Turk J Agric For
29 (2005) 237-242

Effects of Salt and Drought Stresses on Germination and Seedling Growth of Pea (*Pisum sativum* L.)

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Received: 20.07.2004

Abstract: The effects of salt and drought stresses at the water potentials of -2, -4, -6 and -8 bars induced by NaCl and PEG 6000 (polyethylene glycol 6000) each, on germination and early seedling growth, were investigated for 3 pea cultivars (Bolero, Sprinter and Utrillo). Electrical conductivity (EC) values of the NaCl solutions were 4.5, 8.8, 12.7 and 16.3 dS m^{-1}. Germination percentage, mean germination time, root and shoot length, and seedling fresh and dry weight were measured in the study. The objective was to determine genotypic differences among pea cultivars in terms of salt and drought stress and to determine factors (salt toxicity or osmotic stress due to PEG) inhibiting seed germination. The germination results revealed that the genotypes significantly differed for salt and drought stress. Bolero appeared to be more tolerant to salt stress, but Sprinter cv. gave higher values under drought stress. Both NaCl and PEG inhibited germination and seedling growth in all cultivars, but the effects of NaCl compared to PEG were less on germination and seedling growth. All cultivars were able to germinate at all NaCl levels without a significant decrease in germination, while a drastic decrease in germination was recorded at -6 bars of PEG. It was concluded that inhibition in germination at equivalent water potentials of NaCl and PEG was mainly due to an osmotic effect rather than salt toxicity.

Key Words: Pea (*Pisum sativum* L.), salt and drought stress, cultivar, germination, seedling growth

Tuz ve Kuraklık Stresinin Bezelye'nin (*Pisum sativum* L.) Çimlenme ve Fide Gelişimi Üzerine Etkileri

Özet: Sodyum klorür ve PEG 6000 (polyethylene glycole 6000) kullanılarak -2, -4, -6 ve -8 bar su tutma gücüne sahip solüsyonlarda tuz ve kuraklığın üç bezelye çeşidinin (Bolero, Sprinter ve Utrillo) çimlenme ve fide gelişimi üzerine etkileri incelemiştir. Sodyum klorür solüsyonlarının elektriksel iletkenlik (EC) değerleri 4.5, 8.8, 12.7 ve 16.3 dS m^{-1} olarak belirlenmiştir. Araştırmada, çimlenme yüzdesi, ortalama çimlenme zamanı, kök ve sürgün uzunluğu, fide yaş ve kuru ağırlıkları incelenmiştir. Araştırmada amaç, tuz ve kuraklık stresleri bakımından bezelye çeşitleri arasındaki farklılık ile tohumların tuzun toksik etkisi veya NaCl tarafından oluşturulan osmotik potansiyel nedeniyle çimlenip çimlenmediğini belirlemektir. Sonuçlar, hem tuz hem de kuraklık stresi bakımından çeşitler arasında farklılık olduğunu ortaya koymuştur. Sprinter kurak şartlarda daha iyi sonuçlar vermesine rağmen, Bolero tuz stresine daha toleranslı görünmüştür. Sodyum klorür ve PEG solüsyonları çeşitlerin fide gelişimini engellemiş ancak, NaCl' nin etkisi PEG' den daha az olmuştur. Üç çeşitte de tüm NaCl seviyelerinde çimlenmede önemli bir azalma olmazken, -6 bar PEG solüsyonunda çimlenme düşmüştür. Aynı su tutma gücüne sahip NaCl ve PEG solüsyonlarında çimlenmenin azalması tuzun toksik etkisinden çok oluşturduğu osmotik etkiden kaynaklandığı sonucuna varılabilir.

Anahtar Sözcükler: Bezelye (*Pisum sativum* L.), tuz ve kuraklık stresi, çeşit, çimlenme, fide gelişimi

Introduction

Pea (*Pisum sativum* L.), a cool season food legume, has a wide variety of uses and is grown in Turkey and other countries of the Mediterranean region as a cheap source of protein. It is commonly used in the form of dry pulses, fresh peas and edible podded type (Bozoğlu et al., 2004). It has been economically grown for the canning industry and traditionally for dry grain or fresh fruit (Alan, 1984; Kaya et al., 2002). It is particularly sensitive to drought stress (Wilson et al., 1985). Lal (1985)

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indicated that earlier growth stages were more sensitive to salinity than subsequent ones. Cerda et al. (1982) found useful variation in the salt tolerance of pea cultivars whose half maximal yield was between 6 and 10 dS m^{-1}.

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 (Brown et al., 1989; Khajeh-Hosseini et al., 2003). Factors adversely affecting seed germination may include sensitivity to drought stress (Wilson et al., 1985), and salt tolerance (Perry, 1984; Sadeghian and Yavari, 2004). Earlier growth stages are more sensitive to salinity than subsequent ones (Lal, 1985). The stand, subsequent growth and final yield of crop plants are decreased when the moisture supply is limited. Seeds sown in seedbeds having unfavorable moisture because of limited rainfall at sowing time yield in poor and unsynchronized seedling emergence (Ghoulam and Fares, 2001; Mwale et al., 2003), affecting the uniformity of plant density with negative effects on yield.

Salinity has also been identified as the major seedbed factor influencing establishment in arid and semi-arid regions (Almansouri et al., 2001). Germination and seedling growth are reduced in saline soils with varying responses for species and cultivars (Bliss et al., 1986; Hampson and Simpson, 1990). 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 Na^+ and Cl^- ions on the germinating seed (Redmann, 1974; Murrillo-Amador et al., 2002; Khajeh-Hosseini et al., 2003).

The relative importance of the osmotic or toxic effects of NaCl on seed germination of pea is not clear and this study was conducted to determine the effect of NaCl on seedling growth and germination, and to determine factors responsible for failure in seed germination under saline conditions comparing various levels of NaCl and PEG.

Materials and Methods

This study was carried out at the Department of Agronomy, Faculty of Agriculture, University of Ankara, Turkey. The pea cultivars Bolero, Spring and Utrillo, obtained from Seed and Seed Certification Institute of Turkey, were used as material, and were treated with fungicide before planting.

Drought stress was induced by polyethylene glycol (PEG 6000) treatment. Four drought stresses with different osmotic potentials of -2, -4, -6 and -8 bars were arranged as described by Michel and Kaufmann (1973). Salt concentrations that had the same osmotic potentials of -2, -4, -6 and -8 bars (electrical conductivities of the solutions were 4.5, 8.8, 12.7 and 16.3 dS m^{-1}, respectively) were adjusted using NaCl (Coons et al., 1990). Distilled water served as a control.

Three replicates of 20 seeds of each cultivar were germinated in 2 rolled Whatman filter papers with 10 ml of respective test solutions. The papers were replaced every 2 days to prevent accumulation of salts (Rehman et al., 1996). In order to prevent evaporation, each rolled paper was put into a sealed plastic bag. Seeds were allowed to germinate at 20 ± 1 °C in the dark for 10 days. To determine the toxic effects of the solutions on germination, non-germinated seeds in each treatment were transferred to distilled water and counted for an additional 3 days. A seed was considered to have germinated when the emerging radicle elongated to 1 mm. Germination percentage was recorded every 24 h for 10 days. Mean germination time (MGT) was calculated to assess the rate of germination (Ellis and Roberts, 1980) as follows:

$$MGT = (fx) / f$$

where f is the number of newly germinated seeds on each day and x is the day of counting. Root and shoot length, and seedling fresh and dry weights were measured on the 10th day. Dry weights were measured after drying samples at 70 °C for 48 h in an oven (Böhm, 1979).

The experimental design was 3 factorial, arranged in a completely randomized design with 3 replications and 20 seeds per replicate. The first factor was cultivars (Bolero, Sprinter and Utrillo), the second was solutions (NaCl and PEG) and the third was solution levels (0, -2, -4, -6 and -8 bars). Data given in percentages were subjected to arcsine transformation before statistical analysis. For all investigated parameters, analysis of variance was performed using the MSTAT-C software package. Significant differences among the mean values were compared by LSD test ($P < 0.05$).

Results

A significant 3 way interaction (cultivar, solution and stress) was found ($P < 0.05$, 60 df) for all investigated characters. Germination percentage was not significantly decreased by NaCl solutions while it declined considerably with decreasing water potential of PEG. The maximum decrease in PEG was determined in Utrillo (93.5%). The control (0 bars) showed differences among the cultivars for germination percentage. The lowest germination percentage (76.7%) was obtained from Utrillo (Table 1). Transfer of non-germinated seeds from PEG solution to the distilled water resulted in 100% germination regardless of osmotic potential (data not shown). It is evident that PEG was not toxic. Mean germination time was delayed by decreasing water potential. Drought affected it more adversely than did NaCl. Bolero was not affected significantly by NaCl (Table 2).

Increasing NaCl resulted in decrease in root length except for in Utrillo. Bolero gave the highest values at all water potentials of NaCl, while Sprinter had higher values in PEG (Table 3). No cultivars were able to grow roots at -8 bars of PEG. Sprinter was superior to the others at -6 bars of PEG and had a root length of 4.31 cm. NaCl had a stimulating effect on the root growth of Utrillo.

The highest shoot length was determined from Bolero in all NaCl concentrations. Shoot length was severely influenced by salt and drought stress but inhibition was greater in PEG (Table 4). No shoot length was recorded for Utrillo at -2 bars, Bolero at -4 bars, or Sprinter at -6 bars of PEG. Water stresses depressed the shoot growth of the cultivars rather than their root growth.

Decreasing water potential by NaCl and PEG caused a remarkable decrease in seedling fresh weight (Table 5). Differences determined among the cultivars were significant. Although the cultivars showed different responses to each NaCl and PEG concentration, the highest values were observed from Bolero in NaCl, and Sprinter in PEG. NaCl reduced the seedling fresh weight of Bolero and Sprinter; however, it increased seedling fresh weight of Utrillo.

Seedling dry weight showed a trend similar to that of fresh weight, and, depending on the decline in seedling fresh weight, dry weight decreased with increasing NaCl and PEG except for in Utrillo (Table 6). Bolero gave the highest values in all NaCl levels, but Sprinter produced a higher seedling dry weight in PEG. Within the cultivars, Sprinter appeared more tolerant to drought stress and Bolero showed superiority against salt stress.

Table 1. Changes in germination percentages of pea cultivars at different osmotic potentials of NaCl and PEG.

Germination Percentage (%)						
	Cultivars					
	Bolero		Sprinter		Utrillo	
Bars	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	100.0^{a}	100.0^{a}	100.0^{a}	100.0^{a}	76.7^{cd}	76.7^{cd}*
-2	96.7^{ab}	98.3^{ab}	100.0^{a}	100.0^{a}	78.0^{c}	65.0^{d}
-4	96.7^{ab}	81.7^{c}	100.0^{a}	94.7^{b}	69.3^{cd}	13.3^{fg}
-6	100.0^{a}	38.3^{e}	98.3^{a}	80.0^{c}	81.3^{c}	11.6^{gh}
-8	95.0^{b}	7.7^{gh}	95.0^{b}	23.3^{f}	73.3^{cd}	5.0^{h}
$LSD_{0.05}$ (Int) = 7.93 (60 df)			$LSD_{0.01}$ (Int) = 10.54			

* Values show the real germination percentages but variance analysis was performed using arcsine transformed values. Means followed by the same letter(s) are not significantly different at P = 0.05

Table 2. Changes in mean germination time of pea cultivars at different osmotic potentials of NaCl and PEG.

	Mean Germination Time (day)					
	Cultivars					
	Bolero		Sprinter		Utrillo	
Bars	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	2.39^{jkl}	2.39^{jkl}	2.58^{i-l}	2.58^{i-l}	3.54^{fgh}	3.54^{fgh}*
-2	2.18^{l}	5.16^{d}	3.21^{g-j}	5.07^{de}	3.74^{fg}	6.81^{bc}
-4	2.29^{kl}	7.25^{b}	3.14^{g-k}	6.16^{c}	3.96^{fg}	8.39^{a}
-6	2.60^{i-l}	8.59^{a}	3.29^{ghi}	7.18^{b}	4.28^{ef}	9.08^{a}
-8	2.81^{h-l}	8.33^{a}	4.22^{ef}	8.44^{a}	4.96^{de}	8.67^{a}

$LSD_{0.05}$ (Int) = 0.85 (60 df)

* Means followed by the same letter(s) are not significantly different at P = 0.05

Table 3. Root length of pea cultivars at different osmotic potentials of NaCl and PEG.

	Root Length (cm)					
	Cultivars					
	Bolero		Sprinter		Utrillo	
Bars	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	13.99^{a}	13.99^{a}	11.69^{bc}	11.69^{bc}	3.19^{lm}	3.19^{lm}*
-2	11.97^{b}	6.35^{gh}	10.29^{d}	9.29^{de}	4.63^{ijk}	2.23^{m}
-4	10.55^{cd}	2.62^{m}	8.61^{ef}	6.59^{gh}	5.35^{h-k}	0^{n}
-6	8.87^{e}	0^{n}	7.28^{fg}	4.31^{jkl}	4.06^{kl}	0^{n}
-8	5.89^{hi}	0^{n}	5.53^{hij}	0^{n}	2.97^{lm}	0^{n}

$LSD_{0.05}$ (Int) = 1.34 (60 df) $LSD_{0.01}$ (Int) = 1.79

* Means followed by the same letter(s) are not significantly different at P = 0.05

Table 4. Shoot length of pea cultivars at different osmotic potentials of NaCl and PEG.

	Shoot Length (cm)					
	Cultivars					
	Bolero		Sprinter		Utrillo	
Bars	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	5.04^{a}	5.04^{a}	4.97^{a}	4.97^{a}	1.56^{fgh}	1.56^{fgh}*
-2	3.90^{b}	1.09^{hi}	3.16^{c}	1.15^{ghi}	2.62^{de}	0^{j}
-4	3.83^{b}	0^{j}	2.88^{cde}	0.91^{i}	1.73^{f}	0^{j}
-6	3.01^{cd}	0^{j}	2.37^{e}	0^{j}	1.73^{f}	0^{j}
-8	2.70^{cde}	0^{j}	1.63^{fg}	0^{j}	1.17^{ghi}	0^{j}

$LSD_{0.05}$ (Int) = 0.509 (60 df) $LSD_{0.01}$ (Int) = 0.677

* Means followed by the same letter(s) are not significantly different at P = 0.05

Table 5. Seedling fresh weight of pea cultivars at different osmotic potentials of NaCl and PEG.

	Seedling Fresh Weight (mg plant^{-1})					
	Cultivars					
	Bolero		Sprinter		Utrillo	
Bars	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	0.67^{a}	0.67^{a}	0.58^{b}	0.58^{b}	0.16^{jk}	0.16^{jk}*
-2	0.51^{c}	0.11^{kl}	0.39^{d}	0.18^{ij}	0.28^{fg}	0^{m}
-4	0.39^{d}	0^{m}	0.35^{de}	0.11^{l}	0.24^{gh}	0^{m}
-6	0.31^{ef}	0^{m}	0.28^{fg}	0^{m}	0.23^{hi}	0^{m}
-8	0.22^{hi}	0^{m}	0.20^{hij}	0^{m}	0.16^{jk}	0^{m}

$LSD_{0.05}$ (Int) = 0.052 (60 df) $LSD_{0.01}$ (Int) = 0.069 (60 df)

* Means followed by the same letter(s) are not significantly different at P = 0.05

Table 6. Seedling dry weight of pea cultivars at different osmotic potentials of NaCl and PEG.

	Seedling Fresh Weight (mg plant^{-1})					
	Cultivars					
	Bolero		Sprinter		Utrillo	
Bars	NaCl	PEG	NaCl	PEG	NaCl	PEG
0	0.054^{a}	0.054^{a}	0.045^{ab}	0.045^{ab}	0.015^{fg}	0.015^{fg}*
-2	0.043^{abc}	0.014^{fg}	0.036^{bcd}	0.022^{def}	0.028^{c-f}	0^{g}
-4	0.037^{bcd}	0^{g}	0.032^{b-e}	0.015^{fg}	0.026^{def}	0^{g}
-6	0.029^{c-f}	0^{g}	0.027^{c-f}	0^{g}	0.025^{def}	0^{g}
-8	0.024^{def}	0^{g}	0.021^{def}	0^{g}	0.020^{ef}	0^{g}

$LSD_{0.05}$ (Int) = 0.016 (60 df) $LSD_{0.01}$ (Int) = 0.022 (60 df)

* Means followed by the same letter(s) are not significantly different at P = 0.05

Discussion

NaCl and PEG adversely affected the germination and seedling growth of pea but PEG had a greater inhibitory effect 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 and seedling growth 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. Mean germination time of the cultivars varied with solutions and doses. Bolero needed less time to germinate while Utrillo needed more. At the same water potential, the lower mean germination time in NaCl than in PEG could be explained by more rapid water uptake in NaCl solutions. Khajeh-Hosseini et al. (2003) found faster germination in NaCl in soybean. Delgado et al. (1994), who studied the effects of salt stress on growth and nitrogen fixation by pea, faba bean, common bean and soybean plants, indicated that pea was the most sensitive legume among them. Root and shoot length, and seedling fresh and dry weight were decreased by increasing NaCl and PEG concentrations. Consequently, seedling growth was inhibited in pea. Differences between NaCl and PEG were significant for all investigated characters. Inhibition of NaCl was less than that of PEG. Our results confirm the findings of Khajeh-Hosseini et al. (2003) in soybean and those of Murillo-Amador et al. (2002) in cowpea. However, our findings showed that NaCl had greater inhibitory effects on seedling growth than on germination because no significant decrease in germination in the 3 cultivars was observed. Furthermore, the germination percentage of Utrillo was encouraged by salinity. At the same water potential, the lower germination time and higher final germination in NaCl than in PEG 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.

In conclusion, in the germination and early seedling growth stages the investigated cultivars showed different responses to water and salt stress; Bolero was more resistant to salt concentrations while Sprinter appeared tolerant. Furthermore, germination failure due to NaCl resulted from an osmotic barrier induced by NaCl. Redmann (1974) found that the osmotic effect of NaCl on germination of alfalfa was more important than the toxic effect. However, seedling growth was more sensitive to salt stress than was germination.

Acknowledgment

The authors are grateful to Prof. Dr. Sebahattin Özcan, Department of Field Crops, University of Ankara, for allowing us to use his laboratory for this study.

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