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Full Length Research Paper

Effects of water and salt stresses on germination and seedling growth in two durum wheat (*Triticum durum* Desf.) genotypes

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To evaluate durum wheat (*Triticum durum* Desf.) tolerance to osmotic stress at the water potential levels induced by polyethylene glycol 6000 and NaCl solutions at an early stage of plant growth. Each, on germination and early seedling stages, were investigated for two durum wheat genotypes differing in drought tolerance (boefi and leucurum) at laboratory, in Iran in 2010. This investigation was performed as factorial experiment under complete randomized design with four replications. Daily and final germination as well as germination and seedling emergence rate, and seedling fresh and dry weight were measured in the study under controlled conditions. Results showed those germination rate was delayed by both solutions in both varieties, with differences between genotypes among growth stages, given that boefi genotype showed a higher germination rate than leucurum genotype in NaCl. NaCl had a lesser effect on genotypes in terms of germination rate and the final germination than did polyethylene glycol (PEG). This conclusively proves that the adverse effect of PEG on germination and early seedling growth was due to the osmotic effect rather than the specific ion. Seedling growth was reduced by both stresses. But NaCl usually caused less damage than PEG to durum wheat seedlings, suggesting that NaCl and PEG acted through different mechanisms. This difference in cultivar's behavior according to the growth conditions is discussed. It was concluded that inhibition in germination at equivalent water potential levels of NaCl and PEG was mainly due to an osmotic effect rather than did salt toxicity. Finally boefi genotype showed that resistance to both NaCl and PEG stress than leucurum genotype.

Keywords: Durum wheat, germination, NaCl, polyethylene glycol (PEG).

INTRODUCTION

For thousands of years, durum wheat (*Triticum turgidum*, L.var.Durum Defs) has been cultivated both irrigated and rain-fed in the west of Iran. Tetraploid durum wheat (*T. durum*) or hard wheat mainly are used to produce semolina flour used in the food industries especially pasta spaghetti. However, under cultivated area of this plant is less than other hexaploid wheats, but their resistance against disease and environmental stressors such as common consistencies, is more and remarkable in drought conditions (Arzani, 2000). Stalinization is the scourge of intensive agriculture (Mer et al., 2000). High concentration of salts has detrimental effects on germination of seeds (Rahman et al., 2000; Kayani and Rahman, 1987), and plant growth (Pandey and Thakrar, 1997). Many investigators have reported retardation of germination and growth of seedlings at high salinity (Bernstein, 1991). However plant species differ in their sensitivity or tolerance to salts (Torech and Thompson, 1993). Drought and soil salinity are major abiotic constraints on crop production and food security, and adversely impact the socio-economic fabric of many developing countries. Water scarcity, declining water quality for irrigation, and soil salinity are problems which are becoming more acute (Flowers, 2004). It is estimated

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Abbreviations: PEG, polyethylene glycol.

that 20% of all cultivated land and nearly half of irrigated land is affected by salt, greatly reducing the yield of crops to well below their genetic potential (Flowers, 2004; Munns et al., 2006; Jones, 2007). Achieving genetic increases in yield under these stresses has always been a difficult challenge for plant breeders (Khayatnezhad et al., 2010). High concentrations of salts in the soil solution impair cell metabolism and photosynthesis but impose an osmotic stress on cell water relations by increasing the toxicity of sodium in the cytosol (sayar et al., 2010). Under stresses conditions, the germination of seeds is affected by creating an external osmotic potential that prevents water uptake due to the toxic effects of Na^+ and Cl^- ions both during imbibition and seedling establishment (Murillo-Amador et al., 2002). The accumulation of soluble salts in soil leads to an increase in osmotic pressure of the soil solution, which may limit the absorption of water by the seeds or plant roots. Salt damage to plants is attributed to reduction in water availability, toxicity or specific ions, and nutritional imbalance caused by such ions (James et al., 2006). Polyethylene glycol (PEG) widely used to induce water stress, is a non-ionic water polymer, which is not expected to penetrate into plant tissue rapidly (Kawasaki et al., 1983). In contrast, Na+ and Cl^- penetrate into plant cells and can be accumulated in the vacuole for the tolerant plants or in the cytoplasm for sensitive cultivars (Genc et al., 2007). The screening of salt tolerant lines/cultivars has been attempted by many researchers on various species at seedling growth stage (Khayatnezhad et al., 2010). The relation of various seedling growth parameters to seed yield and yield component under saline conditions are important for the development of salt tolerant cultivar for production under saline conditions. Powerful new molecular tools for manipulating genetic resources are becoming available (Munns, 2005). The same authors have reported that this approach identified several markers linked to a gene at a quantitative trait locus (QTL) designated Nax1 (Na^+ exclusion). Munns et al. (2006) has mentioned also that a region on the long arm of chromosome has been shown to contain a QTL for Na^+ exclusion and K^+/Na^+ discrimination. Major increases in salt tolerance of plants would be possible by introducing new genes either by crossing with new donor germplasm or by transformation with single genes. In Tunisia, durum wheat is commonly grown on marginal soils under rainfed (natural and non-irrigated) conditions. It suffers often from drought and salt stresses. It is worth noting that most of the high-yielding durum wheat varieties that are being introduced into the country from various sources are not sufficiently salt and drought tolerant; hence, there is a need for the development of a specific durum wheat breeding programs for salt and drought tolerance. The study presented here deals with the response of two cultivars of wheat to NaCl and water stress at germination and early seedling growth stage.

MATERIALS AND METHODS

This study was carried out at laboratory condition in Ardabil located in north-western of Iran using two durum wheat genotypes namely boefi and leucurum which appear to be drought susceptible and tolerant, respectively (Gholamin et al., 2010), On the basis of preliminary results of a field trial involving 22 durum wheat genotypes were selected for this study. Study was performed in a factorial experiment based on completely randomized design with four replications. Germination and early seedling growth (10 days) of these genotypes were studied in two experiments using distilled water (control) and osmotic potentials (-0.2, -0.4, -0.6 and -0.8 MPa), which were prepared by adding NaCl or PEG-6000 to distilled water according to Van't Hoff's equation (Lang, 1967) to have the same osmotic potential in both NaCl and PEG-6000 solutions. The seeds sterilized with Sodium hypochlorite solution, containing 5% active chlorine, for 5 min. The seeds were then washed three times with sterilized distilled water. All the experiments were conducted in 12 cm petri plate on filter paper beds in growth chambers. 20 grains were sown in 12 cm diameter petri plate on filter paper beds, irrigated with 5 ml solution of respective treatment and incubated at 25°C. The dishes were moistened with equal amounts of desired osmotic solutions (NaCl or PEG-6000 solutions, osmotic potentials of 0, -0.2, -0.4, -0.6 and -0.8 MPa). The number of germinated seeds was recorded daily and the final germination percentage was determined after 10 days. The germination rate was calculated using Maguire's equation (1962): M (GR) = n1/t1 + n2/t2 + … + n7/t7; where n1, n2, ..., n7 are the number of germinated seeds at times t1, t2,…, t7 (in days).

Five seedlings were taken randomly and seedling growth was measured by fresh and dry weights of the different parts of the seedling, on the tenth day after emergence, the height and leaf area. Dry weight was determined for each plant after drying the samples in a forced-air dryer at 80°C for 48 h. The experimental design and statistical analyses were similar to those used for the germination test.

Data were subjected to statistical analysis using ANOVA, a statistical package available from SPSS16 and MSTATC.

RESULTS

The results of ANOVA for germination rate and germination percent showed that genotypes and interaction between genotype and osmotic potential was significant for both traits in 0.01% level. Osmotic potential and three-way interaction was significant at 0.05% level for both traits, and interaction between genotypes and solution was significant at 0.05% level only for germination percentage.

The germination rate decreased with a decrease in osmotic potential in both NaCl and PEG solutions but the inhibition was greater under PEG for both genotypes (Figure 1A). In NaCl solution, leucurum was more affected, although it increased the germination rate in -0.4 and again decreased in -0.6 and -0.8 MPa (Figure 1B), while the rate in boefi did not decrease proportionally as osmotic potential increased under NaCl solution. boefi genotype decreased germination rate at PEG solution, but leucurum genotype increased in -0.2 and then decreased for other levels.

However, the effect of osmotic agent (NaCl, PEG) was found. Both genotypes showed different germination

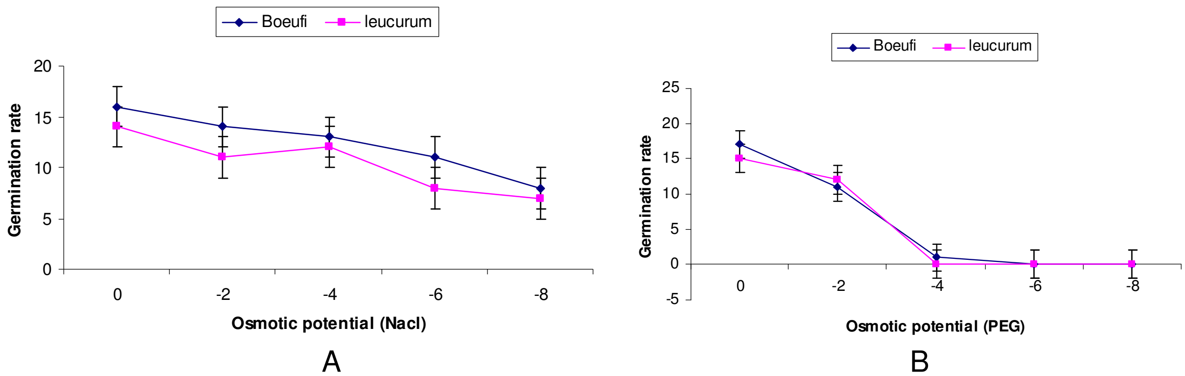

Figure 1. Germination rate of two durum wheat genotypes under decreasing external osmotic potential levels induced by NaCl and PEG solutions.

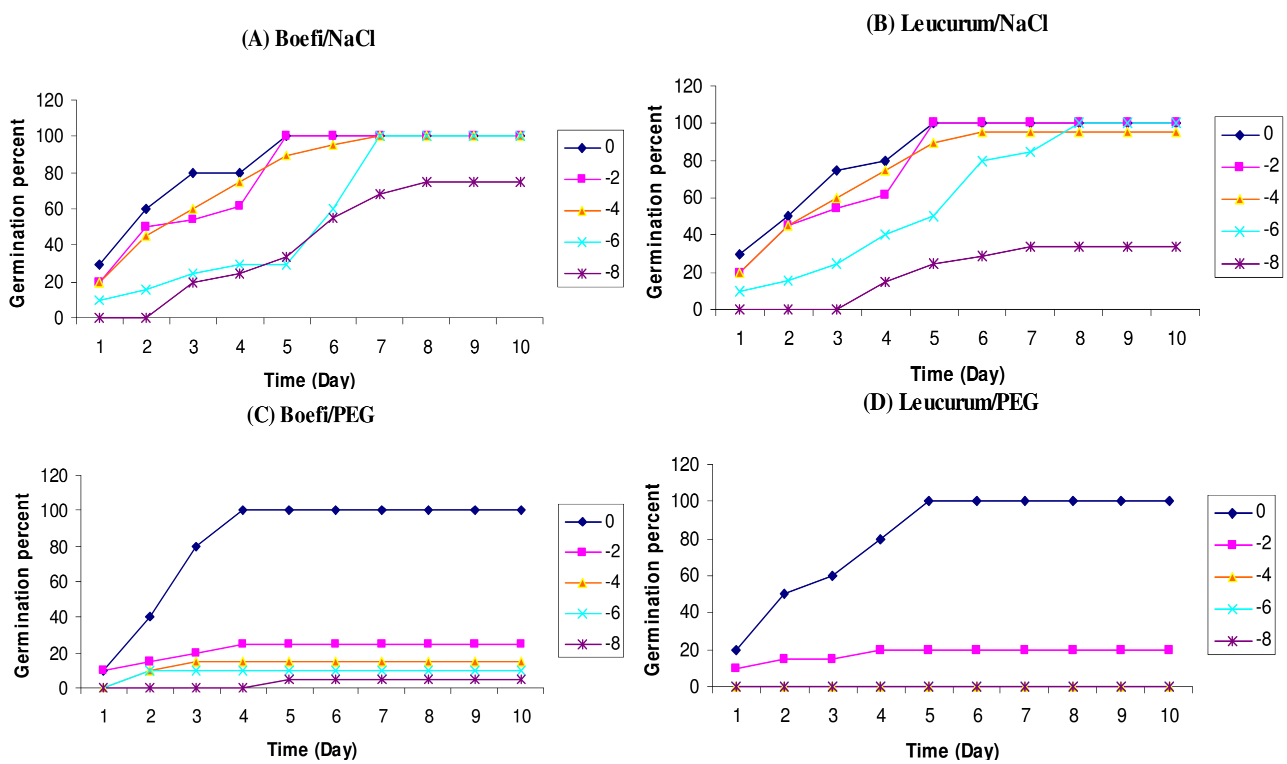

Figure 2. Germination of durum wheat seeds as a function of time and decreasing external osmotic potential levels.

(expressed as cumulative percentage) in all osmotic potential levels of NaCl solution (Figure 2A and B), and their final germination percentage decreased, and delayed as the osmotic potential increased at PEG solutions (Figure 2C and D). Between genotypes, Buefi was better than leucurum at -0.8 levels of Nacl and PEG.

The analysis of variance showed significant differences between genotypes, solutions and osmotic potential for fresh weight shoot height and dry weight (Table 1. In these traits also, the interactions between the examined factors were mostly significant. In leaf area and shoot height, however, the effects of osmotic potential and three-way interaction were not significant but the effect of different solutions (different osmotic potential levels of NaCl or PEG) was highly significant.

PEG significantly reduced the seedling fresh-weight of both cultivars (Figure 3A and B), but boefi fresh weight in control levels of PEG was higher than NaCl control levels. The seedling dry-weight showed that both genotypes dry-weight were less affected than PEG

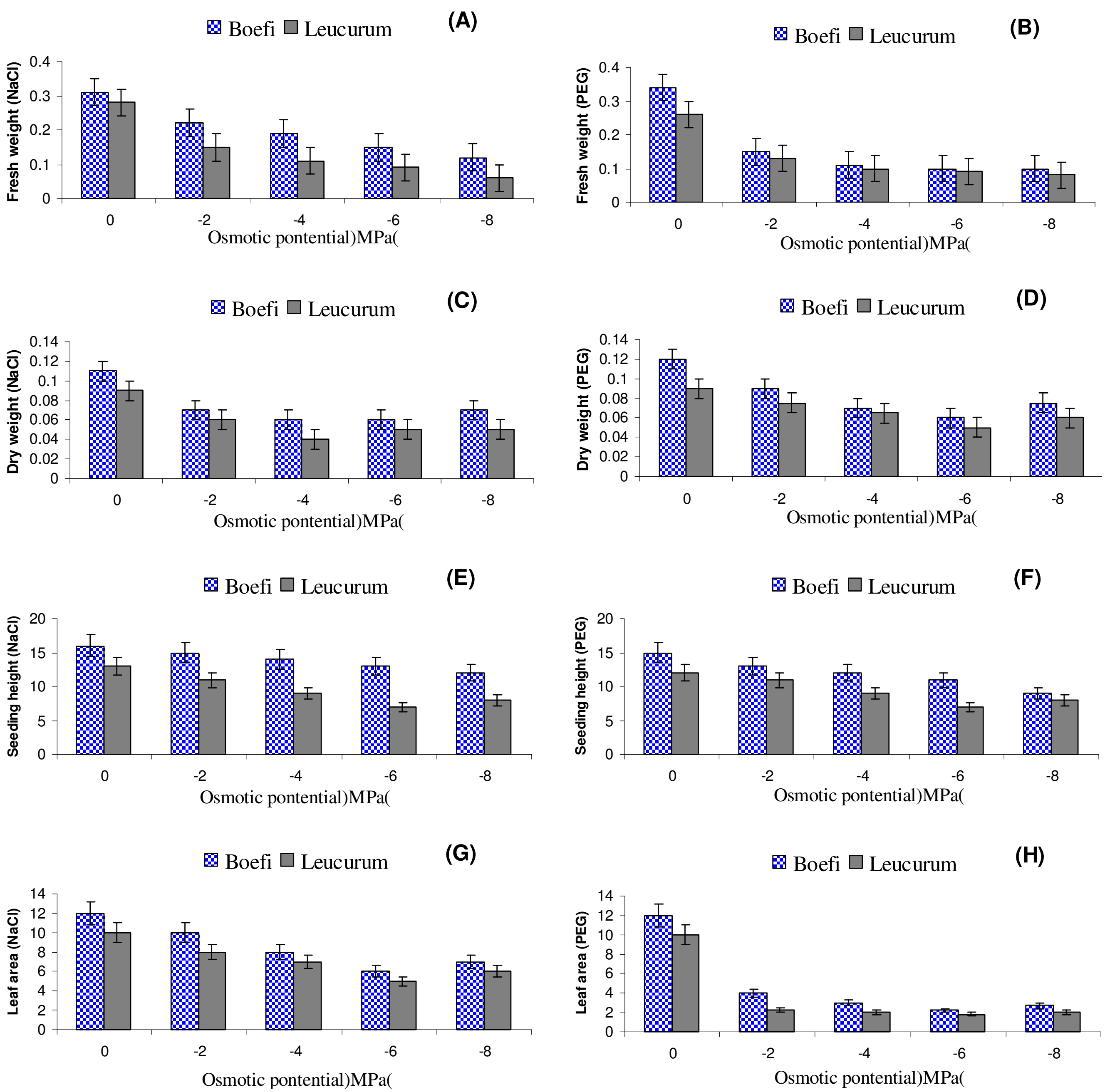

Figure 3. Effects of NaCl and PEG on the growth of 10-days-old seedlings examined for fresh weight (A and B g/plant), dry weight (C and D g/plant), height (E and F cm) and leaf area (G and H cm²) in two durum wheat genotypes; the bars represent the standard error of the mean ($P < 0.05$).

solutions (Figure 3C and D). Seedling height was higher in PEG than in NaCl solution in both genotypes. Boefi showed higher seedling height in NaCl than leucurum but in PEG both genotypes decreased as osmotic potential increased (Figure 3E and F) Leaf area was more affected by PEG than NaCl. Boefi was better than leucurum in both solutions and all osmotic potential levels (Figure 3G and H). In both genotypes, leaf area decreased linearly as osmotic potential levels in PEG increased. In general, PEG at isoosmotic concentration was more harmful to seedling growth than NaCl. The higher inhibition of all growth variables in PEG treated plants than in NaCl shows osmotic dehydration in the factor affecting seedling growth.

DISCUSSION

Water and salt stress due to drought is probably the most

Table 1. Effects of NaCl and PEG on the germination rate and germination percentage.

SOV	df	MS	
		Germination rate (GR)	Germination percentage(GP)
Genotypes (G)	1	**	**
Solutions (S)	1		
Osmotic potentials (O)	4	*	*
GS	1		*
GO	4	**	**
SO	4		
GSO	4	*	*
Error	54	0.84	12.58

** and * significant at the 0.01 and 0.05 levels, respectively.

Table 2. Effects of NaCl and PEG on the fresh and dry weight, plant height and leaf area of durum wheat.

SOV	df	MS			
		Fresh weight	Dry weight	Plant height	Leaf area
Genotypes (G)	1	**	**	**	**
Solutions (S)	1	**	**	**	**
Osmotic potentials (O)	4	**	**	*	
GS	1	**	**	**	*
GO	4	**	**	*	**
SO	4	**	**	**	**
GSO	4	**	**		
Error	54	0.098	0.154	1.59	2.87

** and * significant at the 0.01 and 0.05 levels, respectively.

significant abiotic factor limiting plant and also crop growth and development (Hartmann, 2005). Drought stresses 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). Because of germination is one of the most important traits in early stage of growth in most plants, it seems that golden west in drought stress condition had more resistant than other cultivars and had more yield potential. In according to results of the present study, it suggested that more experiments were carried out on the similar cultivars and further investigation be done on golden west. Results of the current study are in agreement with those obtained by Kalefetoglu et al. (2009), on wheat.

In this study, two durum wheat genotypes were compared with regard to their drought and salt resistance to an imposed water stress in controlled conditions during germination and the early seedling growth stage. Results showed that, although planted under the same conditions, the two genotypes display distinct responses to salinity and drought stress. In this sense, genetic variability within a species offers a valuable tool for studying mechanisms of salt and drought tolerance. One of these mechanisms depends on the capacity for osmotic adjustment, which allows growth to continue under saline conditions. This is basically true for water stress, although osmotic adjustment is not achieved in the same way under both stresses. Under salt stress, this process is accomplished by uptake and accumulation of inorganic ion, mainly Na^+ and Cl^-. Under water stress, it is achieved by synthesis and accumulation of organic compatible solutes (Alian et al., 2000). Significant differences were observed between the exa-mined genotypes, solutions, osmotic potential levels and their interactions with regard to germination rate and final germination percentage (Table 1). Salinity (NaCl) may also affect germination by facilitating the intake of toxic ions, which may change certain enzymatic or hormonal activities of the seed (Smith and Comb, 1991). These physicochemical effects upon the seed seem to result in a slower and/or lower rate of germination or emergence. Both osmotic and toxic effects of salts have been implicated in inhibition of seed germination (El-Hendawy et al., 2005). The cultivar boefi showed a higher germination rate at NaCl solution, but both cultivars decreased in PEG as osmotic potential increased (Figures 1A and B). Since seed germination is more sensitive to salinity and drought stress than the emergence or growth of established seedling (Freeman, 1973), the greater tolerance to salinity and drought of durum wheat during emergence would be an adaptive feature of this species to saline or drought environments. Previous research according to Levitt (1980) indicated the germination test were usually not good indicators f differences in salt or drought tolerance among cultivars. Rhoades (1990) reported that some plants are relatively tolerant during germination, but be-come more sensitive later. In the same way, germination and seedling emergence from laboratory results does not necessarily represent germination and seedling emergence from field soils. Still the most important agronomic question is whether the observed differences in salt tolerance during early stages are representative of the salt tolerance of the cultivars during the whole growth cycle. The fresh and dry weights seedling height and leaf area of the seedlings 10 days after imbibitions showed that, growth was inhibited by both NaCl and PEG. Apparently, the presence of NaCl or PEG in the germination and emergence medium reduces the uptake of water by the seedlings and inhibits the mobilization of the seed reserves to the growing embryonic axis. These data are in agreement with others studies of germination in the presence of NaCl or non ionic osmotic solutions such as mannitol or PEG (Kawasaki et al., 1983; Sayar et al., 2010). The results obtained in this study revealed that dry weights were less affected than fresh weight and are in agreement with the results obtained by Murillo-Amador et al. (2002) in cowpea. These results are also consistent with those found by Sayar et al. (2010) in wheat, who affirmed that growth medium salinity or drought may affect seed germination by decreasing the ease with which the seeds take up water because the activity and events normally associated with germination get either delayed and/or proceed at a reduced rate. The effect of PEG on leaf area of 10-days-old plants was greater than that on fresh and dry weights and plant height (Figure 3H). The decline in leaf growth is the earliest response of glycophytes exposed to salt or drought stress (Shabala et al., 2006). Finally boefi genotype showed that resistance to both NaCl and PEG stress that Leucurum.

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