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Selection of open pollination progenies in some pear species in order to achieve dwarf and drought tolerant rootstocks

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Abstract: One of the important products in Iran and Isfahan province is the pear that its cultivation has been limited by drought stress and global warming in recent years. The use of drought-tolerant rootstocks is one of the available solutions for pear orchards in semi-arid regions. In addition, the lack of dwarf or semi-dwarf rootstocks, which are appropriate and compatible with Iran climatic conditions, limited high density pear orchards. In order to obtain drought tolerant rootstocks, in this research fruit of *Pyrus glabra*, *Pyrus syriaca,* and *Pyrus salicifolia*, along with *P. communis* cv. Spadona, Dargazi, as well as Khoj n. 1 and n. 2 species were collected from different regions of Iran in August and September of 2016. The seeds were separated from the flesh and dried at room temperature. The seeds were cultivated in uniform and light texture of soil in November in the field condition to break the seed dormancy. Seedlings were irrigated regularly for three months in order to establish in the soil; drought stress began in July. In order to apply drought stress, irrigation time was considered based on 80% of allowed water depletion. Morphological traits of seedlings were recorded before stress and at the end of the stress period (late September). The viability percentage of seedlings after drought stress was between 14.28% (*P. communis* cv. Dargazi progenies) to 82.55% for *P. salicifolia*. Comparison of the means and cluster analysis, among populations showed that the three populations of *P. salicifola*, *P. glabra* and *P. communis* cv. Khoj n. 2 had the lowest height and were placed in the same group. After studying single genotypes in these three populations, genotypes no. 31, 32, 41, 57 and 12 from *P. salicifolia*, genotypes 10, 11, 7, 3 and 9 of *P. glabra* and genotype 4 of *P. communis* cv. Khoj n. 2 populations were selected as drought tolerant and dwarf genotypes and were taken to the propagation phase for future evaluation.

1. Introduction

Pear is from the *Rosaceae* family and the *Pyrus* genus. This species has been cultivated in Iran since ancient times, and Iran is one of the earliest areas of pear distribution and variation in the world (Abdollahi, 2011). Drought stress is the most important environmental stress that occurs annually with extreme damage to crops, especially in arid and semi-arid regions (Xoconostle-Cazares *et al*., 2010). In dry and semi-arid regions of Iran, the amount of annual evaporation is higher than precipitation. In recent years, the cultivation of fruit trees, such as pears, has been limited due to climate change and reduce rainfall. Considering that the drought tolerance of the rootstocks is transmitted to the scion (Landsberg and Jones, 1981), the choice of drought tolerant rootstocks with a low water requirement is one of the solutions to drought problem of pear orchards in arid and semi-arid regions such as Iran (Cheruth *et al*., 2009).

Another problem in the cultivation of pears in Iran is the lack of suitable dwarf or semi-dwarf rootstocks that are compatible with cultivars and climatic conditions of the country for high density orchards. The pear and quince are used as rootstocks for pear trees. The quince rootstocks are very dwarf (EM-C) and semi-dwarf (BA29), but these rootstocks could not overcome the problem of graft incompatibility with some pear cultivars. These rootstocks are not also tolerant to winter frosts and chlorosis caused by iron deficiency in the calcareous soils (Harotko, 2007). In recent years, a number of clonal rootstocks have been introduced into Iran such as Pyrodwarf, Fox-11 and some of the American rootstocks of the Old home × Farmingdale series (Abdollahi, 2011). The use of native pear species as a seed or clonal rootstocks can be a solution for access to proper rootstocks with good adaptability to the climatic conditions of the country. Some of these species are dwarf and have a high tolerance to unfavorable environmental conditions that can be used to produce clonal rootstocks (Abdollahi *et al*., 2012).

In the pear genus, 22 species have been identified in the world that are mainly native to Europe, Africa and Asia. Of the 22 identified species, there are 12 species in Iran (Sabeti, 1995). In some parts of the world, these species are used as the rootstock for pear commercial cultivars. For example, a large number of pear cultivars in Turkey are grafted on *P. elaeagrifolia*, in Syria and Lebanon on *P. syriaca*, in ancient Yugoslavia, Turkey and Greece on *P. amygdaliformis*, in the south of Russia on *P. salicifolia* and in Algeria and Morocco are grafted on *P. longipes* (Henareh, 2015).

Wild germplasm has evolved in natural dry ecosystems for tolerance of stress conditions such as high temperatures, drought and salinity. Identification of wild pear germplasm is very important to use as a rootstock in semi-arid regions (Zarafshar *et al*., 2014). According to this, three wild pear genotypes from *P. syriaca* were exposed to four irrigation treatments. The results showed that the Coile wild genotype (*P. syriaca*) was more tolerant to drought conditions compared with other genotypes due to its high relative water content during drought stress and non-decreasing dry weight. Drought stress reduced leaf photosynthesis, stomatal conductance and transpiration in a number of pear species (Javadi and Bahramnejad, 2011).

In another study, drought tolerance was evaluated in three populations of wild pear germplasm (*P. boisseriana*) in greenhouse conditions. Among the three populations studied, the collected population from semi-arid regions showed higher drought tolerance than the other two populations that were collected from semi-humid areas, and recovered more rapidly after irrigation. These seedlings were introduced as promising sources for use as rootstock for commercial pear cultivars in drought conditions (Zarafshar *et al*., 2014). In a research carried out by Ghasemi *et al*. (2014), chlorophyll index was significantly different between studied pistachio rootstocks. In these rootstocks, chlorophyll index in stress conditions was lower than drought conditions. So, the reduction of chlorophyll content can be caused by chlorophyll degradation under drought stress conditions, which leads to reduced pure photosynthesis.

The rootstocks of European pear species are from very vigor to relatively dwarf. These rootstocks are compatible with all the pear tree cultivars and have considerable tolerance to the adverse conditions of soil and fire blight disease. Other species of *Pyrus* genus, such as *P. syriaca* Boiss is compatible with commercial pear cultivars and is currently used in some countries (Radnia, 1996).

Seed cultivation after cross or open pollination is one of the breeding methods that is frequently used in breeding programs. In this way, it is possible to achieve a wide variation for choice of new rootstocks and cultivars. For example, the Manon cultivar was obtained from open pollination of Beurre Bosc cultivar. The Pib-BU3 dwarf pear rootstock was obtained from open pollination of *P. longipes*, while Pi-BU4 and Pi-BU7 rootstocks were obtained from open pollination of the *P. pyrifolia* species (Mohan Jain and Priyadarshan, 2009).

Currently, most pear cultivars in Iran are grafted on different seedling rootstocks of the *P. communis* species. Considering that wild pear genotypes grow on rocks and dry or low moisture soils, as well as some of them have a small growth in the form of shrubs, so some of them can be used as drought tolerant and dwarf rootstocks for commercial pear cultivars (Ashraf and Karimi, 1991; Henareh, 2015). The aim of this research was an evaluation of drought stress tolerance in some wild pear species, and then selection of the dwarf genotypes among the drought tolerant genotypes.

2. Materials and Methods

Plant materials

In this research, seeds from open pollination of *Pyrus syriaca* Boiss., *Pyrus salicifolia* Pall. and *Pyrus. glabra* Boiss. along with *P. communis* L. cv. Spadona and Dargazi as well Khoj including large fruit (Khoj no. 1) and small fruit (Khoj no. 2) were evaluated. *P. syriaca* Boiss. is distributed from west Azarbaijan to Fars in the Zagros Mountains and northwest of Iran (Abdollahi, 2011). This species has shown compatibility with Spadona and Kochia pear cultivars and have improved the growth of the scion in calcareous soils. It is currently used as a rootstock in some countries (Fallouh *et al*., 2008). *P. glabra* species is known as Anchuchek in Iran, and has spread mainly in the Zagros Mountains. The seeds of this species are large and are consumed as snacks in Fars province (Abdollahi, 2011). *P. communis* is more commonly known as Khoj and is scattered in the forests of the north, West Azarbaijan, Sardasht and Baneh in Kordestan province. The fruit of this species is very diverse. Two types of native Khoj including large fruit (Khoj n. 1) and small fruit (Khoj n. 2) were studied in this research (Moazedi *et al*., 2014). *P. communis* cv. Dargazi is native commercial cultivar of Iran that has been introduced as tolerant rootstock (Mansouryar *et al*., 2017). *P. communis* cv. Spadona is high yielding and has high resistance to chlorosis due to iron deficiency. It is also relatively tolerant to psylla and fire blight. *P. salicifolia* species spread in the northwest of Iran, including west and east Azerbaijan provinces (Abdollahi, 2011).

Cultivation of seeds

Fruits of studied species were collected in August and September of 2016 from different regions of West Azarbaijan and Isfahan provinces as well as northern regions of Iran and transferred to the laboratory. The seeds were separated from the flesh and kept in a cool and dry place in paper bags after washing and drying, In order to eliminate the chilling requirement, seeds were cultivated in separate rows in the nursery soil with a sandy loam texture at Isfahan Agricultural and Natural Resources Research Center, in December. From each of these species, 500 seeds were cultivated at intervals of 2-3 cm in the nursery and each produced seedlings were studied as a genotype. During chilling period, adequate moisture was provided and prevented from drying of the culture bed.

Irrigation of seedlings

After emergence of seeds, the seedlings were irrigated for three months in order to establish in the nursery soil. Determination of irrigation time based on allowed water depletion of pear trees is after a 50% decrease in humidity, but because of the seedling roots had not yet been sufficiently developed, and needed enough moisture for better growth, so allowed water depletion was considered 35%. In the irrigation intervals, soil moisture at various depths of the soil was measured up to a depth of 100 cm by the Time Domain Reflectometry device (TDR, Trase6050X1) (Doorenbos and Pruitt, 1977; Alizadeh, 2006).

To obtain the amount of irrigation (irrigation volume), first, net irrigation depth was calculated according to formula 1.

$$In = \sum [(\theta FCi - \theta BLi) \times Di] \quad (1)$$

In this formula, In is the net irrigation depth (mm), θFCi is the moisture content of the field capacity for each layer, θBLi is the soil moisture before irrigation for each layer, Di is the root development depth (mm), and i is the number of each soil layer. Then, according to formula 2, gross irrigation depth was calculated.

$$Ig = In/(1-Lr) \times Ei \quad (2)$$

In this formula, Ig is the gross irrigation depth (mm), In is the net irrigation depth (mm), (1-Lr) is the amount of leaching and Ei is the irrigation efficiency (usually 80-90% for drip irrigation).

Irrigation volume was obtained from the multiplication of gross irrigation depth in the irrigation plot area. This volume was controlled by the installed counter on the pipe before irrigation plot. For irrigation of seedlings leaked tubes with drippler at 10 cm intervals were used. The outlet flow of each drippler was measured in one liter per hour at an appropriate pressure. In this study, the efficiency of the drip system and the leaching requirement was considered 90% and 10% respectively.

Drought stress

Drought stress began in July, which coincided with the beginning of the stress period in Isfahan region. Irrigation frequency was changed in order to apply drought stress, and irrigation time was considered based on 80% of allowed water depletion. Table 1 shows the volume of pure and impure irrigation water and number of irrigation per month.

Evaluated traits

The morphological traits of seedlings were recorded separately for each genotype at the end of the stress period (late September). These traits included height and diameter of seedlings, number and length of internode, crown width, number of suckers and branches, chlorophyll index and leaf dimensions. Before applying stress, seedling height and diameter at 5 cm above the soil surface were recorded. The difference in both seedling height and diameter before and after stress were also calculated. Qualitative traits, including leaf chlorosis and trichome, as well as seedling growth vigor were recorded after stress using national guideline for distinctness, uniformity and stability in pear (DUS guileline) in pears. The abbreviation and measurement method of evaluated traits are given in Table 2.

Data analysis

Mean comparison was calculated with SAS software (version 9.1). Descriptive statistics including mean, minimum, maximum and coefficient of variation and also cluster analysis were performed by Ward method based on Squared Euclidean Distance with SPSS software (version 15).

3. Results

Viability percent

Viability percent of seedlings of the studied pear species after the stress was shown in Table 3. According to the results, *P. salicifolia* showed the highest survival in drought stress conditions. After that, *P. communis* cv. Khoj n. 1 and 2 were placed in the next rank. The lowest percentage of seedling via-

Table 3 - Seedlings viability percent of pear cultivars and species after drought stress

Species	Viability percent
Pyrus communis cv. Spadona	14.77
Pyrus communis cv. Dargazi	14.28
Pyrus communis cv. Khoj n. 1	55.17
Pyrus communis cv. Khoj n. 2	43.79
Pyrus syriaca	20.33
Pyrus salicifolia	82.55
Pyrus glabra	17.74

Table 1 - Number of irrigation, net and gross volume of irrigation water

Irrigation	February	March	April	May	June	July	August	September	October	November	Total
Number of irrigation	3	3	5	7	8	1	1	1	1	1	32
Net volume of irrigation water ($m^3.ha^{-1}$)	540	540	900	1260	1440	180	180	180	180	180	5580
Gross volume of irrigation water ($m^3.ha^{-1}$)	666.6	666.6	1111.1	1555.5	1777.7	222.2	222.2	222.2	222.2	222.2	6888.2

Table 2 - Symbol and measurement method for recorded traits (based on DUS guideline)

Characteristic	Symbol	Unit	Measurement method
Leaf chlorosis	LC	Code	No chlorosis (1), low chlorosis (3), medium chlorosis (5), high chlorosis (7), very high chlorosis (9)
Leaf trichome	LT	Code	No trichome (1), low trichome (3), medium trichome (5), high trichome (7), very high trichome (9)
Seedling growth vigor	GV	Code	Very low (1), low (3), medium (5), high (7), very high (9)
Seedling height	SE	cm	Ruler
Seedling diameter	SD	mm	Caliper
Height difference	HD	cm	Calculation
Diameter difference	DD	cm	Calculation
Internode number	IN	Number	Counting
Internode length	IL	cm	Ruler (average of internodes in branches)
Crown width	CW	cm	Meter
Number of secondary branches	NSB	Number	Counting
Number of suckers	NS	Number	Counting
Chlorophyll index	CI	-	Chlorophyll meter (Spad)
Leaf length	LL	cm	Ruler (average of 10 leaves)
Leaf width	LW	cm	Ruler (average of 10 leaves)

bility during drought stress belonged to *P. communis* cv. Spadona and Dargazi.

Traits before applying stress

The mean comparison of recorded traits among populations before applying drought stress is presented in Table 4. According to the results, *P. communis* cv. Spadona had the highest seedling height. The seedling height in the wild species of *P. glabra*, *P. salicifolia* and *P. communis* cv. Khoj n. 2 was the lowest. *P. communis* cv. Khoj n. 2 and *P. glabra* produced seedlings with the lowest diameter. The largest seedling diameter belonged to *P. communis* cv. Spadona and *P. syriaca* (Table 4). Generally, without drought stress conditions, growth of *P. glabra*, *P. salicifolia* and *P. communis* cv. Khoj n. 2 were lower than *P. communis* cv. Spadona, Khoj n. 1, Dargazi and *P. syriaca*.

Table 4 - The mean comparison of the seedling height and diameter among populations of pear species before applying stress

Species	Seedling height (cm)	Seedling diameter (mm)
Pyrus communis cv. Spadona	33.52 a	3.44 a
Pyrus communis cv. Khoj n. 1	23.38 b	3.15 ab
Pyrus communis cv. Dargazi	24.23 b	3.15 ab
Pyrus syriaca	20.92 b	3.31 a
Pyrus salicifolia	12.80 c	2.62 bc
Pyrus communis cv. Khoj n. 2	11.83 c	1.74 d
Pyrus glabra	7.54 c	2.25 cd

Similar letters in each column indicate no significant difference (LSD).

Traits after applying stress

The mean comparison of recorded traits among populations after stress is presented in Table 5. *P. communis* cv. Spadona had the highest seedling height (36.73 cm) after stress. The seedling height of *P. glabra*, *P. salicifolia* and *P. communis* cv. Khoj n. 2 were lower than other species. Similarly, *P. communis* cv. Spadona showed the highest height difference before and after applying stress.

The seedling diameter of *P. communis* cv. Spadona, Khoj n. 1, Dargazi and *P. syriaca* was more than the other seedling diameter, So that *P. glabra* and *P. communis* cv. Khoj n. 2 had the lowest seedling diameter. No significant difference was observed among the seedling diameter of the species before and after drought stress.

P. communis cv. Spadona had the highest number of internode after the end of drought stress. The lowest number of internode belonged to *P. glabra* and *P. communis* cv. Khoj n. 2. *P. salicifolia* produced the crown with the widest (24.71 cm) width, compared to other species. It should be noted that *P. communis* cv. Spadona, Khoj n. 1 and *P. syriaca* did not show any significant difference with *Pyrus salicifolia*. *P. communis* cv. Khoj n. 2 had the lowest crown width (4.75 cm).

P. salicifolia did not produce a secondary branch. There were no significant differences in the number of secondary branches among the other species. *P. salicifolia* showed the highest chlorophyll index, but did not show significant differences with *P. communis* cv. Spadona, Khoj n. 2, *P. syriaca* and *P. glabra*. The lowest chlorophyll index belonged to *P. communis* cv. Dargazi (Table 5).

P. communis cv. Spadona , with a length of 6.06 cm and a width of 2.7 cm had the highest leaf length and width compared with other species. After *P. communis* cv. Spadona, *P. communis* cv. Khoj n. 1 had the highest leaf length and width. The lowest leaf length was related to *P. communis* cv. Khoj n. 2 with an average of 2.01 cm. *P. syriaca* also produced the leaves with the lowest width (1.37 cm).

P. communis cv. Spadona and Dargazi had the longest internode, but did not show significant differences with *P. communis* cv. Khoj n. 1 and *P. syriaca*.

Table 5 - Mean comparison of some traits in the seedling populations of pear species after applying stress

Species	Seedling height (cm)	Height difference (cm)	Diameter (mm)	Diameter difference (mm)	Internode number	Crown width (cm)	Number of secondary branches	Chlorophyll index	Leaf length (cm)	Leaf width (cm)	Internode length (cm)
Pyrus communis cv. Spadona	36.73 a	3.20 a	4.34 a	0.89 a	20.23 a	10.03 ab	0.23 ab	44.99 ab	6.06 a	2.70 a	1.91 a
Pyrus communis cv. Khoj n. 1	26.65 b	2.80 ab	4.00 a	0.84 a	14.56 b	10.62 ab	0.68 a	42.48 b	5.45ab	2.23 b	1.21 ab
Pyrus communis cv. Dargazi	25.83 b	1.60 ab	3.81 a	0.66 a	14.66 b	7.83 b	0.66 a	42.02 b	4.73 bc	2.10 bc	1.93 a
Pyrus syriaca	21.60 b	1.41 ab	4.02 a	0.70 a	12.91 bc	9.16 ab	0.25 ab	49.77 ab	4.50 c	1.37 d	1.34 ab
Pyrus salicifolia	14.21 c	0.67 b	3.56 ab	0.94 a	10.05 cd	11.24 a	0.00 b	53.83 a	4.30 c	1.73 c	0.94 b
Pyrus communis cv. Khoj n. 2	13.67 c	0.84 b	2.63 c	0.88 a	8.31 de	4.75 c	0.21 ab	46.53 ab	2.01 d	1.97 bc	0.71 b
Pyrus glabra	10.22 c	0.68 b	2.92 bc	0.66 a	5.27 e	8.31 b	0.36 ab	48.85 ab	3.98 c	2.10 bc	0.73 b

Similar letters in each column indicate no significant difference (LSD).

The lowest internode length belonged to *P. salicifolia*, *P. glabra*, and *P. communis* cv. Khoj n. 2 (Table 5).

Cluster analysis of populations

According to the results of cluster analysis, species were classified into three groups at five squared Euclidean distance basis of the Ward method (Fig. 1). *P. communis* cv. Spadona and Dargazi were placed in the first group. *P. syriaca* and *P. communis* cv. Khoj n. 1 in the second group, while *P. communis* cv. Khoj n. 2, *P. salicifolia* and *P. glabra* species in the third group.

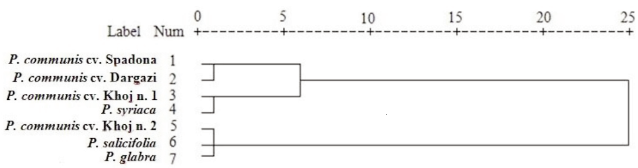

Fig. 1 - Grouping of pear species based on measured characteristics by Ward method.

Study of single genotypes

The mean and the range of traits for each of the examined genotypes, as well as the coefficient of variation in each trait are presented in Table 6. Each genotype was identified by a number and genotypes with minimum and maximum values were presented. Internode length (66.12%), seedling height before stress (60.45%) and seedling height after stress (56.85%) had the highest and chlorophyll index (14.34%) had the lowest coefficient of variation.

In order to achieve dwarfing genotypes, the trait of seedling height was considered. The three pear populations, including *P. salicifolia*, *P. glabra* and *P. communis* cv. Khoj n. 2 had a lower mean seedling height than other populations, so the height of single genotypes of them after stress was shown in figures 2, 3 and 4, respectively. In *P. salicifolia*, genotypes no. 31 (4.5 cm), 32 (5 cm), 41 (5.5 cm), 57 (6 cm) and 12 (6 cm) had the lowest seedling height. Genotypes

Table 6 - Mean, Range and coefficient of variation in studied traits

Characteristics	CV (%)	Standard deviation	Mean	Minimum		Maximum	
				Genotype (number of genotypes)	Rate	Genotype (number of genotypes)	Rate
Seedling height before stress	60.45	9.83	16.26	*P. glabra* (42)	1.4	*P. communis* cv. Spadona (23)	53.9
Seedling diameter before stress	28.35	0.76	2.68	*Py. communis* cv. Khoj n. 2 (33)	0.75	*P. communis* cv. Spadona (64)	6.3
Seedling height after stress	56.85	10.28	18.08	*P. glabra* (10 & 11)	3	*P. communis* cv. Spadona (2)	55
Seedling diameter after stress	26.47	0.94	3.55	*P. communis* cv. Khoj n. 2 (8)	1.6	*P. salicifolia* (8)	7.28
Height difference	55.32	2.19	1.82	*P. salicifolia* (24 & 28)	0	*P. communis* cv. Khoj n. 1 (9) & *P. syriaca* (3, 4,5,6 & 7)	19
Diameter difference	55.17	0.48	0.87	*P. salicifolia* (47)	0.04	*P. salicifolia* (66)	3.06
Internode number	44.65	5.00	11.20	*P. glabra* (7,10 & 11)	3	*P. communis* cv. Khoj n. 1 (14)	26
Crown width	30.67	3.11	10.14	*P. communis* cv. Khoj n. 1 (5) & *P. glabra* (10)	4	*P. communis* cv. Khoj n. 1 (8)	30
Number of secondary branches	37.22	0.67	0.18	Many genotypes	0	*P. communis* cv. Khoj n. 2 (4)	4
Chlorophyll index	14.34	7.81	54.46	*P. communis* cv. Khoj n. 1 (8)	23.2	*P. salicifolia* (36)	69.8
Leaf length	31.93	1.37	4.29	*P. communis* cv. Khoj n. 2 (15)	1	*P. communis* cv. Spadona (13)	7.2
Leaf width	26.70	0.51	1.91	*P. syriaca* (5)	1	*P. communis* cv. Spadona (2)	3.3
Internode length	66.12	0.82	1.24	*P.salicifolia* (30)	0.3	*P. communis* cv. Spadona (1)	10

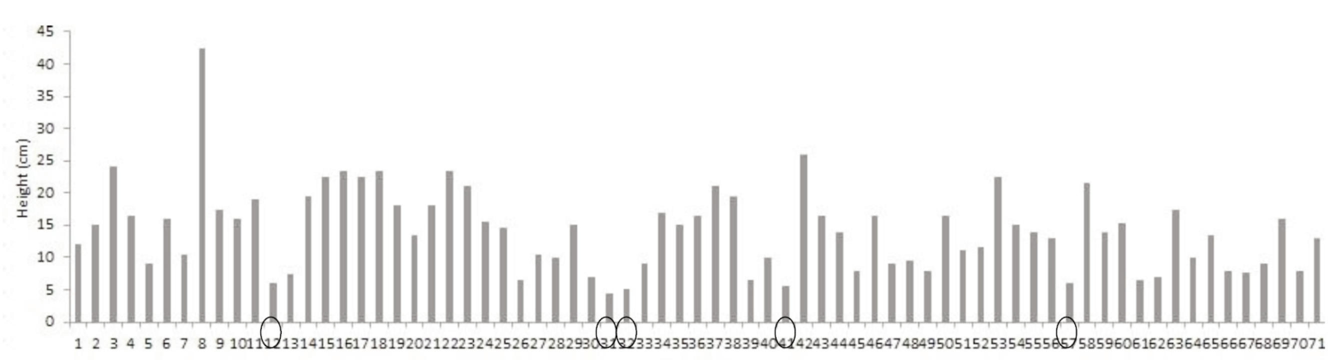

Fig. 2 - Height of single genotypes in *P. salicifolia* after stress.

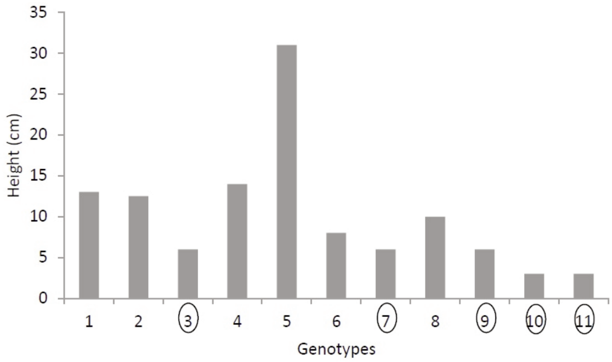

Fig. 3 - Height of single genotypes in *P. glabra* after stress.

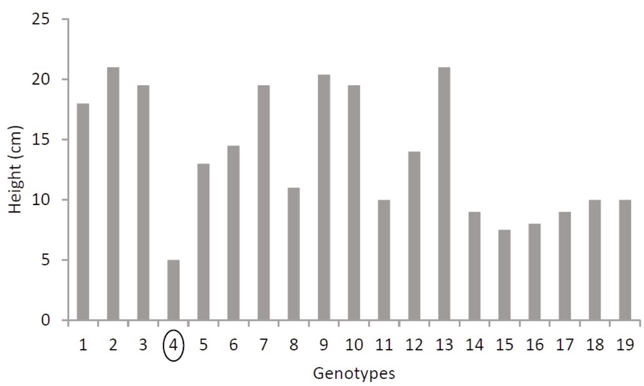

Fig. 4 - Height of single genotypes in *P. communis* cv. Khoj n. 2 after stress.

no. 10 (3 cm), 11 (3 cm), 3 (6 cm), 9 (6 cm) and 7 (6 cm) showed the lowest seedling height in the *P. glabra* species. Genotype no. 4 had the lowest height from *P. communis* cv. Khoj n. 2 population.

Cluster analysis of single genotypes based on all studied traits

Cluster analysis among the single genotypes of the *P. salicifolia*, *P. glabra* and *P. communis* cv. Khoj n. 2 populations was shown in figures 5, 6 and 7. Genotypes no. 12, 31, 32, 41 and 57 of *P. salicifolia* (Fig. 5) and genotypes no. 10, 11, 3, 9 and 7 of *P. glabra* (Fig. 6) were placed in the groups close to each other.

Qualitative traits for selected genotypes were presented in Table 7. All genotypes had a small trichome and had low or very low growth potentials. Leaf chlorosis was not observed in genotypes except for the genotype no. 31 from *P. salicifolia* and genotype no. 3 from *P. glabra* that had low chlorosis. Genotypes no. 12 and 32 of *P. salicifolia* were green and very green, respectively. Genotypes no. 9, 7, 10 and 11 of *P. glabra* and genotype no. 4 of the *P. communis* cv. Khoj n. 2 preserved their green color after applying drought stress.

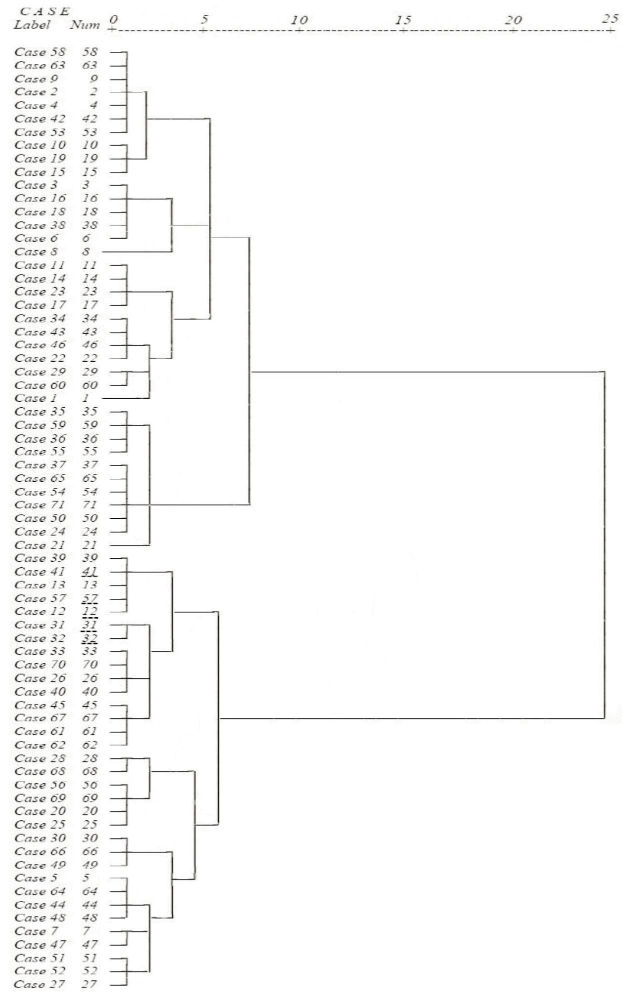

Fig. 5 - Grouping of drought tolerant genotypes in *P. salicifolia* species based on measured traits by Ward method.

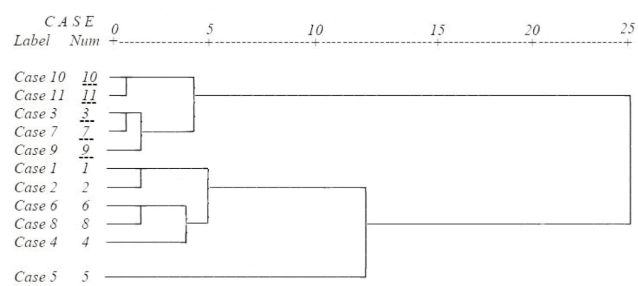

Fig. 6 - Grouping of drought tolerant genotypes in *P. glabra* species based on traits measured by Ward method.

4. Discussion and Conclusions

Viability percent

After the drought stress period, 35.51% of the genotypes survived, and the rest of them were dried. Most surviving genotypes belonged to *P. salicifolia* species. *P. communis* cv. Spadona and Dargazi

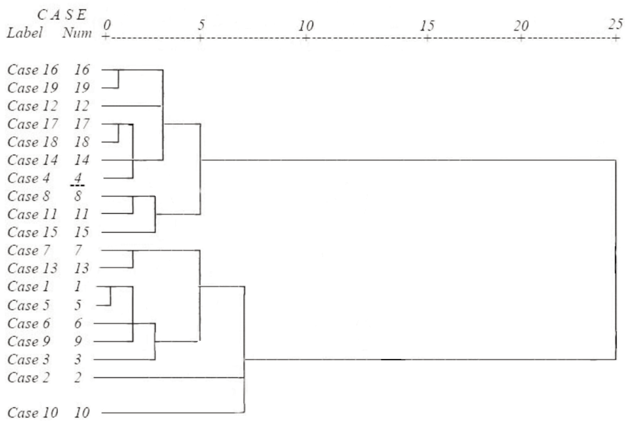

Fig. 7 - Grouping of drought tolerant genotypes in *P. communis* cv. Khoj n. 2 based on the measured traits by the Ward method.

showed the lowest percentage of survival (Table 3). For a long time, wild pear genotypes have been considered in Iran's plateau due to the tolerance to biotic and abiotic stresses (Javadi *et al*., 2005). The adaptation of wild pears with rocky areas and dry or low moisture soils can lead to more tolerance of them under drought stress conditions compared with domestic and commercial rootstocks (Henareh, 2015).

Traits before and after applying stress

The analysis of variance showed that the investigated species had a significant difference in most of the studied traits, which is due to the diversity among populations, so it is possible to select species for different values of a trait. According to table 4, in normal conditions, seedling height and diameter of *P. glabra*, *P. salicifolia* and *P. communis* cv. Khoj n. 2 were less than *P. communis* cv. Spadona, Khoj n. 1, Dargazi and *P. syriaca*. Similarly, after applying drought stress, the seedling height and diameter of *P. glabra*, *P. salicifolia* and *P. communis* cv. Khoj n. 2 species were lower than other populations (Table 5). Morphological adaptations in plants can be one of the adaptive mechanisms under drought stress (Pire *et al*., 2007). The first reaction of plants against drought stress is a reduction in their vegetative growth. Drought stress affects the vegetative characteristics of trees, including their height (Higgs and Jones, 1990). Due to height difference among populations before and after stress, it seems that the effect of drought stress on the seedling height trend of these populations *before and after applying the stress is almost* same.

Before drought stress, the seedling diameter of *P. communis* cv. Spadona, Khoj n. 1, Dargazi and *P. syriaca* was higher than the seedling diameter of *P. salicifolia*, *P. glabra* and *P. communis* cv. Khoj n. 2, while the diameter difference before and after drought stress among compared species did not show a significant difference, therefore, it can be concluded that the effect of drought on the seedling diameter among populations was not the same. Other results also showed that the negative effect of drought stress on seedling diameter was less than its effect on seedling height (Haghighatnia *et al*., 2013).

Growth of branch and internode length is an appropriate index for detecting the effect of drought stress on the plants, so that the occurrence of drought stress can be observed even before the change in the water potential of the leaves (Grimplet *et al*., 2007). Among the remaining genotypes after the stress, *P. communis* cv. Spadona had the highest and *P. glabra* and *P. communis* cv. Khoj n. 2 had the lowest number and length of internode.

The chlorophyll meter indicates the relative

Table 7 - Growth vigor, trichome and chlorosis of leaf in selected genotypes

Species	No. genotype	Leaf chlorosis	Leaf trichome	Seedling growth vigor
Pyrus salicifolia	12	No chlorosis	Low trichome	Low
	31	Low chlorosis	Low trichome	Low
	32	No chlorosis	Low trichome	Low
	41	No chlorosis	Low trichome	Low
	57	No chlorosis	Low trichome	Low
Pyrus glabra	3	Low chlorosis	Low trichome	Low
	9	No chlorosis	Low trichome	Low
	7	No chlorosis	Low trichome	Low
	10	No chlorosis	Low trichome	Very low
	11	No chlorosis	Low trichome	Very low
Pyrus communis cv. Khoj n. 2	4	No chlorosis	Low trichome	Low

chlorophyll concentration, based on the difference between the light transmittance in two red and infrared wavelengths, which correlates with the chlorophyll content of the leaves (Hoel and Solhaug, 1998). In the present study, *P. salicifolia* and *P. communis* cv. Dargazi showed the highest and the lowest chlorophyll index, respectively. Preservation and not decomposition of chlorophyll in *P. salicifolia* during drought stress indicates the tolerance of that species to this stress (Tarahomi *et al*., 2010).

Cluster analysis of populations

Drought tolerance in plants has a direct or indirect relationship with a complex of traits, therefore, all the traits should be considered for selection of tolerant plants. For this reason, in this research cluster analysis was used to facilitate the selection of drought tolerant species. Cluster analysis classified species into three groups at five squared Euclidean distance (Fig. 1). *P. communis* cv. Spadona and Dargazi were placed in the first group that had the lowest survival rate after drought stress. The highest seedling diameter belonged to the plants of this group. In the second group, *P. syriaca* and *P. communis* cv. Khoj n. 1 were placed, which showed low to moderate survival rate after drought stress. In total, the most crown width, primary and secondary seedling height and diameter, as well as the highest number of internode were observed in the species of the first and second groups. *P. communis* cv. Khoj n. 2, *P. salicifolia* and *P. glabra* species formed the third group. These populations had the lowest seedling diameter and height. The lowest seedling growth vigor was also found in these species; therefore, selection of dwarf and drought tolerant genotypes in this group was more possible than other groups. It seemed that traits such as seedling height and diameter were the most effective grouping traits. In the research carried out by Aran *et al*. (2012), seedling height was also one of the important traits in seedling grouping. Of course, different values of some traits were seen in this group. For example, the crown width and viability percentage were observed at low, medium and high levels in this group.

Study of single genotypes

The mean, the range of changes, and the coefficient of variation of each trait were shown in Table 6. Coefficient of variation shows the extent of variability in relation to the mean of the population. In the traits with a high coefficient of variation has provided a higher selection range. Genetic variation helps the plant to overcome environmental changes and also provides more chance for selection of new cultivars (Liu, 2006). In this research, high variation was observed for internode length and seedling height before and after stress. The variability of some traits in 15 cultivars of *Vitis vinifera* L. was previously investigated by Mousazadeh *et al*. (2014). They reported that leaf traits had the highest diversity among the studied traits. Doulati Baneh *et al*. (2013) and Tahzibihagh *et al*. (2012) also observed a high variation in the morphological characteristics of the leaves of grape and pear cultivars. In the current research, the coefficient of variation in leaf dimensions was not high compared to other traits, because of less scatter in relation to the mean of the population. The high diversity coefficient for internode length and seedling height before and after stress indicates the high range of variation in these traits among the studied seedlings, so it is possible to select genotypes based on these two traits.

Survived genotypes after drought stress were selected in order to dwarfing. For this purpose, seedling height of each genotype was investigated separately. Considering that the average height of three populations including *P. salicifolia*, *P. glabra* and *P. communis* cv. Khoj n. 2 were lower than other populations, therefore selection was carried out among genotypes of these populations. The highest number of genotypes was selected from *P. salicifolia*. The least seedlings height was belonged to *P. glabra*. Only a dwarf genotype was selected from the *P. communis* cv. Khoj n. 2 population (Figs. 2, 3 and 4). Finally, 11 dwarf genotypes were selected from these species.

Cluster analysis of single genotypes

Cluster analysis was performed for three populations with a lower seedling height. According to this, five selected genotypes of *P. salicifolia* (Fig. 5), five genotypes of *P. glabra* (Fig. 6) and a genotype of the *P. communis* cv. Khoj n. 2 population (Fig. 7) were classified in the same subgroups. These genotypes had lower seedling height and internode number. Leaf width was also lower in these genotypes.

Qualitative traits of 11 selected genotypes showed that all genotypes had low trichome. The survival and tolerance of these genotypes to drought stress was not along with the increase of leaf trichome. The growth vigor of genotypes was low, and two genotypes 10 and 11 from *P. glabra* had very low growth vigor. These genotypes also had the lowest seedling height. In fact, their growth vigor was correlated with the seedling height. This positive correlation was observed between seedling height and growth vigor in bitter cherry seedlings (Mahlab) (Ganji-Moghadam and Khalighi, 2006). Under drought stress, studied genotypes did not show chlorosis, only genotypes 3 of *P. salicifolia* and 31 of *P. glabra* were greenish-yellow after drought stress. Selected genotypes were transferred to a propagation phase through cutting and layering.

After studying single genotypes in these three populations, genotypes no. 31, 32, 41, 57 and 12 from *P. salicifolia*, genotypes 10, 11, 7, 3 and 9 of *P. glabra* and genotype no. 4 of *P. communis* cv. Khoj n. 2 populations were selected as drought tolerant and dwarf genotypes and were taken to the propagation phase for future evaluation.

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