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Full Length Research Paper

Screening maize (*Zea mays* L.) hybrids for salt stress tolerance at germination stage

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Plant breeding may provide a relatively cost effective short-term solution to the salinity problem by producing cultivars that are able to remain productive at low to moderate levels of salinity. To determine the most tolerant hybrid to salinity stress, an experiment was performed as factorial form under completely randomized design (CRD) with three replications. Hybrid factor consisted of fifteen hybrids and five levels of stress (0, -0.3, -0.6, -0.9 and -1.2 MPa). Results indicated that significant decrease was observed in germination percentage, germination rate, root length, shoot length, seedling length and seed vigour traits in stress conditions. Hybrid K166B×K47/2-2-21-2-1-1-1 showed the highest germination percentage, root length, shoot length, seedling length and seed vigour traits in salinity conditions. Results of cluster analysis using the data for all measured traits under different levels of salinity stress (Ward's minimum variance method) showed clustered hybrids in three groups; Hybrid K166B×K47/2-2-21-2-1-1-1 in the third cluster, hybrids K18×K47/2-2-21-2-1-1-1 and K3651/1×K166A in the second cluster, and twelve other hybrids were in the first cluster. Hybrid K166B×K47/2-2-21-2-1-1-1 was found to be tolerant, while hybrids of the first cluster were sensitive to salt.

Key words: Cluster analysis, germination indices, maize, NaCl stress.

INTRODUCTION

Nearly 10% of the earth's land is salt-affected and an estimated 10 million hectare of agricultural land is lost annually due to salinization and water logging. Salinity of soil and water resources is a serious threat (Amer, 2010). Soil salinity may affect the germination of seeds either by creating low osmotic potential to the seeds, thus preventing water uptake or through the toxic effects of Na^+ and Cl^- ions on germinating seed (Golbashy et al., 2010; Khajeh-Hosseini et al., 2003; Atak et al., 2006; Kaya et al., 2006).

Maize (*Zea mays* L.) is the third most important cereal in the world after wheat and rice, and grows under a wide range of climatic conditions. It is moderately sensitive to salinity and considered as the most salt-sensitive of the cereals (Maas and Hoffman, 1977; Ashraf and McNeally, 1990). Besides, maize is a highly cross-pollinated crop. In consequence, it has become highly polymorphic for the natural and domesticated evolution and thus contains enormous variability (Paterniani, 1990; Maiti et al., 1996) in which salinity tolerance may exist. The response to salinity varies with the stage of development of maize plant (Maas and Hoffman, 1983; Pasternak et al., 1985). Tolerance to stresses as salinity of plants can be determined by using different growth parameters like root length, shoot length, dry mass of root and shoot (Cicek and Cakirlar, 2002). It has also been reported that under saline conditions, germination ability of seeds differ from one crop to another and even a significant variation is observed amongst the different varieties of the same crop (Asana and Kale, 1965, Maas and Hoffman, 1977). Water stress acts by decreasing the percentage and rate of germination and seedling growth in senna (Delachiave and De Pinho, 2003), in maize (Farsiani and Ghobadi, 2009; Khayatnezhad et al., 2010), in wheat (Gholamin and Khayatnezhad, 2010) and in safflower (Mostafavi, 2011).

Plant breeding may provide a relatively cost effective short-term solution to the salinity problem by producing cultivars that are able to remain productive at low to moderate levels of salinity. The availability of genetic variation, both at intra or inter-specific level, is a prerequisite for the success of breeding programs (Ashraf

Table 1. Used amounts of sodium chloride to obtain different levels of water deficit.

Osmotic potential (MPa)	NaCl (gL^{-1} of distilled water)
0	Distilled water
-0.3	3.51
-0.6	7.01
-0.9	10.52
-1.2	14.03

et al., 1987; Maas, 1986). Genetic variability for salt tolerance was reported in alfalfa (McKimmie and Dobrenz, 1991), *Trifolium* (Ashraf et al., 1987), sunflower (Francois, 1996), rice (Hakim et al., 2010), safflower (Mostafavi, 2011) and maize (Sharif et al., 1999; Khan et al., 2003; Giaveno et al., 2007). Development of salt tolerance in crops depends ultimately on two factors. Availability of genetic variation by screening and selection of those plants with superior performance when exposed to such stress is very important (Epstein et al., 1980).

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. The objective of this research was to classify maize hybrids under salinity stress and determine the most tolerant hybrid to salinity stress.

MATERIALS AND METHODS

In order to study the effects of salinity stress on germination and early seedling growth in maize hybrids, experiment was conducted in factorial form using a completely randomized design with three replications. In this experiment, fifteen maize hybrids - the combination of selected inbred lines - were used in five levels of salinity treatment (distilled water as control, -0.3, -0.6, -0.9 and -1.2 MPa) by using different NaCl concentrations (Table 1). This experiment was carried out at Seed Laboratory, Islamic Azad University - Shoushtar Branch, Iran, in 2011.

Evaluation of parameters

In each level of stress, twenty seeds of each hybrid were selected and sterilized in sodium hypochlorite (1%) and then washed in distilled water for two times. The seeds of hybrids were germinated in Petri dishes on two layers of filter paper in an incubator maintained at 25°C. Daily, germination rate was measured and need to replace the filter papers and add the NaCl soluble were performed. Seeds were considered germinated when the emergent radicle reached 2 mm length. After 7 days, germination percentage was measured by ISTA (1996) standard method. At end of the seventh day, the germination percentage, mean germination time (MGT) (Ellis and Robert, 1981), germination rate, root length, shoot length, seedling length and seed vigour index were also measured and evaluated using the following formulas;

$$GP = \frac{SNG}{SN0} \times 100 \qquad (1)$$

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).

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

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).

Seed vigour = germination percentage × seedling length (3)

Statistical analysis

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%. Plots were drawn using software EXCEL. All investigated traits were subjected to hierarchical cluster analysis using procedure Ward's minimum variance method as a clustering algorithm. Ward's minimum method is a hierarchical clustering procedure in which similarity used to join clusters is calculated as the sum of squares between the two clusters summed over all variables (Hair et al., 1998). It minimizes them within cluster sums of squares across all seven partitions.

RESULTS AND DISCUSSION

Analysis of variance showed that there were significant differences between salinity stress levels. The results of this study revealed 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).

Table 2. Analysis of variance on mean of squares of measured traits maize hybrids under salinity stress.

Source of variance	Df	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Root length (cm)
Salinity levels	4	5578.30**	4.08**	0.02**	90.15**
Hybrid	14	465.08**	4.68**	0.02**	2.29**
Salinity levels × Hybrid	56	118.64**	2.26**	0.01**	1.41**
Error	150	43.66	0.33	0.00	0.62

Source of variance	Df	Shoot length (cm)	Seedling length (cm)	Seed vigour
Salinity levels	4	57.13**	289.83**	700324.44**
Hybrid	14	1.14**	4.82**	21403.77**
Salinity levels × Cultivar	56	0.58**	2.05**	7291.68**
Error	150	0.21	1.05	2867.93

**Significant at 1% probability level.

Germination percentage and germination rate

The differences between the means hybrids (Table 3) and salinity stress levels (Figure 1) were compared by Duncan multiple range test. It was observed that in all of hybrids, there was a decrease in germination percentage because increment of salinity stress and maximum germination percentage was delayed. While in this experiment, different hybrids had different response to the salinity stress. Among the maize hybrids, K166B×K47/2-2-21-2-1-1-1 had the highest germination percentage and there was no significant difference with hybrid K18×K47/2-2-21-2-1-1-1. However, maximum reduction in germination percentage was observed at the highest level- that is, -1.2 MPa of NaCl (Figure 1A). Results of means comparison, using Duncan multiple range test, showed that germination percentage and germination rate were decreased by decrease in osmotic potential, while the maximum germination rate and percentage were obtained at 0 MPa level (control treatment) (Figure 1A, C). Hybrid K18×A679 showed the highest germination rate (Table 3). This agreed 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. Other studies indicated that stress can contribute to improving germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao et al., 1999), enhancement of ATPase activity, RNA and acid phosphatase synthesis (Fu et al., 1988), and also by increasing amylases, proteases or lipases activity (Ashraf and Foolad, 2005).

Mean germination time

Among the maize hybrids, K166B × K3640/5 and K166B×K19 had the highest mean germination time. The mean germination time increased with increase in the concentration of NaCl solution (Figure 1B). In NaCl treatments, the mean germination time was delayed by stress conditions. Alebrahim et al. (2008) reported that with a decrease in the osmotic potential in PEG and NaCl solutions, the mean germination time in lines of MO17 and B73 increased. Also, Mostafavi (2011) in a study on 6 genotypes of safflower reported that the mean germination time increased with a decrease in the osmotic potential in NaCl solution.

Root, shoot and seedling length

The root length provides an important clue to the response of plants to salinity stress. A special reduction in the root length, the shoot length and the seedling length of all hybrids of maize was observed because of salt stress. Among the maize hybrids, hybrid K166B×K47/2-2-21-2-1-1-1 and K3651/1×K166A had the longest root length and seedling length and hybrids K166B×K47/2-2-21-2-1-1-1, K3651/1×K166A and K166B×K19 showed the highest shoot length (Table 3). Results of this study showed that root length, shoot length and seedling length decreased with increasing salinity levels in all hybrids. The most effective levels in reducing these attributes were -0.9 MPa of NaCl (Figure 1D to F). Best level of NaCl concentration in root length, shoot length and seedling length was in control treatment

Table 3. Mean comparison of main effects of maize hybrids under salinity stress.

Hybrid	Germination (%)	Mean germination time (day)	Germination Rate (number in day)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Seed vigour
K166B×K47/2-2-21-2-1-1-1	79.8[a]	3.3[c]	0.30[c]	2.9[a]	2.1[a]	5.0[a]	399[a]
K18×K47/2-2-21-2-1-1-1	70.2[ab]	2.9[b]	0.34[bc]	2.1[b]	1.6[c]	3.7[b]	250.7[b]
K3640/5×K47/2-2-21-2-1-1-1	37.7[e]	2.7[b]	0.37[b]	2.2[b]	1.8[b]	4.1[ab]	154.6[c]
K166A×K47/2-2-21-2-1-1-1	34.1[f]	3.0[b]	0.33[bc]	1.6[cd]	1.5[c]	3.1[b]	105.7[d]
K3651/1×K166B	48.2[c]	3.5[bc]	0.296[c]	1.9[c]	1.6[c]	3.5[b]	168.7[c]
K3651/1×K3640/5	48[c]	3.5[bc]	0.30[c]	1.6[cd]	1.7[b]	3.2[b]	153.6[c]
K3651/1×K166A	46.3[cd]	3.6[bc]	0.28[cd]	2.8[a]	2.2[a]	5.0[a]	231.5[bc]
K166B×K3640/5	44.9[d]	4.1[a]	0.24[d]	2.1[b]	2.0[ab]	4.1[ab]	185.2[c]
A679×K3640/5	44.3[d]	3.9[ab]	0.26[cd]	2.1[b]	2.0[ab]	4.0[ab]	177.2[c]
K166B×K19	40 d[e]	4.0[a]	0.25[d]	2.5[ab]	2.1[a]	4.6[ab]	184.0[c]
A679×K19	36.3[e]	3.9[ab]	0.27[cd]	1.6[cd]	1.3[d]	2.9[c]	105.3[d]
K18×A679	39.5[de]	2.1[c]	0.48[a]	1.6[cd]	1.56[c]	3.3[b]	131.5[cd]
K18×K19	30.8[f]	3.6[a]	0.29[c]	2.2[b]	1.3[d]	3.5[b]	107.8[d]
K18×K3651/1	43.5[d]	3.0[b]	0.34[bc]	1.8[c]	1.7[b]	3.5[b]	152.25[c]
K18×K166B	39.9[de]	3.4[a]	0.30[c]	2.0[bc]	1.6[c]	3.6[b]	143.6[c]

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

(Figure 1D to F). Water stress acts by decreasing the percentage and rate of germination and seedling growth in senna (Delachiave and De Pinho, 2003); in maize (Farsiani and Ghobadi, 2009; Khayatnezhad et al., 2010), in wheat (Gholamin and Khayatnezhad, 2010) and in safflower (Mostafavi, 2011). Khayatnezhad et al. (2010) and Mostafavi et al. (2011) in a study of four maize hybrids in drought stress conditions reported that hybrid golden west and KSC704 produced the highest root length, shoot length and seedling length, respectively.

Seed vigour

Among the hybrids, K166B×K47/2-2-21-2-1-1-1 was affected the least by salinity stress because it gave the lowest reduction rate for seed vigour (Table 3). Seed vigour decreased with increase in concentration of NaCl solution. Best level of NaCl concentration in seed vigour was the control treatment (Figure 1G). Of all hybrids, K166B×K47/2-2-21-2-1-1-1 produced highest seed vigour at all salt regimes. Mostafavi et al. (2011) in a study of four maize hybrids in drought stress conditions reported that hybrid KSC704 produced the highest seed vigour.

Classification of maize hybrids

Cluster analysis was done using the data for all measured traits under different levels of salinity stress. Results of cluster analysis (Ward's minimum variance method) clustered hybrids in three groups; hybrid K166B×K47/2-2-21-2-1-1-1 in the third cluster, hybrids K18×K47/2-2-21-2-1-1-1 and

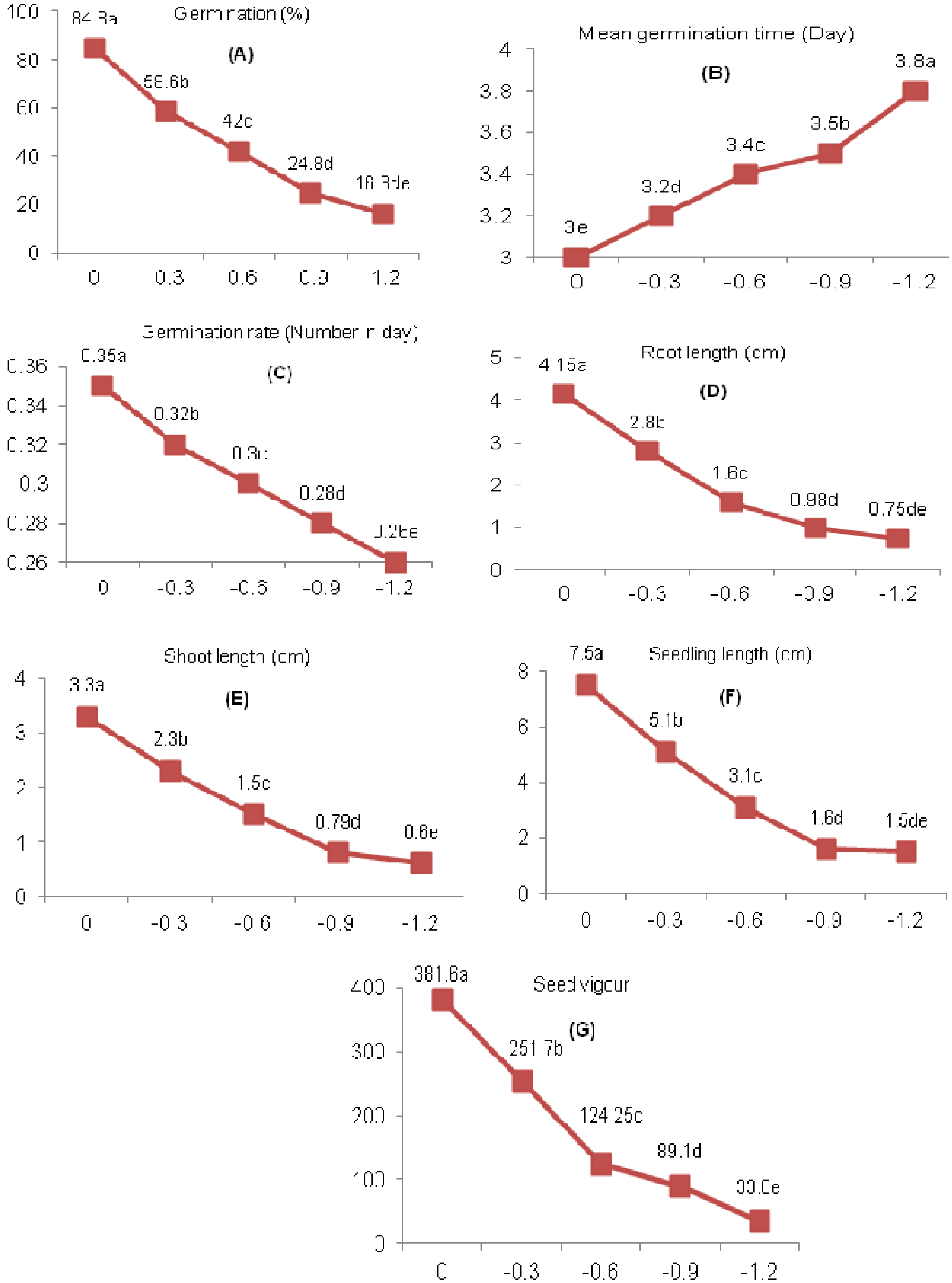

Figure 1. Results of salinity stress levels on the different parameters evaluated.

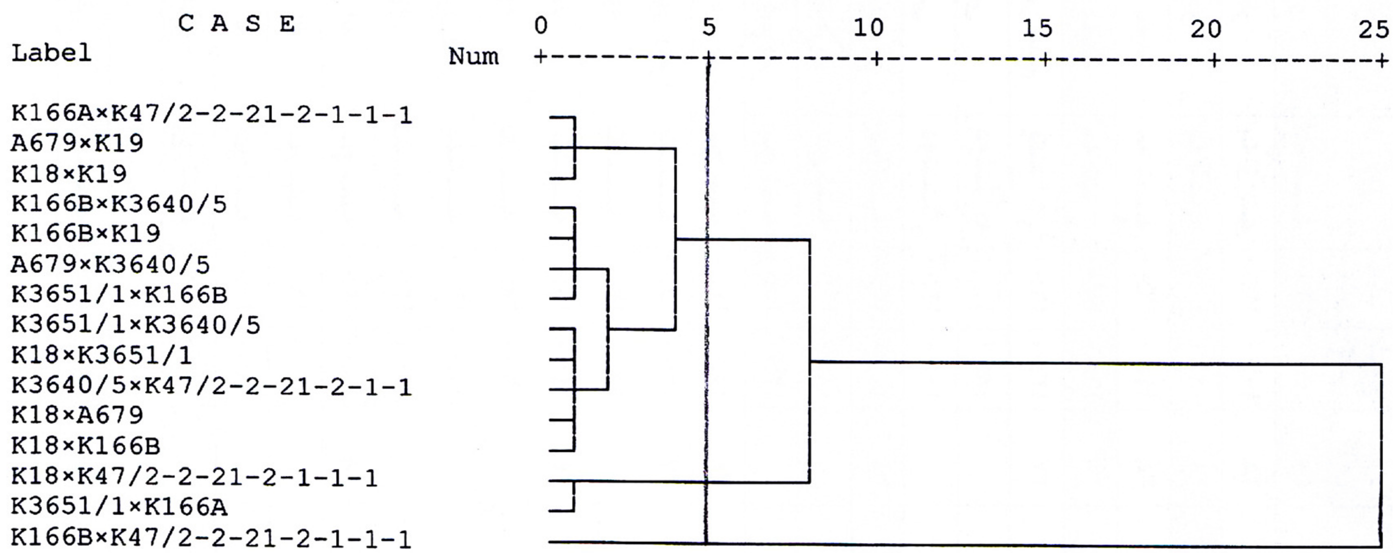

Figure 2. Cluster analysis of maize hybrids under different levels of salinity stress using Ward's minimum variance method.

K3651/1×K166A in the second cluster and hybrids, K166A×K47/2-2-21-2-1-1-1, A679×K19, K18×K19, K166B×K3640/5, K166B×K19, A679 ×K3640/5, K3651/1×K166B, K3651/1×K3640/5, K18×K3651/1, K3640/5×K47/2-2-21-2-1-1-1, K18×A679, K18×K166B were in the first cluster. Hybrid K166B×K47/2-2-21-2-1-1-1 was found to be tolerant, while hybrids of first cluster were sensitive to salt (Figure 2). Giaveno et al. (2007) reported genetic variability among fourteen tested hybrids under salt stress. Khayatnezhad et al. (2010) in a study of four maize cultivar reported that the golden west cultivar was the most resistant in stress conditions that possessed the highest levels of all traits. Mostafavi et al. (2011) in a study of four maize hybrids under drought stress conditions reported that hybrid KSC704 was tolerant, while KSC500 was sensitive to drought. Mostafavi (2011) in a study on six safflower also reported that at the highest salt level (-1.5 MPa) classified all genotypes into three group. According to the obtained results, Kose was the most resistant and KM5, KM8 and KM47 were the most sensitive genotypes. Ajmal Khan and Weber (2006) found that resistance to stress at germination stage and primary growth of seedling is independent from next growth stages and evaluation of stress tolerance need more experiment at next growth stages. Screening of available germplasm of a crop is a feasible means of identifying salt tolerant genotypes or genotypes that could maintain a comparatively reasonable yield on salt affected soils (Ashraf et al.,1987).

Conclusion

In this study, salt stress adversely affected the germination percentage, germination rate, mean germination time, root length, shoot length, seedling length and seed vigour of fifteen hybrids of maize, and a significant variation in salt tolerance was observed among all the hybrids. Obviously, acceptable growth of plants in arid and semiarid lands which are under exposure of salinity stress is related to ability of seeds for best germination under unfavorable conditions, hence evaluation of salinity tolerant genotypes is important at primary growth stage. In this research, hybrid K166B×K47/2-2-21-2-1-1-1 had the highest germination percentage, root length, shoot length, seedling length and seed vigour traits, and therefore was found to be tolerant to salt stress. While hybrids, K166A×K47/2-2-21-2-1-1-1, A679×K19, K18×K19, K166B×K3640/5, K166B×K19, A679×K3640/5, K3651/1×K166B, K3651/1×K3640/5, K18×K3651/1, K3640/5×K47/2-2-21-2-1-1-1, K18×A679 and K18×K166B were sensitive to salt stress. Ranking of the genotypes was done using the data for all measured traits at all levels of salt. These results can be related to some earlier studies in which hybrids identified as salt tolerant at the earlier growth stages showed tolerance when tested at the later growth stages. Although, a considerable magnitude of variation for salt tolerance was observed in the 15 hybrids of maize while screening them at germination stages, further studies need to be carried out to assess whether the genotypes marked as salt tolerant at the initial growth stages maintain their degree of salt tolerance when tested as adult.

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