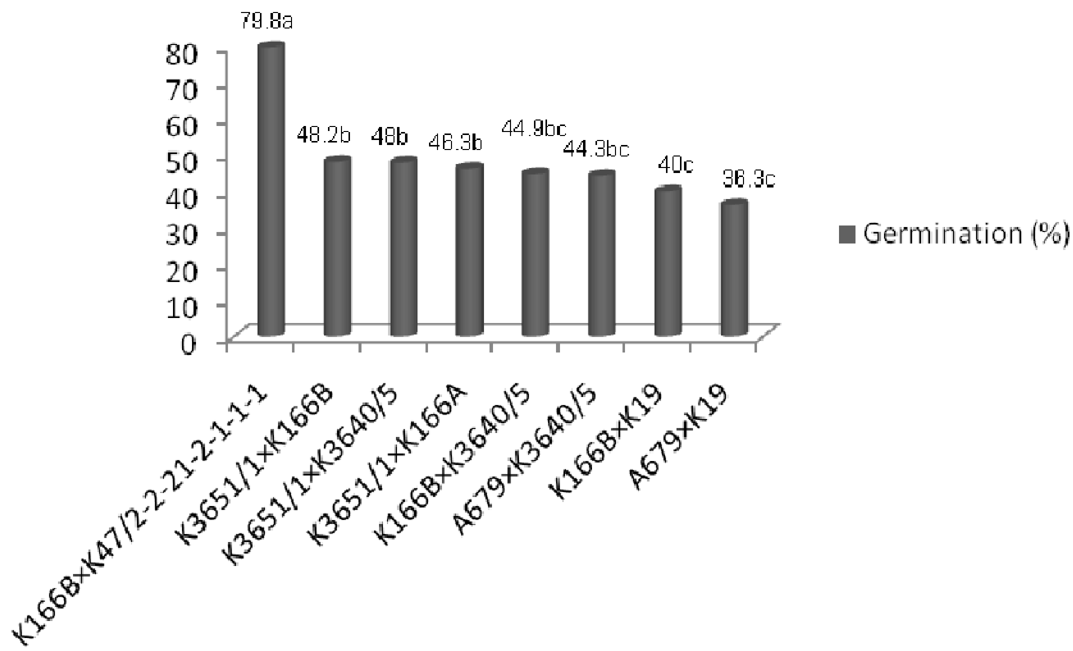

Figure 2. Germination percentage of maize hybrids under different salinity stress.

Table 3. Mean comparison of main effects of salinity stress levels and hybrids.

Treatment	Mean germination time (day)	Germination rate (number in day)	Length of radicle (cm)	Length of plumule (cm)	Length of seedling (cm)	Seed vigor
K166B×K47/2-2-21-2-1-1-1	3.3 c	0.30 a	2.9 a	2.1 a	5.0 a	399 a
K3651/1×K166B	3.5 bc	0.296 a	1.9 c	1.6 cd	3.5 c	168.7 c
K3651/1×K3640/5	3.5 bc	0.30 a	1.6 c	1.7 bc	3.2 d	153.6 c
K3651/1×K166A	3.6 bc	0.28 ab	2.8 a	2.2 a	5.0 a	231.5 b
K166B×K3640/5	4.1 a	0.25 d	2.1 bc	2.0 ab	4.1 bc	185.2 c
A679×K3640/5	3.9 ab	0.26 cd	2.1 bc	2.0 ab	4.0 bc	177.2 c
K166B×K19	4.0 a	0.25 d	2.5 ab	2.1 ab	4.6 ab	184.0 c
A679×K19	3.9 ab	0.27 bcd	1.6 c	1.3 d	2.9 d	105.3 d
Control	3.0 d	0.34 a	4.2 a	3.6 a	7.75 a	671.9 a
60 mM	3.5 c	0.29 b	2.9 b	2.2 b	5.1 b	314.2 b
120 mM	3.8 bc	0.27 bc	1.8 c	1.6 c	3.4 c	167.6 c
180 mM	4.1 ab	0.25 cd	1.04 cd	0.9 d	1.94 d	58.1 d
240 mM	4.2 a	0.23 d	0.86 d	0.78 d	1.7 d	33.3 de

Means with similar letter(s) in each trait is not significantly different at 5% probability level according to Duncan's Multiple Range Test.

were linearly decreased by decrease in osmotic potential of NaCl solution, while the maximum germination rate and percentage were obtained at zero level of applied salts (Figure 1 and Table 3). Results also deciphered that germination rate had the most important effect on stand establishment and plan density under laboratory and green-house conditions. This is also in agreement with the results of Farsiani and Ghobadi (2009) and Khayatnezhad et al. (2010) in maize, Gholamin and Khayatnezhad (2010) in wheat and Mostafavi (2011) in safflower. Some studied referred that stress can contribute to improve germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao et al., 1999), enhance-ment of ATPase activity, RNA and acid phos-phathase synthesis (Fu et al., 1988), also by increase of amylases, proteases or lipases activity (Ashraf and Foolad, 2005). The decreasing tendency of germination rate due to increasing salinity was in the conformity with the reports of early researchers (Mohammed et al., 1989; Khan et al., 1997). The reduction of germination rate at high salt levels might be mainly due to osmotic stress (Heenan et

Table 4. Supplementary analysis of interaction effects.

Salinity level (mM)	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Length of radicle (cm)	Length of plumule (cm)	Length of seedling (cm)	Seed vigor
Control	690.1**	1.6*	0.08*	2.1*	1.2*	4.1*	34975.7**
60	340.56*	1.0*	0.05*	1.2*	0.65**	0.62*	8392.4**
120	293.9**	0.93*	0.01*	3.68**	0.72*	5.3**	6721.9*
180	88.5*	0.09**	0.09**	0.44**	0.24**	0.22*	249.7*
240	124.2*	0.07**	0.05**	2.39ns	0.475ns	4.4ns	1139.7**

ns, * and **:non significant, significant at 5% and 1% probability levels, respectively.

al., 1988).

Among the maize hybrids, K166B×K19 and K18×K3640/5 had the highest mean germination time. The mean germination time increased with decrease in the osmotic potential in NaCl solution (Table 3). In NaCl treatments, the mean germination time was delayed by stress conditions. There were between hybrids significant differences for mean germination time in all salinity levels (Table 4). Alebrahim et al. (2008) reported that with decrease in the osmotic potential in PEG and NaCl solutions, the mean germination time in lines of MO17 and B73 increased. Mostafavi (2011) with study on 6 genotypes of safflower reported that the mean germination time increased with increase in the osmotic potential in NaCl solution.

The length of radicle is one of the most important traits for salinity stress because roots are in contact with soil and absorb water from soil. For this reason, the length of radicle provides an important clue to the response of plants to salinity stress. A marked reduction in the length of radicle, the length of plumule and the length of seedling of all hybrids of maize was observed due to salt stress. Among the maize hybrids, hybrid K166B×K47/2-2-21-2-1-1-1 and K3651/1×K166A had the highest length of radical, plumule and seedling while, hybrid A679×K19 had the lowest length of radical, plumule and seedling (Table 3). Results of this study showed that length of radical, plumule and seedling decreased with increasing salinity levels in all hybrids (Table 3). Also, it was clearly determined that there were no significant differences between studied hybrids at salinity level 240 mM for the length of radicle, the length of plumule and the length of seedling (Table 4).

The most effective levels in reducing these attributes were 180 mmol of NaCl (Table 3). Best level of NaCl concentration in radicle length, plumule length and seedling length was control treatment. This results are in agreement with many researches (Gholamin and Khayatnezhad, 2010; Farsiani and Ghobadi, 2009; Mohammadkhani and Heidari, 2008; Jajarmi, 2009; Khayatnezhad et al., 2010). Reduction of seedling height is a common phenomenon of many crop plants grown under saline conditions (Javed and Khan, 1995; Karim et al., 1992; Amin et al., 1996). Kramer (1974) reported that the first effect measurable due to water deficit was the growth reduction, caused by the declining in the cellular expansion. The cellular elongation process and the carbohydrates wall synthesis were very susceptible to water deficit (Wenkert et al., 1978) and the growing decrease was a consequence of the turgescence laying down of those cells (Shalhevet et al., 1995).

Seed vigor decreased with decrease in osmotic potential of NaCl solution. Best level of NaCl concentration in seed vigour was the control treatment (Table 3). A significant inter-genotype variation was observed under salt stress. Of all hybrids, K166B×K47/2-2-21-2-1-1-1 produced highest seed vigor at all salt regimes, while A679×K19 produced lowest seed vigor (Table 3). There were between hybrids significant differences for seed vigor in all salinity levels (Table 4). Mostafavi (2011) reported that seed vigor increased in osmotic potential until -3 bars but decreased in -5 bars.

Conclusion

In the present study, the best level of salinity for all investigated traits was control. Salt stress adversely affected the germination percentage, germination rate, mean germination time, length of radical, plumule and seedling and seed vigour of 8 hybrids of maize. A significant variation in salt tolerance was also observed among all the studied hybrids. Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to the ability of seeds for best germination under unfavourable conditions, so necessity of evaluation of salinity tolerant genotypes is important at primary growth stage. In this research, with attention to that hybrid K166B×K47/2-2-21-2-1-1-1 had the highest germination percentage, germination rate, radical, plumule and seedling length and seed vigour, therefore could be rated as salt stress tolerant hybrid whereas, hybrid A679×K19 is the most sensitive hybrid to ward salinity stress.

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