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Screening of Almond Hybrids for Drought Tolerance Using some Morphological and Physiological Traits

Arvin Abdini[1], Ali Imani[*2], Mousa Rasouli[3], Mehrshad Zinalabdini[4], Vahid Abdoosi[1]

[1] Department of Horticulture Science, University of Tehran, Science and Research Branch, Tehran, Iran

[2]Temperate Fruit Research Center, Horticultural Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

[3] Department of Horticulture and Landscape Engineering, Faculty of Agriculture, Malayer University, Malayer, Hamedan, Iran

[4] Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

A R T I C L E I N F O

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Electrolyte leakage;
Leaf relative water content;
Prunus dulcis Mill.

A B S T R A C T

In this research, twenty-two selected almond hybrids with their parents ‘A1-99’ (drought-sensitive) and ‘Tuono’ (relatively tolerant against drought), were investigated for screening drought tolerance using some morphological and physiological traits. This research was carried out in a complete randomized design with three replications at the Temperate Fruit Research Center of Horticultural Sciences Research Institute (HSRI) in years of 2014 and 2015. The results showed that by applying severe drought stress (30% FC), some morphological and physiological characteristics such as chlorophyll index (ChI), relative water content (RWC) of leaf decreased, while electrolyte leakage (EL) and Quantum efficiency of the photosystem II (F_v/F_m) increased. According to results, there were significant differences among genotypes, for example range of F_v/F_m among the selected hybrids varied from 550 to 879. Frequency of drought tolerance showed that 77. 27% of hybrids were susceptible to relatively susceptible and 22.72% were tolerant to relatively tolerant. Finally, according to the results from this study, ‘DT19’ hybrid was recognized as the most sensitive and ‘DT1’ hybrid was known as the most tolerant hybrid that could be introduced as promising and tolerant to drought stress for utilizing in the breeding programs.

Introduction

Drought stress is one of the main problems of agriculture in the world that limits growth and yield of crops (Egea *et al.*, 2010; Samandari Gikloo and Elhami, 2012; Shamshiri *et al*., 2015; Alaei, 2019). Increasing negative osmotic potential is a crucial problem in salty and dry soils that results in reduction of water absorption and consequently growth cease. Although main effect of prevention of water uptake is increasing the concentration of solution mater in soil. Some solutions which bear magnesium salts has toxic effect beside osmotic effect, and when exceeds determined amount reduce growth more drastically (Razouk *et al.*, 2013). Meanwhile, negative osmotic of soil solution affects osmotic potential of leaf (Ψs), relative water content (RWC) and leaf water potential (Ψ_W) and leaf turgor potential (Ψp) (Munné-Bosch *et*

[*]**Corresponding author**: Email address: imani_a45@yahoo.com or a.imani@areeo.ac.ir
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al., 2003, Hernández *et al.*, 2004). Furthermore, drought stress leads to overproduction of ROS and subsequent oxidative damage (Abbaspour *et al*., 2016; Mohammadi *et al*., 2020). Almond is a nut crop which is planted in arid and semi-arid areas due to drought tolerance and low water content condition. However, for economical production it requires irrigation in dry season, although almond shows physiological compatibility in water shortage to survive, but drought tolerance varies among different cultivars (Samandari Gikloo and Elhami, 2012; Romero *et al.,* 2004; Karimi *et al.,* 2013; Ryan, 2013).

To prevent the yield loss of agricultural crops during drought periods, developing plants that tolerate undesirable conditions is essential (Ashrafi *et al*., 2018). Therefore, one of the approaches of managing drought stress is the cultivation of drought tolerant rootstocks and cultivars. Drought stress tolerance is the result of the interaction of morphological, physiological and biochemical traits (Lotfi *et al.*, 2019). Therefore, instead of a simple feature, combination of different attributes that direct relationship with drought tolerance can be used as criteria for selecting the ideal selections (Vahdati *et al.*, 2009, Momenpour *et al.,* 2011, Nikoumanesh *et al.,* 2011).

Comparing the response of almonds 'Lauranne' and 'Masbovera' to drought stress, it has been shown, 'Masbovera' has more osmotic compatibility and water potential, lower evaporation and transpiration rate, higher photosynthesis rate, higher water use efficiency and lower hydraulic root resistance than 'Lauranne'. It had better compatibility with no irrigation condition in Mediterranean climate (De Herralde, 2000, 2001, 2003). Drought response is different between almond species (Zokaee-Khosroshahi *et al*., 2014). Some reports have indicated that leaves of *scoparia* specie fallen under stress but in *lycoides* specie some leaves survive and capable to do photosynthesis (Rouhi *et al.,* 2007) which indicates the direct effect of drought stress on photosynthesis in some cultivars of plants (Barzegar *et al*., 2017). Indeed, drought stress accumulates osmotic regulators in leaves of tolerant genotypes which has crucial role in leaves turgor protection (Torrecillas *et al.,* 1996). Water stress is a crucial environmental factor that restricts photosynthesis. After stomata closure, intercellular CO_2 decreases and consequently results in accumulation of energetic electron carriers, free radical formation, low light complexes and reduction of photosynthesis efficiency (Griffiths and Parry, 2002). Water requirement of almond during growth season and phenologic growth are not the same. As a result, recognizing sensitive courses in water requirement aspect to prevent any growth arrest and negative physiological responses is important (Germana 1997, Goldhamer and Smith 1995). Few researches have been carried out regarding drought tolerance heritage and some morphological and physiological traits and most studies accomplished to determine almond trees` water requirement. Therefore, this study aimed to evaluate the effect of drought stress on the heritage, morphological, and physiological response of the selected almond hybrids to identify parameters that may be indicators of tolerance to these stresses.

Materials and Methods

This research carried out at the Temperate Fruit Research Center of Horticultural Sciences Research Institute (HSRI) in years of 2014 and 2015. In this research, twenty two hybrids obtained from hybridization between 'A1-99' (maternal parent: drought-sensitive) with 'Tuono' (father parent: relatively tolerant to drought) with their parents base on completely randomized design under severe drought stress with 3 replications for each treatment. The morphological vegetative traits such as trunk diameter, tree height, scion height, rootstock diameter, trunk diameter in the lower part of graft location, the main branch's diameter of scion, density and the number of foliage, density and the number of leaves in 20 centimeters of selected branch with identical diameter in all hybrids and parents were evaluated.

Also, length of leaf, width of leaf, length of petiole, and the length of current branches' growth were measured. Some physiological traits such as chlorophyll index (ChI) based on SPAD criterion, the leaf relative water content (RWC), electrolyte leakage (EL), and measuring the rate of chlorophyll's fluorescence were investigated. For this work, they (hybrids with their parents) were grafted on vegetative uniform rootstock (GF 677) in 15-liter plastic pots (height= 34 centimeters and the inset's diameter= 32 centimeters, one plant per pot) containing of soil and washed sand mixture (3:1) and soil analyses is shown in Table 1.

Table 1. Physical and chemical characteristics of soil mixture.

Physical and chemical trait	Unit	Value	Physical and chemical trait	Unit	Value
Depth	(cm)	0-30	Text	-	Loam
S.P	(%)	39	Ca	(ppm)	1217
EC	(ds m^{-1})	1.28	Mg	(ppm)	315.5
pH	-	7.4	T.N.V	(%)	13.7
N	(%)	0.15	Cu	(ppm)	2.12
O.C	(%)	1.43	Zn	(ppm)	4.85
$P_{avr.}$	(ppm)	102.9	Fe	(ppm)	28.04
Sand	(%)	45	$K_{avr.}$	(ppm)	687
Silt	(%)	32	Mn	(ppm)	15.86
Clay	(%)	23	Na	(ppm)	91.95

These plants were normally and equally irrigated and fed for four months (until the beginning of treatments of drought stress) when they were completely established. During this time, the plants were not exposed to any kind of environmental and nutritive stress. Evaluation of the treatments were performed in severe drought stress (30% FC). Drought treatments were started in late July and continued for 12 weeks. Field capacity (FC) of soil in pots was determined by weighing method before transferring plants. For determining FC, a number of pots containing the tested beds were saturated with water so that water gets out from the bottom of the pots. In order to avoid evaporation, the pot surface was covered by aluminum foil. The weight of the pots was evaluated each day until it reached a stable level, then the soil of each pot was mixed to make a uniform mixture and then some soil was removed to record its wet weight (WW). For determining the soil dry weight (DW), it was placed in the oven for 24 hours at 72°C. Then the field capacity was calculated from the calculated from the following equation (Liberato *et al.*, 2006):

$$FC = \frac{(WW-DW)}{DW} \times 100$$

Maintenance of the pots water was made by weighing of the pots and replacing the water lost by transpiration using a precision scale. Irrigation for pots was performed due to changes their weight and leaching requirement (Liberato *et al.*, 2006). After applying drought stress treatments and the end of experiment, for screening the selected almond hybrids for drought tolerance, some of morphological traits as well as physiological indices were considered as suitable criteria for assessing the resistance or susceptibility to drought stress as well as heritability response under severe drought stress condition.

Measuring the morphological features and heritage attribute

A digital caliper and meter were used for measuring the all of the morphological features in relation with vegetative growth. In order to estimate the heritability of traits in the hybrids compared to the parents, after measuring the traits studied in all hybrids and parents, the mean of these traits in the total hybrids was calculated and compared with the average traits studied in parents (Nikoumanesh *et al.,* 2011).

Measuring the chlorophyll index (CI) based on SPAD criterion

CI was evaluated in the upper fully expanded young leaves without destruction of vegetative tissues in summer and when applying the drought treatment with a Field Scout CM1000 chlorophyll meter. The Field Scout CM1000 chlorophyll meter estimates chlorophyll content based on ratios of the amount of ambient and reflected light at 700 and 840 nm. Measurements were made on a clear day between 9 pm and 11 am.

Measuring the leaf relative water content (RWC)

For determination of RWC, samples of leaves of fully developed and without petiole were separated were weighed with a digital scale of 0.0001 (FW). Immediately immersed in 15 ml of distilled water and after 24 hours storing in a refrigerator and 4°C, they were distilled from water and removed from the surface of the two layers of tissue paper tissue and weighed again (TW). After 48 hours, they were completely dried in oven at 70 ° C and reweighted (**DW**) for 48 hours. Finally, the percentage of relative water content was calculated using Ratio2-5 (Yamasaki and Dillenburg, 1999).

$$\mathrm{RWC} = \frac{\mathrm{W} - \mathrm{DW}}{\mathrm{TW} - \mathrm{DW}} \times 100$$

FW = Fresh leaf weight, DW = Leaf dry weight and TW = Leaf weight in saturated state

Measuring the electrical conductivity (EC)

For determination of relative ionic content, 0.5 gr of each sample, was put into tubes with 25 ml of distilled water and kept at 25 c° for 24 hours on shaker with the speed of 120 r/min and then, electrical conductivity (EC) of the medium was detected using a conductivity meter (conduct meter; Radiometer, Copenhagen). Following the initial reading (Lt), samples were autoclaved for 20 minutes to kill leaf tissues and then kept at 25°C for 2 hours on shaker with speed 120 in/min and a final reading (Lo) was obtained. Finally, relative ionic percentage was calculated via formulae (Lt/Lo) ×100, (Lutts *et al.,* 1995).

Measuring the fluorescence parameters.

The device known as chlorophyll fluorescence measurement (made in Opti-Science American Company, OS-30p version) was used for studying the rate of changes in chlorophyll's fluorescence. For this purpose, first, the clips of chlorophyll measurement device were connected to leaves. These clips have a special shape on which the diode of device is easily anchored. The clips have valves, and by closing them the considered part of leaf is placed in the darkness. Also, the leaves of the samples that are under the drought treatment are placed in absolute darkness for 30 min using light exclusion clips (Maxwell and Johnson, 2000), and then the active light radiated on the leaf by using the fluorescence measurement device. The chlorophyll's fluorescence increased and got the level of F_o (optimum fluorescence) after placing the leaf against this light. In F_o level, there is maximum ability to use the excited energy, and when the light intensity is enough, the rate of fluorescence increases from the F_o to its maximum rate that is F_m (maximum fluorescence). As soon as the flash of saturated light is applied, the rate of fluorescence increases from the optimum fluorescence to maximum fluorescence. This increase indicates the gradual growth of fluorescence operation and decreasing the speed of photochemical reactions. One of the other important parameters of chlorophyll fluorescence is F_v that obtains in the form of F_m-F_o. In these conditions, the first receiver of electron (QA) has been completely reduced. By measuring these parameters, the maximum quantum efficiency of photosystem

(ΦPSII) was obtained by using the following equation:

$$Fv/Fm = \frac{Fm - Fo}{Fm}$$

The amount of F_o (optimum rate of fluorescence) and F_m (maximum rate of fluorescence) were read by fluorescence measurement. The amount of F_v was calculated from the difference between F_m and F_o (Grant *et al.*, 2010).

Statistical analysis

After being sure of the uniformity of data, the analysis of variance and comparing the mean were performed on them by using Duncan's multiple range test method at P < 0.01 and P < 0.05 by Minitab software. 17.3and SAS 9.4 software, and the related diagrams were drawn by Excel software.

Results

Analysis of variance of morphological traits as well as some physiological indices evaluated in almond hybrids in relation with their parents under severe drought stress conditions (Table 2) showed that the effect of drought stress on all morphological traits except petiole length was significant (P < 0.01). Also, the effect of drought treatment on replication was not significant in all morphological indices (Table 2).

Table 2. Analysis of variance of morphological traits evaluated in almond hybrids in relate with their parents under severe drought stress conditions

Source	df	Height	Rootstock diameter	Trunk diameter under the graft	Number Leaf density in 20 cm	Branch diameter of the scion	Leaf length	Leaf width	petiole length	Current shoot length
Cultivar	23	888.58**	35.38**	23.43**	108.2**	15.28**	3.79**	1.63**	1.54^{ns}	11.21**
Error	48	99.04	6.63	3.70	11.05	2.62	0.97	0.32	0.43	0.71

ns: not significant; *: P<0.05; **: P< 0.01

The results of the physiological indices evaluated in almond hybrids in relation to their parents under severe drought stress conditions in Table 3 indicated that the effect of drought stress on chlorophyll index was significant based on SPAD criteria (P<0.01). According to the results of Table 3, it is observed that the relative water content of leaves is affected by drought stress and is significant (P<0.01).

Table 3. Analysis of variance of physiological traits evaluated in almond hybrids in relate with their parents under severe drought stress conditions.

Source	df	Mean of Square			
		ChI	RWC	EL	F_v/F_m
Cultivar	23	45.745**	255.3*	142.86*	0.009829*
Error	48	9.457	158.8	54.84	0.005421

ns: not significant; *: P<0.05; **: P< 0.01

As stated by the results of Table 3, the EL index and also the chlorophyll fluorescence index [fluorescence variable to fluorescence maximum ratio (F_v /F_m)] in almond hybrids and their parents was significantly affected by drought stress (P<0.01). In Table 4 mean comparison of some of the morphological characteristics and the photosynthesis parameters of almond hybrids/cultivars has been presented. Also heritability and frequency of some of the morphological characteristics and the photosynthesis parameters in almond hybrids with their parents under severe drought stress conditions in Fig.s 1-5 has been presented.

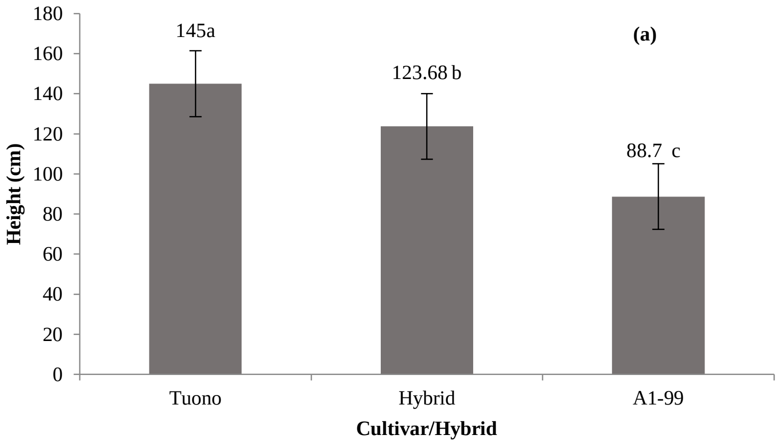
Table 4. mean comparison of some of the morphological characteristics and the photosynthesis parameters of almond hybrid/ cultivar

Cultivar/Hybrid	Height (cm)	Rootstock Diameter (mm)	Trunk diameter under the graft (mm)	Branch diameter of the scion (mm)	Number Leaf Density in 20cm	Leaf length (cm)	Leaf width (cm)	(cm)	New shoot Length (cm)	Chlorophyll index (Chl) (%)	RWC (%)	EL (%)	Fv/Fm
'Tuono'	145 a[!]	25.54b	23 bc	19.54ab	17.33d	5.6c	2.4c	1.973b	4.33c	46.53 a	71.02 b	56.00bc	0.818b
'A1-99'	88.77i	20.50c	19.99c	18.71b	30.33b	8.66a	3.41 b	2.16b	3.13d	34.13cd	61.41c	67.09ab	0.702c
'DT1'	146.67a	25.03b	20.923c	22.83a	42a	8 a	3.5b	2.27b	7.19a	48.79a	79.27a	52.68c	0.879a
'DT2'	139.12b	27.54ab	30.49a	20.90a	18.57d	6.58c	3.32b	1.474c	3.40cd	39.47a-d	79.27a	64.38b	0.689cd
'DT3'	134 c	23.00bc	19.93bc	16.38b	29.66b	6.58b	3.23b	3.46a	6.33ab	43.93ab	66.02bc	70.87a	0.759bc
"DT4"	102.33h	25.4 b	22.24bc	17.74b	26.33bc	5.16cd	1.68 d	1.66c	3.5cd	44.03ab	59.03c	58.71b	0.648d
"DT5"	116f	25.91b	23.26bc	21.1a	29b	5.483cd	3.033b	2.08b	1.7 ef	46.36a	56.95c	47.58ab	0.711c
"DT6"	127bc	19.93c	21.41bc	16.91b	28.93b	5.34cd	2.89a-f	1.85bc	3.5cd	40.8b	76.04ab	41.729b	0.787b
"DT7"	131c	31.79a	25.46b	19.11a-f	29.66b	6.33c	3.33b	2.46b	6.16ab	43.93ab	70.80b	48.65ab	0.687cd
"DT8"	118.33f	26.19b	20.82c	17.95b	29.33b	5.830cd	4.330a	2.00 b	6.16ab	46.90a	79.18a	59.42b	0.740b
"DT9"	104h	24.24bc	19.2c	17.69b	28.00b	5.16cd	1.43d	2.10b	5.16b	46.73a	63.61c	50.57c	0.730 c

Cultivar/Hybrid	Height (cm)	Rootstock Diameter (mm)	Trunk diameter under the graft (mm)	Branch diameter of the scion (mm)	Number Leaf Density in 20cm	Leaf length (cm)	Leaf width (cm)	(cm)	New shoot Length (cm)	Chlorophyll index (Chl) (%)	RWC (%)	EL (%)	Fv/Fm
"DT10"	127.24cd	26.89ab	22.84bc	19.94ab	27.35b-d	6.91bc	2.26bc	1.81bc	3.35cd	47.09a	68.95bc	60.24b	0.598ab
"DT11"	110g	27.78ab	23.09bc	19.11ab	32.33b	6.60bc	3.80ab	1.79bc	4.53bc	42.73a-c	74.76b	65.64ab	0.743bc
"DT12"	127.93c	26.74ab	21.80bc	22.33a	30.21b	7.62ab	3.69b	2.13b	2.99d	41.56b	59.92bc	63.08b	0.646d
"DT13"	144.98a	28.52ab	21.00bc	18.72ab	18.33cd	4.61d	1.80d	1.86bc	1.59 ef	42.36b	78.95a	45.82b	0.621de
"DT14"	125.66d	27.9ab	21.00bc	23.10a	21.66c	7.76b	1.96cd	1.50c	2.11e	42.40b	61.31c	64.74b	0.649d
"DT15"	144.85a	27.70ab	25.13b	22.78a	29.66b	8.46a	2.9a-f	2.14b	3.35cd	37.76c	78.50a	63.98b	0.650d
"DT16"	93.66h	25.15b	21.89bc	19.54ab-f	27.33b	6.16c	2.16c	2.16b	4.26c	44.46ab	71.84b	59.37b	0.764bc
"DT17"	119.33e	22.32bc	20.31bc	17.54b	27.66b	8.19a	2.43c	2.31b	2.93d	39.86b	75.6ab	58.70b	0.644d
"DT18"	133.33c	25.12b	20.43bc	16.4bc	31.66b	5.16cd	1.68cd	1.56c	1.50ef	39.86b	6250c	63.14b	0.671cd
‘DT19’	138.66b	22.54bc	19.34c	14.80c	13.33d	5.60cd	2.90bc	1.97bc	0.98f	32.70c	49.60d	59.69b	0.550e
"DT20"	114.33f	22.38bc	19.88c	17.39b	26.00bc	7.00b	3.16b	2.06b	3.50cd	44.80ab	71.52b	56.94bc	0.760bc
"DT21	109.66g	27.19a	24.94b	16.62c	27.33b	6.66bc	3.1	2.30b	5.16b	41.20b	70.6	53.93c	0.683cd

Cultivar/Hybrid	Height (cm)	Rootstock Diameter (mm)	Trunk diameter under the graft (mm)	Branch diameter of the scion (mm)	Number Leaf Density in 20cm	Leaf length (cm)	Leaf width (cm)	(cm)	New shoot Length (cm)	Chlorophyll index (Chl) (%)	RWC (%)	EL (%)	Fv/Fm
"		b					0b				9b		
DT22	133.66c	25.57b	23.94bc	21.90a	26.33bc	7.10b	2.41c	2.10b	5.15b	45.16ab	69.96b	57.32bc	0.744bc

Values followed by the same letter within the same columns do not differ significantly at P<0.01 according to DMRT



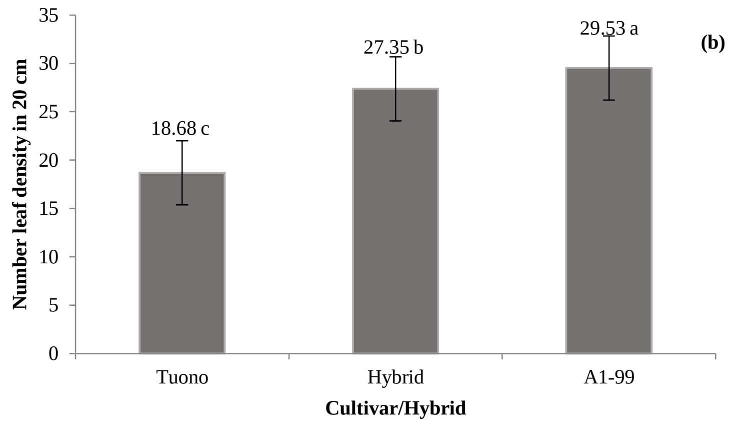


Fig. 1. The height (a) and leaf density (b) in almond hybrids and their parents under severe drought stress conditions.

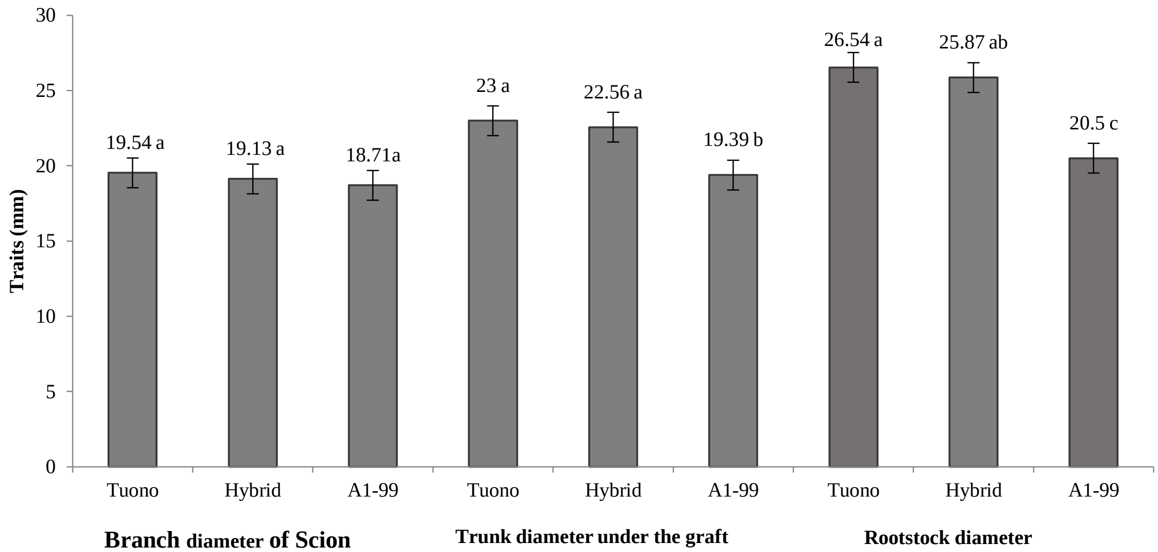


Fig. 2. The branch diameter of scion, trunk diameter under the graft and rootstock diameter in almond hybrids and their parents under severe drought stress conditions.

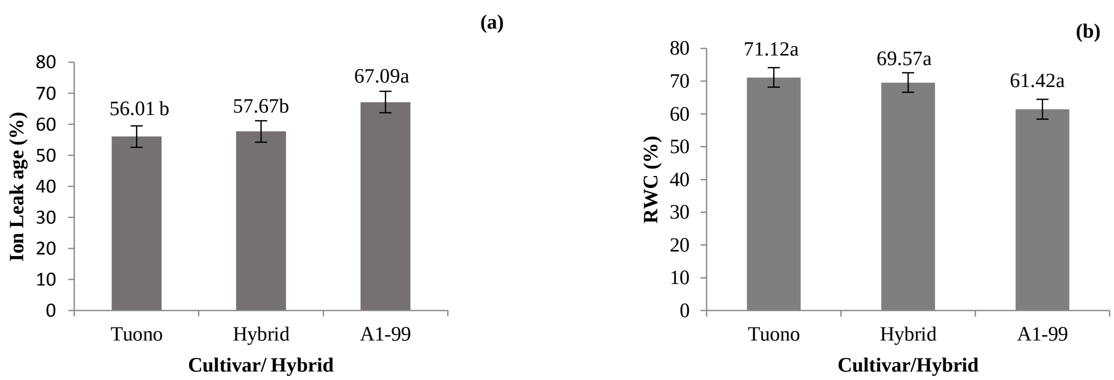


Fig. 3. The percent of ion leakage (a) and RWC (b) in almond hybrids and their parents under severe drought stress conditions.

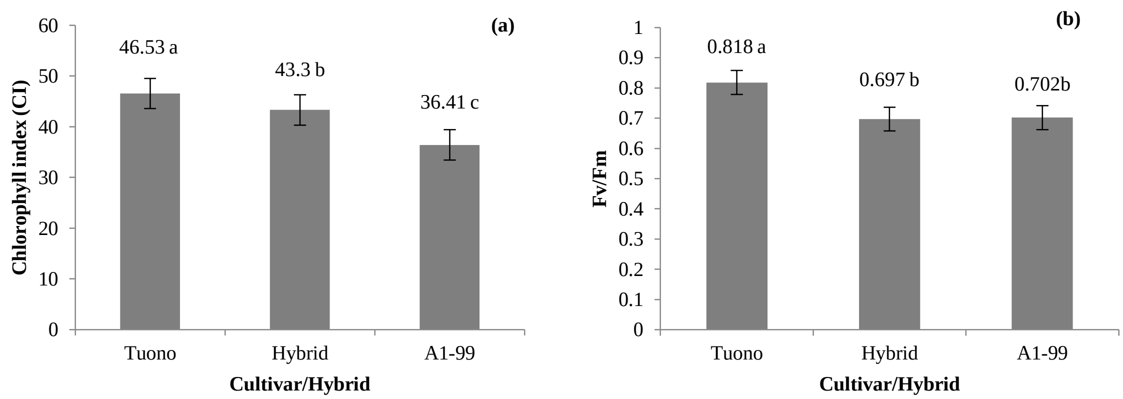


Fig. 4. Heritability and frequency of some photosynthesis parameters (a=chlorophyll index and b=FV/Fm) in almond hybrids and their parents under severe drought stress conditions.

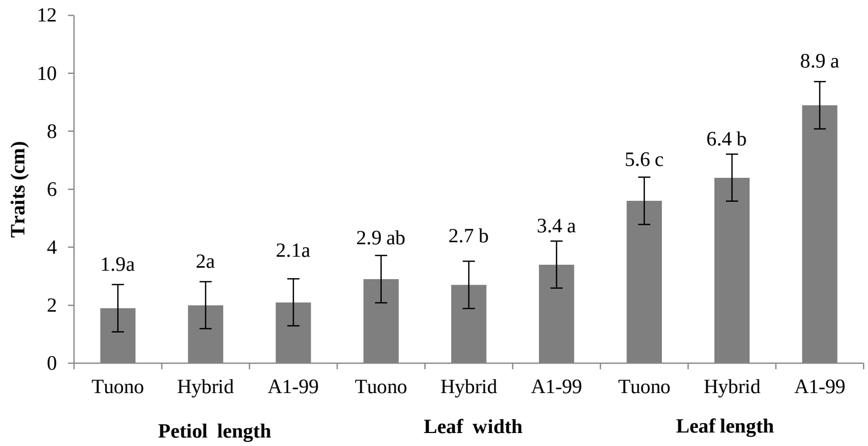


Fig. 5. The leaf traits in almond hybrids and their parents under severe drought stress conditions.

According to Table 4, by applying drought stress on selected almond hybrids and their parents, their responses were diferent. The highest height was 146.67 cm for ‘DT1’ and the lowest height for ‘A1-99’ (mother parent) was 88.77 cm. According to the results of Table 4, the average effect of drought treatment on trunk diameter under the grafting site in selected almond hybrids was determined that ‘DT2’ was 30.49 mm higher than other truncated trunks under the grafting line, in relation to the diameter of the main branch diameter of almond hybrids, the results showed that "DT14" had 23.10 mm thick branches and ‘DT19’ was 14.80 mm with the smallest diameter of the main branch of the scion had been given. Studied results of the number of leaves in 20 cm from the branch tip showed that ‘DT1’ with the mean of 42 leaves per 20 cm has the highest amount among other hybrids. ‘DT19’ with the average of 13.33 leaves has the lowest value. Also ‘A1-99’ had the highest leaf length (8.66 cm) while "DT13" (4.61 cm) had the smallest size among other hybrids/cultivars. Considering leaf width, the "DT8" hybrid with 4.33 cm has the highest leaf width in the selected cultivars, while the "DT9" has the lowest leaf width of 1.43 cm.

In relation to the growth rate and the length of the new branch, according to Table 4 ‘DT1’ hybrid with 7.19 cm

In this study, the chlorophyll index based on the SPAD criteria showed that this index in the ‘DT1’ hybrid the highest rate (48.79) was observed among other hybrids. While the ‘DT19’ hybrid showed the lowest chlorophyll index (32.70). Leaf relative water content: According to the average comparison results among the selected hybrids of the hybrids studied in this study, "DT13" with 78.95% had the highest relative water content among the other cultivars, while the lowest amount was in ‘DT19’ with 49.60%.

According to Table 4, EL increased in different cultivars in response to droughtand the increase showed a significant difference among different cultivars, so that ‘DT3’ with 70.87% had the highest EL value.

Quantum efficiency of the photosystem II (F_v/F_m): based on the results obtained (Table 4), F_v/F_m in ‘DT19’ hybrid had the lowest (550) value while the ‘DT1’ hybrid with 879, had the highest value under the drought stress conditions.

Descriptive statistics of studied morphological and physiological characteristics in selected almond hybrids under severe drought stress condition in Table 5 has been presented.

Table 5. Descriptive statistics of studied morphological and physiological characteristics in selected almond hybrids under severe drought stress condition.

Variable	Minimum	Maximum	Mean	Std. Deviation	Diversity index (%)
Height (cm)	83.00	160.00	122.73	19.29	15.71
Rootstock diameter(mm)	15.00	39.20	25.30	3.98	15.76
Trunk diameter under the graft	17.09	36.51	22.26	3.66	16.44
Branch diameter of the scion	13.87	26.27	19.13	2.61	13.65
Number of leaf in 20 cm	12.00	45.00	26.56	6.89	25.95
Leaf length(cm)	4.00	10.00	6.44	1.44	22.43
Leaf width(cm)	1.00	5.00	2.79	0.89	32.08
Petiole length(cm)	1.000	4.100	2.11	0.70	33.53
Current shoot length(cm)	0.00	8.300	3.80	2.06	54.11
SPAD	28.80	52.00	43.05	4.68	10.88
RWC	50.00	152.94	69.27	13.77	19.87
EL	30.83	82.47	58.17	9.93	17.07
F_v/F_m	0.452	0.8270	0.701	0.08	11.55

The results of drought tolerance trait in 22 hybrids in Table 6 showed that in terms of frequency, there were 17 hybrids with susceptible to relatively susceptible that contained 77.27% hybrids (Fig. 6). On the other hand, five hybrids with tolerant to relatively drought tolerant (Table 5) were, which included 22.72% of the hybrids (Fig. 6).

Table 6. Assemblage of almond hybrids and their parents base on drought stress susceptible to tolerance under severe drought stress conditions

Cultivar/Genotype	tolerant to relatively tolerant	susceptible to relatively susceptible
'Tuono'	+	-
'A1-99'	-	+
'DT1'	+	-
'DT2'	-	+
'DT3'	-	+
"DT4"	-	+
"DT5"	-	+
"DT6"	+	-
"DT7"	-	+
"DT8"	-	+
"DT9"	+	-
"DT10"	-	+
"DT11"	-	+
"DT12"	+	-
"DT13"	-	+
"DT14"	-	+
"DT15"	+	-
"DT16"	+	-
"DT17"	-	+

Table 6. Continued.

"DT18"	-	+
'DT19'	-	+
"DT20"	-	+
"DT21"	-	+
"DT22"	-	+

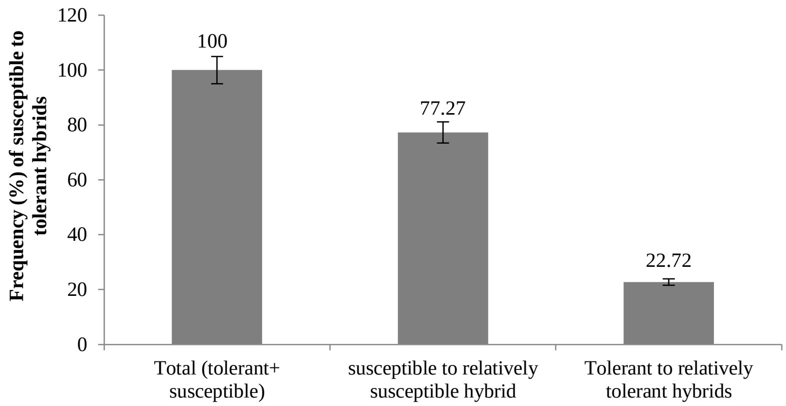


Fig 6. Frequency of susceptible to tolerance hybrids under severe drought stress conditions.

Discussion

Considering the significance of the effect of drought stress on growth and height of different hybrids/cultivars (Table2, 4), this result suggests that when trees was exposed to drought and dehydration conditions, the height of plants decreases with increasing drought stress. Stocker (1960) reported that drought stress reduced stem length and due to dwarf in plants. Mosavi (2014) reported that the activity of indole acetic acid oxidase (IAAO) enzyme in fast growing plant tissues very low, however, the activity of this enzyme has increased in drought stress conditions and causes the auxin hormone to decompose in the plant. Similar results (Zokaee-Khosroshahi *et al.,* 2014) showed that the selected species of almond under drought stress, the branch length and the height is affected, so that the least amount of branch length was observed in *P. eburnea* and *P. eleagnifolia* species. Rigling *et al.,* (2003) reported that water shortage affects the growth of cells and affects the formation of the cells of xylem adversely and reduces its amount. Studied results of the density of leaves in selected branch of hybrids/cultivars showed that range of this item from 42 up to 13.33 leaves varied. McMichael *et al.* (1973) reported a decrease in the number of leaves, an increase in ethylene production due to stress and leaf loss. On the other hand, during the stress period, plants tended to reduce transpiration by reducing the number of leaves in which in this study was evident (Jones and Cortlett 1992). Similar results by Rajabpoor *et al.* (2014) has been reported that, under certain osmotic stress conditions, some of the wild almond species *in vitro* exhibit some morphological, biochemical, physiological and pathological changes.

In relation to the growth rate and the length of the current branch, according to Table 3 some of hybrids/cultivars such as 'DT1' showed the highest growth under drought stress conditions, indicating that this hybrid has better resistance and compatibility in drought stress than others. In contrast to 'DT19' hybrid among all cultivars/hybrids, had the lowest growth, which designates that this hybrid is susceptible to drought conditions. Nikoumanesh *et al.* (2011)

examined the morphological and molecular diversity of 55 Iranian almond genotypes. Their results indicated that there was difference between leaf characteristics including length, width, and leaf area with tree growth ability which is consistent with the results of this study.

The results of chlorophyll index (ChI), which is used as a non-destructive and rapid method to measure changes in chlorophyll content and related to the effect of stress on plants, showed that 'DT1' hybrid had the highest amount ChI among others. Similar results were presented by Samandari Gikloo and Elhami (2012). According to their results, there was a significant difference in the chlorophyll index in drought stress between almond cultivars, so that " Tuono "cultivar showed a much higher chlorophyll index than the " Princess ". This can be explained by the direct effect of dryness on the leaf chlorophyll content index, which reduces it, and subsequently the yield decreases (Schlemmer *et al.,* 2005).

Leaf relative water content is referring as an indicator of the degree of stress and wilt (Maclagan 1993) and evaluating for the tolerance to drought stress (Faraloni *et al.,* 2011). Based on comparison of results from hybrids in this study, "DT13" had the highest relative water content among the others in which indicating the relative tolerance of this hybrid to stress conditions. While the lowest amount was in 'DT19' as sensitive to drought stress. A similar result was reported by Faraloni *et al.* (2011) show different amounts of relative water content in different species of almond under severe drought stress conditions, so as to *P. eburnean* species had the highest relative water content in leaves which indicates that this species has a good resistance to drought stress.

These results show that sensitive genotypes are less capable of absorbing or retaining water within their leaves due to drought and dehydration condition. It can be pointed out that with increasing the intensity of water stress, water absorption conditions for plants as a result, the amount of water in the tissues of the plant tissue is distanced from the state of tungsten, and the reduction of water content has a negative effect on cell division and plant growth. According to studies conducted on the leaf relative water content of different plants, it was recommended that this index can help the breeder for screening (Krause *et al.*, 1993, Gradziel *et al.*, 2001).

According to Table 4, by rising the severity of drought stress on the hybrids/cultivars, El increased, but the rate of increase in different hybrids/cultivars was significantly different, for instance 'DT3' had the highest EL rate with 70.87%. Therefore, protecting the plant cell membrane from stress damage depends on their morphological and physiological characteristics. In this regard the cell membrane stability plays a pivotal role in tolerance to drought stress and heat, because it directly relates to the production of heat shock proteins, photosynthetic system features, key enzymes and TLC membranes (Sairam *et al.,* 2009). For example, under stress conditions, sugars protect membrane stability through osmotic regulation and turgid pressure of the cells (Bartels and Sunkar, 2005, Lotfi *et al.*, 2009). According to a report (Akbarpour *et al.,* 2017), EL in different cultivars was significant ($P < 0.01$), indicating a different amount of cell membrane stability in different cultivars and genotypes in almonds and is consistent with results of the present study.

Quantum efficiency of the photosystem II (F_v/F_m) (F_V/F_m) indicate that 'DT1' hybrid is more tolerant to drought stress than other hybrids (Table 4). This may be due to (Yuan *et al.,* 2005) the effect of drought stress as one of the most important environmental factors limiting photosynthesis, thus by closing the stomata, the intracellular CO_2 is reduced, resulting in the accumulation of energetic electron carriers, the formation of free radicals, the disturbance of light-picking complexes and the loss of photosynthesis efficiency. For other reasons, this can be noted in the report of Naumann *et al.* (2007) on drought stress, which stated that various stresses, including drought stress, by reducing the consumption of electron transfer chain (NADPH and ATP) products, increased the level of ferredoxin and reclamation of free radicals, resulting in the destruction of proteins in the thylakoids membrane of cells, which results in reduction of electron transfer from photosystem II and the reduction of the Maximum quantum yield and increasing the chlorophyll fluorescence (Belkhodja *et al.,* 1994).

The results from frequency of drought tolerance trait of hybrids to recognizing hybrids from the most sensitive to the most resistant was investigated (Table 4). In the case of parents, the ‘Tuono’ cultivar as the father parent was more tolerance than ‘A1-99’ as the mother parent. Among the hybrids," DT1" and ‘DT19’ were the most resistant and the most sensitive and located in groups of tolerant to relatively tolerant and susceptible to relatively susceptible respectively. Achievement to tolerance hybrids is important in terms of breeding goals, since as much as a drought tolerant, it is an advantage. So that drought tolerant varieties performed better during drought stress which led to increased productively (Gradziel *et al.,* 2001, Vargas *et al.,* 2001).

Screening and frequency magnitude indicates the reliability with which the genotype will be recognized by its phenotype expression (Chandrabau and Sharma 1999). Finally, an estimate of screening and frequency for different traits could be useful in breeding programs because direct selection for characters with high performance will be effective. The results of this study showed the range of frequency and variability for some traits in almond hybrids, which can be used in the next experiments for improving almond hybrids.

Evaluation of diversity index in morph-physiological characteristics of almond genotypes indicate that almond current shoot length (54.11%) and petiole length (0.7%) had the highest and the lowest diversity index respectively (Table 6).

The results showed a wide morpho-physiological diversity among the studied genotypes, so that the selected genotypes in this study can be categorized into specific groups for exploitation in breeding programs, and these important factors in distinction of selected genotypes. Considering the differences in the traits of the studied genotypes , significant variation in important traits was found which suggests suitable genotypes for use in almond breeding programs, and provides information about inheritance of these traits that have been considered by almond breeders (Kester 1965, Gradziel *et al.,* 2001).

Conclusions

In conclusion, the results showed that by applying severe drought stress, some morphological characteristics such as height, diameter of the main branch of the scion, leaf number density, growth of leaf length and width, and growth of new branches; physiological characteristics including chlorophyll index, leaf relative water content, EL and maximum photochemical efficiency (F_v/F_m) decreased but there are variations in term of these traits among genotypes. For example range of F_v/F_m among the selected hybrids was varied from 550 to 879. Finally, according to the analysis of data obtained from this study, ‘DT19’ was recognized as the most sensitive and ‘DT1’ was known as the most tolerant hybrid that could be introduced as promising and tolerant to drought stress for utilizing in the breeding programs.

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