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Full Length Research Paper

Evaluation of drought stress effects on germination and early growth of inbred lines of MO17 and B73

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To study the effect of polyethylene glycol (PEG) stress on germination and early growth stages on two inbred lines of maize, experiment were laid out at seed laboratory, in Iran in 2012. This investigation was performed as factorial experiment under completely randomized design (CRD) with three replications. Inbred line factor consist of two inbred lines (MO17 and B73) and four levels of stress (0, -3, -6 and -9 bar). The principal aim of current study was to compare the two lines of maize in relative to the stress conditions. Results indicated that significant decrease was observed in germination percentage (92.7%), germination rate (59.4%), length of radicle (89%), length of plumule (98.7%), length of seedling (92.5%) and seed vigour (99.6%). The mean germination time (MGT) and radicle/plumule length ratio increased with decrease in the osmotic potential of PEG solution. Inbred line MO17 produced the highest germination percentage, length of radicle, length of seedling, radicle/plumule length ratio and seed vigour; therefore, this line was tolerant higher than inbred line B73 to drought stress.

Key words: Early growth, germination, MO17 and B73, polyethylene glycol (PEG) stress.

INTRODUCTION

Drought stress is one of the most important environmental factors in reduction of growth, development and production of plants. It can be said that it is one of the most devastating environmental stresses. Iran, with an annual rainfall of 240 mm, is classified as one of those dry regions (Jajarmi, 2009).

Maize being nutritionally an important crop has multiple functions in the traditional farming system; it is being used as food and fuel for human being and feed for livestock and poultry (Wattoo et al., 2009). Maize is 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 study maize lines with high genetic capacity to tolerate abiotic stress.

Among the stages of the plant life cycle, seed germination and seedling emergence, and establishment are key processes in the survival and growth of plants (Hadas, 2004). Germination is regulated by duration of wetting and the amount of moisture in the growth medium (Schutz and Milberg, 1997; Gill et al., 2002).

Water stress acts by decreasing the percentage, rate of germination and seedling growth (Delachiave and De Pinho, 2003). Water stress do not only affects seed germination but also increases mean germination time (MGT) in maize plants (Willanborb et al., 2004).

The adverse effect of water shortage on germination and seedling growth has been well reported in different crops such as maize (Mohammadkhani and Heidari, 2008; Farsiani and Ghobadi, 2009; Khayatnezhad et al., 2010; Mostafavi et al., 2011; Khodarahmpour, 2011).

Polyethylene glycol is the best solute that we are aware of for imposing a low water stress that is reflective of the type of stress imposed by a drying soil (Verlues and Bray, 2004; Verslues et al., 1998; Van der Weele et al., 2000).

Maize is a protandrous plant (anthesis is sooner than silking); hence, to achieve the synchronization of silking and anthesis stages of parental lines, delayed sowing dates have been suggested for male lines. In this situation, accelerated germination and uniform emergence are essential. Any unfavorable environmental factor such as drought during germination may interfere with this

Table 1. Value PEG for stress levels.

Drought level	PEG (g/lit)
0	Distilled water
-3	138
-6	189
-9	251

Table 2. Pedigree/origin of studied inbred lines of maize.

Inbred line	Pedigree sources/origin
Lancaster sure crop (LSC)	
MO17	CI. 187–2 × C103
Reid yellow dent (RYD)	
B73	BSSS C5 (Iowa stiff stalk synthetic)

synchronism (Janmohammadi et al., 2008). Therefore, the principal aim of present study was to compare the effects of drought stress induced on germination and early seedling stages of two lines of maize.

MATERIALS AND METHODS

Effect of drought stress induced by different osmotic potential levels [(distilled water) 0, -3, -6 and -9 bar] polyethylene glycol (PEG) 6000 treatments (Table 1) on germination and early seedling development of maize were studied. Two inbred lines of maize were used (Table 2). This investigation was performed as factorial experiment under completely randomized design (CRD) with three replications (for each line and salinity level) at Seed Laboratory, Islamic Azad University - Shoushtar Branch, Iran in year 2012.

This study was performed in Petri-dishes (11 cm) containing 2 layered filter paper (90 mm). The selected seeds of each line were first sterilized in sodium hypochlorite (1%) solution and then washed twice in deionized distilled water. Then Petri-dishes containing double layer filter paper were moistened by respective prepared PEG solutions. Thereafter, a selected number of seeds of each line 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 PEG solutions were added when required. Seeds were considered germinated when the emergent radicle reached to 2 mm length. After 10 days, germination percentage was measured by International Seed Testing Association (ISTA), (1996), standard method. By the end of 10^{th} day, the germination percentage, MGT (Ellis and Robert, 1981), germination rate, the length of radicle and plumule of seeds and length of seedling, radicle/plumule length ratio and seed vigour were also measured.

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

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

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

GR, Germination rate; N, the number of germinated seeds; n, number of germinated seed on growth day; g, number of total germinated seeds (Ellis and Robert, 1981).

Seed vigour = germination percentage × seedling length (3)

For statistical analysis, the data of germinating percentage were transformed to arcsin $\sqrt{\frac{X}{100}}$. Analyses were done using the MSTAT-C software (Michigan State University). Differences between means were determined by Duncan's multiple range tests (DMRT) at probability level 1%. Drawings were made using Excel computer software.

RESULTS

The results of this study revealed that various concentrations of PEG had a significant effect on all the measured traits. For inbred lines, there were significant differences for all traits, except germination percentage, length of plumule, radicle/plumule length ratio and seed vigour. Also, analysis of variance showed that, interaction effects were significant for all investigated traits except germination percentage, germination rate, length of radicle, radicle/plumule length ratio and seed vigour (Table 3).

Mean comparison results indicated that for all traits except mean germination time, germination rate and length of plumule in drought stress, inbred line MO17 had more than inbred line B73 (Table 4).

Results showed that the germination is affected by the PEG concentrations; it means that inbred lines MO17 and B73 of maize showed a reduction in germination with an increase in PEG concentrations induced water deficit (Table 4 and Figure 1A). Results of means comparison showed that germination percentage (92.7%) was decreased by decrease in osmotic potential. It should be noted that in this experiment drought level of -9 bar of PEG was also considered, but the data not harvested for inbred line B73 at this level (Figure 1A). Mean germination percentage for lines was 59.3% for control (0 bar) but, 4.4% was at osmotic potential of -9 bar (Table 4). Germination percentage of lines was adversely affected due to the application of different levels (-3, -6 and -9 bar) of PEG. It was observed that, in inbred lines there was a decrease in germination percentage due to drought stress increment and maximum germination percentage was delayed (Figure 1A).

The MGT increased with a decrease in the osmotic potential in PEG solution (Table 4 and Figure 1B). MGT for lines was 3.21 days for control (0 bar) but, 7.7 days was at osmotic potential of -9 bar (Table 4). In PEG treatments, the MGT was delayed by stress conditions.

Table 3. Analysis of variance on mean of square germination and seedling growth in drought stress.

Source of variance	Df	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Length of radicle (cm)	Length of plumule (cm)	Length of seedling (cm)	Radicle/plumule length ratio	Seed vigour
Drought levels	3	2781.43**	19.75**	0.04**	6.27**	30.96**	63.90**	23.09**	338809.84**
Inbred line	1	81.45ns	11.25**	0.02**	4.33*	0.02ns	4.98*	13.59ns	22273.58ns
Drought levels× Inbred line	3	12.61ns	1.46*	0.002ns	0.93ns	1.36*	4.40*	4.76ns	16730.41ns
Error	16	161.36	0.38	0.001	0.76	0.22	0.99	3.65	14485.18

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

Table 4. Comparison of means simple effect of lines and drought stress levels on germination and seedling growth.

Treatment	Germination (%)	Mean germination time (day)	Germination rate (number in day)	Length of radicle (cm)	Length of plumule (cm)	Length of seedling (cm)	Radicle/plumule length ratio	Seed vigour
MO17	27.61a	5.2a	0.23a	2.151	1.74a	3.88a	1.24a	179.75a
B73	22.18b	5.99a	0.17a	1.23b	1.66a	2.89b	0.74b	124.95b
0	59.30a	3.21c	0.32a	3.34a	5.24a	5.85a	0.64b	521.08a
-3 bar	21.18b	5.33b	0.20b	1.73ab	0.81b	2.55b	2.96b	56.32b
-6 bar	14.64b	7.0a	0.15bc	1.3b	0.65b	1.95b	2.75b	27.94b
-9 bar	4.35b	7.7a	0.13c	0.37c	0.07c	0.44c	5.29a	1.91c

*Means with similar letter(s) in each trait is not significantly different (P= 1% Duncan's multiple range test).

Minimum MGT was for inbred line MO17 under control level (Figure 1B).

The germination rates were decreased by increase in PEG concentrations (Table 4 and Figure 1C). There were no significant differences between lines for germination rate (Table 4). Results of means comparison showed that germination rates (59.4%) were decreased by decrease in osmotic potential, while the maximum germination rates were obtained at control treatment (Table 4). Germination rate for lines was 0.32 for control (0 bar) but, 0.13 was at osmotic potential of -9 bar (Table 4).

A special reduction in length of radicle, length of plumule and length of seedling of lines of maize was observed because of drought stress (Table 4). Best level of PEG concentration in length of radicle, length of plumule and length of seedling was control treatment (Table 4). The radicle/ plumule length ratio increased with a decrease in the osmotic potential in PEG solution (Table 4 and Figure 1G).

At the control level, length of radicle and plumule, and length of seedling reached their highest values. For length of radicle and length of seedling, inbred line MO17 was higher than inbred line B73 (Table 4 and Figures 1D and F).

Inbred line MO17 affected the least by drought stress because it gave the lowest reduction rate for seed vigour (Table 4). Seed vigour decreased (99.8%) with increase in concentration of PEG solution. Best level of PEG concentration in seed vigour was control treatment (Table 4 and Figure 1H).

DISCUSSION

In this study, we concluded that PEG adversely affected the germination and seedling growth of inbred lines of maize. Distinct genetic differences were found among the lines with respect to germination and seedling growth subjected to PEG. It seems that inbred line MO17 in drought stress condition had more tolerant than B73 line. Khodarahmpour (2011) indicated that water potential significantly reduced germination percentage (71.2%), germination rate (24.2%),

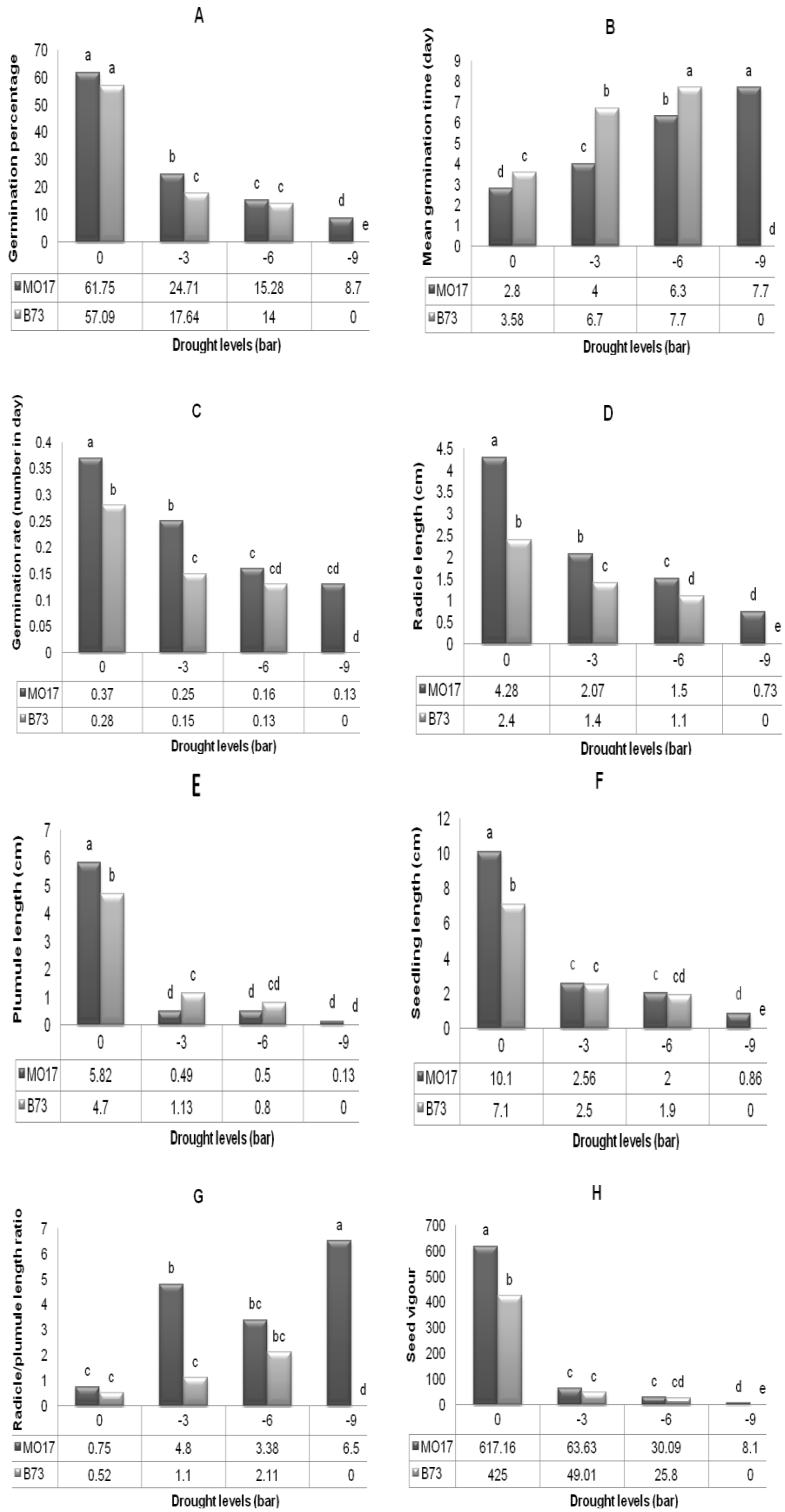

Figure 1. Comparison of means interaction effect of lines and drought stress levels on germination and seedling growth (P= 1% Duncan's multiple range test).

root length (60%), shoot length (89.8%), seedling length (71.2%) and seed vigour (91.7%). The MGT and root/shoot length ratio increased with decrease in the osmotic potential of PEG solution. Hybrid K3651/1×K166B produced the highest germination percentage, germination rate, root length, seedling length and seed vigour; hence, this hybrid was the most tolerant hybrid to drought stress. Mostafavi et al. (2011) with study of four maize hybrids in drought stress conditions reported that hybrid KSC704 was tolerant, while KSC500 was sensitive to drought. Khayatnezhad et al. (2010) with study of four maize hybrids in drought stress conditions reported that hybrid golden west produced the highest radicle length, plumule length and seedling length.

Water stress do not only affects seed germination but also increases MGT in crop plants (Willanborb et al., 2004). Alebrahim et al. (2008) reported that with a decrease in the osmotic potential in PEG and NaCl solutions, the MGT in inbred lines MO17 and B73 increased.

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), enhancement of ATPase activity, RNA and acid phosphathase synthesis (Fu et al., 1988), also by increase of amylases, proteases or lipases activity (Ashraf and Foolad, 2005).

Kramer (1974) reported that the first effect measurable due to water deficit was the growth reduction caused by the decline 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).

Water stress due to drought is probably the most significant abiotic factor limiting plant and also crop growth and development (Hartmann et al., 2005). Drought stress is physiologically related, because induce osmotic stress and most of the metabolic responses of the affected plants are similar to some extent (Djibril et al., 2005). Water deficit affects the germination of seed and the growth of seedlings negatively (Van Den Berg and Zeng, 2006).

Emergence rate is an important criteria in breeding for high yield. The case of under drought conditions is special because the seedlings with high emergence rate will have an edge in competition for space, light and water resources, and will eventually have the highest yield as compared to others.

Drought reduced emergence index in most of the populations, and those showing high emergence rate with low susceptibility might be helpful in evolving better performing maize cultivars under drought conditions. This agreed with the results of Khayatnezhad et al. (2010), Khodarahmpour (2011) and Mostafavi et al. (2011). According to Ayaz et al. (2001), decrease of seed germination under stress conditions is due to the occurrence of some metabolic disorders. It seems that, decrease of germination percentage and germination rate is related to reduction in water absorption into the seeds at imbibitions and seed turgescence stages (Hadas, 1977).

According to results of the present study, it suggested that more experiments were carried out on the lines and further investigation should be done on MO17 line. Results of the current study were in agreement with other experiments in maize including that of Khayatnezhad et al. (2010), Mostafavi et al. (2011) and Khodarahmpour (2011).

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