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Effects of Drought Stress on Almond Cultivar's Responses Grafted on Different Rootstocks

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A R T I C L E I N F O

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Drought stress;
Rootstock

A B S T R A C T

In this study, the response of selected almond cultivars on different rootstocks under drought stress base on Morpho-physiological traits using a factorial experiment in a randomized complete block design with three replications was investigated. The experimental was carried out at the Temperate Fruit Research Center of Horticultural Sciences Research Institute (HSRI) in 2016. The factors included cultivars in five levels (Supernova, Texas, Marcona, Shokoufeh and K13-40), rootstocks in three levels: GF-677, GN-22) (Peach × almond hybrids) and seedlings of bitter almond No.32 (Somewhat resistant to drought stress) and drought stress in four levels: irrigation intervals of 3 (control), 5, 10 and 15 days. The factors such as leaf abscission, leaf area (LA), cell membrane stability index (MSI), chlorophyll fluorescence (CF) and chlorophyll content index (CCI), minimal fluorescence (F_0), maximal fluorescence (F_m), variable fluorescence (F_v) and maximum quantum yield of photosystem II (F_v/F_m) were measured. The results showed that the interaction between the cultivar and the rootstock for $F0$ and for CCI was significant at 1% level. Interactions of cultivar and drought stress were significant for F_m and F_v at the 5% level and for CCI, F_0, F_v/F_m at the 1% level. Interactions of rootstock × drought were significant for CCI, F_0, F_v/F_m at the 1% level. Drought decreased F_v with increasing F_0 and decreasing F_m, in the evaluated cultivars and reduced the F_v/F_m in sensitive cultivars on seedling rootstock and GN-22 from 0.82 to 0.66 but in resistance cultivar Shokoufeh on GF-677 was from 0.818 to 0.789. As a general result, all of the cultivars on the GF-677 rootstock showed greater resistance to drought stress, and Shokoufeh and Marcona cultivars, especially on the GF-677 rootstock, tolerated drought stress better, and these combinations of rootstock - scion were superior to present experiment.

Introduction

Almond is the most important nut species in worldwide (Kodad *et al*., 2018). Germplasms of almond are a valuable genetic source for important physiological characteristics such as drought tolerance that can be identified and used for breeding programs (Yadollahi *et al*., 2011; Akbarpour *et al*., 2017). According to available statistics, more than 45% of the world's cultivated lands are subject to drought stress insistently

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or on continuous (Bao *et al.*, 2009). Drought stress generally has significant effects on plant physiology, and in particular the productivity and growth of almonds. Plant physiological characteristics such as photosynthesis and transpiration rate are dependent on the severity and duration of drought stress (Rouhi *et al*., 2007). Evaluation and identification of the tolerant cultivars of fruit trees are very important for drought stress and their ability to grow under these conditions (Yadollahi *et al*., 2011). In terms of almond production, Iran ranked fourth after the United States, Spain and Australia, with a cultivated area of 70,000 hectares, average yield of 1428 kg ha^{-1} and a production of 100,000 tons (FAO, 2014). Genetic differences in drought tolerance have been observed in various plant species (Bota *et al*., 2001). Today, can be trying to use the rootstocks for fruit trees, which, in addition to uniformity and increase in yield, can also tolerate different stresses and reduce the need for irrigation. Except domesticated almonds (*P. dulcis*), which are used commercially as rootstock, some almond species are sometimes used as the rootstock for domestic almonds in Iran and other countries (Zokaee Khosroshahi, 2013). However, due to the separation of traits in the seedling rootstocks, vegetative rootstock is used to maintain the genetic and uniformity of fruit trees. Peach × almond rootstocks such as GF-677, GN-22 and GN-15, such as seed rootstock, have been shown to be well tolerated to dry and low irrigated conditions (Felipe, 2009). This means that water absorption and protection efficiency is high.

Reducing the number of developed leaves is another defense mechanism of the plants in the face of stress that helps them absorb less light and reduce their transpiration (Sivritepe *et al*, 2008; Akbarpour*et al*, 2017). In many studies, leaf area is used as an indicator for assessing the effects of dehydration. Generally, plants tend to lower leaf abscission and produce smaller leaves to reduce water losses. Plants of dry and semi-arid regions, drought tolerance are obtained by reducing the transpirational organs through leaf abscission. Leaf abscission during drought stress is largely due to increased ethylene synthesis and plant sensitivity to this hormone (Taiz and Zeiger, 2006; Jangpromma *et al*., 2010). This mechanism has been reported in the *Zygophyllum dumosum* plant (Sundberg, 1985) and almond (Sundberg, 1985). Drought induced early aging in the leaves prevents cell proliferation and thus reduces leaf area (Kramer and Boyer 1995). It was reported that almond Nonpareil cultivar grafted on the seedling rootstock of bitter almonds had a higher leaf area than the same cultivars linked to the peach seed rootstock (Sharma and Joolka, 2004). The GF-677 rootstock, such as seedling almond, is a drought resistant, and can be used in areas where there is dehydration (Momenpour *et a*l., 2015). Plants have methods for protecting cellular macromolecules and membranes without sacrificing those (Jangpromma *et al*., 2010). A plant may have several strategies for stress (Taiz and Zeiger, 2006). The relative importance of each solution depends on the duration, severity of stress and type of plant species. Measuring the effects of drought stress with different methods and for different vegetative and physiological traits of the plant. CF and CCI, which are non-destructive degradable, fast and usually reliable, can be used to measure the stress impact on plants. CF is a valid physiological indicator for detecting changes induced in the photosynthesis device (Mehta *et al*., 2010). In many plant species, F_v/Fm is about 0.83 and lower amounts are a sign of the effect of stress on plants (Maxwell and Johnson, 2000).

The F_v/F_m index has been used in many studies related to the effect of stress in plants. This parameter has a good ability to estimate and study the photochemical efficacy and the degree of damage to photosystem II due to stress (Lotfi and Ghassemi-Golezani, 2015). Genotypes with higher F_v/F_m exhibit higher photosynthetic efficiency under severe stress conditions (Khanizadeh and Dewell, 2002). The CCI is a non-destructive and rapid method for measuring changes in the amount of chlorophyll and the effect of stress on plants. Drought affects the CCI of the leaf and decreases it, as results yields also decrease (Schlemmer *et al.*, 2005). Significant differences were observed in the CCI between almond cultivars so that the Tuono was significantly higher than the Princess in drought stress (Samandari Gikloo and Elhami, 2012). Despite the key role of interactions, between the rootstock, cultivars and environmental stresses, and their recognition to deal with the destructive effects of drought stress in semi-arid Iran, little research has been done. Therefore, the present study was conducted to investigate the interaction of rootstock, cultivar and drought stress with two non-destructive methods such as chlorophyll meter and SPAD, with the aim of assessing the potential of the rootstock and cultivars graft combinations under deficit irrigation for identifying and using in almond breeding programs.

Materials and Methods

Plant material and experiment design

This experiment was carried out in a factorial arrangement of 5 × 3 × 4 in a randomized complete block design with 3 replications as pot. The cultivar factor was selected on 5 levels of selective almond cultivars of different regions of the world, which included the cultivars of Shokoufeh and K13-40, Iran, Marcona, Spain, Texas, USA, Supernova, Italy, and from the maternal orchard of the Temperate Fruits Center in Karaj were prepared. Also, the rootstock factor was 3 levels including peach × almond hybrids (GF-677 and GN-22) and seedlings of bitter almond No.32 (sensitive to medium to drought stress). One-year plants of GF-677 and GN-22 were obtained from Ita-sadra tissue culture plant in Fars province, Iran. One-year old seedlings of bitter almonds 32 were also from the Temperate Fruits Center in Karaj (51° East latitude, 35° 48 'north latitude, elevation 1320 m above sea level, average annual temperature 13.7° C the average rainfall was 254.5 mm per year). Drought stress due to irrigation intervals in 4 levels included irrigation intervals of 3 days (control), 5, 10 and 15 days (Rostami Shahraji *et al*., 2010). Rootstocks were planted in late March 2015 in 20 kg pots (45 × 35 × 25 cm) and the scions were grafted by Chip budding in June 2016 at 15 cm height from the soil surface of the pot. In each experimental unit, 7 pots were placed, 3 pots as control and 4 pots for each level of drought stress. The soil in the pots has a loam texture composed of 46% sand, 34% silt and 20% clay. Plant roots and soil before planting with benomyl two per thousand were disinfected. In September 2016 (two months after grafting), the application of drought treatments began and lasted for 15 days. During the experiment, control plants were irrigated every 3 days and For other plants, irrigation interval treatments of 5, 10 and 15 days were applied alternately (Rostami Shahraji *et al*., 2010).

Measured parameters

Chlorophyll Content Index (CCI) or (SPAD)

The CCI was measured with a CCI meter (Model 502, Minolta Inc., Osaka, Japan), after standardization for each replicate and at each treatment level without degradation of plant tissues from the midpoint of the tenth leaf fully developed from the top and bottom and the middle of each branch, was measured at 11 to 12 hours and the mean value was determined as the CCI (Mujdeci *et al*., 2011).

Chlorophyll fluorescence (CF)

Measurement of CF parameters in each plant by sampling the 10th leaf developed from the top of the shoots and at 10 to 12 hours (about two hours after the sun was exposed to the plants), in the first instance, CF (Model, English Hansatech Instrument) was attached to the leaves so that a portion of the leaf was placed under a clip and in the dark for 30 minutes, then, using a fluorescence measuring apparatus, Act. Light was applied to the leaf, F_0 and F_m values were read. The F_v value of the difference between F_m and F_0 and the ratio of F_v to F_m were also calculated (Grant *et al*., 2010).

Cell membrane stability index (MSI)

To measure the MSI, from the tenth leaf developed from the end of the main branch of each cultivar, the discs of the same size and freshly separated and prepared in two groups, from each group, 0.1 g after being washed with distilled water in the test tubes contain 10 ml of water twice distilled. The first group was kept for 30 minutes at 40°C in a warm bath and after removing the hot water bath, reducing its temperature to 25°C, its electrical conductivity was measured (C1). The second group was placed in a hot water bath at 100° C for 10 minutes. After cooling (25°C), its electrical conductivity was measured (C2). The MSI was then calculated using the following equation (Sairam *et al*., 2009).

$$MSI= [1 - (C_1/C_2)] \times 100$$

C1 = electrical conductivity after exposure to a temperature of 40°C

C2 = electrical conductivity after exposure to a temperature of 100°C

Leaf abscission

In order to measure leaf abscission in each stage of drought stress, the number of leaves was counted and the number of leaves was deducted in the previous step and the amount of reduction was calculated as percentage of leaf abscission (Momenpour *et a*l., 2015).

Leaf area (LA)

In order to measure leaf area, using leaf area meter (Leaf Area Meter England Company) calculated a total area of 6 leaves without petiole and the mean of them was determined as the final leaf area.

Data analysis

The data was analyzed using SAS 9.4 software, description was performed for traits with double and triple interactions that were significant. The comparison of the meanings was done by Duncan's multiple range tests at 5% and 1% levels with SAS 9.4 software.

Results

Chlorophyll Content Index (CCI)

The results of analysis of variance of data (Table 1) showed that the interaction of cultivar and rootstock, cultivar and drought stress as well as rootstock and drought stress in relation to CCI was significant at 1% level. But the three-fold interactions of the factors were not significant for this trait. Interactions effects of the cultivar and the rootstock (Table 2) showed all cultivars on the GF-677 had more values than CCI compared to other rootstocks. For example, amount of CCI in the Marcona cultivar on the GF-677 was 39.017 while this amount for the same cultivar on seedling rootstock was 35.967 units.

Table 1. Results of variance analysis of cultivar, rootstock and drought stress effects on MSI, CF and CCI in almond.

Source S.O.V	df	Mean Square							
		CCI	F_v	F_v/F_m	F_0	F_m	MSI (%)	LA (cm^2)	Leaf Abscission (%)
Replication	2	0.796	637.32	0.0001	88.94	1021.41	23.15	1.1	0.24
Cultivar	4	95.61**	53997.05**	0.0048**	784.02**	46017.93**	646.43**	3797.3**	2632.0**
Rootstock	2	248.68**	152406.67**	0.0169*	4191.61**	107536.61**	2243.03**	1072.7**	4487.6**
Stress	3	594.27**	743991.29**	0.0748**	20551.40**	517623.06**	8503.42**	14707.8**	8461.0**
Cultivar×Rootstock	8	15.48**	4445.65**	0.0003**	44.22ns	4405.95**	28.88ns	20.2**	758.4**
Cultivar×Stress	12	13.91**	12844.17**	0.0011**	152.25**	11592.09**	175.23**	425.4**	1376.0**
Rootstock×Stress	6	48.81**	41761.20**	0.0053**	1322.69**	28577.93**	635.24**	533.7**	2348.7**
Cultivar×Rootstock×Stress	24	3.05ns	1460.71**	0.0002**	57.06*	1293.57**	16.95ns	16.7*	399.8**
Error	118	2.338	505.401	0.0001	46.357	465.75	16.93	2.133	0.815
CV%		4.128	2.405	1.079	2.592	1.802	4.696	3.342	9.577

ns: Non-significant, *: significant at %5 **: significant at %1

Table 2. Results of interaction effects of cultivar and rootstock on - CF and CCI in almond.

Cultivar	Rootstock	Mean			
		CCI	F_V	F_v/F_m	F_m
K13-40	GN-22	35.667ef	898.75fg	0.763efg	1169.6fg
	GF-677	39.400ab	978.92bc	0.789c	1240.1bc
	Seedling	34.300fg	880.33gh	0.753g	1155.5gh
Supernova	GN-22	34.25fg	865.42h	0.761fg	1127.7h
	GF-677	38.008bcd	974.42bc	0.793bc	1227.3bd
	Seedling	33.383g	875.08gh	0.758g	1143.2gh
Texas	GN-22	36.658cde	865.58h	0.757g	1132.3h
	GF-677	38.875ab	959.42cd	0.785cd	1218.3ce
	Seedling	33.100g	868.17gh	0.754g	1140.4gh
Marcona	GN-22	38.975ab	957.17cd	0.788c	1214.3ce
	GF-677	39.017ab	1005.75b	0.801ab	1253.9b
	Seedling	35.967def	936.67de	0.775de	1205.3de
Shokoufeh	GN-22	38.458bc	994.67b	0.792bc	1254.9b
	GF-677	40.658a	1039.75a	0.807a	1288.5a
	Seedling	38.958ab	919.67ef	0.771ef	1188.8ef

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test.

Also, the interactions effects of cultivar × drought stress (Table 3) showed that cultivar Shokoufeh and after that, Marcona suffered most of the cultivars to drought stress but in severe stress treatments with irrigation intervals of 10 and 15 days, all cultivars had a significant difference with control (Table 3). The results of interactions rootstock × drought stress (Table 4) also showed that in control, the highest amount of CCI (41.093) was found in the GN-22 rootstock, and the rootstocks had no significant difference.

Table 3. Results of interaction effects of cultivar and drought stress on MSI, CF and CCI in almond.

Cultivar	Drought stress (day)	Mean					
		CCI	F_v	F_v/F_m	F_0	F_m	MSI (%)
K13-40	3	40.567ab	1042.89a	0.802a	255.00fg	1297.89a	100.00a
	5	39.356abc	1040.11a	0.803a	255.56fg	1295.67a	97.53ab
	10	34.322f	872.00c	0.762c	269.78de	1141.78d	79.05e
	15	31.578gh	722.33e	0.707e	295.89a	1018.22f	63.72f
Supernova	3	39.422abc	1035.22a	0.811a	239.44h	1274.67ab	100.00a
	5	38.389bcd	1025.56a	0.808a	243.11h	1268.67ab	95.98ab
	10	33.822fg	833.67cd	0.753cd	270.22de	1103.89e	75.23e
	15	29.233h	725.44e	0.709e	291.56ab	1017.00f	64.31f
Texas	3	40.444ab	1032.56a	0.808a	242.56h	1275.11ab	100.00a
	5	39.467abc	1023.11a	0.803a	248.56gh	1271.67ab	95.63ab
	10	34.444ef	833.33d	0.749d	278.78cd	1112.11de	79.91e
	15	30.489h	701.89e	0.701e	293.78ab	995.67f	63.60f
Marcona	3	40.189ab	1039.33a	0.812a	240.44h	1279.78a	100.00a
	5	39.878ab	1032.44a	0.809a	243.11h	1275.56ab	98.59ab
	10	36.944cde	946.67b	0.780b	265.33ef	1212.00c	87.90cd
	15	34.933ef	847.67cd	0.750cd	282.89bc	1130.56de	78.56e
Shokoufeh	3	41.222a	1058.44a	0.816a	240.44h	1298.89a	100.00a
	5	40.789ab	1043.78a	0.808a	249.11gh	1292.89a	98.65ab
	10	39.0abd	977.78b	0.788b	262.33ef	1240.11bc	91.91bc
	15	36.422def	858.78cd	0.748d	285.56abc	1144.33d	81.72de

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test.

Table 4. Results of interaction effects of rootstock and drought stress on MSI, CF and CCI in almond.

Rootstock	Drought stress (day)	Mean					
		CCI	F_v	F_v/F_m	F_0	F_m	MSI (%)
GN-22	3	41.093a	1042.27a	0.811a	242.00f	1284.27a	100.00a
	5	40.107abc	1033.13a	0.807a	247.07ef	1280.20a	97.23ab
	10	35.17e	861.80d	0.759c	269.60d	1131.40d	79.59d
	15	30.847g	728.07e	0.710e	295.00b	1023.07e	63.67e
GF-677	3	40.407ab	1046.73a	0.810a	244.47f	1291.20a	100.00a
	5	40.360ab	1041.00a	0.808a	247.00ef	1288.00a	98.81a
	10	39.213bc	981.67b	0.794b	254.53e	1236.20b	93.62b
	15	36.787de	897.20c	0.767c	269.87d	1167.07c	85.90c
Seedling	3	39.607abc	1036.07a	0.808a	244.27f	1280.33a	100.00a
	5	38.260cd	1024.87a	0.803a	249.60ef	1274.47a	95.79ab
	10	32.740f	834.60d	0.746d	283.73c	1118.33d	75.19d
	15	29.960g	688.40f	0.691f	304.93a	993.33f	61.58e

Similar letters in each column shows non-significant difference according to Duncans Multiple Range Test.

Chlorophyll fluorescence (CF)

The results of analysis of variance showed that the interactions of cultivar × rootstock × drought stress (Table 1) for all parameters of CF (F_m, F_0, F_v/F_m and F_v) were significant at 1% level. Therefore, with investigation the three-way interactive effects resulted in the following results. The results of analysis of the effects of cultivar, rootstock and drought stress (Figs. 1, 2, 3 and 4) on CF maximum in leaf of almond cultivars adapted to dark conditions showed that in drought stress (irrigation intervals of 5 days) there was no significant differences between different rootstock and scion combinations with control (Figs. 1, 2, 3 and 4).

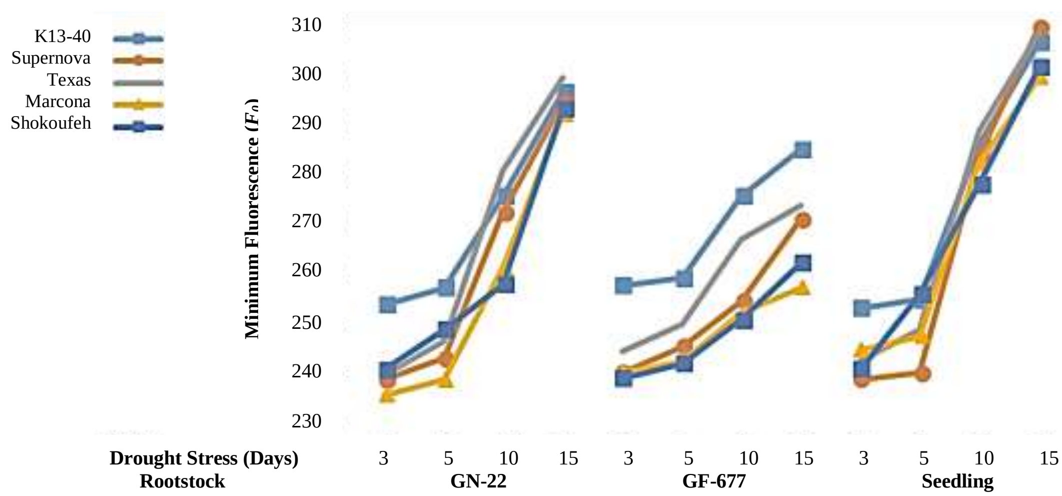

Fig. 1. Results of interaction effects of cultivar × rootstock × drought stress Minimum florescence (F_0) in almond.

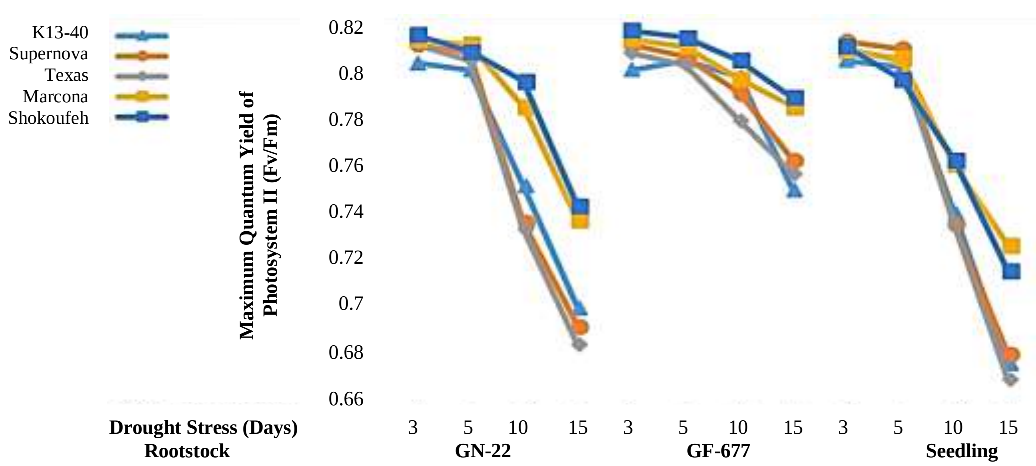

Fig. 2. Results of interaction effects of cultivar × rootstock × drought stress on Maximum quantum yield of photosystem II (F_v/F_m) Variable in almond.

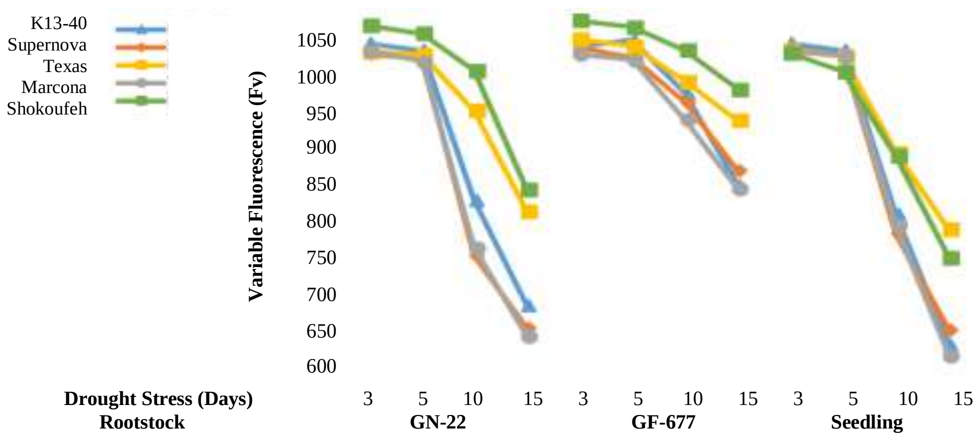

Fig. 3. Results of interaction effects of cultivar × rootstock × drought stress on florescence (F_v) in almond.

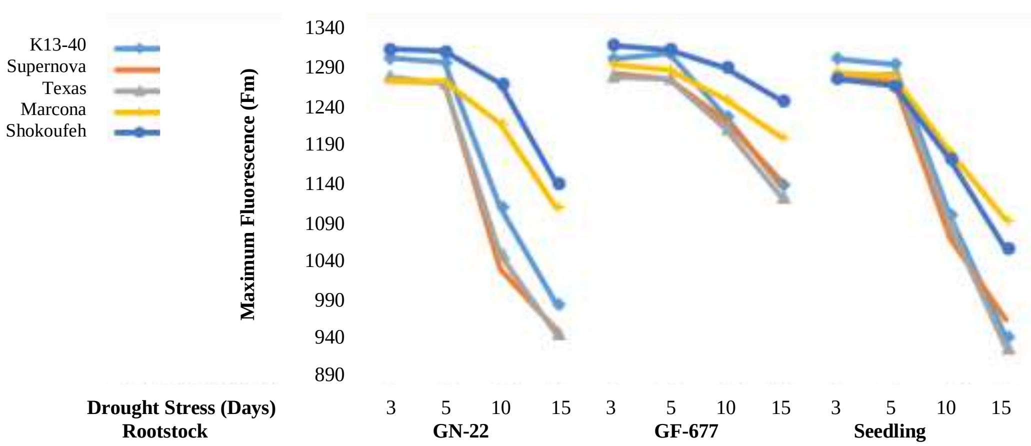

Fig. 4. Results of interaction effects of cultivar × rootstock × drought stress on Maximum florescence (F_m) in almond.

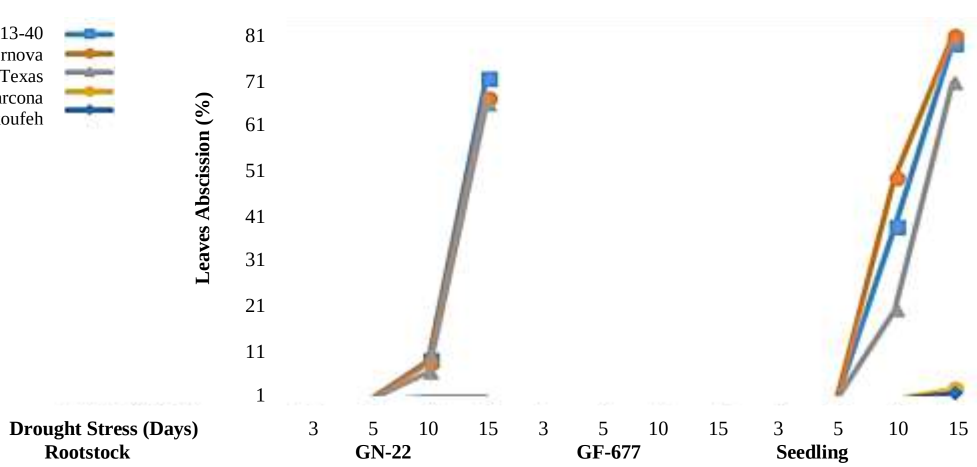

Fig. 5. Results of interaction effects of cultivar × rootstock × drought stress on Leaves abscission in almond.

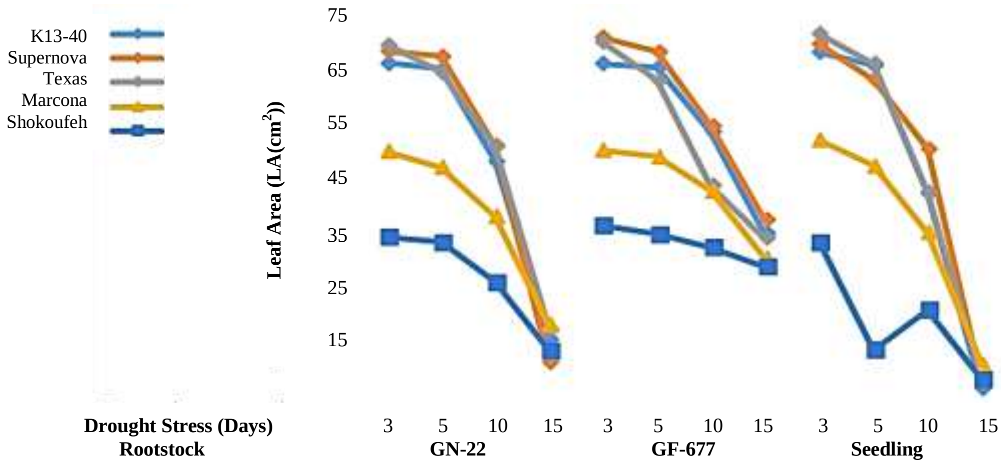

Fig. 6. Results of interaction effects of cultivar × rootstock × drought stress on Leaf Area (LA (cm^2)) in almond.

Minimal fluorescence (F_0)

According to the results of interaction the effects of cultivar × rootstock × drought stress (Fig. 1), the F_0 content in the leaf adapted to dark conditions increased with increasing drought stress in all rootstock and scion combinations. In all cultivars, the rate of F_0 increase was determined on the rootstock of GF-677, then GN-22 and seedlings, respectively. The highest increase was observed in severe stress (irrigation intervals of 15 days) in all cultivars on the seedling rootstock. In this parameter, the Marcona and Shokoufeh cultivars were the lowest on the GF-677 rootstock (7.07% and 9.61% respectively), and the highest increase in F_0 (29.43%) was in the combination of Supernova cultivar on the seedling rootstock.

Maximum quantum yield of photosystem II (F_v/F_m)

The results of interaction the effects of cultivar, rootstock and drought stress (Fig. 2) showed that the PSΠ quantum efficiency in all rootstock and scion combinations had an indirect relationship with drought stress, so that, with increasing drought stress (irrigation treatments), the F_v/F_m decreased. The highest quantum efficiency of the photosystem II (0.818) was found in the Shokoufeh cultivar on the GF-677 rootstock in control and the lowest (0.667) in Texas cultivar on seedling rootstock under severe stress (15 days irrigation interval). In 10 days irrigation stress, Shokoufeh and Marcona cultivars did not have significant differences with the control. While, at level of irrigation intervals for 15 days, all cultivars on all rootstocks with control showed a significant difference at 1% level. so, in the irrigation interval of 10 days, decrease of F_v/F_m in Shokoufeh cultivar on GN-22 was 2.45% and in the irrigation interval of 15 days 9.07% compared to the control, while this decrease for K13-40 on the same rootstocks was 6.04% and 11.96% respectively.

Variable fluorescence (F_v)

The results of averaging and interactions of cultivar, rootstock and drought stress (Fig. 3) showed that F_v of all cultivars evaluated on different rootstocks decreased with increasing drought stress levels. All cultivars on GF-677 showed the lowest reduction in F_v content, so that Shokoufeh and Marcona cultivars did not different significantly with the control on GF-677 in irrigation intervals of 10 days. Under irrigation interval of 15-day was the smallest and the highest F_v, respectively, for the Texas compound on a seedling rootstock (676.61), and a combination of the Shokoufeh cultivar on the GF-677 rootstock (67881), respectively.

Cell membrane stability index (MSI)

According to the results of variance analysis (Table 1), simple effects of cultivar, rootstock and drought stress were significant for MSI at 1% level. Dual interaction effects of drought stress × cultivar, and rootstock × drought stress were significant at 1% level. Based on the results of the Means comparison of the effects of drought stress × cultivar (Table 3), in different cultivars under drought stress with irrigation intervals of 5 days were not significantly different from the control (3 days irrigation intervals). In this level of drought stress, despite the decrease in MSI, the Shokoufeh cultivar with the value of 98.68% had the highest MSI while the Texas cultivar decreased by 4.37 %. In all cultivars, drought stress with irrigation intervals of 10 days caused a significant reduction in MSI at 1% level compared to control. At this level of drought stress, Shokoufeh had the lowest decrease 8.08% and Supernova had the highest 24.77% membrane damage. Results of interaction effects of cultivar and drought stress (Table 4) showed that in severe drought stress (irrigation interval of 15 days), K13-40 had the least MSI (63.72%) and highest MSI (81.72% and 78.56%) were observed respectively in Shokoufeh and Marcona cultivars.

Leaf abscission

The results of analysis of variance about relation to leaf abscission percentage (Table 1) showed that the simple effects of cultivar, rootstock and stress factors, interactions effects of rootstock × cultivar, cultivar × stress and rootstock × stress, as well as cultivar × rootstock × stress ratio was significant at 1% level. The results of the triple effects of cultivar × rootstock × stress (Fig. 5) showed that the different rootstock-scion combinations in response to stress in terms of percentage loss were significantly different.

The highest percentage of leaf abscission in K13-40, Texas, and Supernova on the seedling rootstock (80.38%, 67.67%, 70%) respectively was observed under drought stress (irrigation intervals of 15 days), whereas these amounts in same cultivars on GN-22 were 71, 67.66 and 33.65 were respectively, which indicates the response of different cultivars to stress when transplanted on different rootstocks.

In the irrigation interval of 3 (control) and 5 days, none of the components rootstocks and scions was evaluated for leaf abscission. In the 10-day irrigation interval, Supernova, K13-40 and Texas cultivars on the seedling rootstock were 49%, 38% and 19.67%, and also on the GN-22 respectively had 8%, 8.67and 6%, but none of the cultivars on the GF-677 rootstock has not a leaf abscission, indicating the rootstock effects on the resistance of different cultivars to relatively severe drought stress in almonds.

Leaf area

According to analysis of variance of data in relation to leaf area (Table 1), simple effects of cultivar, rootstock, stress and double effects of rootstock × cultivar, stress × cultivar and stress × rootstock were significant at 1% level. Also, the interactions of three cultivars × rootstock × drought stress were significant at 1% level. Means of the interactions between the cultivars × rootstock × drought stress factors (Fig. 6) showed that drought stress reduced the leaf area in all combinations of rootstocks - scions. As in any combination of rootstock - scion, the highest and lowest leaf area was associated with the control plants and was under severe drought stress (15 days irrigation interval, respectively).

In the irrigation interval of 3 days (control), the different rootstock-scion combinations had a significant difference compared to each other. The highest and lowest leaf area (71.5 and 33.13 cm^2) was related to the combination of Texas cultivar and then the Shokoufeh on the rootstock of bitter almond seedling.

In the irrigation interval of 5 days, leaf area changes showed a decreasing trend, however, there was no reliable pattern for identifying the effects of irrigation on evaluated rootstocks - scions combinations. In the irrigation cycle, the maximum and minimum leaf area reduction was observed for the 5 days in control plants (9.9% and 0.81%), respectively, in the compounds of Texas and then K13-40 on the GF-677, respectively. In the irrigation interval of 10 days, all the compounds of rootstocks - scions evaluated in terms of leaf area showed decreasing significantly compared to the control plants. In this level of drought stress, the highest and the lowest leaf area reduction 40.75% and 10.55% respectively was observed in the combination of Texas on the bitter almond seedling and Shokoufeh cultivar on GF-677 compared to the control plants.

Discussion

Chlorophyll content index (CCI)

with the development of drought stress, the GF-677 rootstock showed less reduction (Respectively -0.047, -1.194 and -3.627 unit) and had the highest CCI in irrigation intervals of 5, 10 and 15 days compared to other rootstocks such as GN-22 rootstock (-1.347, -6.967 and -9.747 unit) and seedling rootstock (-0.986, -5.923 and -10.246 unit)), although there was a significant difference in the stress level at 1% level with control. According to reports, the relationships between CCI readings and extractable leaf pigments in various plant species is not universal and varies with measurement procedure, sensor type, leaf characteristics, plant species and environmental factors (Markwell et al., 1995; Xiong et al.,2016; Yuan et al.,2016)

Chlorophyll fluorescence (CF)

In drought stress (irrigation intervals of 10 days) there was a significant difference between the cultivars grafted on different rootstocks, so that the cultivars of Shokoufeh and Marcona had the highest F_m (Fig. 4) on the GF-677 rootstock (1286.33 and 1245 respectively) and there was no significant difference with the control. Other cultivars on the GF-677 rootstock were less affected by F_m than the other two, although their difference was significant with the control. In intensive drought stress (15 days irrigation interval) Shokoufeh cultivar on GF-677 rootstock was the best combination and there was no significant difference with control. Therefore, considering the results of MSI, it could be maintained by maintaining cell wall structure, tolerate the destructive effects of stress (Khanizadeh and Dewell, 2002).

Minimal fluorescence (F_0)

In this parameter, the Marcona cultivar was the lowest on the GF-677 rootstock, while the highest increase in F_0 was in the combination of Supernova cultivar on the seedling rootstock. Changes in F_0 could be interpreted in different ways. F_0 represents an estimate of the relative size of the antenna pigments of the PSII complex (Huang et al., 2004). Similarity result was reported by Baker and Rosenqvist (2004), they also suggested that an increase in F_0 has been shown to be a symptom of damage to the PSII reaction center, resulting in a reduction in absorbed light and a subsequent increase in unused emitted light.

Maximum quantum yield of photosystem II (F_v/F_m)

Variations of the *Fv/Fm* for the Shokoufeh cultivar on the GN-22 rootstock in the irrigation interval of 10 days were -6.59% and in the irrigation interval 15 days -13.18% compared to the control and for the K13-40 on the same rootstock in irrigation intervals 10 days -8.2%

and in irrigation intervals of 15 days -16.27% decreased compared to the control. In general, the combination of cultivars on the GF-677 rootstock was more tolerant to drought stress and GN-22 and seedling. Momenpour *et al.* (2015) reported the similar results of drought stress. They stated different stresses, including dryness, by reducing the consumption of electron transport chain products (NADPH and ATP), increase the of fredoxin and free radicals, resulting in degradation of the thylakoid membrane, resulting in the transfer of electrons from the receptive site of photosystem II and yield maximum of photosystem II reduce and CF increases (Peper *et al.,* 2007).

Variable fluorescence (F_v)

F_v of all cultivars evaluated on different rootstocks decreased with increasing drought stress levels. So that. Under severity stress, the highest F_v, for the Texas compound on a seedling rootstock, and the it's lowest in combination of the Shokoufeh cultivar on the GF-677 rootstock. This important parameter was also a suitable criterion for detecting the superior rootstock and scion composition resistant to drought stress. Genotypes with higher F_v/F_m exhibit higher photosynthetic efficiency under severe stress conditions (Khanizadeh and Dewell, 2002)

Cell membrane stability index (MSI)

Results Table 4 showed that the lowest changes in MSI on the GF-677 rootstock occurred with the trend of increasing drought stress, so that in severe stress (15 days irrigation interval only 14.10 percentage of cell MSI decreased, while on GN-22 and seedling rootstocks, 36.33% and 38.42% of the MSI was reduced respectively, which was very destructive for leaf cells. Due to drought stress and heat (which is common in semi-arid regions such as Iran in the summer), cell membranes have lost their stability, and if the leaves of such plants are in aqueous solution, the solutes of the cell are leaked, thereby maintaining the MSI can be measured (Sairam, 2002).

In different stresses, cultivars that can maintain their cell MSI will have the least damage that depends on their morpho-physiological characteristics and the cell MSI plays a pivotal role in tolerance to drought stress and heat because it directly relates to the production of heat shock proteins, the characteristics of photosynthetic system, key enzymes and thylakoid membrane (Bewley, 1979). For example, it was reported that under stress conditions, MSI is protected by sugars through osmotic regulation and turgeration of the cells (Bartels and Sunkar, 2005). The highest survival percentage of scions was found on the GF-677 rootstock, which produced the highest proline content during drought stress (Shokouhian *et al*., 2015). Cultivars such as Shokoufeh and Marcona with smaller leaves are more resistant to drought stress. Ionic leakage was significant at 1% probability level in different cultivars (Akbarpour *et al*., 2017) which indicates the difference of the cell MSI in different cultivars and genotypes in almonds and in line with the current research.

Leaf abscission

In irrigation interval of 15 days, all cultivars grafted on the bitter almond seedling, which were lower in the Shokoufeh and Marcona cultivars than in other cultivars, indicating the effects of the cultivar in stress resistance. In general, in the present experiment, the combination of all almond cultivars evaluated on the GF-677 rootstock had a greater resistance to drought stress in terms of shelf life than the main photosynthetic unit and guaranteed the survival of the plant under severe conditions (Fig. 5). The similarity results were reported that the number of developed leaves in different genotypes of almond under in vitro drought stress (Akbarpour *et al*., 2017) and in various species of almond (Rouhi *et al*.,2007) under greenhouse drought stress compared to the control is significantly decreased which is consistent with the results of the present study.

Leaf area

In general, all the cultivars evaluated when they were grafted on GF-677 had a minimum reduction in leaf area compared to control, but the same cultivars on the bitter almond seedling and GN-22 had the highest reduction in leaf area than the control respectively (Fig. 5).

Reduction of leaf area has been reported as one of the most important factors affecting the survival of plants such as almonds by De Herralde *et al* (2003) and Zokaee Khosroshahi (2013). According to Zokaee Khosroshahi (2013), almond species showed different responses to drought stress due to changes in leaf area, leaf length and width, and total leaf area, so that the resistant species *of P. dulcis* and *P.* eburnean Showed the least decrease in leaf area Compared to the sensitive species such as P. scoparia and P. eleagnifolia which is consistent with the results of this research. Because firstly, different combinations of rootstock-scion showed a different response to drought stress, and secondly, the combination of Skokofeh on the GF-677, which was resistant to drought stress, showed a minimum reduction in leaf area compared to the relative to susceptible compounds such as Supernova, Texas and K13-40 on the rootstock of bitter almond seedlings.

The smaller leaf area causes less transpiration relative compared to the larger leaf area, and having small leaves may help reduce water loss (Bacelar *et al*., 2004). Of course, there must be a balance between decreased transpiration and leaf critical level for photosynthesis, otherwise, the advantage of reducing transpiration is eliminated by inadequate access to absorbent materials. It can be argued that cultivars on GF-677 rootstock in compassion of the same cultivars on the Seedling in potted conditions could tolerate more drought stress. Because they produced smaller leaves and decreased transpiration and, by preserving them (the least abscission), controlled their vital activities, such as photosynthesis, and were the most tolerant to drought stress.

Conclusions

The results of this study showed that drought stress reduced the CCI by decreasing the MSI, resulting in a reduction in F_v/F_m. With increasing drought stress, significant differences were observed between the cultivars for each trait. So, interactions of cultivar × drought stress were significant for F_m and F_v at the 5% level and for CCI, F_0, F_v/F_m at the 1% level. Interactions of rootstock × drought were significant for CCI, F_0, F_v/F_m at the 1% level. Drought decreased $F_v$with increasing F_0 and decreasing F_m, in the evaluated cultivars and reduced the F_v/F_m in sensitive cultivars on seedling rootstock and GN-22 from 0.82 to 0.66 but in resistance cultivar Shokoufeh on GF-677 was from 0.818 to 0. 789. As a general result, all of the cultivars on the GF-677 rootstock showed greater resistance to drought stress, and Shokoufeh and Marcona cultivars, especially on the GF-677 rootstock, tolerated drought stress better, and the combinations of rootstock and scion was superior to present experiment.

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