Response of Almond Genotypes/Cultivars Grafted on GN15 'Garnem' Rootstock in Deficit-Irrigation Stress Conditions

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Abstract

This study was conducted to evaluate the response of Iranian promisinglate blooming almond genotypes to deficit-irrigation stress on GN15 rootstock. One-year old plants subjectedto three deficit-irrigation, including moderate and severe stress (soil water potential, Ψ_{soil} = -0.8 and -1.6 MPa, respectively) and a control treatment (Ψ_{soil}= -0.33 MPa), were applied for six weeks to five grafting combinations. A factorial experiment was conducted with a CRD which included three irrigations factors, five genotype factors and three replications. Genotypes/cultivarsincluded: 'K3-3-1', 'H', '13-40', 'Sahand' and 'Ferragness' grafted on GN15 rootstock. Deficit-irrigation stress caused a significant reduction in plant growth parameters such as fresh and dry weights of plant organs, leafnumber, and total leafarea and leaf relativewatercontent in all almond genotypes and cultivars. Specific leaf weight (SLW) and leafabscission also significantly increased in drought-treated plants compared to the control group. Total shoot length, individual leaf area, leaf dimension (length and width), stomatal size and frequency were decreased in response to deficit-irrigation treatments. In response to stress, the'Ferragnes' and 'Sahand' cultivars on GN15 rootstock showed the highest relative water content (RWC) among the genotypes and showed the smallest decrease in fresh and dry weights of organs. The '13-40' and 'K3-3-1' genotypes showed the greatest leaf abscission and a decrease in the total leaf area, (the most reduction in transpiration area).

Keywords: GN15,Growth parameters, RWC, SLW.

Introduction

Almond (*P. dulcis* Mill, syn: *P. amygdalus* Batsch) is the 7th crop, after pistachio, grape, date, apple, walnut and orange in terms of the cultivated area in Iran, with 185,000 -ha and annual production of 155, 527 Tons (FAO stat, 2013). Water limitation is an important factor in the reduction of agricultural crop production, which is related to global warming and climate changes. On the other hand, most stone fruits have limitations around the world as a result of the prevalence of calcareous soils, whereas most rootstocks show lime-induced chlorosis. Thus, the need for rootstocks to overcome these shortcomings has become essential for growing stone fruit in many regions. 'Felinem=GN9', 'Garnem = GN15', and 'Monegro=GN22' have been released as

potential rootstocks for several stone fruit species that are grown in soils having these limitations (Felipe, 2009). This clones tolerant tosoil chemical limitation, as a result of alkaline soils (pH =8.0 to 8.5) that contain high levels of active lime (10% to 12%) (De la Guardia *et al*., 1995), iron chlorosis similar to 'GF677' and 'Adafuel' (Felipe, 2009) and a high level of resistance to the main root-knot nematode species attacking *Prunus* (Pinochet *et al*., 1999). The adaptation of 'Felinem' and 'Garnem' to poor soils is mostly good if the soils are well drained. The breedingof fruit tree rootstocks for environmental stress tolerance is difficult and time-consuming. Some wild almonds currently grow in their native habitats all over the world, and their products are used locally. For example, *P. eleagnifolia*is is used as a rootstock for the plum (Gholami *et al*., 2010). Many of species have been directly used as rootstocks for almonds, usually for use under non-irrigated conditions (Sorkheh*et al.*, 2012). 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).

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). Almond is a drought resistant species and highly adapted to a wide range of soil water availability (Isaakidis*et al*., 2004). The tolerance of almond trees to water stress is related to adaptive mechanisms present in their leaves or rootstock. 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. In 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 al.* (2001) reported a generally high positive correlation between sensitivity to drought and stomatal length and a negative regression with stomatal frequency in plants. Fanizza and Reina (1990) showed a lower sensitivity to water stress in *Prunuswebbii*than cultivated almonds because of its morphological and physiological characteristics such as lower leaf area, stomatal density and size, and lower leaf water potential. 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 (Manuela *et al*., 2003). 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. It is possible to improve almond rootstocks through the screening of wild species and/or by conducting hybridization programs (Kester and Gradziel, 1996). The aim of the present study was to evaluate the effects of different level of drought stress on some morphological traits of young cultivated almond seedlings grafted on GN15 'Garnem' rootstock under greenhouse conditions. This research will provide documentation to improve our understanding of mechanisms involved in the response of young almond plants on GN15 rootstock to drought stress as well as breeding and selecting higher drought resistant genotypes.

Materials and Methods

Experimental Site

This study was conducted in the Horticultural Station of Sahand (46◦ 45´ E, 38◦ 15´ N), East Azerbaijan Agricultural and Natural Resources Research and

Education Center (Iran) during the 2014-2015 growing season.The plant materials, which were used in this experiment, were five almond (*P. dulcis* Mill.) genotypes including:1) 'K3-3-1':open pollinated of Tardy Nonpareil,2) 'H':HybridShokofeh×Feragness, 3) '13-40 :selection of native almonds, 4) commercial Iranian almond cultivar named `Sahand' cultivar `from Horticultural Station of Sahand and 5) commercial almond cultivar named 'Ferragnès' cultivar, which were obtained from the almond collection of Seed and Plant Improvement Institute (SPII) and grafted onto GN15 (almond × peach) hybrid rootstock (Felipe, 2009) in 25th August, 2014 and were controlled during the 2014 growing season. Grafted seedlings were transplanted on March 5, 2015, into the 20L containers (one seedling per container) in the experimental glasshouse. Each container was 0.40 m in diameter and 0.35 m deep and a hole at the bottom for drainage. Day and night temperatures were 25 - 40 and 20 - 25°C, respectively. The relative humidity of the greenhouse was 55-65% . The soil was silty loam consisted of humus, soil and sand (1:1:1). The soil comprised of silt (6 - 8%), clay (22 - 40%)and sand (50 -70%), pH 7.4 - 7.8, Mg 371.9 mg^{-L}, Na 1155 mg^{-L}, Ca 489.9 mg^{-L}, K 545 mg^{-L}, Fe 210 µg$^-$L, Zn 184 µg^{-L}.

Plants were supplied with a soluble 20:8:12 N: P: K fertilizer and well watered before beginning of measurements, until plants reached 30 cm in height. A factorial experiment was conducted with a randomized complete block design which included three irrigations factors, five genotype factors and three replications. On June 22, 2015, treatments were applied based on Ψ_{soil} from soil moisture content curve based on results obtained from the soil samples (Soil and Water Research Institute, Tehran, Iran). The soil-water balance was analyzed in fully irrigated trees. The control pots were irrigated based on drainage lysimetery every two days.

Plants were kept in the nominated Ψ_{soil} (soil water potential) for six weeks. Treatments were: T1 = control pots were watered regularly to field capacity (well irrigated, Ψ_{soil} = -0.33 MPa), T2 = seedlings kept in Ψ_{soil} = -0.8 MPa as moderate drought stress, T3 = seedlings kept in Ψ_{soil} = -1.6 MPa as severe drought stress. Drought stress treatments in the experiment ended on 6th of August 2015, a total of six weeks. For further analysis, plants were harvested and divided into leaves and roots, washed with tap and distilled water and immediately frozen in liquid nitrogen. Plant materials were then transferred to the University of Tabriz.

Evaluation of morphological changes

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 (LAi) were determined using image j 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). The leaf disc (1cm diameter from the middle of the lamina at 14:00 h) masses (FM) of each treatment

were recorded. They were then hydrated for 24 hours at 5°C in darkness. This was followed by a state of water saturation (constant mass obtained) which was finally weighed (TM). Leaf discs were oven-dried at 75°C for 72 hours and dry mass (DM) was then recorded. RWC was calculated according to the following expression.

RWC [%] = (FM − DM)/(TM − DM) × 100

Stomatal characteristics

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

Analyses of variance of the data were carried out using the SPSS program (Version20, IBM Institute, USA), and the means were compared by Duncan's multiple range tests ($P \leq 0.05$).

Results

Fresh weight (FW)

Drought-stressed plants had significantly lower root, stem, leaf, and whole plant fresh weight (FW)values compared to the control. For all genotypes, the mean FW of each organ and the root/stem FW ratio (fR/fS) under the severe drought stress (Ψ_{soil} = -1.6 MPa) were analyzed as percentages of the control treatment. The greatest decrease in whole plant FW (33%) was found in '13-40'genotype. This reduction was mainly caused by the strong decrease in root (35.7%), and leaves (38.9%). The FW of stemsand roots were minimally reduced (2.6% and 23.9% reduction, respectively in 'Sahand' and 'Fragness' cultivars). The lowest decrease in leaf FW (24.2% and 26.2%) occurred in the 'Fragness' and 'Sahand' cultivars, and the fR/fSratio was greatly reduced in '13-40' and 'K3-3-1'(Table 1). This result relates mainly to the strong reduction in root FW. On the other hand, the lowest decrease in whole plant FW (13.4%) was found in 'Fragness' cultivar, indicating a higher level of drought resistance on GN15 rootstock among the evaluated genotypes.

Table 1. Decrease in fresh and dry weights (%) of plant organs under severe drought stress (Ψs= -1.6 MPa) relative to the control (Ψs= -0.33 MPa) in five almond genotypes on GN15 rootstock.

Genotypes	Roots		Stems		Leaves		Whole plant		F_r/F_s	
	FW(%)	DW(%)	FW(%)	DW(%)	FW(%)	DW(%)	FW(%)	DW(%)	FW(%)	DW(%)
Sahand	-25.3	-25.4	-2.6	-6.00	-26.2	-32.9	-14.1	-17.3	-6.9	+12.7
Fragness	-23.9	- 24.4	-3.9	-11.6	-24.2	-22.3	-13.4	-14.6	-4.6	+20.3
13-40	-35.7	-35.4	-29.9	-13.9	-38.9	-39.2	-33.0	-33.0	-12.4	-11.9
H	-27.9	-31.3	-32.2	-12.6	-37.2	-25.2	-30.7	-30.1	-7.7	-6.3
K3-3-1	-27	-27.2	-36.4	-13.6	-34.4	-33.4	-30.8	-31.1	-11.3	-10.7

Dry weight (DW)

Genotypes and drought treatments significantly influenced the dry weight (DW) of plant organs ($P \leq 0.05$) for all genotypes. The DW of organs and subsequently the whole plant was decreased compared to control group. The greatest decrease in DW of the whole plant (33%) was observed in '13-40' which might

be a reason for the higher sensitivity of this genotype to drought stress. The greatest reduction in root DW (35.4%) and the least decrease in leaf DW (22.3%) were found in '1340' and 'ferragness,' respectively. In addition, the greatest change in fR/fS ratio (11.9% decrease) was observed in '13-40' genotype, 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 (24.4% and 11.6%, respectively) was found in 'Ferragness'. The fR/fS ratio for severe drought treatment had a 20.3% increase as compared to the control, showing that stem DW was influenced by drought stress more than root DW in this cultivar on GN15 rootstock. Although the highestreduction in leaf DW (39.2%) was observed in '13-40'(because of leaf shedding), the whole plant DW for this genotype was less affected by drought. The least reduction in whole plant DW (14.6%) occurred in 'Ferragness,' showing a

higher level of drought tolerance than the other genotypes.

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 'Ferragnes' and '13-40', respectively (Fig. 1). Significant changes in total shoot lengths were observed in response to drought stress treatments. Therefore, it seems that this trait may be used as a drought stress marker in young seedlings of almond genotypes.
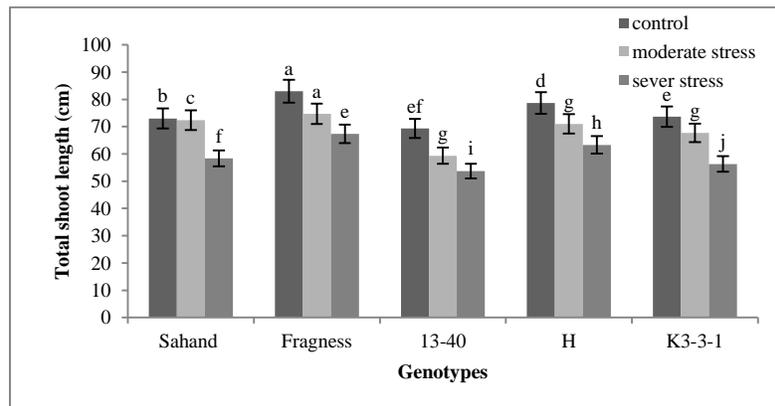

Fig. 1.Effects of drought treatments on total shoot length of five almond Genotypes on GN15 rootstock.
Vertical bars indicate ± standard error (SE) of three replications.

Leaf number and total leaf area (LAt)

Drought stress caused significant reductions in the leaf number and LAt of plant in all genotypes (Fig. 2). Significant differences among genotypes and treatments (P ≤ 0.05) were observed in both examined factors. The results showed that seedlings of '13-40'and 'K3-3-1'had the largest decrease in leaf number with a 76.8% and 83% reduction, respectively, with increasing of the soil

water potential from -0.33 MPa to -1.6 MPa. On the other hand, the least reduction in leaf number occurred in 'Fragness' and Sahand(33 and 35.7%), respectively. The greatest and the least reduction in LAt was also found in 'Ferragness'(51.7%) and 'H'(37%), respectively.
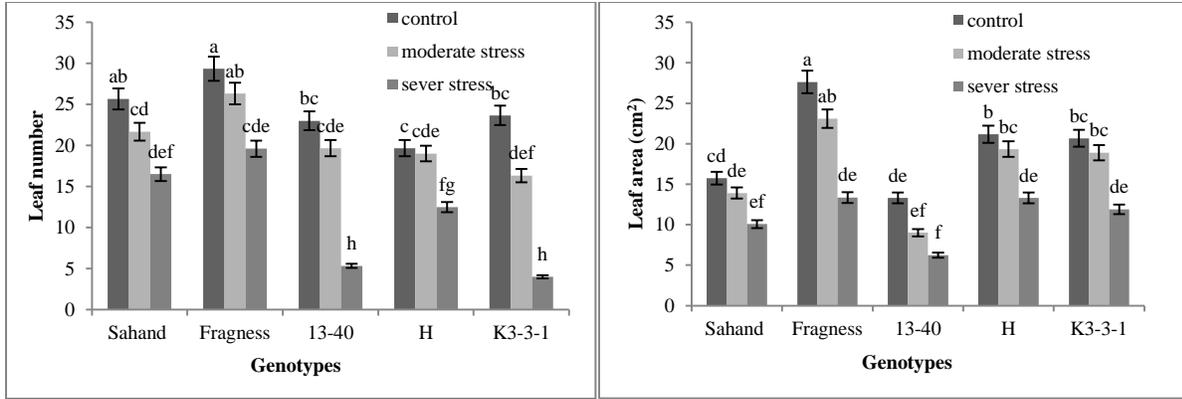
Fig. 2. Effects of drought treatments on leaf number (left) and total leaf area (right) of five almond Genotypes on GN15 rootstock. Vertical bars indicate ± standard error (SE) of three replications. Values by the same letter do not differ significantly according to the Duncan's multiple range test (*P*≤0.05).

Specific leaf weight (SLW)

There were significant differences ($P \leq 0.05$) in specific leaf weight (SLW) among the genotypes, with 'Ferragness' having the highest SLW compared to the other genotypes. There were not significant differences between sever stress and control treatment in 'Sahand' and '13-40' genotypes at measurement time. The lowest amounts of SLW were found in '13-40' and 'K3-3-1' compared to the control. Drought stress caused a significant increase in SLW (Fig. 3), so that the lowest and highest values of SLW for all genotypes and all measurement times occurred at -0.33 and -1.6 MPa, respectively.
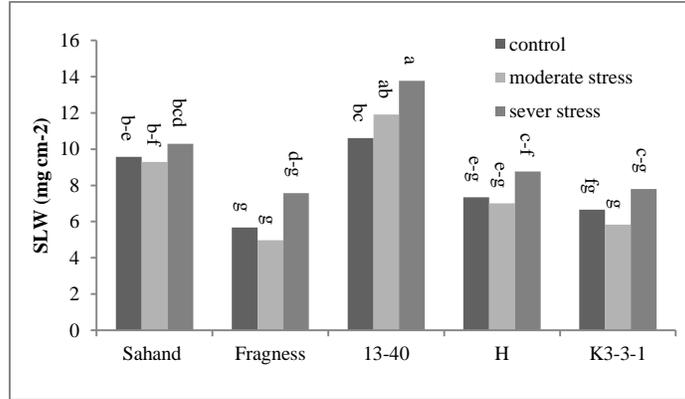

Fig. 3.Effects of drought treatments on specific leaf weight (SLW) of five almond genotypes at end of drought stress.Values by the same letter do not differ significantly according to the Duncan's multiple range test (*P*≤0.05).

Relative water content (RWC)

The results presented in Fig. 4 showed that statistically significant differences ($P \leq 0.05$) were observed in the RWC of leaves between the genotypes on GN15 rootstock. A comparison of RWC values in different genotypes under severe drought stress showed that 'Ferragnes' on GN15 rootstock had the highest leaf RWC, which suggested that it was more resistant to water stress than the other genotypes. The mean RWC of the samples (average of two measurements during drought period) rankedthe five examined genotypes in the Fig. 4.
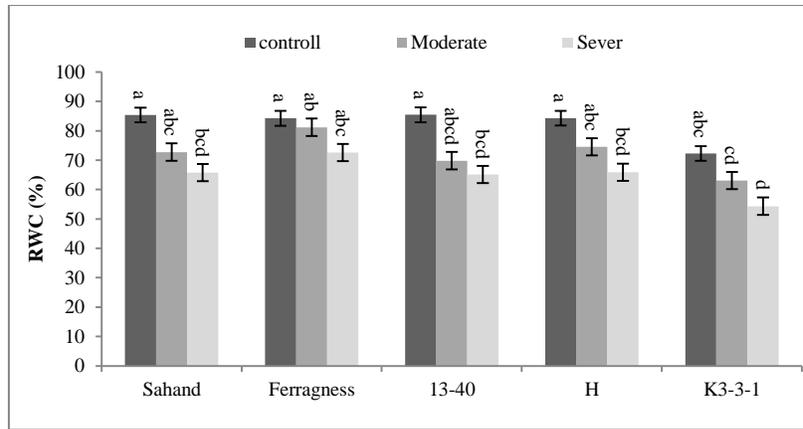
Fig. 4.Effects of drought treatments on relative water content (RWC) of five almond genotypes at end of drought stress. Vertical bars indicate ± standard error (SE) of three replications

Leaf RWC was significantly reduced ($P \leq 0.05$) in response to drought treatments in all genotypes (Fig. 4). The control plants (-0.33Mpa) and sever stress(-1.6 MPa) treated ones showed the highest and lowest RWC of leaves, respectively. Differences between treatments were gradually reduced. There was not a significant difference between the 'Sahand' and 'H' genotypes at the highest level of drought stress. A comparison of changes in leaf RWC of plants treated with -1.6 MPa relative to controls (Fig. 4) showed that the RWC of 'Ferragnes' was less influenced and that of 'K3-3-1'was more influenced by drought stress than the other genotypes.

Leaf characteristics

Almond genotypes had a significant effect ($P \leq 0.05$) on leaf dimensions (length and width) and individual leaf area on GN15 rootstock (Fig. 5). At measurement time, 'Ferragness' had the greatest and '13-40' had the least values for the above-named parameters. The results showed that decreasing osmotic potential of the soil down to -0.8 MPa had no significant effect on leaf characteristics compared to the controlin allgenotypesexcept '13-40'. In leaf characteristics under different levels of drought stress changes showed no clear pattern. The 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 genotypes. For example, 'Fragness' cultivar on GN15 rootstock with large leaves had a good tolerance to drought stress.
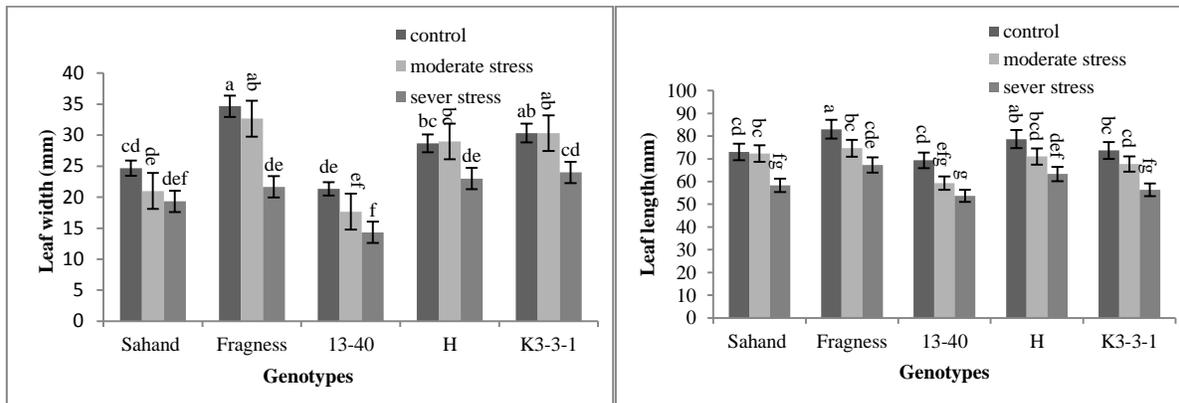

Fig. 5. Means comparison of leaf characteristics for five almond genotypes under different levels drought stress. Values by the same letter do not differ significantly according to the Duncan's multiple range test ($P \leq 0.05$).

Stomatal parameters

The results showed that there was significant difference ($P \leq 0.05$) between the cultivars in the level of stomata size (length and width). The greatest (24.59 μm) and the lowest (21.51 μm) length of stomata pore were observed in the leaves of 'K3-3-1'and 'Ferragnes,' respectively. Furthermore, 'H' and 'Ferragnes' had the

greatest (10.2 μm) and the least (9.4 μm) stomatal widthamong the examined genotypes, respectively. Stomatal density was significantly influenced by genotypes as well, and 'K3-3-1' and 'Ferragnes' had the highest (235.51 mm^{-2}) and the lowest (170 mm^{-2}) stomata, respectively (Table 2).

Table 2. Effects of drought treatments on leaf area and stomatal characteristics of five almond cultivar/genotypes grafted on GN15.

Main factors	leaf area (cm^2)	Stomata		
		Frequency (n/mm^2)	Length (µm)	Width (µm)
Drought Stress				
Control	19.7 a	213.4 a	24.8 b	12.118 a
S1	16.84 b	196 b	23.1 a	11.48 b
S2	10.97 c	185.4 c	20.92a	11.32 b
Cultivar/genotypes				
Sahand	10.073bc	177.66c	22.8bc	11.6ab
Ferragness	13.340ab	170.33c	21.5bc	9.4c
13-40	6.223d	211b	23.8ab	12.4a
H	13.320ab	197.66bc	22.2bc	10.2bc
K3-3-1	11.897abc	234.66a	24.5a	11.6ab

C: control (Ψ_{soil}= -0.33MPa), S1: moderate (Ψ_{soil}= -0.8Mpa) and S2: severe stress (Ψ_{soil} =-1.6 MPa) and five almond cultivar/genotypesincludes: 'Sahand', 'Ferragnes', '13-40', 'H' and 'K3-3-1' on GN15. Values by the same letter do not differ significantly according to the Duncan's multiple range test ($P \leq 0.05$).

Discussion

Genotypes and drought stress treatments had significant effects on the fresh weight (FW) and dry weight (DW) of plant organs ($P \leq 0.05$), although absolute values varied by genotypes. In comparison to other Prunus species, it seems that almond has different mechanisms for water stress resistance. Sardabi *et al*., (2005) reported that water stress caused greater difference in root dry weight than shoot dry weight among five genotypes of *P. dulcis* and two ecotypes of Amygdalus scoparia. Hence, almond ecotype and almond genotypes have potential to develop roots that have greater resistance to water stress. Nevertheless, it has been reported that root system characters alone were less closely associated with drought resistance in some Prunus species (Rieger *et al.*, 2003). In all genotypes studied, root DW reduced in both water stresses.

Although the greatest reduction in leaf FW (13.8%) occurred in 'K3-3-1' and '13-40' genotypes, these genotypes had a lower tolerance to drought than the other genotypes. Due to the adaptive mechanism of leaf shedding under drought conditions, thesegenotypes also had the highest reduction in total leaf area among the examined genotypes (Fig. 2). A decrease in freshweight (FW) and dry matter may be due to the considerable reduction of plant growth due to the reduction of photosynthesis (Shao *et al*., 2008). Changing resource pools (e.g., water or nutrient availability caused by drought) may also affect the distribution of biomass. Karimi *et. al* (2013) also reported that increasing PEG level in the medium significantly reduced fresh weight and leaf growth indices of the explants. Meanwhile drought sensitive genotypes such as 'B-124 ', 'Sepid',

'Mamaei' showed stunted growth with high rate of leaf abscission under osmotic stress.

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 genotypes, the mean DW of each organ as well as root/stem DW ratio (fR/fS) under the highest level of drought stress (Ψs= -1.6 MPa) was analyzed as a percentage of the control (Table 1). Confirming the results of previous studies on fruit trees such as 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 lack of water. During water stress, depending on the intensity and duration of the drought, plants tend to minimize water loss caused by transpiration by reducing their number of leaves (Yadollahi *et al.*, 2011). In this study, both leaf number and LAt decreased in all genotypes as the drought stress level increased. For each of five genotypes, leaf number and LAt at the severe stress (-1.6Mpa) was compared with those of the control (Fig. 2). Since a reduction in individual leaf area, length, and width were not affected by drought treatments, the reduction in LAt was mainly due to leafabscission 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 'K3-3-1' was more pronounced and started earlier than in the other genotypes, especially in severe drought stress (approximately at the end of the third week of the drought period), and continued until the end of the experiment, whereas in the other genotypes (such as Ferragness and Sahand) it started near the sixth week of the drought period. Thus, defoliation in these genotypes may represent a quick response and a morphological adaptation to reduce water loss and redistribute

resources under severe drought stress conditions. However, since leaves on the almond are essential for photosynthesis and productivity, 'Ferragness' and 'Sahand' cultivars are important.

Specific leaf weight indicates leaf dry mass per area and 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, Dichio *et al*., 2007). 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. As competition between fruits and leaves decreases, the accumulation of dry masses in leaves and subsequently leaf weight per area increases. Some researchersbelieve that changes in SLW under drought conditions may be induced by anatomical and morphological changes in leaves. It is found that mild drought increased SLW by increasing leaf and cuticle thickness and the amount of surface waxes. Given thatcell 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).

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 species, such as almond, might be less influenced by drought stress than sensitive ones. In this study, drought stress caused a slight increase in the SLW of almond genotypes. 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 (Fig. 3) showed that SLW for '13-40', 'Sahand' and 'H' genotypes is less influenced by drought stress than other genotypes. This may indicate a higher drought tolerance in these genotypes.

Moderate water stress had no significant effect on RWC but five genotypes showed different responses to severe water stress. 'K3-3-1'showed a reduction in RWC immediately after applying water stress, while none of them reduced in those genotypes reduction in RWC might activate resistance mechanisms. It shows that almond leaves actively synthesis compatible solutes that lead to absorbing the water. It proves the strong mechanisms in almond leaves to keep them active in stress conditions. A rapid recovery in water can be correlated with a greater physiological tolerance to drought stress. This pattern has been observed in almond cropsby Fereres *et al.* (1981). This response has been observed in other plants under water stress conditions, and it indicates that the pattern to promote stomata1opening may thus be related to other processes, not only related to leaf water potential (Massai and Gucci, 1997), such as the transmission from the roots of chemical signals, that reflect thesoil-root conditions. In addition, Karimi *et al.,* (2013) reported that under osmotic stress, leaf water content were significantly higher in the leaves of tolerant genotypes.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. Because of their role in transpiration and photosynthesis, stomata can influence water loss, water use efficiency, and plant yield (Manuela *et al.*, 2003). Aasamaa *et al.* (2001) found a negative correlation between sensitivity to drought and stomatal frequency in temperate deciduous trees. Thiscorrelation can be seen well in 'Ferragnes', 'Sahand' and 'H' genotypes. In our study, the lowest number of stomata per leaf area

(170.85 stomata mm^{-2}) was found in 'Ferragnes', and 'Sahand' genotype with 177.07 stomata mm^{-2} was ranked second. Therefore, these two genotypes may have a higher resistance to drought stress than the others (Table 2). 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 stress (Tanaka *et al.*, 2005). 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, the 'Ferragness' cultivarhad the smallest stomata 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 conditions, indicating that drought adaptation could occur (Martinez, 2010). However, in this study drought stress treatments had significant effect on stomatal size or density in leaves of almond cultivars(Table 2). In this study, by reducing the leaf area, stomatal size of the stressed plants decreased but had no effect on stomatal parameters of leaves that had already been developed since the duration of the drought period was six weeks.

Conclusions

All genotypes had similar responses to drought stress treatments, but the intensity of responses was different in genotypes. There were morphological differences among the genotypes. Drought stress caused an increase in SLW and a decrease in RWC of leaves, fresh and dry weight of plant organs, number of leaves per plant, and total leaf area. An increase in SLW of 'Sahand' was less than that of the other genotypes, which may indicate that the 'Sahand' leaves are less sensitive to drought stress. 'Ferragness' had the highest RWC, the least stomatal density and stomatal length of leaves under severe drought stress. This may act as an

adaptive mechanism to undesirable environmental conditions, in particular water deficit. It seems that traits related to leaf morphology such as individual leaf area, leaf length, and leaf width may not be good markers for drought stress. Considering the above results and observations, it can be concluded that the 'Ferragness' and 'Sahand' cultivars on GN15 rootstock possessed a higher level of resistance to drought stress compared to the other genotypes.

Acknowledgements

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