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Water Stress Induced by Polyethylene Glycol 6000 and Sodium Chloride in Two Maize Cultivars

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Abstract: Responses of growth and germination to water stress induced by PEG 6000 and NaCl in two maize (*Zea mays* L.) cultivars 704 and 301 were studied. Water stress was generated by additions of PEG 6000 or sodium chloride to the root medium. Water potentials were: zero as control and -0.15, -0.49, -1.03 and -1.76 MPa as treatments. After 24 h treatment, the roots and shoots length and dry weight were of plants determined. In water stress, roots and shoots length and dry weight decreased at both treatments in both varieties. The germination is inversely proportional to the NaCl and PEG concentrations, it means that 704 and 301 cultivars of maize showed a reduction in germination with an increasing in NaCl or PEG concentrations induced water deficit, but this reduction in NaCl treatment were higher than PEG treatment. At treatment by PEG, the germination was severely decreased at -1.03 MPa. At treatment by NaCl no germination occurred at -1.03 in 301 var. and germination was very low at -1.03 MPa in 704 var., no germination occurred at -1.76 MPa in both varieties at both treatments. Decrease of germination in 704 variety was higher than 301 variety.

Key words: Germination, growth, polyethylene glycol (PEG) 6000, sodium chloride, water potential, *Zea mays* L.

INTRODUCTION

Drought can be simply defined as a period of below normal precipitation that limits plant productivity in a natural or agricultural system. In the field, drought can cause a number of plant stresses including temperature, light and nutrient stresses. However, the stress component that defines drought is a decrease in the availability of soil water. This decreased water availability can be quantified as a decrease in water potential (Kramer and Boyer, 1995). Decreased water potential (decreased free energy of the water) makes it more difficult for the plant to take up water and this in turn elicits a range of responses that allow the plant to avoid water loss, allow water uptake to continue at reduced water potential or allow the plant to tolerate a reduced tissue water content. The physiological mechanisms involved in cellular and whole plant responses to water stress therefore generate considerable interest and are frequently reviewed (Smith and Griffiths, 1993; Kramer and Boyer, 1995; Neumann, 1995, 1997; Turner, 1997).

Shoot growth slowed. Plant leaves grow by increasing cell numbers through division and then expanding these cells using water in much the same way as air is used to blow up a balloon. When water is limiting cell expansion is reduced.

Seed germination is usually the most critical stage in seedling establishment, determining successful crop production (Almansouri *et al.*, 2001). Crop establishment depends on an interaction between seedbed environment and seed quality (Khajeh-Hosseini *et al.*, 2003). Factors adversely affecting seed germination may include sensitivity to drought stress (Wilson *et al.*, 1985) and salt tolerance (Sadeghian and Yavari, 2004). Seeds sown in seedbeds having unfavorable moisture because of limited rainfall at sowing time yield in poor and unsynchronized seedling emergence (Mwale *et al.*, 2003), affecting the uniformity of plant density with negative effects on yield. Salinity may also affect the germination of seeds by creating an external osmotic potential that prevents water uptake or due to the toxic effects of sodium and chloride ions on the germinating seed (Khajeh-Hosseini *et al.*, 2003).

Water availability and movement into the seeds are very important to promote germination, initial root growing and shoot elongation (Bewley and Black, 1994). Only, highly negative water potential, especially in early germination, may influence the seeds water absorption, making germination not possible (Braccini *et al.*, 1996).

To allow germination, there is minimum moisture that the seed should get and it depends on its chemical composition and of the permeability of the tegument.

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With water absorption, tissues will be rehydrated and consequently starting intensification of breathing and of all the other metabolic activities, that culminate in the necessary supply of energy and nutrients to restart the embryonic axis growth (Carvalho and Nakagawa, 1988). In order for the process of germination to start, it is necessary for the seed to reach an adequate level of hydration, which will permit a reactivation of the metabolic processes (Cordoba *et al.*, 1995).

Induced water deficit by polyethylene glycol showed similar values to that observed in the fields (Thill *et al.*, 1979), permitting also vigour evaluation. In similar potential ranges, germination patterns may be different between species or even between varieties of the same species (Therios, 1982). Some species, as maize are sensitive to sodium chloride during germination.

Polyethylene glycol of high molecular weight range (6000 or above) can not enter the pores of plant cells (Oertli, 1985) and thus causes cytorrhysis rather than plasmolysis. Polyethylene glycol is also a better choice for imposing low water potential than the often used solute mannitol because mannitol has been shown to be taken up by plant cells and can cause specific toxic effects on growth (Hohl and Schopfer, 1991; Verslues *et al.*, 1998).

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 (Verslues and Bray, 2004; Verslues *et al.*, 1998; Van der Weele *et al.*, 2000).

In this study, the effect of induced water deficit, either by polyethylene glycol or by sodium chloride, was evaluated observing the germination of two maize cultivars. We wanted to know which of these materials had higher inhibitory effects in growth and germination of maize plants.

MATERIALS AND METHODS

Plant materials and growth conditions: This study was conducted at biochemistry laboratory, Department of Biology, Urmia University, Iran, during the spring of 2007. Two genotypes of maize (*Zea mays* L.) var. 704 and var. 301 were used. The seeds of both cultivars were germinated in Petri dishes on two layers of filter paper at 25°C in an incubator. After three days, the seedlings transferred to plastic pots (15 cm diameter, 20 cm depth) filled with sand and irrigated with half strength of Hoagland nutrient solution. Six days seedlings were transferred to hydroponics culture of aerated test tubes containing polyethylene glycol (PEG) 6000 or sodium chloride (NaCl) solutions with osmotic potential -0.15,

Table 1: Used amounts of sodium chloride and polyethylene glycol 6000 to obtain different levels of water deficit

Osmotic potential (Mpa)	NaCl (g L^{-1} of distilled water)	Polyethylene glycol 6000 (g L^{-1} of distilled water)
0	0.00	0
0.15	1.76	10
0.49	5.74	20
1.03	12.07	30
1.76	20.88	40

-0.49, -1.03 and -1.76 MPa, respectively (Table 1) (Burlyn and Mirrill, 1973; Steuter *et al.*, 1981; Nicholas, 1989) as treatments and aerated test tubes containing half strength Hoagland nutrient solution which served as control. Stress was applied for 24 h. Then, roots and shoots length and dry weight of both varieties were measured.

Roots and shoots length and dry weight measurements: The length of roots and shoots were measured and after that plant were dried at 105°C until reached constant weight for the determination of dry weight (Fletcher, 1988).

Germination test: Seeds were submitted to germination, using include osmotic potentials (0, -0.15, -0.49, -1.03, -1.76 MPa) of sodium chloride and polyethylene glycol 6000 (Braccini *et al.*, 1996). Germination test was conducted with four replications per treatment with 25 seeds each. Seeds were put in Petri dishes included three paper towels, moistened to 2.25 its weight with one of the solutions mentioned before (Krzyzanowski, 1991). Germination was evaluated at day 3 after cultivation. The number of seeds germinated was counted regularly and after final germination the germination percentage was estimated. Only normal seedlings were counted to determine germination percentage (Brasil, 1992).

Statistical analysis: Mean values were taken from measurements of four replicates and Standard Error of the means was calculated. Differences between means were determined by One-way ANOVA and Turkey's multiple range tests ($p < 0.05$). Analyses were done using the SPSS (version 13.0).

RESULTS AND DISCUSSION

Five water potential was used: 0, -0.15, -0.49, -1.03 and -1.76 MPa. Results of shoot and root length (Table 1, 2) were similarly affected by sodium chloride and polyethylene glycol induced water deficit. At zero potential, both shoot and root lengths reached their highest values. All other treatments gradually reduced the seedling growing. Kramer (1974) reported that the first

Table 2: Effects of osmotic potential induced by PEG 6000 and NaCl on roots length (mm) of two maize cultivars*

Osmotic potential (Mpa)	PEG 6000		NaCl	
	704 var.	301 var.	704 var.	301 var.
	(mm)			
0	92.8000±1.01003	105.8750±0.51539	94.4250±0.21747	102.6250±0.23936
0.15	87.4500±0.72169	98.0000±1.22474	88.5750±2.14801	96.3750±0.23936
0.49	81.6250±0.94373	90.0000±1.02062	75.5750±0.27801	86.0000±0.40825
1.03	77.6500±0.54237	83.1250±1.19678	64.5000±0.28868	71.5000±0.28868
1.76	68.1000±1.28906	74.8750±0.96555	55.5000±0.28868	61.0000±0.40825

*: Results are shown as mean±standard error (p<0.05), obtained from four replicates

Table 3: Effects of osmotic potential induced by PEG 6000 and NaCl on shoots length (mm) of two maize cultivars*

Osmotic potential (Mpa)	PEG 6000		NaCl	
	704 var.	301 var.	704 var.	301 var.
	(mm)			
0	82.7500±1.85585	113.1250±1.19678	85.6250±0.23936	113.0000±0.40825
0.15	67.1250±0.96036	92.5000±1.44338	68.7500±5.42179	88.0000±0.40825
0.49	62.2500±0.77728	80.5000±0.67700	58.0000±0.40825	75.2750±0.11087
1.03	58.5500±0.49413	66.8750±1.19678	44.7500±0.25000	59.0000±0.40825
1.76	54.2250±0.70873	62.5000±1.02062	40.7500±0.32275	52.5000±0.28868

*: Results are shown as mean±standard error (p<0.05), obtained from four replicates

Table 4: Effects of osmotic potential induced by PEG 6000 and NaCl on dry weight of roots (g/10 seedlings) of two maize cultivars*

Osmotic potential (Mpa)	PEG 6000		NaCl	
	704 var.	301 var.	704 var.	301 var.
	(g/10 seedlings)			
0	0.2150±0.00289	0.2650±0.00645	0.2000±0.00000	0.2475±0.00250
0.15	0.1450±0.00289	0.1950±0.00645	0.1300±0.00000	0.1825±0.00250
0.49	0.1175±0.00250	0.1600±0.00913	0.1050±0.00289	0.1425±0.00479
1.03	0.0950±0.00289	0.1325±0.00479	0.0825±0.00250	0.1125±0.00250
1.76	0.0750±0.00289	0.1125±0.00479	0.0450±0.00289	0.0900±0.00408

*: Results are shown as mean±standard error (p<0.05), obtained from four replicates

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).

Dry weight of shoot and root were affected by water deficit (Table 3, 4), but the roots affected higher than shoots and sodium chloride has higher effect than PEG 6000. Water deficit at -0.15 and -0.49 MPa showed higher root dry weight, at -1.03 and -1.76 MPa there were a significant reduction in root dry weight for both cultivars at both treatments. According to Marur *et al.* (1994), water restriction acted slowing physiological and biochemical processes and seedlings at low water deficit showed a weak growing leading to a lower accumulation of dry matter.

Braccini *et al.* (1996) reported that soybean roots exposed to water deficit were well developed than the roots that grew without water deficit. But, in our experiment, roots length decreased with increasing PEG and NaCl concentrations. It seems that increase of root length is because of uptake water from water resource and

in our experiment we had drought stress in water solutions of NaCl or PEG (physiological drought), therefore, roots of both varieties decreased.

Results showed that the germination is inversely proportional to the NaCl and PEG concentrations, it means that 704 and 301 cultivars of maize showed a reduction in germination with an increasing in NaCl or PEG concentrations induced water deficit (Table 5, 6), but this reduction in NaCl treatment were higher than PEG treatment. At treatment by PEG, the germination was severely decreased at -1.03 MPa, no germination occurred at -1.76 MPa. At treatment by NaCl no germination occurred at -1.03 and -1.76 MPa in 301 var. and germination was very low at -1.03 MPa and no germination occurred at -1.76 MPa in 704 var. According Mayer and Poljakoff-Mayber (1989) results like this could be attributed to absence of energy to start the germination process, as energy was obtained by increments in the respiratory pathway after the imbibition and in low levels of water potential tax water absorption was processed slowly.

At control plants (*Zero potentia* L.), 301 var. presented a low germination than 704 var. In water stress, germination decreased at both treatments in both

Table 5: Effects of osmotic potential induced by PEG 6000 and NaCl on dry weight of shoots (g/10 seedlings) of two maize cultivars*

	PEG 6000		NaCl	
	704 var.	301 var.	704 var.	301 var.
Osmotic potential (Mpa)		(g/10 seedlings)		
0	0.2100±0.00000	0.2700±0.00913	0.2250±0.00289	0.2575±0.00250
0.15	0.1650±0.00289	0.2325±0.00854	0.1775±0.01436	0.2050±0.00289
0.49	0.1425±0.00629	0.1850±0.00645	0.1150±0.00289	0.1550±0.00289
1.03	0.1250±0.00289	0.1625±0.00629	0.1100±0.00408	0.1350±0.00289
1.76	0.1025±0.00250	0.1300±0.00408	0.0875±0.00250	0.1050±0.00289

*: Results are shown as mean±standard error (p<0.05), obtained from four replicates

Table 6: Effects of osmotic potential induced by PEG 6000 and NaCl on seed germination (%) of two maize cultivars*

	PEG 6000		NaCl	
	704 var.	301 var.	704 var.	301 var.
Osmotic potential (Mpa)		(%)		
0	94.5000±1.04083	82.7500±1.10868	95.2500±0.47871	88.0000±0.40825
0.15	82.5000±0.95743	75.0000±1.08012	88.5000±2.53311	83.0000±0.40825
0.49	75.7500±0.85391	64.7500±1.18145	79.0000±0.40825	70.7500±0.47871
1.03	24.7500±1.25000	9.7500±0.85391	4.2500±0.25000	0.0000±0.00000
1.76	0.0000±0.00000	0.0000±0.00000	0.0000±0.00000	0.0000±0.00000

*: Results are shown as mean±standard error (p<0.05), obtained from four replicates

varieties. At treatment by PEG, in 704 var. germination were 94.5% in control plants (*Zero potentia* L.) and decreased to 24.75% in PEG 30% (-1.03 MPa). In 301 var. germination were 82.75% in control plants (*Zero potentia* L.) and decreased to 9.75% in PEG 30% (-1.03 MPa), but the germination decreased to zero in PEG 40% (-1.76 MPa) in both varieties. At treatment by NaCl, in 704 var. germination were 92.25% in control plants (*Zero potentia* L.) and decreased to 4.25% in -1.03 MPa. In 301 var. germination were 88% in control plants (*Zero potentia* L.) and decreased to 0% in -1.03 MPa, the germination decreased to zero in -1.76 MPa in both varieties.

The percentage results presented drastic decrease as the potential went from -0.49 to -1.03 MPa in PEG and NaCl solutions. Analysing the results of percentage in PEG and NaCl solutions, it was seen that in low water potential (under -0.5 MPa), the largest reduction found with PEG solutions compared to NaCl treatment, was also observed by Nassiff and Perez (1997) in seeds of *Pterogyne nitens.*

When the potential was sufficiently low, such as -0.5 MPa, the seeds could contain sufficient water to start the germination process (Phase I and II) without however, passing to root cell growth (Phase III). The process of elongation and the cellular wall synthesis are highly sensitive to water deficiency (Wenkert *et al.*, 1978) and reduction in growth could be due to the decrease of turgor of these cells.

Water deficit worked decreasing velocity and seed germination percentage and for each species there was water potential, that under it germination did not occur (Adegbuyi *et al.*, 1981). Germination patterns could be different between species and between different varieties in the same species (Therios, 1982).

These results could be attributed to the reduction of the osmotic potential. Van der Mozel and Bell (1987) related that NaCl could affect germination, as by the ionic effect, as by ion cell reaching toxic levels or for combination of both. At -1.76 MPa both cultivars failed to germinate. Santos *et al.* (1992) reported that soybean seed germination, with high vigour, was null, when germinated in solutions of NaCl at -1.5 MPa.

There was significance in the interactions between cultivars and NaCl induced water deficit. Shoot dry weight gradually decreased with water deficit increase. Similar results were obtained by Santos *et al.* (1992). There was significance in the interaction between cultivars and NaCl induced water deficit, which led to reduction in shoot and root length, especially at -1.03 and -1.76 MPa. Both cultivars showed very good germination at osmotic potential zero, they were more sensible than PEG to salt imposed water deficit. However, the results obtained with NaCl did not match with the one observed for polyethylene glycol and NaCl has higher effect than PEG, meaning that there was another kind of factor acting in this case. It suggesting that NaCl and PEG acted through different mechanisms.

Sodium chloride can be a strong osmotic agent, but it affects the development just by increasing the sodium concentration in the growing medium.

Sodium is a small ion that can pass easily throughout cellular membranes and cells must pump it out expending energy to do that, otherwise the water activity decrease and all the metabolic pathways can be disturbed or disrupted, causing some misbalance in the energy production-consumption.

According Braga *et al.* (1999), potentials between -0.4 and -0.6 MPa declined all parameters (germination percentage, size and seedling weight), in common bean

plants and seed with low physiological quality showed higher decrease when submitted to lower water potentials. Germination, in strongly negative water potentials, especially at the beginning of the imbibition could influenciate water absorption by the seeds and this event could turn not viable the germination process (Bansal *et al.*, 1980).

Water deficit, induced by sodium chloride or polyethylene glycol, affected germination and seedling development. Germination was severely affected at -1.76 MPa. Parameters that evaluated seedling development were more affected by water deficit than germination. Beginning at -0.15 MPa seedling started shoot and root growth reduction. Dry weight shoot and root affected highly by water stress at both treatments.

For shoot length, cultivars showed the best result at no water deficit, decaying highly at -1.76 MPa, what was according to obtained data of Torres *et al.* (2000), where the increase in water deficit represented a reduction in seedlings.

We conclude that NaCl and PEG adversely affected the germination and seedling growth of maize. In low water stress PEG had a greater inhibitory effect in germination than did NaCl. Our results agree with those given by Murillo-Amador *et al.* (2002), who observed that NaCl had a lesser effect on the germination of cowpea than did PEG and Sadeghian and Yavari (2004), who stated that seedling growth was severely diminished by water stress in sugar beet.

Moreover, distinct genetic differences were found among the cultivars with respect to germination and seedling growth subjected to NaCl and PEG.

Root and shoot length and seedling fresh and dry weight were decreased by increasing NaCl and PEG concentrations. Consequently, seedling growth was inhibited in maize. Differences between NaCl and PEG were significant. Growth inhibition of NaCl was higher than that of PEG. However, our findings showed that NaCl had greater inhibitory effects on seedling growth than on germination. Higher germination in NaCl than in PEG in low water stress could be explained by more rapid water uptake in NaCl solutions and achievement of a moisture content that allowed germination. Khajeh-Hosseini *et al.* (2003) suggested that the achievement in NaCl solutions was due to rapid imbibition in soybean seeds.

Stress inhibition of germination could not be attributed to an inhibition of mobilisation of reserves and that the main effect of PEG occurred via an inhibition of water uptake while detrimental effects of NaCl may be linked to effects of accumulated toxic ions.

In conclusion, in the germination and early seedling growth stages the investigated cultivars showed different responses to water stress induced by PEG and NaCl. However, seedling growth was more sensitive to NaCl than was germination.

We suggest that effect of water stress induced by mannitol, sucrose or other materials on growth and germination study in maize cultivars and compare with our results.

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