Morphological Changes in Response to Drought Stress in Cultivated and Wild Almond Species

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Abstract
This study was undertaken to identify morphological changes in young seedlings of 5 Iranian almond species (*Prunus dulcis*, *P. eburnea*, *P. eleagnifolia*, *P. haussknechti,* and *P. scoparia*) under polyethylene glycol-induced drought stress. Drought stress caused a significant reduction in plant growth parameters such as fresh and dry weights of plant organs, leaf number, total leaf area, and leaf relative water content in all almond species. Specific leaf weight also increased significantly in drought-treated plants compared to control. No significant changes in shoot length, individual leaf area, leaf dimension (length and width), or stomatal size and frequency were observed in response to drought treatments. *P. eburnea* had the highest relative water content among the species and showed the smallest decrease in fresh and dry weights of organs and greatest decrease in leaf number and total leaf area (the most reduction in transpiration area) as an adaptive mechanism to drought stress.

Key words: Growth parameters, seedling, stone fruits, water requirement.

Introduction

Plant responses to water deprivation are usually monitored through selected morphological and physiological parameters which have been proven to be good indicators of drought in different studies (Sircelj *et al.*, 2007). Some of the most important standards for evaluating plant genotypes under drought stress are measurements of morphological parameters such as growth, leaf characters, stomatal properties, and water relations. The leaf characteristics of some *Prunus* species have been found to generally reflect drought adaptation. For example, individual leaves of xerotic *Prunus* spp. are smaller than those from a more mesic environment (Rieger and Duemmel, 1992). Yadollahi *et*

al. (2011) reported that seedlings of six almond genotypes had different reactions to water stress, showing the ability to tolerate moderate and severe drought conditions. They concluded that root dry weight/leaf area, lower stomatal size, and lower SLA (specific leaf area) may be related to drought resistance in cultivated almonds. Both the leaf water potential and the relative water content of two olive cultivars decreased when levels of drought stress increased (Boussadia *et al.*, 2008). Zamani *et al.* (2002) studied different irrigation regimes on Iranian almond seedlings, and their results showed that leaf area, shoot length, and dry weight of stem and root were decreased in all cultivars when irrigation intervals increased. In a study of 31 apple cultivars, drought-stressed plants showed significant declines in tree height, trunk diameter, biomass production, and total leaf area (Liu *et al.*, 2012). Aasamaa *et*

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al. (2001) reported a generally high positive correlation between sensitivity to drought and stomatal length, but a negative regression with stomatal frequency in plants. *Prunus webbii* showed a lower sensitivity to water stress than cultivated almonds because of its morphological and physiological characteristics such as lower leaf area, stomatal density and size, and lower leaf water potential (Fanizza and Reina, 1990).

In plants, water deficit induces the expression of different genes involved in stress tolerance at both cellular and molecular levels. Several major classes of genes that are altered in response to water-deficit stress have been described in the *Prunus* species. These genes are entangled in signaling and gene regulation and in the transcription of gene products that support cellular adaptation to water-deficit stress (Campalans *et al.*, 2001; Manuela *et al.*, 2003). Recently, Alimohammadi *et al.* (2013) used the cDNA-AFLP technique to screen for candidate transcripts which are differentially expressed under control and stress conditions in wild almond *P. scoparia* under water-deficit stress. Their results highlighted the importance of starch synthesis, sugar, and ABA mediated signaling pathways as well as the mRNA splicing and epigenetic response of *P. scoparia* in resistance to water deficit. These results will be useful in exploring the functions of these multiple signal-inducible genes in order to unveil the relationship and crosstalk between different signaling pathways involved in *Prunus* resistance to water-deficit.

Non-domesticated germplasm and exotic landraces represent sources of genetic diversity from which important characteristics, such as drought tolerance, can be identified and then utilized in breeding programs (Sorkheh *et al.*, 2012). Wild almond species currently grow in their native habitats all over the world, and their products are used locally. For example, the kernel of *P. scoparia* is used as an edible nut; *P. eleagnifolia* is used as a rootstock for the plum (Gholami *et al.*, 2010). Moreover, many of the above-named species have been used directly as rootstocks for almonds, usually for use under non-irrigated conditions (Sorkheh *et al.*, 2009). Because wild almonds are highly adaptable to unfavorable environmental conditions, these species can be used as rootstock for commercial almond growing. Subsequently, their effects on scion productivity, nut quality, and tolerance to soil-born diseases can be screened (Baninasab and Rahemi, 2007). Furthermore, wild almond species represent valuable germplasm sources for breeding (Gradziel *et al.*, 2001). It is possible to improve almond rootstocks through the screening of wild species and/or by conducting hybridization programs (Kester and Gradiziel, 1996). The aim of the present study was to evaluate the effects of different osmotic potentials of nutrient mediums (caused by PEG-6000) on some morphological traits of young seedlings of five cultivated and wild almonds under greenhouse conditions. This research will provide documentation to improve our understanding of mechanisms involved in the response of young almond plants to drought stress as well as breeding/selecting higher drought resistant genotypes.

Materials and Methods

Plant material and experimental design
A 4×5 factorial experiment (4 stress levels and 5 species) based on a completely randomized design was conducted with three replications. The experiment was carried out at the Faculty of Agriculture, Bu-Ali Sina University (Iran) during 2011–2012. A local genotype of *Prunus dulcis* and four Iranian wild almonds including *P. eburnea*, *P. eleagnifolia*, *P. haussknechti,* and *P. scoparia* were selected as the plant material. Three of the wild species are shrubs exclusively native to Iran, and *P. scoparia* is a tree or shrub species native to Iran and Turkmenistan. Seeds were sown in 15-L plastic pots containing a 1:1:1 (by volume) mix of soil, sand, and manure.

Seedlings were grown in the greenhouse for 6 months (June-November 2011) and then transferred outdoors for natural leaf-shedding. After 2.5 months, pots were again transferred to the greenhouse, and plants were individually transplanted into 7.5-L pots containing a 1:1 (by volume) mix of perlite and cocopeat. Seedlings were supplied with half-strength Hoagland nutrient solution (300 ml a day) until drought treatments were begun.

Drought treatments consisted of a control treatment (osmotic potential of the nutrient solution; Ψs=-0.1 MPa) and three drought stress levels (Ψs=-0.6, -1.1, and -1.6 MPa, respectively). Drought levels were obtained by adding different concentrations of PEG-6000 to the nutrient solution, according to the method of Michel and Kaufmann (1973). Drought levels were maintained for 2 weeks (drought period); then nutrient solutions of all treatments were replaced by that of the control treatment (Ψs=-0.1 MPa), and this level was kept for 4 weeks more (recovery period).

Evaluation of morphological changes

Total shoot length, individual leaf area (LA_i), leaf dimensions, specific leaf weight (SLW), and relative water content (RWC) were measured four times, including one and two weeks after the beginning of drought treatments as well as two and four weeks after the recovery period. Stomatal characteristics were determined at the end of the drought period only, and the other measurements were carried out at the end of the experiment. Analyses of variance of the data were carried out using the SAS program (Version 9.1, SAS Institute Inc., Cary, North Carolina, USA), and the means were compared by Duncan's multiple range tests ($P \leq 0.05$).

Growth parameters. To obtain the total shoot length of each plant, the lengths of all branches were measured. Leaf dimensions (length and width of blade) and individual leaf area (LA_i) were determined using ImageJ software, version 1.32j (National

Institutes of Health, USA). At the end of experiment, all plants were harvested, their green leaves were separated, and data for leaf number per plant and total leaf area (cm^2) were recorded. Fresh and dry weights of root, stem (including branches), leaves, and the whole plant were measured and the root/stem weight ratio was calculated.

Specific leaf weight (SLW). Mature leaves of each plant were sampled and the area of each leaf was measured using ImageJ software (version 1.32j). In order to calculate the SLW (the ratio of leaf dry weight to leaf area expressed as mg cm^{-2}), the same leaves were dried and weighed.

Relative water content (RWC). Leaf RWC was determined as described by Kirnak *et al.* (2001), using small sections of leaves taken from the middle of the lamina.

Stomatal characteristics. The impression approach described by Meister and Bolhar-Nordenkampf (2001) was used to determine the stomatal characteristics of leaves. One fresh and fully-expanded leaf from each replicate of each treatment was selected. Stomatal frequency and size were obtained by examining imprints of the leaves.

Results and Discussion

Fresh weight

Genotypes and drought treatments had significant effects on the FW of plant organs ($P \leq 0.05$). Drought-stressed seedlings had significantly lower root, stem, leaf, and whole plant FW values compared to the control, although absolute values varied by species. For all species, the mean FW of each organ and the root/stem FW ratio (f_R/f_S) under the highest level of drought stress (Ψs=-1.6 MPa) were analyzed as percentages of the control (Table 1). The largest decrease in whole plant FW (50.9%) was found in *P. scoparia*. This reduction was caused mainly by the strong decrease in root FW (75.8%), since the FW of stems and roots were reduced minimally (8.4% and 1.4% reduction, respectively). The lowest decrease in leaf FW (1.4%) occurred in *P. scoparia*, and the f_R/f_S ratio was greatly

reduced in this species (73.4%). This result relates mainly to the strong reduction in root FW. On the other hand, the lowest decrease in whole plant FW (6.5%) was found in *P. eburnea*, indicating a higher level of drought resistance in this species. The highest reduction in leaf FW (13.8%)

occurred in *P. eburnean,* meaning that this species has a higher tolerance to drought than the others. Due to the adaptive mechanism of leaf shedding under drought conditions, *P. eburnea* also had the highest reduction in total leaf area among the examined species (Table 2).

Table 1. Decrease (%) in fresh and dry weights of plant organs under severe drought stress
(Ψs= -1.6 MPa) relative to the control (Ψs= -0.1 MPa) in five almond species

species	roots		stems		leaves		whole plant		f_R/f_S	
	FW	DW	FW	DW	FW	DW	FW	DW	FW	DW
P. dulcis	-44.7	-40.0	-30/9	-22.3	-11/9	-4.6	-39.6	-34.3	-16.6	-21.1
P. eburnea	+0.2	-2.5	+4.7	-22.2	-13.8	-5.9	-6.5	-16.9	-4.2	+30.0
P. scoparia	-75.8	-62.4	-8.4	-34.0	-1.4	-1.0	-50.9	-46.2	-73.4	-35.8
P. haussknechti	-22.1	-27.5	-41.5	-39.5	-13.3	-4.1	-32.2	-35.9	+32.0	+15.1
P. eleagnifolia	-42.4	-42.0	-32.8	-1.1	-2.4	-1.6	-28/2	-38.2	-11.8	-21.8

Table 2. Reduction (as unit and %) in leaf number and total leaf area under severe drought stress
(Ψs= -1.6 MPa) compared to the control in five almond species

species	leaf number		total leaf area	
	unit (n)	%	unit (cm^2)	%
P. dulcis	9.3	10.3	201.2	30.9
P. eburnea	31.7	33.3	268.1	51.9
P. scoparia	14.7	36.7	11.4	24.6
P. haussknechti	7.3	9.0	99.4	16.8
P. eleagnifolia	13.0	27.3	22.1	29.4

Dry weight

Genotypes and drought treatments significantly influenced the DW of plant organs ($P \leq 0.05$). For all species, the DW of organs and subsequently the whole plant decreased as drought stress levels increased. A decrease in dry matter may be due to the considerable reduction of photosynthesis and plant growth (Shao *et al*., 2008). Changing resource pools (e.g., water or nutrient availability) may also affect distribution of biomass. As suggested by Arji and Arzani (2000), decreasing root DW under drought conditions may be caused by a decrease in the accumulation of

root carbohydrates. Therefore, plants with high amounts of dry mass under drought stress can be considered as drought tolerant genotypes. For all species, the mean DW of each organ as well as root/stem DW ratio (f_R/f_S) under the highest level of drought stress (Ψs=-1.6 MPa) was analyzed as a percentage of the control (Table 1). The largest reduction in DW of the whole plant (46.2%) was observed in *P. scoparia*, which might be a reason for the higher sensitivity of this species to drought stress. The greatest reduction in root DW (62.4%) and the least decrease in leaf DW (1.0%) were also found in *P. scoparia*. In addition,

the greatest change in d_R/d_S ratio (a 35.8% decrease) was observed in this species, indicating that root DW was affected by drought stress more than stem DW. On the other hand, the lowest reduction in the DW of roots and stems (2.5% and 22.2%, respectively) was found in *P. eburnea*. The d_R/d_S ratio for severe drought treatment had a 30% increase as compared to the control, showing that stem DW was influenced by drought stress more than root DW in this species. Although the strongest reduction in leaf DW (5.9%) was observed in *P. eburnea* (because of leaf shedding), the whole plant DW for this species was not affected as much by drought. The least reduction in whole plant DW (16.9%) occurred in *P. eburnea*, showing that *P. eburnea* had a higher level of drought tolerance than the other species.

Total shoot lengths
The results of ANOVA showed that genotypes had a significant effect on total shoot length, and the highest and lowest shoot lengths were observed in *P. eburnea* and *P. eleagnifolia*, respectively, at all measurement times. No significant changes in total shoot lengths were observed in response to drought stress treatments. Therefore, it seems that this trait may not be used as a drought stress marker in young seedlings of almond species.

Leaf number and total leaf area (LA$_t$)
Drought stress caused significant reductions in the leaf number and LA$_t$ of seedlings in all species (Fig. 1). Significant differences among genotypes and treatments ($P \leq 0.05$) were observed in both examined parameters, confirming the results of previous studies on fruit trees such as almonds (Zamani *et al*., 2002), grapevines (Gomez-del *et al*., 2002), peaches (Rieger *et al*., 2003), olives (Bacelar *et al*., 2009), and apples (Liu *et al*., 2012). Theoretically,

the loss of leaf area is an important stress avoidance strategy and is considered a plant's first defensive mechanism against drought stress (Levitt, 1980). During water stress, depending on the intensity and duration of the drought, plants tend to minimize transpirational water loss by reducing their number of leaves (Jones and Cortlett, 1992). In this study, both leaf number and LA$_t$ decreased in all species as the drought stress level increased. For each of five species, leaf number and LA$_t$ at the highest level of drought stress was compared with those of the control (Table 2). The results showed that seedlings of *P. scoparia* and *P. eburnea* had the largest decrease in leaf number with a 36.7% and 33.3% reduction, respectively, with increasing Ψs of the nutrient solution from -0.1 MPa to -1.6 MPa. On the other hand, the least reduction in leaf number occurred in *P. haussknechti* (9%) and then *P. dulcis* (10.3%). The greatest and the least reduction in LA$_t$ was also found in *P. eburnea* (51.9%) and *P. haussknechti* (16.8%), respectively. Since individual leaf area, length, and width were not affected by drought treatments, the reduction in LA$_t$ was due mainly to leaf abscission and the reduction in number of leaves per plant, especially under high levels of drought stress. It is interesting to note that leaf abscission in *P. eburnea* was more pronounced and started earlier than in the other species, especially in severe drought stress (approximately at the end of the first week of the drought period), and continued until the end of the experiment, whereas in the other species it started near the beginning of the recovery period. Thus, defoliation in this species may represent a quick response and a morphological adaptation to reduce water loss and redistribute resources under severe drought stress conditions.
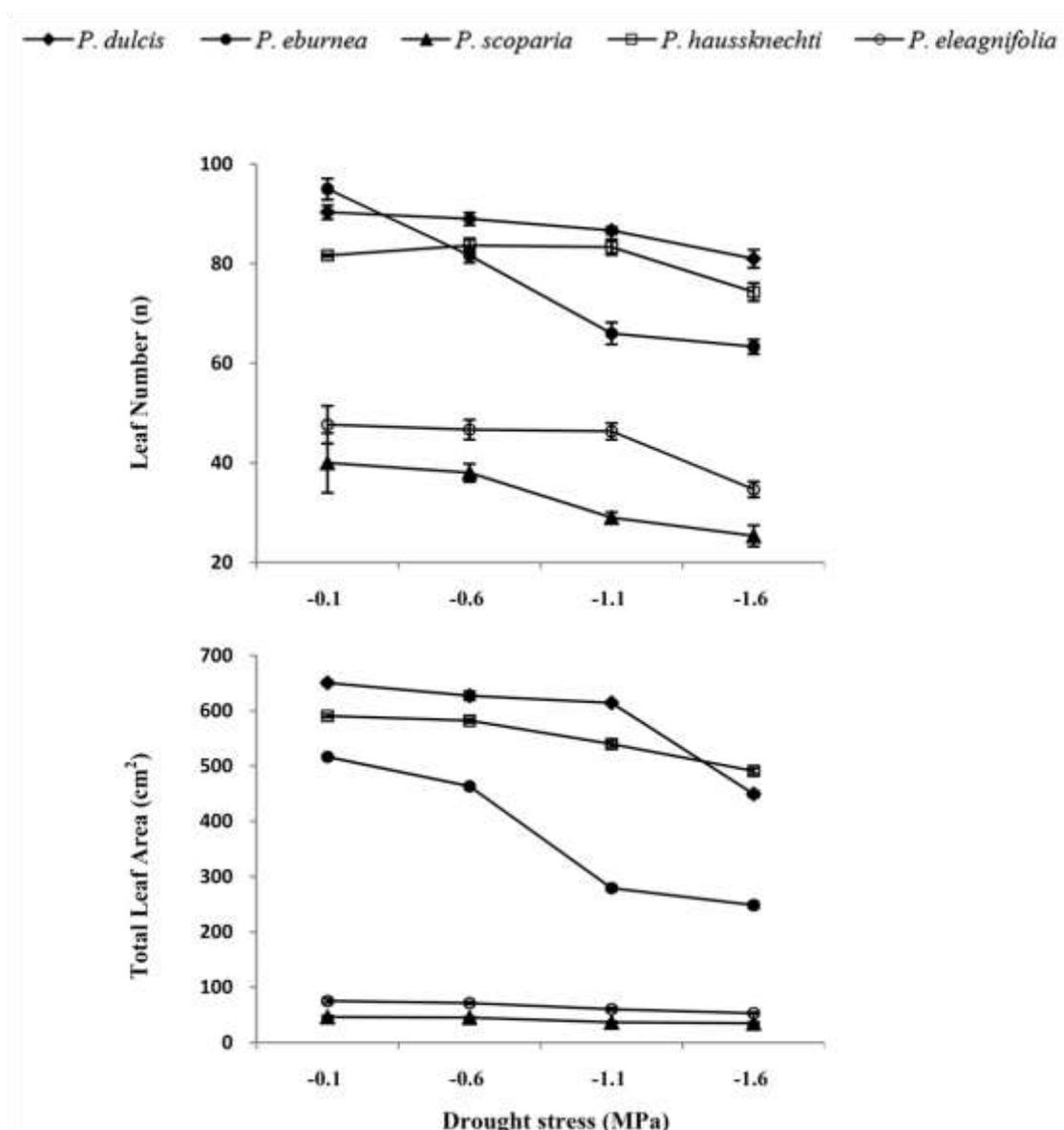
Fig. 1. Effects of drought treatments on leaf number (top) and total leaf area (down) of five almond species. Vertical bars indicate ± standard error (SE) of three replications

Specific leaf weight (SLW)

There were significant differences ($P \leq$ 0.05) in SLW among the species, with *P. eburnea* having the highest SLW compared to the other genotypes at all measurement times. The lowest amounts of SLW were found in *P. dulcis* at the 1st and 2nd, in *P. scoparia* at the 3rd, and in *P. haussknechti* at the 4th measurement time. At the two latter times, there were no significant differences between *P. scoparia*, *P. haussknechti,* and *P. dulcis*. Drought stress caused a significant increase in SLW (Fig. 2), so that the lowest and highest values of SLW for all species

and all measurement times occurred at -0.1 and -1.6 MPa, respectively.

Specific leaf weight indicates leaf dry mass per area. It has been widely exploited as a reliable morpho-physiological marker contributing to drought tolerance for various crop plants (Ali et al., 2011). Drought stress was found to have caused an increase in SLW in almost all studies. Increases in SLW under drought conditions have also been reported in some fruit trees such as peaches (Martinez, 2010) and kiwifruits (Gucci et al., 1996). Xu and Zhou (2005) suggested that variations in SLW

under drought conditions may be caused by variations in the concentration of carbohydrates such as starch. Dichio et al. (2007) concluded that SLW increased by drought stress in peach trees is due to the decrease in the fruit's competition. As competition between fruits and leaves decreases, the accumulation of dry masses in leaves and subsequently leaf weight per area increase.

Some authors believe that changes in SLW under drought conditions may be induced by anatomical and morphological changes in leaves. Kramer (1983) found that mild drought increased SLW by increasing leaf and cuticle thickness and the amount of surface waxes. Krause *et al*. (1993) suggested that because cell division is apparently more sensitive to low water availability than photosynthesis, assimilates are used for differentiation products. It has been also reported that drought stress causes an increase in sclerenchyma cells and cell wall thickness and thereby increases SLW (Krause *et al*., 1993).
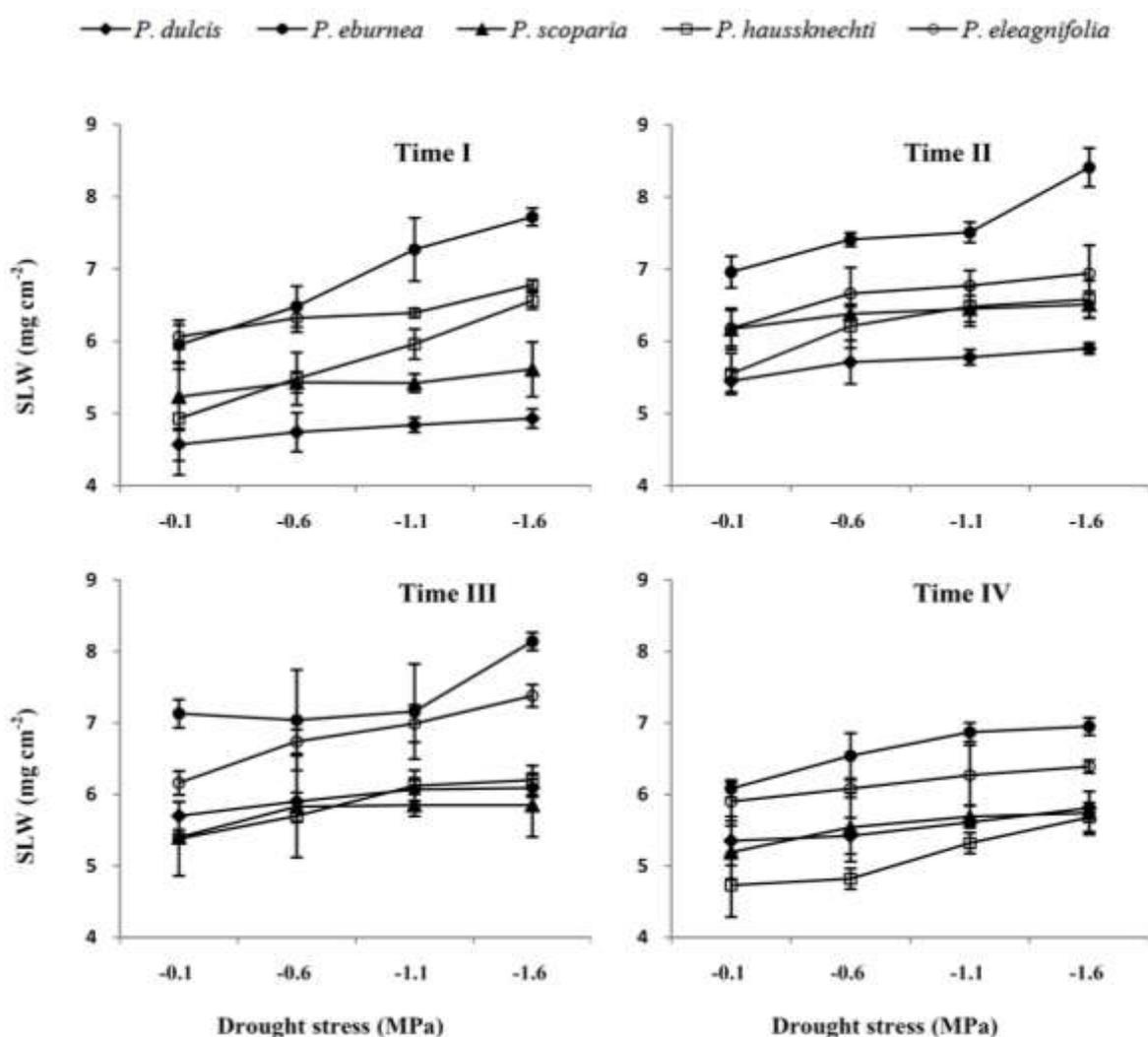

Fig. 2. Effects of drought treatments on specific leaf weight (SLW) of five almond species at different measurement times. Times I and II are respectively one and two weeks after the beginning of drought treatments, and times III and IV are two and four weeks after the recovery period, respectively. Vertical bars indicate ± standard error (SE) of three replications

Although to date no comparison of drought-resistant and drought-sensitive plants has been done, with due attention to the results of similar studies on drought stress, it can be expected that the SLW in drought-resistant genotypes might be less influenced by drought stress than sensitive ones. In this study, drought stress caused an increase in the SLW of almond species. The control plants of all samples had the lowest SLW values, and the highest values were observed in plants treated with the highest level of drought stress (-1.6 MPa). A comparison of the differences in SLW values between these two groups of plants (Table 3) showed that SLW for *P. dulcis* is less influenced by drought stress than other species. This may indicate a higher drought tolerance in *P. dulcis*.

Table 3. Variations of specific leaf weight (mg cm^{-2}) and relative water content (%) of five almond species under severe drought stress (Ψs= -1.6 MPa) compared with the control (Ψs= -0.1 MPa) at different measurement times

species	SLW (mg cm^{-2}) at different measurement times					RWC (%) at different measurement times				
	I	II	III	IV	average	I	II	III	IV	average
P. dulcis	0.36	0.45	0.39	0.46	0.42	-7.03	-6.26	-2.75	+2.28	-3.44
P. eburnean	1.77	1.45	1.01	0.87	1.28	-7.72	-8.05	-3.19	+1.23	-4.43
P. scoparia	0.38	0.34	0.45	0.55	0.43	-9.23	-10.80	-4.36	-0.98	-6.34
P. haussknechti	1.63	1.03	0.82	0.95	1.11	-8.03	-8.71	-3.31	+1.88	-4.54
P. eleagnifolia	0.72	0.76	1.22	0.49	0.80	-15.65	-16.18	-9.72	-3.40	-11.24

Relative water content (RWC)

Relative water content is considered an alternative measurement of water status, and a plant's drought resistance is related to its ability to maintain high RWC in leaves under stress (Faraloni *et al.*, 2011). Statistically significant differences ($P \leq 0.05$) were observed in the RWC of leaves between the species. A comparison of RWC values in different species at the highest level of drought stress showed that *P. eburnea* had the highest leaf RWC under severe drought stress, which suggests it is more resistant to water stress than the other species. The mean RWC of the samples (average of two measurements during drought period) ranks the five examined species in the following order: *P. eburnea* (83.9%), *P. dulcis* (83.5%), *P. haussknechti* (81.5%), *P. scoparia* (79.8%) and *P. eleagnifolia* (75.9%).

Leaf RWC was significantly reduced ($P \leq 0.05$) in response to drought treatments in all species (Fig. 3). The control plants and the -1.6 MPa treated ones showed the highest and lowest RWC of leaves, respectively. Water status of plants, however, was improved after drought was removed, so the differences between treatments were gradually reduced. No significant difference between treatments was observed at the end of the recovery period. A comparison of changes in leaf RWC of plants treated with -1.6 MPa relative to controls (Table 3) showed that the RWC of *P. dulcis* was less influenced and that of *P. eleagnifolia* was more influenced by drought stress than the other species.
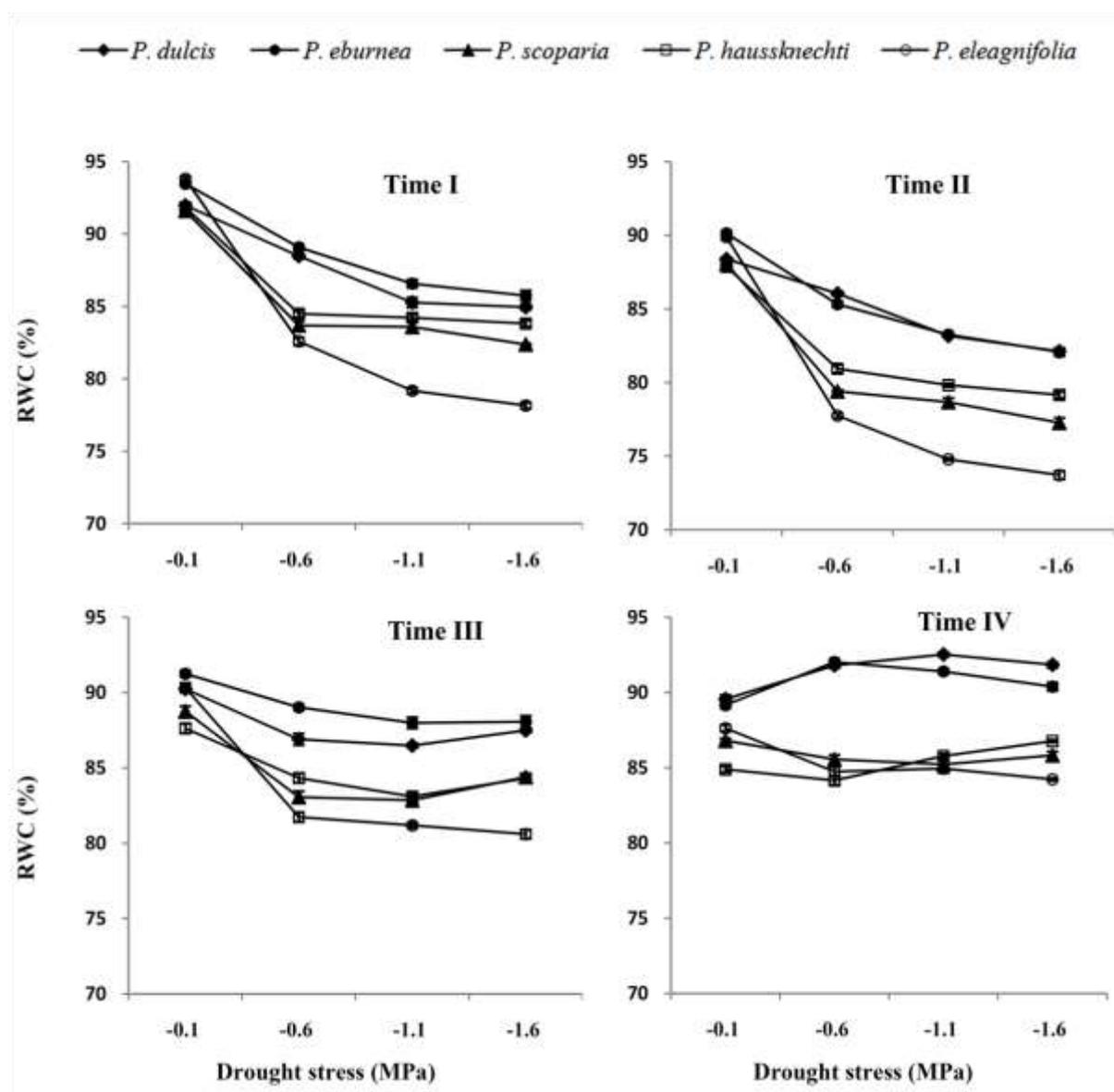
Fig. 3. Effects of drought treatments on relative water content (RWC) of five almond species at different measurement times. Times I and II are one and two weeks after the beginning of drought treatments, respectively, and times III and IV are two and four weeks after the recovery period, respectively. Vertical bars indicate ± standard error (SE) of three replications

Leaf characteristics

Genotypes had a significant effect ($P \leq 0.05$) on leaf dimensions (length and width) and individual leaf area (Table 4). At all measurement times, *P. haussknechti* had the greatest and *P. scoparia* had the least values for the above-named parameters. On the other hand, results showed that decreasing osmotic potential of the nutrient solution down to -1.6 MPa had no significant effect on leaf characteristics compared to the control. Changes in leaf characteristics under different levels of drought stress showed no clear pattern. This might be due to the relatively short time of the experiment which did not allow new leaves (probably with different characteristics) to develop. However, results of this study showed that parameters related to leaf morphology such as leaf length, leaf width, and area of lamina were not suitable indexes to evaluate drought resistance or sensitivity in almond species.

Table 4. Results of the analysis of variance and means comparison of leaf characteristics for five almond species under different levels of PEG-induced drought stress

	leaf length (mm)				leaf width (mm)				leaf area (mm^2)			
	I	II	III	IV	I	II	III	IV	I	II	III	IV
							factor A (genotypes)					
G1	49.3[a]	51.1[a]	47.3[a]	49.0[b]	17.0[b]	17.7[b]	16.6[b]	16.6[b]	596.1[b]	647.6[b]	572.0[b]	591.4[b]
G2	49.0[a]	46.0[b]	47.7[a]	42.8[c]	19.2[ab]	18.9[ab]	18.8[a]	17.8[b]	681.4[a]	614.3[b]	652.2[ab]	571.6[b]
G3	21.1[c]	20.2[c]	22.1[c]	19.9[d]	6.6[d]	6.6[d]	6.7[d]	6.4[c]	107.7[d]	101.1[c]	115.2[c]	124.0[c]
G4	50.7[a]	53.6[a]	45.4[a]	54.4[a]	19.5[a]	19.5[a]	19.3[a]	19.6[a]	742.6[a]	768.0[a]	677.9[a]	768.8[a]
G5	27.5[b]	23.3[c]	27.2[b]	21.5[d]	10.0[c]	8.7[c]	9.5[c]	7.9[c]	204.0[c]	153.4[c]	194.7[c]	151.2[c]
						factor B (stress levels; MPa)						
-0.1	39.0[a]	38.1[ab]	38.1[a]	36.1[b]	14.7[a]	14.0[a]	14.3[a]	12.9[a]	477.4[a]	444.8[ab]	463.3[a]	411.1[b]
-0.6	40.4[a]	36.2[b]	38.4[a]	35.1[b]	14.3[a]	13.9[a]	14.5[a]	13.3[a]	484.0[a]	427.2[b]	482.0[a]	417.0[b]
-1.1	39.6[a]	39.8[ab]	38.4[a]	37.6[ab]	14.5[a]	14.3[a]	13.9[a]	14.5[a]	459.4[a]	489.6[a]	409.8[a]	442.6[ab]
-1.6	39.0[a]	41.3[a]	36.8[a]	41.3[a]	14.4[a]	15.0[a]	14.0[a]	13.9[a]	444.7[a]	466.0[ab]	414.6[a]	494.9[a]
						P values						
A	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
B	0.9425	0.0568	0.8650	0.0172	0.9771	0.1863	0.7936	0.2049	0.6186	0.1173	0.1249	0.0607
A×B	0.1453	0.1420	0.0401	0.3023	0.2110	0.0001	0.0001	0.0078	0.0001	0.0001	0.0001	0.0001
CV	17.65	13.26	15.70	14.46	19.57	9.93	12.33	15.37	19.08	15.82	21.99	20.52

Different letters within the columns indicate significant differences ($P < 0.05$). I, II, III, and IV: measurement times including one and two weeks after the beginning of drought treatments and two and four weeks after the recovery period, respectively; G1: *P. dulcis*; G2: *P. eburnea*; G3: *P. scoparia*; G4: *P. haussknechti*; G5: *P. eleagnifolia*; CV: coefficient of variation.

Stomatal parameters

Stomatal size (length and width) was significantly influenced ($P \leq 0.05$) by genotypes (Fig. 4). The greatest (42.59 μm) and the lowest (22.61 μm) length of stomatal pore were observed in the leaves of *P. dulcis* and *P. haussknechti*, respectively. Moreover, *P. haussknechti* and *P. scoparia* had the greatest (19.51 μm) and the least (13.87 μm) stomatal width, respectively, among the examined species. Stomatal density was significantly influenced by genotypes as well, and *P. scoparia* and *P. haussknechti* had the highest (251.51) and the lowest (197.85) stomata mm^{-2}, respectively.

Because of their role in transpiration and photosynthesis, stomata can influence water loss, water use efficiency, and plant yield (Manuela *et al*., 2003). Stomatal size and frequency differ among various plant species. The number of stomata per leaf area may be a good criterion for identifying and selecting drought resistant genotypes. A study by Blum and Sullivan (1986) on millet indicated that there is a direct correlation between low stomatal density and resistance to drought stress. Aasamaa *et al*. (2001) found a generally high negative correlation between sensitivity to drought and stomatal frequency in temperate deciduous trees. In our study, the lowest number of stomata per leaf area (197.85 stomata mm^{-2}) was found in *P. haussknechti*, and *P. eburnea* with 198.07 stomata mm^{-2} was ranked second. Therefore, these two species may have a higher resistance to drought stress than the others.
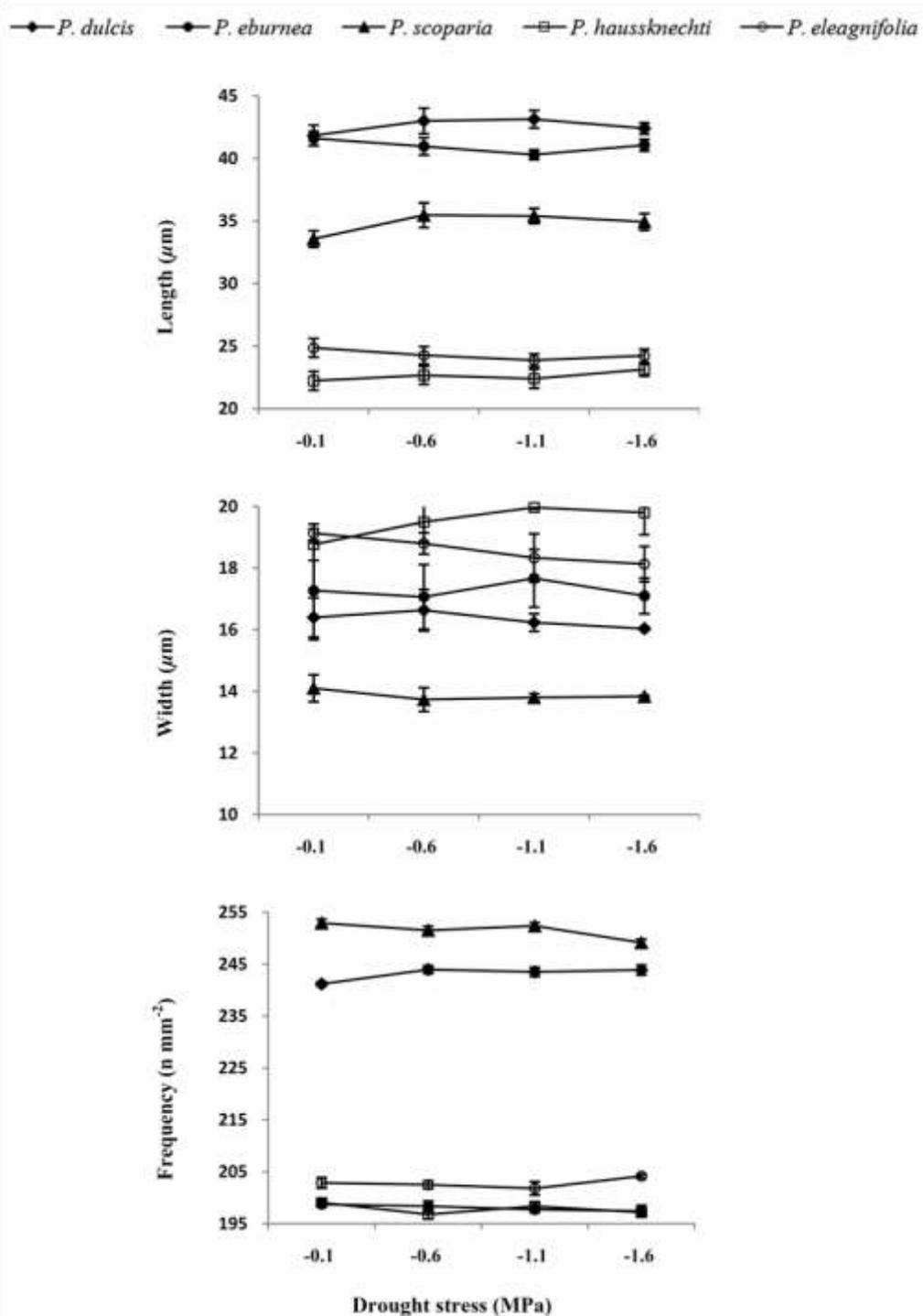
Fig. 4. Effects of drought treatments on stomatal characteristics of five almond species. Vertical bars indicate ± standard error (SE) of three replications

Large and small stomata respond differently to water deficit. As light intensity or water status of the plant changes, larger stomata tend to open faster and to close later than smaller ones; therefore, they are more sensitive to drought deficit (Tanaka *et al*., 2005). Aasamaa *et al*. (2001) found a positive correlation between sensitivity to drought and stomatal length in temperate deciduous trees. Fanizza and Reina (1990) suggested that the lower sensitivity of *P. webbii* to

water stress compared to cultivated almonds is at least partly due to its lower stomatal density and size. It was also concluded by Yadollahi *et al.* (2011) that lower stomatal size might be related to drought resistance in cultivated almonds. In our study, *P. haussknechti* had the smallest stoma length.

Environmental factors such as moisture can alter stomatal size and density. For example, early reports showed an increase in stomatal density and a decrease in cell size under water deficit, indicating that drought adaptation could occur (Palasciano *et al.*, 2005; Martinez *et al.*, 2007). However, in this study drought stress treatments had no significant effect on stomatal size or density in leaves of almond species. In this study, drought stress had no effect on stomatal parameters of leaves that had already been developed since the duration of the drought period was only 15 days, and no new leaves developed during the experiment.

Conclusion

Drought stress induced by PEG-6000 caused an increase in SLW and a decrease in RWC of leaf, fresh and dry weight of plant organs, number of leaves per plant, and total leaf area, but had no significant effects on total shoot length, individual leaf area, leaf dimensions, stomatal size and density, or root/stem fresh and dry weight ratios. The almond species examined in this study showed significant differences in most of the measured parameters, but no significant differences were observed among these species in terms of total shoot length, root/stem fresh weight ratio, and root/stem dry weight ratio. It seems that the latter three parameters are not appropriate indexes for use in evaluating drought resistance or sensitivity in young seedlings

of almond species. It was also demonstrated that traits related to leaf morphology such as individual leaf area, leaf length, and leaf width may not be good markers for drought stress.

All examined species had similar responses to drought stress treatments, but the intensity of these responses was different in the various species. For example, the increase in SLW of *P. dulcis* was less than that of the other species under drought treatments. This probably means that *P. dulcis* leaves are less sensitive to drought stress. Furthermore, there were morphological differences among the examined species. For example, *P. haussknechti* had the least stomatal density and stomatal length among the species. This may act as an adaptive mechanism to undesirable environmental conditions, in particular water deficit.

In conclusion, *P. eburnea* had the highest RWC of leaves under severe drought stress compared to the other species, the least reduction in fresh and dry weights of roots, stems and the whole plant, and the greatest reduction in total leaf area (mainly because of its quick abscission of its leaves under drought conditions). Considering the above results and observations, it can be concluded that *P. eburnea* possesses a higher level of resistance to drought stress compared to the other species.

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