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Research Note

Effects of drought stress on germination indices of corn hybrids (*Zea mays* L.)

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Abstract:
Water deficit is one of the most wide spread constraint to crop productivity in the world and it is of prime necessity to try to find suitable genotype resistant to drought stress conditions.. In order to study the effect of drought stress on germination and early seedling growth of corn, an experiment was designed with four hybrids of corn (KSC704, KSC475, KSC500 and Consor) by using five concentrations of PEG 6000 (0, -4, -8, -12 and -16 bar). The experiment was laid out in completely randomized design with four replications in Seed Technology Laboratory of College of Abuoraihan, University of Tehran. Results of analysis of variance showed significant differences among hybrids, stress levels and its interaction for most investigated traits, which demonstrates high diversity among hybrids that enabled us to screen drought tolerant cultivar. To find the best tolerant hybrid to drought stress conditions, taking all traits into account in this study, we found that KSC704 is the most resistant and KSC500 is the most sensitive hybrid.

Keywords: Polyethylene glycol (PEG), seed vigor, germination stage, early seedling growth

Among various environmental stresses, drought stress has become a critical problem worldwide due to its dramatic effects on plant physiology and performance (Janmohammadi *et al.,* 2008). 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 a dry region of the world (Jajarmi 2009).

Water deficit, extreme temperatures and low atmospheric humidity lead to drought and are limiting factors for better plant performance and higher crop yield (Hirt and Shinozaki, 2003 and Szilgyi, 2003). Therefore, plants that use water more efficiently can produce higher yields in water limited environments (Dodd and Donovan, 1999 and Oomah *et al.,* 2006). However, depending upon plant species, certain stages such as germination, seedling or flowering could be the most critical stages for water stress (Ahmadi *et al.,* 2009). Germination is a critical stage of the plant life and resistance against drought during the germination makes a plant stable (Jajarmi 2009). The adverse effects of water shortage on germination and seedling growth have been well reported in different crops such as wheat (Dhanda *et al.,* 2004), sorghum (Gill *et al.,* 2002) and sunflower (Mohammad *et al.,* 2002). Water availability of the soil is considered one of the principal causes of low germination in seeds (Mian and Nafziger 1994 and Sebei *et al.,* 2007). Water stress acts by decreasing the percentage and rate of germination (Delachiave and Pinho, 2003). The study of the influence of the drought using osmotic solutions is one of the methods in the study of resistance during the germinal phase (Radhouane 2007). Polyethylene Glycol (PEG) solution is commonly used to control water potential in seed germination studies.

Corn (*Zea mays* L.) is a main food and economical crop throughout the world and, it is urgent to increase maize yields even under the unfavorable conditions (Tida *et al.,* 2006). Maize grain is extensively used for the preparation of corn starch, corn syrup, corn oil dextrose, corn flakes, gluten, grain cake, lactic acid and acetone which are used by various industries such as textile, foundry, fermentation and food industries (Hussain 2009). Global demand for maize will increase from 526 million tons to 784 million tons from 1993 to 2020, with most of the increased demand coming from developing countries (Rosegrant *et al.,* 1999). Maize requires 500-800 mm of water during life cycle of 80 to 110 days (Critchley and Klaus, 1991). Under such environments evolution of high yielding, drought tolerant maize varieties are reliable option to cope with the menace of water shortage. Physiological parameters may be used as selection criteria that must be simple and rapidly measurable, heritable, responsive to selection and related to crop growth and yield to attain the desired result (Richads 1978). Farsiani and Ghobadi (2009) showed that all considered traits except length of radicle and plumule in sweet corn SC403 had significant differences compared to flint corn SC704. These authors found that sweet corn SC403 in drought and salt stress conditions had more resistant than flint corn SC704 and had more yield potential.

The principal aim of present study was to compare the effects of induced drought stress on germination and early seedling growth stage of four hybrids of corn.

In order to study the effects of water stress on germination indices in corn hybrids, an experiment was conducted in factorial form, using a completely randomized design with four replications. In this experiment, four corn genotypes (KSC704, KSC475, KSC500 and Consor) were evaluated in five levels of drought treatment (distilled water as control,-4, -8, -12 and -16 bars). This experiment was carried out in Seed Technology laboratory of College of Abuoraihan, University of Tehran. PEG 6000 was applied to induce stress and it was prepared by dissolving the required amount of PEG in a beaker with distilled water placed on a shaker bed (at 25°C) for 16 h.

The seeds were sterilized by soaking in a 5 per cent solution of hypochlorite sodium for 5 min. Twenty seeds were washed with distilled water and were put in Petri dishes (with 9cm diameter) with moistened filter paper in 4 replications. The Petri dishes were covered to prevent the loss of moisture by evaporation. The Petri dishes were put into an incubator for 12 days at 25°C temperature. Every 24 hours after soaking, germination percentage and other traits were recorded daily. After 12 days of incubation, shoot length, root length, seedling length, shoot fresh weight, root fresh weight, seedling fresh weight, seed vigor, root to shoot fresh weight and root to shoot ratio of germinated seeds were measured. Seeds were considered germinated when the emergent radical reached 2 mm length. Rate of germination and coefficient of velocity of germination were calculated using the following formulae:

$$GP = S_{NG}/S_{N0} \times 100\%$$

Where GP is germination percentage, S_{NG} is the number of germinated seeds, and S_{N0} is the number of experimental seeds with viability (Close and Wilson, 2002 and Danthu *et al.,* 2003).

$$GR=\sum N/\sum (n\times g)$$

Where GR: Germination rate; n: number of germinated seed on g^{th} day and g: Number of total germinated seeds

Seed Vigor = [seedling length (cm) × germination percentage]

The data were subjected to analysis of variance (ANOVA) using SAS release 9.1 software package. Means were compared using LSD at 5% probability level. Cluster analysis was performed by using the STATISTICA software.

Analysis of variance showed that, there were significant differences between hybrids, drought stress levels and its interaction. The results of this study reveal that various concentrations of PEG had a significant effect on all the measured traits (Table 1). The differences between the means (hybrids and drought stress levels) were compared by LSD and are shown in Table 2. It was observed that, in all the hybrids there was a reduction in germination percentage due to drought stress increment and germination was delayed. Under conditions of highest drought stress that is -16 bar, all hybrids had 40 per cent germination after 12 days. Drought stress can contribute to improved germination rate and seedling emergence in different plant species by increasing the expression of aquaporins (Gao *et al.,* 1999), ATPase activity enhancement, RNA and phosphatase acid synthesis (Fu *et al.,* 1988) and also by increasing proteases or lipases activity analysis (Ashraf and Foolad, 2005). According to Ayaz *et al.* (2000), seed germination reduction under stress conditions is due to 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 imbibition and seed turgescence stages (Hadas 1977). Root length is one of the most important characters for drought stress because roots are in contact with soil and absorb water from soil. For this reason, root length provides an important clue to the response of plants to drought stress. Among the hybrids, the maximum root length, shoot length, seedling length and seed vigor were detected in KSC704 with 7.24, 3.02, 10.26 and 953.07 cm respectively, while the minimum was observed in KSC500.

In general, shoot length diminished with increasing drought levels in all hybrids (Table 2). The highest and the lowest seedling fresh weight were observed in KSC704 and KSC500 hybrids, respectively (Table 2). Among the hybrids, KSC704 was affected the least by drought stress because it gave the lowest reduction rate for seed vigor. Although the hybrids showed different responses to drought levels, the highest value in control was observed in KSC704 hybrid. In addition, it was clearly determined that there were no statistical differences between measured hybrids at high drought levels for germination rate and root to shoot fresh weight ratio (Table 2).

It seems that, PEG concentration (drought stress) affects seed germination via limitation of water absorption by seeds (Dodd and Donovan, 1999), excessive use of nutrient pool (Bouaziz and Hicks, 1990) and creation of disorders in protein synthesis. PEG concentrations resulted in a decline of both shoot length and root length. In all drought levels, KSC704 gave the highest root to shoot ratio while the lowest values were observed in KSC500. Generally, all measured traits declined with increasing drought levels, which showed that a greater reduction in root length occurred than that in shoot length. On the other hand, it means that the roots were more adversely affected than shoots by PEG concentration. Cluster analysis was done using the data for all measured traits in discriminating the genotypes. Results of cluster analysis (Ward's minimum variance method) showed that hybrid KSC704 was found to be tolerant, while KSC500 sensitive to drought (Fig. 1). 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. Polyethylene glycol (PEG) causes osmotic stress and could be used as a drought simulator (Turhan 1997). In the present experiment PEG-6000 was used to create the osmotic stress, as Hu and Junes (2004) utilized it for the development of water deficit environment in laboratory studies. The variation among hybrids showed that germination percentage decreased with the increase in PEG-6000 concentration in all the hybrids. However KSC704 performed better than others. Many reports indicated that germination percentage and seed vigor can be utilized as screening criteria for stress tolerance. In the present study, the findings are very similar to the (Hu and Junes, 2004 and Turhan 1997) in which germination decreased due to the increase in PEG-6000 concentration. Present study strongly supports that germination indices can be utilized to screen corn hybrids for drought tolerance at germination and early seedling growth stage.

Osmotic solutions are used to impose water stress reproducibly under in vitro conditions (Pandey and Agarwal, 1998). Polyethylene glycol molecules with a Mr _ 6000 (PEG 6000) are inert, non ionic and virtually impermeable chains that have frequently been used to induce water stress and maintain a uniform water potential throughout the experimental period (Lu and Neumann, 1998).

Molecules of PEG 6000 are small enough to influence the osmotic potential, but large enough to not be absorbed by plants (Saint-Clair 1980). Because PEG does not enter the apoplast, water is withdrawn from the cell. Therefore, PEG solution mimic dry soil more closely than solutions of low Mr osmotica, which infiltrate the cell wall with solutes (Veslues *et al.,* 1998). Our findings were in line with Ghorbanpour *et al* (2011), Jajarmi (2009), Demir and Aril (2003) and Mauromicale and Licandro (2002) who indicated that drought stress severely reduces seed germination and early seedling growth. But the hybrids having genetic potential to maintain the higher growth under stress conditions are drought tolerant. On the basis of the results of this study, corn hybrids can be classified into three groups, KSC704 and Consor had the best performing under drought conditions, KSC475 and KSC500 the hybrids having medium tolerance to water stress.

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Table 1- Analysis of variance of traits of corn hybrids under drought stress treatment.

Source	df	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Seedling Fresh Weight (g)
Hybrids	3	22.25**	153.80**	288.72**	0.018**	0.018**	0.070**
Stress	4	25.62**	120.74**	255.13**	0.017**	0.014**	0.063**
Interaction	12	2.25**	12.27**	23.95**	0.001**	0.001**	0.003**
error	60	0.37	1.14	2.37	0.0002	0.0001	0.0006

Source	df	Seed Vigor	Germination Percentage	Root to Shoot Length Ratio	Root to Shoot Weight Ratio	Germination Rate
Hybrids	3	2862099.20**	21756.32**	11.70**	1.09**	5.56**
Stress	4	1734829.43**	1707.78**	1.75**	0.12ns	6.17**
Interaction	12	195371.43**	232.22ns	0.49**	0.14**	6.82**
error	60	32162.77	162.22	0.18	0.05	0.98

*, **, ns: significant at 5%, 1% level and not significant, respectively

Table 2- Mean comparison of corn hybrids traits

Hybrids	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Seedling Fresh Weight (g)
SC704	3.02	7.24	10.26	0.08	0.09	0.17
SC475	2.88	5.05	7.92	0.07	0.05	0.12
K	2.69	5.48	8.17	0.06	0.05	0.12
SC500	0.77	0.71	1.48	0.02	0.01	0.03
LSD 5%	0.38	0.67	0.97	0.009	0.007	0.01
drought stress						
0	4.20	8.09	12.29	0.11	0.09	0.20
-4	2.92	6.51	9.43	0.07	0.07	0.14
-8	1.99	4.46	6.45	0.05	0.05	0.10
-12	1.68	2.76	4.44	0.04	0.03	0.07
-16	0.91	1.29	2.19	0.02	0.02	0.04
LSD 5%	0.43	0.75	1.08	0.01	0.008	0.01

Hybrids	Seed Vigor	Germination Percentage	Root to Shoot Length Ratio	Root to Shoot Weight Ratio	Germination Rate
SC704	953.07	89.33	2.4174	1.05	3.94
SC475	479.21	52.00	1.6304	0.74	4.68
K	619.11	68.00	1.9501	0.82	4.55
SC500	40.09	11.33	0.6126	0.48	3.56
LSD 5%	113.44	8.05	0.27	0.14	0.62
drought stress					
0	940.97	67.50	1.89	0.88	3.95
-4	746.03	60.83	1.92	0.83	3.99
-8	509.64	55.83	1.82	0.73	3.48
-12	285.39	51.67	1.49	0.66	5.15
-16	132.32	40.00	1.15	0.75	4.34
LSD 5%	126.83	9.00	0.30	0.16	0.70

Table 3- Interaction mean comparison of measured traits (LSD 5%)

Hybrids	Drought Level	Shoot Length	Root Length	Seedling Length	Shoot Fresh Weight	Root Fresh Weight	Seedling Fresh Weight
KSC475	-16	0.92	1.36	2.28	0.02	0.02	0.04
KSC475	-12	1.73	1.66	3.39	0.04	0.02	0.06
KSC475	-8	1.72	3.19	4.91	0.04	0.04	0.08
KSC475	-4	4.28	9.01	13.29	0.10	0.08	0.18
KSC475	0	5.72	10.03	15.76	0.14	0.09	0.23
KSC500	-16	0.24	0.13	0.38	0.00	0.00	0.01
KSC500	-12	0.67	0.38	1.04	0.01	0.00	0.01
KSC500	-8	0.17	0.19	0.36	0.01	0.00	0.01
KSC500	-4	0.78	0.63	1.41	0.01	0.01	0.02
KSC500	0	1.99	2.23	4.22	0.05	0.05	0.09
KSC704	-16	1.31	2.49	3.80	0.04	0.04	0.08
KSC704	-12	2.76	5.56	8.31	0.07	0.07	0.14
KSC704	-8	3.07	8.39	11.46	0.09	0.09	0.18
KSC704	-4	3.82	9.62	13.44	0.09	0.10	0.19
Concor	0	4.14	10.16	14.30	0.13	0.13	0.26
Concor	-16	1.16	1.17	2.32	0.02	0.02	0.03
Concor	-12	1.56	3.47	5.02	0.03	0.03	0.06
Concor	-8	2.99	6.08	9.07	0.07	0.06	0.13
Concor	-4	2.79	6.79	9.58	0.08	0.07	0.15
Concor	0	4.94	9.92	14.87	0.12	0.10	0.22

Hybrids	Drought Level	Seed Vigor	Germination Percentage	Root to Shoot Length Ratio	Root to Shoot Weight Ratio	Germination Rate
KSC475	-16	74.96	30.00	1.52	0.91	6.37
KSC475	-12	180.00	53.33	0.97	0.48	4.48
KSC475	-8	273.67	50.00	1.81	0.83	4.19
KSC475	-4	894.78	66.67	2.10	0.78	4.39
KSC475	0	972.67	60.00	1.76	0.69	3.99
KSC500	-16	3.78	3.33	0.27	0.34	1.52
KSC500	-12	17.00	13.33	0.48	0.39	7.28
KSC500	-8	3.56	3.33	0.57	0.20	1.85
KSC500	-4	14.11	6.67	0.61	0.48	3.03
KSC500	0	162.00	30.00	1.13	1.00	4.11
KSC704	-16	300.78	76.67	1.88	0.96	4.14
KSC704	-12	628.78	76.67	2.13	0.98	4.09
KSC704	-8	1061.33	93.33	2.86	1.09	3.87
KSC704	-4	1344.44	100.00	2.57	1.19	3.88
Concor	0	1430.00	100.00	2.65	1.01	3.72
Concor	-16	149.78	50.00	0.92	0.80	5.34
Concor	-12	315.78	63.33	2.37	0.80	4.75
Concor	-8	700.00	76.67	2.07	0.78	4.01
Concor	-4	730.78	70.00	2.39	0.87	4.67
Concor	0	1199.22	80.00	2.00	0.84	3.97

Table 4- Analysis of variance of interaction effects corn hybrids traits

Drought Level	df	Shoot Length (cm)	Root Length (cm)	Seedling Length (cm)	Shoot Fresh Weight (g)	Root Fresh Weight (g)	Seedling Fresh Weight (g)
-16	3	0.89ns	3.73*	7.87*	0.001**	0.0008**	0.004**
-12	3	2.94**	20.28**	37.28**	0.003**	0.0030**	0.011**
-8	3	7.40**	50.55**	95.21**	0.005**	0.0056**	0.021**
-4	3	9.69**	67.41**	127.10**	0.006**	0.0065**	0.024**
0	3	10.35**	60.93**	117.04**	0.007**	0.0051**	0.023**

Drought Level	df	Seed Vigor	Germination Percentage	Root to Shoot Length Ratio	Root to Shoot Weight Ratio	Germination Rate
-16	3	64661ns	3851.87**	1.99**	0.32**	17.53**
-12	3	269305**	2977.79**	3.30**	0.31**	8.37**
-8	3	869876**	6174.08**	3.61**	0.57**	4.78**
-4	3	1221551**	6114.80**	3.20**	0.35**	2.07ns
0	3	1218191**	3566.67**	1.57**	0.09ns	0.10ns

*, **, ns: significant at 5%, 1% level and not significant, respectively