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Full Length Research Paper

Effect of drought stress induced by polyethylene glycol (PEG) on germination indices in corn (*Zea mays* L.) hybrids

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Drought stress is one of the most important environmental factors in reduction of growth, development and production of plants. Germination is one of the main growth stages and success in this stage is dependent on moisture content of soil at time of planting. This study examined germination characteristics of seven corn hybrids under five levels of osmotic potential (0, -3, -6, -9 and -12 bar) by polyethylene glycol (PEG) 6000 using a factorial experiment based on a completely randomized design with three replications. Results indicated that water potential significantly reduced germination percent (71.2%), germination rate (24.2%), root length (60%), shoot length (89.8%), seedling length (71.2%) and seed vigour (91.7%). The mean germination time and root/shoot length ratio increased with decrease in the osmotic potential of PEG solution. Hybrid K3651/1×K166B produced the highest germination percent, germination rate, root length, seedling length and seed vigour, hence this hybrid was the most tolerant hybrid to drought stress.

Key words: Corn, drought stress, early growth, germination, PEG.

INTRODUCTION

Corn is an important cereal crop grown all over the world (Farhad et al., 2009). Also, it is a stable food and commercial crop (Ti-da et al., 2006). On the other hand, 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). 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 and rate of germination and seedling growth (Delachiave and De Pinho, 2003). Water stress not only affects seed germination but also increases mean germination time in crop plants (Willenborb et al., 2004). The adverse effect of water shortage on germination and seedling growth has been well reported in different crops such as corn (Mohammadkhani and Heidari, 2008; Farsiani and Ghobadi, 2009; Khayatnezhad et al., 2010; Mostafavi et al., 2011), wheat (Dhanda, 2004; Jajarmi, 2009), sorghum (Gill et al., 2002), sunflower (Mohammed et al., 2002). Mostafavi et al. (2011) in a study of four corn hybrids in drought stress condition reported that hybrid KSC704 was tolerant, while KSC500 was sensitive to drought. Khayatnezhad et al. (2010) and Mostafavi et al. (2011) in a study of four corn hybrids in drought stress conditions also reported that hybrid golden west and KSC704 had the highest root, shoot and seedling length, respectively.

Solutions of high molecular weight, polyethylene glycol, are often used to control water potential in seed germination studies (Hardegree and Emmerich, 1990). The polyethylene glycol (PEG)-induced inhibition of germination has been attributed to osmotic stress (Dodd and Donovan, 1999; Sidari et al., 2008). The aim of this study was to investigate the effects of osmotic stress generated by PEG on germination characteristics and seedling growth of corn hybrids. The primary objective of the present study was to compare seven maize hybrids to drought stress.

MATERIALS AND METHODS

Effect of water stress was induced by different osmotic potential levels [(distilled water) 0, -3, -6, -9 and -12 bar] PEG 6000 treatments on germination and early seedling development of corn were studied. Seven hybrids of combination selected inbred lines (K18, K19, K166B, K3651/1, K47/2-2-21-2-1-1-1, A679 and K3640/5) of corn were used. This investigation was performed as factorial experiment under completely randomized design (CRD) with three replications at Seed Laboratory, Islamic Azad University, Shoushtar Branch in Iran in 2011. In each level of stress, twenty seeds of any 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 2 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 PEG soluble was performed. Seeds were considered germinated when the emergent radicle reached 2 mm length. After 7 days, germination percent was measured by International Seed Testing Association (ISTA) standard method. At the end of the seventh day, the germination percent (Formula 1), mean germination time (MGT) (Ellis and Robert, 1981), germination rate (Formula 2), root, shoot, seedling, root/shoot length ratio and seed vigour (Formula 3) were also measured.

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

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

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

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

Formula 3: Seed vigour = Germination percent × Seedling length

Statistical analysis

For statistical analysis, the data of germinating percent 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 was also carried out.

RESULTS AND DISCUSSION

Analysis of variance showed that there were significant differences between drought stress levels. The results of this study revealed that various concentrations of PEG had a significant effect on all the measured traits. For hybrids, there were significant differences for all traits except germination rate and shoot length. Also analysis of variance showed that interaction effects were significant for all investigated traits (Table 1).

Germination percent and rate

According to results of mean comparison, hybrid K3651/1 × K166B (66.3%) had the highest germination percent and there was no significant difference with hybrid K18 × K166B (62.75%). But hybrids A679 × K19 (29%) and A679 × K3640/5 (31.2%) had the lowest germination percent (Table 2). Germination percent of all hybrids was adversely affected due to the application of different levels (0, -3, -6, -9 and -12 bar) of PEG. It was observed that in all of hybrids there was a decrease in germination percent due to drought stress increment and maximum germination percent was delayed. In this experiment, different hybrids had different response to the drought stress. Mean germination percent for all hybrids was 73% for control (0 bar), but 23.1 and 21% was at osmotic potentials of -9 and -12 bar, respectively (Figure 1A). At the highest PEG level (-12 bar), hybrid K18 × K166B produced maximum germination percent. Also with attention to results of Table 3, between hybrids there were significant differences for germination percent in all drought levels (Table 3) in agreement to the results of Mostafavi et al. (2011) on corn under drought stress conditions.

Hybrid K3651/1 × K166B had the highest germination rate. Results of means comparison showed that germination percent and rate were decreased by 71.2 and 24.2%, respectively by decrease in osmotic potential. While the maximum germination rate and percent were obtained at 0 bar level (control treatment) (Figures 1A, C). There were significant differences between hybrids for germination rate in drought levels of -9 and -12 bar (Table 3). Emergence rate is important criteria in breeding for high yield and special is the case under drought conditions because the seedlings with high emergence rate will have edge in competition for space, light and water resources, and eventually will have highest yield 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 corn cultivars under drought conditions. This agreed with the results of Khayatnezhad et al. (2010) and Mostafavi et al. (2011) in corn, Jajarmi (2009) in wheat and Mostafavi (2011) in safflower. 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 rate is related to reduction in water absorption into the seeds at imbibitions and seed turgescence stages (Hadas, 1977).

Table 1. Analysis of variance for measured traits in corn hybrids under control and different levels of osmotic potential (PEG-6000).

Source of variance	Df	Germination percent	Mean germination time (Day)	Germination rate (Number in day)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root/shoot length ratio	Seed vigour
Drought levels	4	3700.5**	3.26**	0.02**	29.37**	25.68**	99.275**	186.27**	484725.66**
Hybrid	6	524.07**	0.935*	0.004^{ns}	5.725**	0.22^{ns}	8.26**	32.635**	57753.22**
Drought levels× Hybrid	24	166.245**	0.91*	0.004*	4.56**	1.26**	7.67**	20.43**	27807.08**
Error	70	43.415	0.41	0.002	1.797	0.37	2.59	6.49	7803.45

ns, * and ** represent non significant, significant at 5% and 1% probability levels, respectively; Df represents degree of freedom.

Table 2. Mean comparison of corn hybrids in drought stress levels.

Treatment	Germination percent	Mean germination time (Day)	Germination rate (Number in day)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root/shoot length ratio	Seed vigour
K3651/1 × K166B	66.3^{a}	3.2^{b}	0.32^{a}	4.7^{a}	1.8^{a}	6.5^{a}	4.4^{b}	430.95^{a}
K18 × K166B	62.75^{ab}	3.7^{ab}	0.28^{ab}	4.1^{ab}	1.4^{a}	5.7^{ab}	4.0^{b}	380.5^{b}
K166B × K47/2-2-21-2-1-1-1	40.1^{c}	4.1^{a}	0.25^{b}	3.6^{ab}	1.5^{a}	5.1^{ab}	5.1^{b}	227.85^{c}
K166B × K19	38.5^{c}	3.85^{a}	0.28^{ab}	3.1^{b}	1.4^{a}	4.3^{b}	5.1^{b}	191.2^{c}
K18 × K19	34.2^{cd}	3.7^{ab}	0.28^{ab}	3.1^{b}	1.6^{a}	4.7^{b}	2.9^{b}	184.3^{cd}
A679 × K3640/5	31.2^{d}	3.6^{ab}	0.28^{ab}	3.9^{ab}	1.6^{a}	5.5^{ab}	8.0^{a}	226.6^{c}
A679 × K19	29.0^{d}	3.7^{ab}	0.28^{ab}	3.7^{ab}	1.5^{a}	5.3^{ab}	3.6^{b}	162.5^{c}

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

Mean germination time

Among the corn hybrids, K166B × K47/2-2-21-2-1-1-1 (4.1 days) and K166B × K19 (3.85 days) had the highest mean germination time and were no significant differences with other hybrids except K3651/1 × K166B (3.2 days) (Table 2), while hybrid K3651/1 × K166B produced the highest germination percent. The mean germination time increased with increase in the concentration of PEG solution (Figure 1B). Also with attention to results of Table 3 between hybrids, there were significant differences for mean germination time except control treatment (Table 3). In PEG treatments, the mean germination time was delayed by stress conditions. Water stress not only affects seed germination but also increases mean germination time in crop plants (Willenborb et al., 2004). Mohammed et al. (2002) reported that by NaCl levels of germination percent decreased and mean germination time increased proportionately. Alebrahim et al. (2008) also 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. Mostafavi (2011) in a study of 6 genotypes of safflower reported that the mean germination time increased with a decrease in the osmotic potential in NaCl solution.

Root, shoot, seedling length and root/shoot length ratio

The root length provides an important clue to the response of plants to drought stress. A special reduction in the root, shoot and seedling length of all hybrids of corn was observed because of drought stress. Among the corn hybrids, hybrid K3651/1 × K166B had the highest root and seedling length. Also, there were no significant differences for shoot length among hybrids

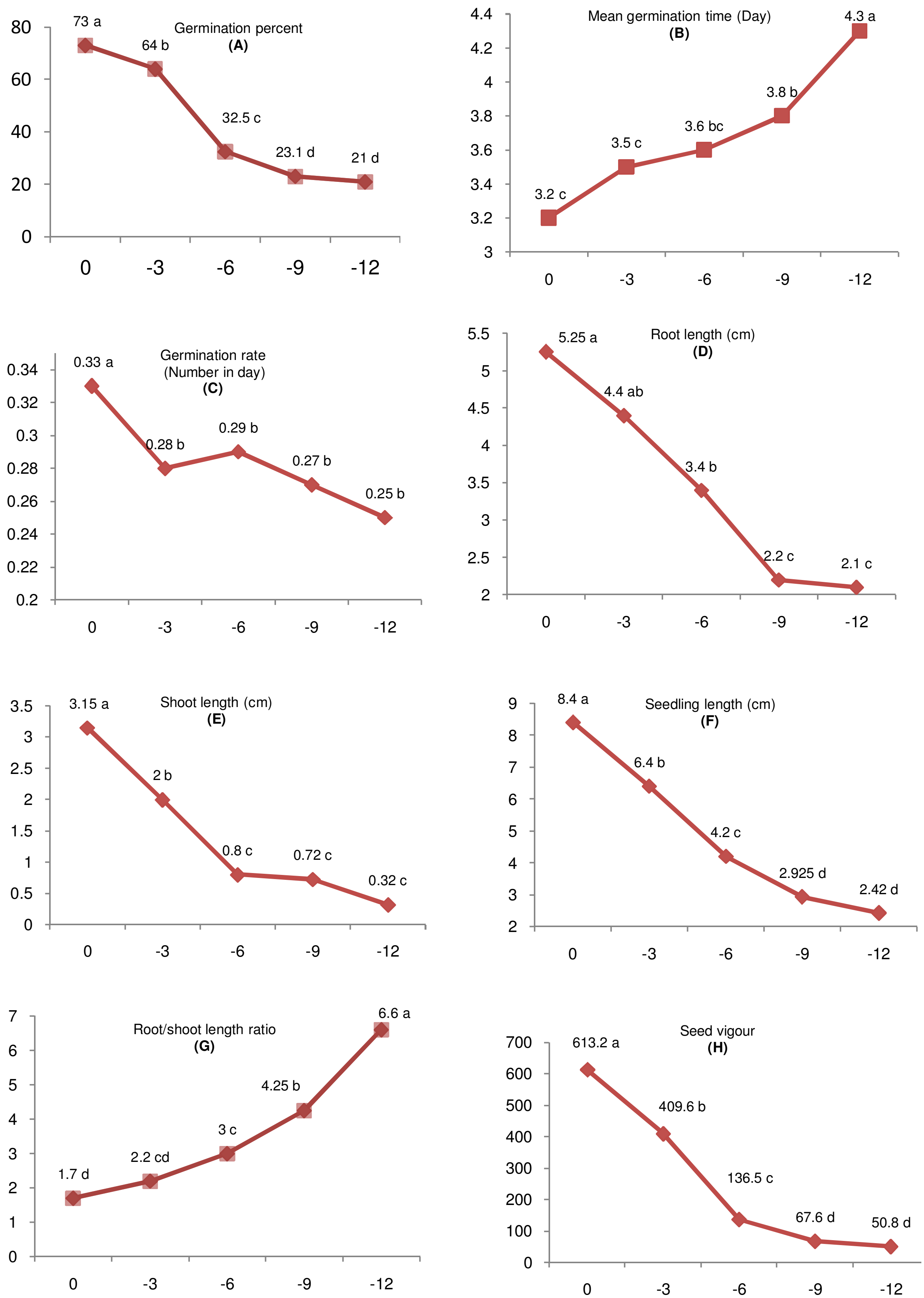

Figure 1. Studied traits of corn hybrids under control and different levels of osmotic potential (PEG-6000).

Table 3. Supplementary analysis of interaction effects between osmotic potential and corn hybrids.

Drought level (bar)	Germination percent	Mean germination time (Day)	Germination rate (Number in day)	Root length (cm)	Shoot length (cm)	Seedling length (cm)	Root/shoot length ratio	Seed vigour
0	410.8**	0.54^{ns}	0.004^{ns}	0.83^{ns}	1.26^{ns}	1.27^{ns}	0.36^{ns}	32908.8**
-3	374.3**	0.37*	0.002^{n}s	12.69*	2.15^{ns}	20.76*	11.01^{ns}	99906.6**
-6	166.06*	0.375*	0.002^{ns}	2.54*	0.54^{n}s	4.72*	7.94ns	5059.19*
-9	103.2*	2.45*	0.008**	5.56**	0.21*	7.76**	74.38**	9017.08**
-12	282.55**	6.83**	0.04**	2.49**	0.46*	4.9**	19.63**	3087.41**

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

(Table 2). Khayatnezhad et al. (2010) and Mostafavi et al. (2011) with study of four corn hybrids in drought stress conditions reported that hybrid golden west and KSC704 produced the highest root, shoot and seedling length, respectively. Results of this study showed that root length (60%), shoot length (89.8%) and seedling length (71.2%) decreased with increasing drought levels in all hybrids. The most effective levels in reducing root and seedling length were -9 and -12 bar of PEG (Figures 1D, F) and there were no significant differences between -6, -9 and -12 for shoot length (Figure 1E). Best level of PEG concentration in root, shoot and seedling length was control treatment (Figures 1D to F). There were significant differences between hybrids for root and seedling length in all drought levels except control treatment but there were significant differences between hybrids for shoot length in drought levels of -9 and -12 bar (Table 3). Furthermore, the hybrid A679 × K3640/5 had the highest root/shoot length ratio and between other hybrids, there were no significant difference. The root/ shoot length ratio increased with decrease in the osmotic potential of PEG solution (Figure 1G). There were significant differences between hybrids for root/shoot length ratio in drought levels of -9 and -12 bar (Table 3).

Water stress acts by decreasing the percentage and rate of germination and seedling growth as reported by Delachiave and De Pinho (2003) in senna, Farsiani and Ghobadi (2009) and Khayatnezhad et al. (2010) in corn, Gholamin and Khayatnezhad (2010) in wheat, and Mostafavi (2011) in safflower. There were reports in the literature of potential drought resistance traits like extensive viable rooting system that could explore deeper soil layers for water (Mirza, 1956; Bocev, 1963). Corn plants with more roots at seedling stage subsequently developed stronger root system, produced more green matter and had higher values for most characters determining seed yield (Bocev, 1963).

Seed vigour

Among the hybrids, hybrid K3651/1 × K166B was affected the least by drought stress because it gave the lowest reduction rate for seed vigour (Table 2). Seed vigour decreased with increase in concentration of PEG solution to 91.7% (Figure 1H). There were significant differences among hybrids for seed vigour in all drought levels (Table 3). Mostafavi et al. (2011) in a study of four corn hybrids in drought stress conditions reported that hybrid KSC704 produced the highest seed vigour and there were significant differences among hybrids for seed vigour in all drought levels except for -16 bar.

Conclusion

Drought affected the morphological behavior of corn hybrids. From present results, it can be concluded that from our experiment that germination of corn hybrids was reduced by only 23.1% under an osmotic potential of -9 bar. The variation among hybrids showed that germination percentage decreased with an increase in PEG-6000 concentration in all the hybrids. Hybrid K3651/1 × K166B produced the highest germination percentage, germination rate, root length, seedling length and seed vigour, hence hybrid K3651/1 × K166B performed better than others. Many reports indicated that germination percentage and seed vigour can be utilized as screening criteria for stress tolerance. In the present study the findings are very similar to the previous case in which germination decreased due to the increase in PEG-6000 concentration. This study strongly supports the assertion that germination indices can be utilized to screen corn hybrids for drought tolerance at germination and early seedling growth stage. There are many reports that are in agreement with the present findings indicating that drought stress severely reduces seed germination and early seedling growth. But the varieties having genetic potential to maintain the higher growth under stress conditions are drought tolerant.

Water stress due to drought is probably the most significant abiotic factor limiting plant and also crop growth and development (Hartman et al., 2005). Growth of plants in arid and semi-arid land is dependent upon plants susceptibility to drought stress and also related to the ability of seeds to achieve optimum germination under these unfavorable conditions. Therefore, it is necessary to identify hybrids tolerance to drought at the primary growth stage. Taking all traits into account, this study found that K3651/1 × K166B was the most tolerant hybrid to water stress conditions.

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