Response of Durum Wheat (*Triticum durum* Desf.) Growth to Salt and Drought Stresses

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Abstract: Two durum wheat (*Triticum durum* Desf.) cultivars were tested for salt and drought tolerance at germination, seedling emergence and early seedling growth in NaCl and PEG-8000 solutions of different osmotic potentials (–0.2, –0.4, –0.6 and –0.8 MPa). Daily and final germination and emergence percentage, as well as germination and seedling emergence rate, seedling growth, fresh and dry weight were recorded under controlled conditions. Results showed that germination and emergence rates were delayed by both solutions in both cultivars, but Omrabia showed higher germination and emergence rates than BD290273 in NaCl while BD290273 was less affected by NaCl and PEG solutions at the emergence stage. Sodium chloride had a lesser effect on both cultivars in terms of germination rate, emergence rate, final germination and emergence percentage than did PEG-8000. This conclusively proves that the adverse effect of PEG-8000 on germination, emergence and early seedling growth was due to the osmotic effect rather than to the specific ion. Seedling growth was reduced by both stresses. However, NaCl usually caused less damage than PEG to durum wheat seedlings, suggesting that NaCl and PEG acted through different mechanisms.

Keywords: durum wheat; emergence rate; germination rate; salt and drought stresses; seedling growth

Drought and soil salinity are major abiotic stress factors affecting seriously crop production and food safety. They reveal adverse impacts on the socio-economic structure of many developing countries. Water scarcity, declining water quality for irrigation, and soil salinity are problems which are becoming really acute (Flowers 2004). It was estimated that 20% of all cultivated land and nearly a half of irrigated land are affected by salt, greatly reducing the yield of crops below their genetic potential (Flowers 2004; Munns 2006; Jones 2007). Achieving genetic increases in yield under abiotic stresses has always been a difficult challenge for plant breeders (Blum 2005).

High concentrations of salts in the soil solution impair cell metabolism and photosynthesis imposing an osmotic stress on cell water relations by increasing the toxicity of sodium in the cytosol (Carden 2003). Moreover, salt and water stresses are responsible for either inhibition or delayed seed germination and seedling establishment (Bewley & Black 1994). Under stress conditions, the germination of seeds is affected by creating an external osmotic potential that prevents water uptake or due to the toxic effects of Na^+ and Cl^- ions both during imbibition and seedling establishment (Murillo-Amador 2002).

The accumulation of soluble salts in soil leads to an increase in the osmotic pressure of soil solution, which may limit the absorption of water by the seeds or by the plant roots. Salt damage to plants is attributed to the reduction in water availability,

toxicity or specific ions, and nutritional imbalance caused by such ions (James *et al.* 2006).

Powerful new molecular tools for manipulating genetic resources are becoming available (Munns 2005). A locus for the low-Na^+ trait was mapped to the long arm of chromosome 2A using a quantitative trait locus (QTL) approach (Lindsay *et al.* 2004). The same authors have reported that this approach identified several markers linked to a gene at a QTL designated Nax1 (Na^+ exclusion). Munns (2006) also mentioned that a region on the long arm of chromosome contains a quantitative trait locus (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, most durum wheat is commonly grown on marginal soils under rainfed (natural and non-irrigated) conditions. It often suffers from drought and salt stresses. It is true that the most of the high-yielding durum wheat varieties 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 programme for salt and drought tolerance. The present study was conducted with the objective of identifying some parental lines and morphological parameters to be used in a breeding programme to develop salt/drought-tolerant wheat cultivars. The underline hypothesis of the study is that salt/drought tolerance correlates positively with root primary number as well as negatively with the aerial part of the plant (Sayar *et al.* 2008). This suggests that this relationship may be exploited as a selection tool in wheat breeding and that using measurements of some growth parameters as physiological markers for salt/ drought tolerance may benefit wheat-breeding programmes.

MATERIALS AND METHODS

Based on preliminary results of a field trial involving 25 durum wheat cultivars (Sayar *et al.* 2007), the cultivars BD290273 and Omrabia that showed sensitivity and tolerance to drought, respectively, were selected for this study. Germination, emergence and early seedling growth (10 days) of these cultivars were studied in two experiments using distilled water (control) and solutions with different osmotic potentials (–0.2, –0.4, –0.6 and –0.8 MPa), which were prepared by adding NaCl or PEG-8000 to distilled water according to Van't Hoff's equation (Lang 1967) to have the same osmotic potential in both NaCl and PEG solutions.

This study was carried out at Kef research station (INRAT) located in a semi-arid zone (36°14'N 8°27'E) in north-western Tunisia.

Germination

Seeds of each cultivar were previously disinfected by immersion in a calcium hypochlorite solution, containing 5% of active chlorine, for 5 min. Seeds were then washed three times with sterilised distilled water. Germination tests were carried out in sterilised Petri dishes (150 × 15 mm) covered at the bottom with a cotton layer. The dishes were moistened with equal amounts of desired osmotic solutions (NaCl or PEG-8000 solutions, osmotic potentials of 0, –0.2, –0.4, –0.6 and –0.8 MPa). Three millilitres of the appropriate solution were added daily to each dish. Germination tests were carried out in the dark growth chamber (Model MB-60B, Percival Manufacturing Company, USA) at 25°C ± 0.5°C and 80% ± 1% of relative air humidity. Seeds were considered germinated when the coleorhizae were at least 2 mm long. The number of germinated seeds was recorded daily and the final germination percentage was determined after 7 days. The germination rate was calculated using MAGUIRE's equation (Maguire 1962): $M = n_1/t_1 + n_2/t_2 + \ldots + n_7/t_7$; where n_1, n_2, .., n_7 represent the number of germinated seeds at times t_1 , t_2, ..., t_7 (in days).

Emergence and seedling growth

In this experiment, the effects of NaCl and PEG-8000 with decreasing external osmotic potentials on both seed emergence and young seedlings were studied. Seeds of each cultivar (BD290273 and Omrabia) were disinfected as previously described. Emergence tests were carried out in plastic trays of 25 cm wide × 50 cm long × 6 cm deep, which were filled with sterilized compost soil (leaf-mould: stable-litter:sand; 1:1:1) under light/dark cycle conditions of 16/8 h at 25°C and 80% relative humidity placed in a growth chamber. Each pot was

moistened daily with uniform amounts of desired osmotic solutions (NaCl or PEG-8000 solutions, osmotic potentials of 0, –0.2, –0.4, –0.6 and –0.8 MPa). A seedling was considered emerged when its coleoptile was visible above the substratum surface. The number of emerged seedlings was recorded daily (seedling rate), and the number of final emerged seedlings (expressed in percentage) was counted after 10 days. The emergence rate was calculated according to Maguire's equation (Maguire 1962): $M = n_1/t_1 + n_2/t_2 + ... + n_{10}/t_{10}$; where n_1, n_2, ..., n_{10} represent the number of emerged seeds at times t_1 , t_2 , ...,t_{10} (in days).

Seedling growth

Five seedlings were taken away randomly and seedling growth was measured by estimating fresh and dry weights of the different parts of seedlings, on the tenth day after emergence, when also the height and the leaf area were measured. Leaf area was determined with the use of a Leaf Area Meter (Model 300A, Li-Cor, USA) and dry weight for each plant after drying the samples in a forced-air drier at 80°C for 48 h. The experimental design and statistical analyses were similar to those used for the germination test.

Experimental design and statistical analysis

Effects of three factors were analysed in these experiments and a completely randomized design with four replications of 20 seeds per replication was used. The first factor (cultivar) had two levels (D290273 and Omrabia), the second one (osmotic agent) had two levels (NaCl and PEG-8000) and the third one (osmotic potential) had five levels (0, –0.2, –0.4, –0.6 and –0.8 MPa). Analyses of variance (ANOVA) of the obtained data were applied using SAS software (SAS 2002).

RESULTS

Germination

The germination rate decreased with the decrease in osmotic potential in both NaCl and PEG solutions, but the inhibition was greater under PEG (Figure 1B) in both cultivars. In NaCl solution, cultivar Omrabia was more affected, although germination rate increased in –0.6 and –0.8 MPa (Figure 1A), while this parameter in BD290273 did not decrease proportionally as the osmotic potential increased under NaCl solution. The ANOVA for germination rate and germination percentage

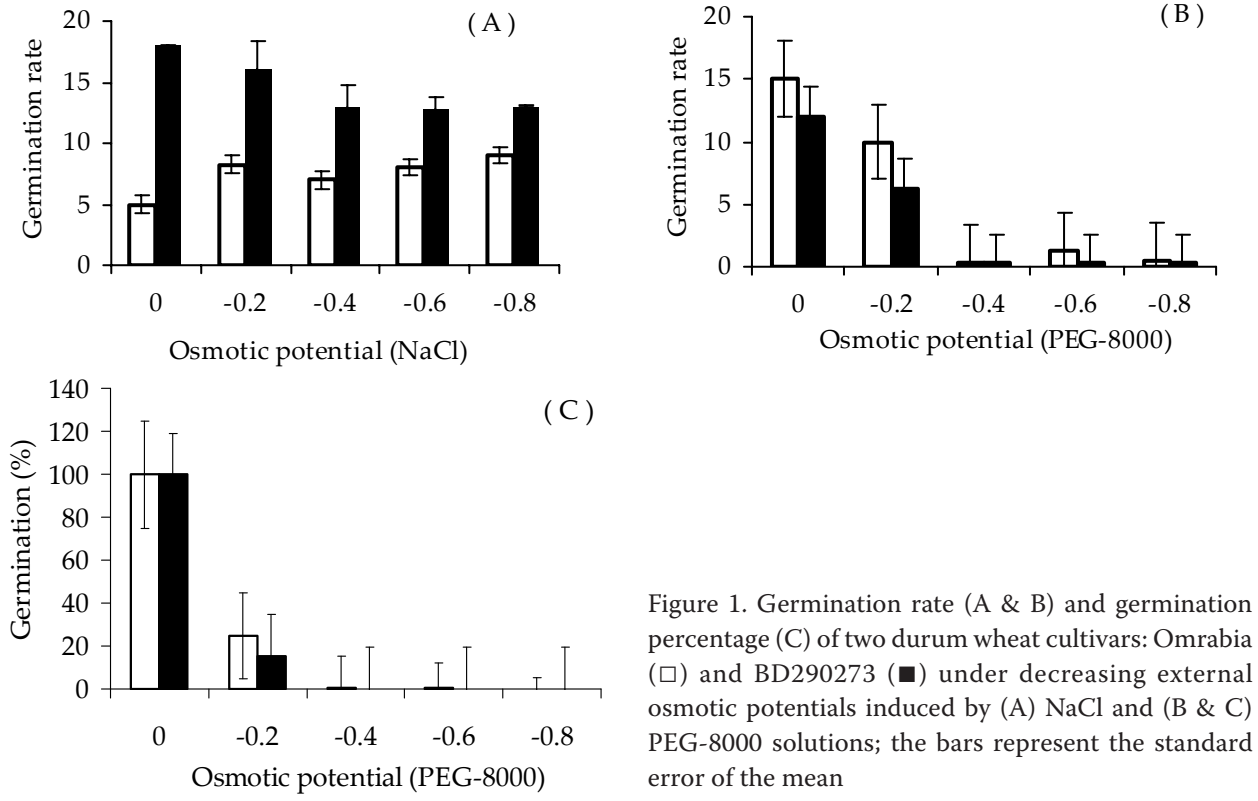

Figure 1. Germination rate (A & B) and germination percentage (C) of two durum wheat cultivars: Omrabia (□) and BD290273 (■) under decreasing external osmotic potentials induced by (A) NaCl and (B & C) PEG-8000 solutions; the bars represent the standard error of the mean

Table 1. Effects of cultivars, osmotic agents (NaCl and PEG-8000) and different osmotic potentials on the germination and emergence rates and percentages: summary of ANOVA results

Source of variation	df	Germination rate			Germination percentage		
		mean square	*F* value	*P* level	mean square	*F* value	*P* level
Cultivar (A)	1	99.013	35.63	0.0094*	2940.313	49.19	0.0060*
Osmotic agent (B)	1	2.112	2.22	0.1420	148.512	3.22	0.0782
Osmotic potential (C)	4	485.706	510.52	0.000*	26308.200	570.96	0.0000*
AB	1	2.112	2.22	0.1420	655.513	14.23	0.0004*
AC	4	10.419	10.95	0.0000*	922.375	20.02	0.0000*
BC	4	2.269	2.38	0.0620*	32.825	0.71	
ABC	4	0.831	0.87		235.075	5.10	0.0015*
Error	54	0.951			46.077		
		Emergence rate			Emergence percentage		
Cultivar (A)	1	12.403	117.61	0.0017*	7605.000	1194.50	0.0001*
Osmotic agent (B)	1	5.671	72.71	0.0000*	6195.200	365.44	0.0000*
Osmotic potential (C)	4	5.256	67.39	0.0000*	5879.675	346.83	0.0000*
AB	1	0.435	5.58	0.0218*	4410.450	260.16	0.0001*
AC	4	0.179	2.29	0.0713	475.063	28.02	0.0000*
BC	4	0.303	3.88	0.0076*	412.887	24.36	0.0001*
ABC	4	0.146	1.87	0.1293	393.700	23.22	0.0000*
Error	54	0.078			16.953		

*$P < 0.05$; other values are not statistically significant; df – degrees of freedom

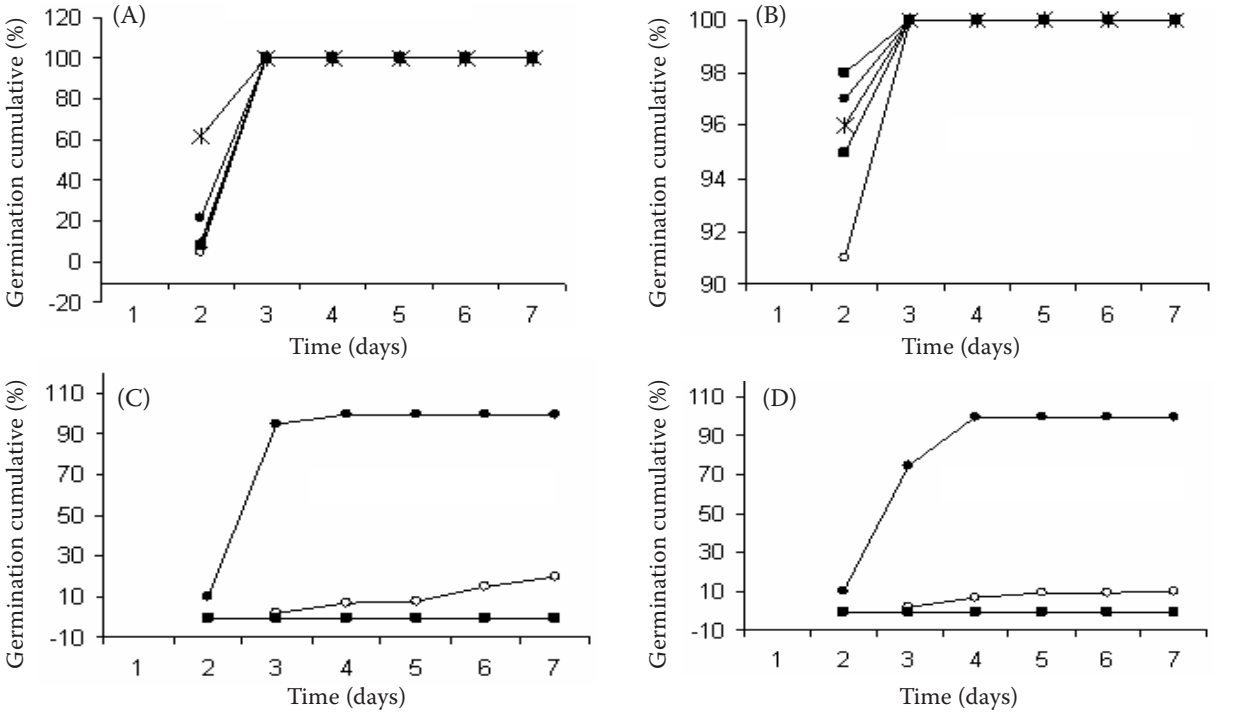

Figure 2. Germination of durum wheat seeds as a function of time and decreasing external osmotic potentials; Omrabia (A) and BD290273 (B) under NaCl, Omrabia (C) and BD290273 (D) under PEG; (●) control; (▼) –0.2; (○) –0.4; (■) –0.6, and (×) –0.8 MPa

showed high significance ($P < 0.01$) of differences between both the cultivars and osmotic potentials, and also significance of interactions between these two factors (Table 1). However, the effect of osmotic agent (NaCl, PEG) was not found.

Both cultivars showed 100% germination (expressed as cumulative percentage) in all osmotic potentials of NaCl solution (Figure 2A, B), but their final germination percentage decreased (Figure 1C) and delayed as the osmotic potential increased at PEG solutions (Figure 2C, D).

Emergence

The ANOVA for emergence rate (ER) and emergence percentage (EP) showed highly significant differences between cultivars, solutions, osmotic potentials and their interactions except for AC and ABC in emergence rate (Table 1).

Figure 3 shows large differences in the emergence rate between osmotic potentials for both cultivars. In both NaCl and PEG solutions, Omrabia showed higher emergence rates than BD290273 (Figures 3A, B). Emergence percentage (EP) for BD290273 was considerably reduced in NaCl, while Omrabia had essentially the same values of this parameter under increasing osmotic potentials (Figure 3C). In PEG solution, EP in BD290273 decreased linearly with the decrease in osmotic potentials, but Omrabia showed 98% at 0, –0.2 and –0.8 MPa and decreased until 90% at –0.4 MPa (Figure 3D).

Seedling growth

The analysis of variance showed significant differences between the cultivars, solutions and osmotic potentials for fresh weight, plant height and dry weight (Table 2). In these traits, also the interactions between the examined factors were mostly significant. In leaf area, however, the effects of cultivars and osmotic agents were not significant but the effect of different solutions (different osmotic potentials of NaCl or PEG) was highly significant.

PEG significantly reduced the seedling fresh weight of both cultivars (Figure 4A). The seedling dry weight showed that cultivar Omrabia was less affected than BD290273 by both solutions. Differences between cultivars showed that dry weight of

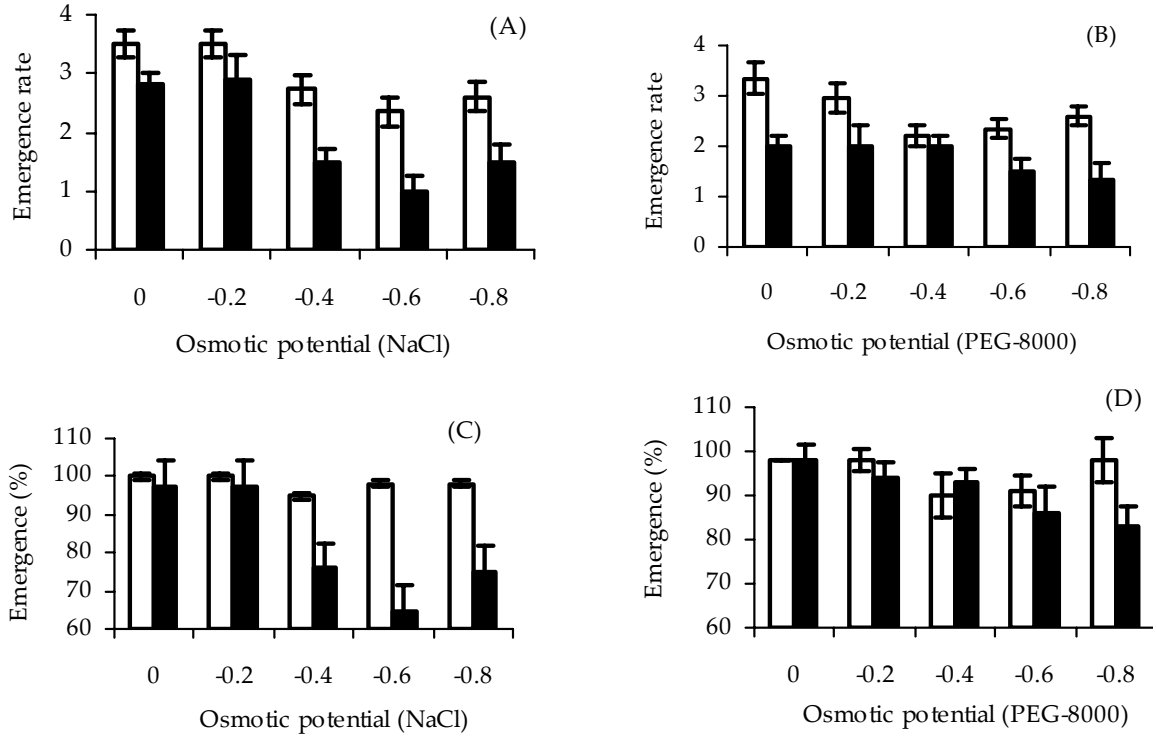

Figure 3. Emergence rate (A & B) and emergence percentage (C &D) of two durum wheat cultivars Omrabia (□) and BD290273 (■) under decreasing external osmotic potentials created by NaCl (A & C) and PEG-8000 (B & D) solutions; the bars represent the standard error of the mean

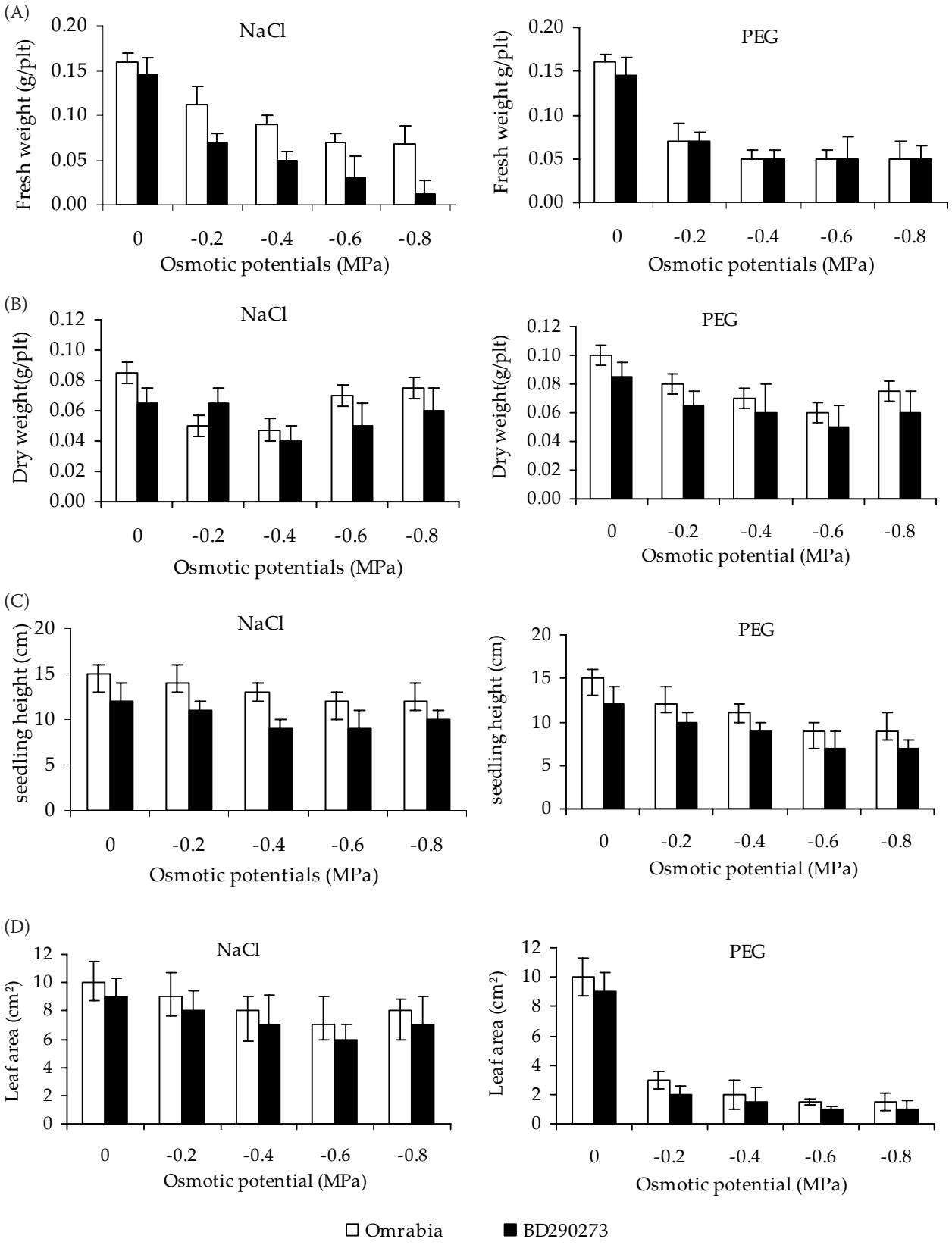

Figure 4. Effects of NaCl and PEG-8000 on the growth of 10-days-old seedlings examined for fresh weight (A, g/plant), dry weight (B, g/plant), height (C, cm) and leaf area (D, cm²) in two durum wheat cultivars; the bars represent the standard error of the mean ($P < 0.05$)

Table 2. Effects of cultivars and osmotic agents (NaCl and PEG-8000) and different osmotic potentials on fresh and dry weight, plant height and leaf area: summary of ANOVA results

Source of variation	df	Fresh weight			Dry weight		
		mean square	*F* value	*P* level	mean square	*F* value	*P* level
Cultivar (A)	1	0.078	819.40	0.0001*	0.005	675.00	0.0001*
Osmotic agent (B)	1	0.032	382.23	0.0000*	0.000*	6.43	0.0141*
Osmotic potential (C)	4	0.009	102.37	0.0000*	0.002*	31.20	0.0000*
AB	1	0.014	162.58	0.0000*	0.001*	13.42	0.0006*
AC	4	0.000	5.40	0.0000*	0.000*	6.80	0.0002*
BC	4	0.007	76.69	0.0000*	0.001*	21.17	0.0000*
ABC	4	0.000	5.23	0.0012*	0.000*	6.22	0.0003*
Error	54	0.000			0.000		
		Plant height			Leaf area		
Cultivar (A)	1	234.612	224.33	0.0006*	5333.378	1.18	0.3561
Osmotic agent (B)	1	23.112	38.67	0.0000*	5402.185	1.21	0.2768
Osmotic potential (C)	4	50.500	84.49	0.0000*	4236.589	0.95	0.0000*
AB	1	1.013	1.69	0.1986	3984.664	0.89	0.0000*
AC	4	2.113	3.53	0.0124*	4507.484	1.01	0.4119
BC	4	18.050	30.20	0.0000*	4549.059	1.02	0.4071
ABC	4	0.762	1.28	0.2909	4521.240	1.01	0.4103
Error	54	0.598			4475.355		

*$P < 0.05$; other values are not statistically significant; df – degrees of freedom

Omrabia was higher in PEG than in NaCl and this cultivar was slightly superior in both solutions than BD290273 (Figure 4B). Seedling height was higher in NaCl than in PEG solution in both cultivars. Omrabia showed higher values of seedling height in NaCl than BD290273, but in PEG the seedling height of both cultivars decreased as osmotic potentials increased (Figure 4C). Leaf area was more affected by PEG than NaCl. Omrabia was superior to BD290273 in both solutions and all osmotic potentials (Figure 4D). In both cultivars, leaf area decreased linearly as osmotic potentials in PEG increased. In general, PEG-8000 was more harmful to seedling growth than NaCl at iso-osmotic concentration. The higher inhibition of all growth variables in PEG-8000 treated plants than in NaCl treated ones showed that osmotic dehydration is the factor affecting seedling growth.

DISCUSSION

In this study, two durum wheat cultivars were compared with regard to their drought tolerance to an imposed water stress in controlled conditions during germination, seedling emergence and at an early seedling growth stage. Results showed that, although planted under the same conditions, the two genotypes displayed 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 (Gregorio *et al.* 2002). Polyethylene glycol (PEG) widely used to induce water stress is a non-ionic water soluble polymer which is not expected to penetrate into the plant tissue rapidly (Kawasaki 1983). In contrast, Na^+ and Cl^- penetrate into plant cells and can accumulate in the vacuole of tolerant plants or in the cytoplasm of sensitive cultivars (Genc 2007). One of the salt tolerance mechanisms depends on the capacity for osmotic adjustment which allows plant growth to continue under saline conditions. This is basically true of water stress, although osmotic adjustment is not achieved in the same way under both stresses. Under salt stress, osmotic adjustment is accomplished by uptake and accumulation of inorganic ions, mainly Na^+ and Cl^-. Under water stress, this process is achieved by synthesis and accumulation of organic compatible solutes

(Alian 2000). However, unfortunately these organic solutes were not measured in this study.

Significant differences were observed between the examined cultivars, solutions, osmotic potentials and their interactions with regard to germination rate and final germination percentage (Table 1). The fact that both cultivars showed 100% germination in all osmotic potentials of NaCl solution (Figure 2A, B), but their final germination percentage decreased (Figure 1C) and delayed as the osmotic potential increased in PEG solution (Figure 2C, D), proves that the adverse effect of PEG-8000 on germination was due to an osmotic effect rather than to a specific ion. These results are consistent with findings of Abogadallah and Quick (2009), 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. 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 & 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 2005). In our study, it appears that sensitivity to PEG and NaCl between cultivars is different at the germination stage compared with the seedling emergence, given that both cultivars showed 100% of final germination percentage in NaCl solutions (Figure 2A, B); however, in PEG solution, this decreased as osmotic potentials increased (Figure 1C). The cultivar Omrabia showed a higher germination rate in NaCl solution, but both cultivars decreased in PEG as the osmotic potential increased (Figure1C, D). At the seedling emergence stage, cultivar Omrabia showed a higher emergence rate and final emergence percentage in both NaCl and PEG solutions than BD290273 (Figure 3). Both cultivars were less affected by osmotic potential at the seedling emergence stage, since both showed the final germination percentage of 0% for solutions with osmotic potential –0.4 MPa and higher values in PEG solution (Figure 1C), whereas at the emergence stage both showed 83% (BD290273) and 96% (Omrabia) of final emergence percentage in PEG solution (Figure 3D). The seedling stage was more sensitive to NaCl than to PEG under iso-osmotic potential treatments than germination, which may be affected by NaCl ionic toxicity. This may well be associated with different permeability of roots to NaCl and PEG. Other possible harmful effects of exposure to low osmotic potentials used during germination and seedling emergence include reduced imbibition, reduced growth of embryonic axes which are actually dehydrated via the toxic concentration of salt ions and membrane damage, among others (Poljakoff-Mayber 1994). Since seed germination is more sensitive to salinity and drought stress than the emergence or growth of established seedlings (Freeman 1973), the greater tolerance of durum wheat during emergence to salinity and drought would be an adaptive feature of this species to saline or drought environments. Previous research according to Levitt (1980) indicated that the germination tests were not usually good indicators of differences in salt or drought tolerance among cultivars. Rhoades (1990) reported that some plants are relatively tolerant during germination, but become more sensitive later. In the same way, germination and seedling emergence from laboratory results do 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 cultivars during the whole growth cycle.

The fresh and dry weights, seedling height and leaf area of the seedlings 10 days after imbibition show that both NaCl and PEG-8000 inhibited growth. 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 other studies of germination in the presence of NaCl or non-ionic osmotic solutions such as mannitol or PEG (Kawasaki 1983).

The results obtained in this study revealed that dry weights were less affected than fresh weights and are in agreement with the results obtained by Murillo-Amador (2002) in cowpea. The effect of PEG on the leaf area of 10-days-old plants was greater than that on fresh and dry weights and plant height (Figure 4). The decline in leaf growth is the earliest response of glycophytes exposed to salt or drought stress (Shabala 2006).

In general, the obtained results can provide a guideline for the selection of salt or drought-

tolerant wheat cultivars at germination, seedling emergence and early growth stages. The result may be useful to breeders and plant physiologists and could be reckoned as an efficient tool for screening new or existing cultivars for their salt/drought tolerance.

Our limited understanding of stress-related metabolic phenomena is a major gap in achieving considerable success in developing highly salt/drought tolerant genotypes. Thus, a comprehensive profiling of stress-responsible metabolites and pathways is necessary for the successful molecular breeding of stress-tolerant crop plants. The exploration of additional stress-associated gene resources in both crop plants and highly salt-tolerant model plants will allow a future molecular analysis of salt/drought tolerance mechanisms in potential crop plants (Gregorio *et al.* 2002; Vinocur & Altman 2005). Certainly, extensive work is needed to elucidate well the genetic, biochemical, and physiological basis of wheat salt/drought tolerance. Future knowledge of components of salt/drought tolerance and the identification and cloning of target genes may allow the transfer of multiple genes to produce highly salt-tolerant transgenic cultivars. With the current advances in genetic transformation technology, it seems possible to transfer multiple genes that may act in combination to improve wheat salt tolerance. Further improvements in salt tolerance will undoubtedly result from close interactions between molecular geneticists, physiologists, breeders and agronomists.

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