Study on Some Morphological Characteristics of Progenies from Controlled Crosses in Almond Commercial Cultivars (*Prunus dulcis* Mill.)

Asghar MOUSAVI[1]
Maryam TATARI[2] (✉)

Summary

Some phenological and morphological characteristics in three populations (150 progenies from each population) were evaluated with cross combinations of 'Sefied' (male)×'Shahrood-16' (female), 'Shahrood-12' (male)×'Shahrood-16' (female) and 'Marcona' (male)×'Shahrood-12' (female). Progenies showed a significant difference in most traits such as seedling height, leaf area, growth vigor, trunk diameter, branch density and angle as well as leafing date. According to the results of descriptive statistics, high diversity coefficients among populations and evaluated traits were observed. The results of simple correlation analysis showed significant correlations between some leaf characteristics and growth parameters, such as seedling height, trunk diameter, and canopy width. There was a significant correlation between branch angle, seedling height, and canopy width with growth habit. The results of the principal component analysis showed that five main and independent components described 71.9% of the variance, and the seedling height, growth vigor, leaf area, canopy width, and leafing date traits showed a high correlation in the main components. Cluster analysis between populations based on all traits showed that the first and second populations with the same female parent ('Shahrood 16') had higher similarity than the third population. The cross-combinations with the same female parent were placed in the same group, which shows the female parent has more effect than the male parent in the inheritance of traits.

Key words

almond, cluster analysis, correlation, diversity, population

[1] Horticulture Crops Research Department, Chahar-Mahal va Bakhtiari Agricultural and Natural Resources Research and Education Center, AREEO, Shahrekord, Iran

[2] Horticulture Crops Research Department, Isfahan Agricultural and Natural Resources Research and Education Center, AREEO, Isfahan, Iran

✉ Corresponding author: mtatari1@gmail.com

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Introduction

Iran is one of the most important centers of the almond production in the world (Chaichi et al., 2003). The main problems of producing almond are the early flowering of local genotypes and damage to flowers due to late spring frost, self-incompatibility phenomenon in almond and no overlap in flowering time.

One of the main problems of almonds in the country is the irregular production of almonds, mainly due to the early flowering of indigenous genotypes and the coincidence of their flowering time with spring cold conditions, lack of pollination and fertilization induced by inactivity pollen insects during the flowering of almonds. There is a self-incompatibility phenomenon in almond and the absence of overlap flowering of cultivars, so growers suffer much damage (Imani, 2010).

Today, this problem has been reduced to some extent by introducing late flowering and cold hardness cultivars. Therefore, in doing research, it is essential to apply ideas concerning agronomic breeding programs, particularly by using hybridization to access late-flowering cultivars, high yielding, precocious and marketable cultivars. In this regard, a large number of commercial cultivars have been selected and introduced for crosses between commercial and late-flowering cultivars with self-compatible cultivars such as Tuno, Supernova, Laurent and Gingco in Italy and Spain (Kester and Gradziel, 1996). In Iran, Harir cultivars (Ai × Nonpariel), Shokoufeh (Ai × Nonpariel), and Azar (Ai × Cristomorto) have been obtained through controlled crossings at Shand Horticultural Research Station in Tabriz (Chaichi et al., 2003). Evaluation of the progenies from controlled crosses between local and foreign cultivars to select desirable cultivars is underway at Sahand Horticultural Research Station of Tabriz (Eskandari and Chaichi, 2000(and Kamal Abad Horticultural Research Station in Karaj (Imani, 2010) where the promising genotypes have been obtained that are at the evaluation stage.

The flowering time of male parent (pollen donor) has little effect on the need for chilling and germination time of almond seeds, and their need for chilling, in this case, is influenced by the flowering time of the maternal parent (pollen receptor) (Garcia Gusano et al., 2005). However, supplementary studies have shown that after removing the shell and covering the almond kernels (tegument), the effect of the paternal parent (pollen) on their germination and dormancy time is higher (Garcia Gusano et al., 2009). Seeds produced by an early-flowering paternal parent have less chilling than seeds from the late-flowering paternal parent, indicating the effect of the internal mechanism of dormancy on the father's parent. In fact, by eliminating parts such as the shell and covering the almond kernels (tegument), which play a role in the external mechanism of dormancy, the role of father's parents and its effect on the germination time of hybrid seeds will be more pronounced (Garcia Gusano et al., 2009).

Morphological traits are the first markers used in the management of germplasms and morphological features are helpful for revealing extensive information about genetic variation in various products (Gitonga et al., 2008). Morphological traits were used to evaluate different genotypes in different species of genus *Prunus*, which can be evaluated for almond genotypes (Chalak et al., 2007; De Giorgio et al., 2007; Zeinalabedini et al., 2007; Imani, 2010; Mousavi et al., 2010; Salim Pour et al., 2013), apricot (Mohammad Zadeh et al., 2013; Asma and Ozturk, 2005), sour cherries (Ganji Moghadam and Khalighi, 2006), plums (Aran et al., 2011; Tamarzizt et al., 2009) and sweet cherries (Khadivi et al., 2010).

It takes several years to complete the process of crossing, obtaining hybrid seeds, eliminating the need for chilling and growing in the greenhouse, moving to the field, and getting to the stage of evaluating the characteristics of the fruit and kernel. It is a time-consuming and costly process (Dicenta et al., 2005; Kester and Gradziel, 1996). In this investigation, the stages of controlling crosses between Iranian and foreign almond commercial cultivars until the transfer of greenhouse seeds to the field, and their preliminary assessment in the vegetative phase have been studied with the aim of searching and achieving correlation between vegetative traits.

In this study, the evaluation was done of the diversity of morphological traits and vegetative growth indices in 450 hybrid seedlings (150 hybrid seedlings of each crossing combination) represented from 1200 hybrids seedlings of three different combinations of a controlled cross between Iranian and foreign almond cultivars.

Materials and Methods

Plant Materials

Three combination compilations were designed to create a first-generation (F1) population in almond breeding in 2021 to achieve late flowering and high yielding cultivars with desirable fruit and kernel characteristics.

Cross Combinations and Population

The First Population (AHP1): The first combination of controlled crosses was done between the cultivars 'Sefied' (as male parent with very early flowering, Softshell and spur type in bearing habit) 'Shahrood 16' (as female parent with very late flowering, Softshell and shoot type in bearing habit) to study the late-flowering trait and to obtain of late-flowering progenies.

The Second Population (AHP2): The second combination of controlled crosses was done between the cultivars 'Shahrood 16' (as a female parent with very late flowering and shoot type in bearing habit) and 'Shahrood 12' (male parent with late flowering and spur type in bearing habit) to obtain late-flowering and high-yielding progenies.

The Third Population (AHP3): The third combination of controlled crosses was done between 'Marcona' (as male parent with middle flowering time and fruitful cultivar with mixed in shoot and spur types in bearing habit) and 'Shahrood 12' (as female parent with late flowering and spur type in bearing habit) with two different bearing habits (Shahrood 12 with spur bearing and Marcona With mixed bearing habits) to achieve high yielding progenies. Some characteristics of 'Sefied' and 'Marcona' (as male parents) and 'Shahrood 12' and 'Shahrood 16' (as female parents) have been reported previously. Some characteristics of the 'Sefied' and 'Marcona' cultivars have been reported as paternal parents and 'Shahhrood 12' and 'Shahrood 16' as mother parents (Mousavi et al., 2010).

After selecting suitable parents, to access the populations of controlled crosses, all stages of collecting pollen, emasculation flowers, and controlled crosses were done according to the standard method (Rasouli et al., 2010; Mousavi et al., 2010) in the collection of almond cultivars in the Saman area affiliated to the Agriculture and Natural Resources Research Center of Shahrekord in ChaharMahal va Bakhtiari province.

At harvest time, fruits obtained from controlled crosses were harvested separately in each cross with a marked label. The hull of the fruits was removed and nuts were dried under sunshine conditions.

The hybrid nuts were soaked in water for 12 to 24 hours (times depending on the hard or soft shell of the hybrid nuts). Then they were panted in perlite and sand (1: 1) mixture in plastic containers with suitable drain and placed in cold storage at 5 °C for 40 days.

After eliminating the chilling requirement, the hybrid seeds were planted in plastic pots with a medium of organic fertilizer + sand + soil (1: 2: 1) and placed in a greenhouse combination.

Their germination was carried out at the appropriate greenhouse condition temperature.

After providing the appropriate environmental conditions, the hybrid seedlings of each cross combination were planted in separate parts with 1 × 3 m intervals in the field at the Horticultural Research Station of Agriculture and Natural Resources Campus of the University of Tehran. Then, all agronomic care and operations were carried out from the hybrid seedlings.

Measurement Traits

For evaluation of morphological characteristics of progeny, 150 hybrids of each hybrid cross were labeled and some growth indices, including seedling height, trunk diameter, canopy width, leaf dimensions, leaf area, growth vigor and other traits based on almond descriptor (UPOV) were measured with slight variation (Table 1). To measure leaf-related traits, ten mature and healthy leaves from each seedling were harvested from the middle part of the annual shoot in four different geographical directions (Farokhzad et al., 2011). Leaf length, leaf width and petiole length were measured and averaged using a ruler. Leaf area was measured simultaneously for each of the ten leaf samples using a leaf area meter, the ΔT model (UK-made), and the mean leaf area was expressed in millimeters. Also, the values of leaf length and width were used to calculate leaf length/width ratio (L/W).

Seedling height was measured in the fall season from the soil surface to the end of the tallest branch (principal axis) using a meter. Canopy width was measured in the east-west direction at the middle height of the canopy by using the meter. The height-to-width ratio of the canopy (HC/DC) was calculated to express the canopy expansion trait. The trunk's diameter was measured by a caliper from a distance of 5 cm above the soil surface and reported in millimeters. The growth vigor of seedlings was estimated as scoring (code) from very poor (1) to very strong (11). Branch density and thickness of annual shoots were also recorded based on scoring. Growth habit of seedling was measured from very high (1) to dangling (5), and anthocyanin content of annual shoots from very low (1) to very high (9).

Table 1. Measured traits, unit and their abbreviation in almond progeny from controlled crosses using Almond Descriptor (International Union for the Protection of New Varieties of Plants (UPOV))

Measured traits	Abbreviation	Unit
Leaf Length	LL	cm
Leaf Width	LW	cm
Leaf Petiole Length	LPL	cm
Leaf Area	LA	mm^2
Leaf Length/Leaf Width Ratio	LL/LW	Ratio
Tree Height	TH	cm
Canopy Diameter (Canopy width)	CD(CW)	cm
Tree Height/Canopy Diameter	TH/CD	Ratio
Trunk Diameter (Stem Diameter)	TD (SD)	mm
Growth Vigor	GV	code
Branch Density	BD	code
Branch Angle	BA	code
Growth Habit	GH	code
One-year Branch Diameter	YBD	code
One-year Branch Anthocyanin	YBA	code
Leafing Date*	LD	day

Note: * - the basal time was one day before the first day of leaf bud opening

The branching angles of the primary (major) branching that play a significant role in tree growth habits were observed from very closed (1) to over-open (9). To increase the accuracy, the average size of the angle of four sub-branches was considered as the branching angle (Table 1). Visits at intervals of two days were recorded on the date of leaf bud opening (leafing date), and the basal time was one day before the first day of leaf bud opening. For this trait, the sprouting of the first leaf tip in at least 50% of each seedling bud was considered as the time of bud opening (leafing date).

Data Analysis

Analyses of variance and mean comparison between populations were performed for the traits measured by SAS version 9.2. Descriptive statistics including mean, minimum, maximum and coefficient of variation as well as Pearson and Spearman correlation coefficients for quantitative and qualitative traits, cluster analysis and factor analysis were used with SPSS software (version 21), respectively. Factor analysis was performed using the factor rotation technique and maximum variance (Varimax). The factors with values higher than one were selected as main factors, and factor coefficients above 0.5 were considered as significant coefficients. Cluster analysis between populations was performed by using the Ward method.

Results and Discussion

Percentage of Fruit Set

The number of emasculated flowers, percentage of initial and final fruit set, number of hybrids, number of seedlings, percentage of seed germination, number of seedlings in the main field, and number of fatality seedlings for each crossing combination are reported in Table 2. The results of controlled crosses combination between almond cultivars showed that the highest percentage of initial and final fruit set crossing of 'Marcona' and 'Shahrood 12' combination was 33% and 25%, respectively, and the lowest percentage of initial and final fruit set crossing of 'Sefied' and 'Shahrood 16' cultivars was 20% and 15%, respectively. The almond cultivars used in this study were self-incompatible, but the selective combinations in controlled crosses were cross-compatible. In a previous study, the percentage of initial and final fruit set in supplemental pollination of self-compatible cultivar 'Supernova' with different almond cultivars (as Paternal parent) was reported to be higher than in the present study (8), probably due to the genetic potential of the cultivars for fruit set. Also it may be due to differences of climatic conditions in place of pollination tests.

According to the results of this study (Table 2), in the combination of a cross between 'Sefied' (parental parent) and 'Shahrood 16', from a total of 2000 pollinated flowers, finally 300 hybrid seeds were obtained that represented 15% of the final fruit set. From 300 hybrid seeds, 285 germinated after chilling (95%) and after transfer to the pot and then to the field, about 9 % of seed losses (fatality seedlings) was found among seedlings. Perhaps one of the reasons for the lower fruit set in this combination ('Sefied' ×'Shahrood' 16) is the reduction in germination due to more extended storage of pollen grain 'Sefied' as very early flowering and use in controlled pollination of a very late flowering ('Shahrood 16') compared to pollen grain 'Marcona' and 'Shahrood 12'. On the other hand, 'Shahrood 16' is a foreign cultivar and the results of previous studies have shown that it is identical with the 'Tardy Nonpareil' (40) which is self-incompatible with S_7S_8 S-alleles (37). The genotyping results of S- alleles in almond cultivars (34) showed that 'Sefied' cultivar had S_7S_{38} S-alleles which strengthened the probability of lower fruit set by the presence of a common allele (S_7).

The high percentage of germination in the progeny of the combination of 'Sefied' with 'Shahrood 16' compared to the other crosses was probably because both parents had a soft shell trait. In contrast, the other combination had one parent or both parents with a hard shell. The chilling requirement for germination of hybrid seeds from crossing between a very early flowering ('Sefied') and very late flowering ('Shahrood 16') cultivar both of which were soft shells was about 30 days. However, it took about 35 days for other combinations. This difference may not be related to the nature of late flowering or early flowering, but it is related to the hardness of the shell to exit rootlet from seeds so that seeds with more rigid shells need a longer time to germinate than soft shell cultivars. The influence of flowering time in parents on the chilling requirements for seed germination from controlled crosses showed that parental flowering time was directly related to the chilling requirement and germination time. Seed germination time was influenced by flowering time in both parents (Garcia Gusano et al., 2009).

The results showed that most of the measured traits among studied populations, including significant height, leaf area, growth vigor, trunk diameter, density and angle of branches and leafing date had significant differences in seedlings. According to the results, the Third Population (AHP3) had significant differences in some traits such as length and width of leaf, leaf area, petiole length, canopy width, branch density, branch angle, anthocyanin content of branch and leafing date from First (AHP1) and Second (AHP2) Populations . In contrast, the First (AHP1) and Second (AHP2) Populations did not show significant differences in some traits such as length, width, leaf area, canopy width, trunk diameter, shoot density and growth habit traits (Table 3). The populations showed significant differences for leaf length, petiole length, branch angle, annual branch thickness, annual branch anthocyanin content and leafing date (Table 3). Leaf width varied from 2.45 to 2.62 cm (Table 3). Significant differences in leaf width were also reported in the four wild *Prunus* species (Zeinalabedinid et al., 2008), which agrees with previously reported results in almonds (Salim Pour et al., 2013).

Table 2. Comparison of the different measured traits in three combinations of controlled cross in almond cultivars

Cross combination		Number of crossed flower	Number of hybrid seeds	Fruit set percentage		Number of seedlings	Germination percentage	Number of seedlings in field	Fatality percentage of seedlings
Male parent	Female parent			Initial	Final				
'Sefied' (S_7S_{38})	'Shahrood 16' (S_7S_8)	2000	300 (15%)	20	15	285	95	259	9.12
'Shahrood 12' (S_1S_3)	'Shahrood 16' (S_7S_8)	2500	600 (24%)	30	24	540	90	450	16.6
'Marcona' ($S_{11}S_{12}$)	'Shahrood 12' (S_1S_3)	2600	650 (25%)	33	25	570	87.7	477	16.3

Table 3. Mean comparision of traits in the studied populations from crossing of almond cultivars

Population	Mean of traits*															
	LL (cm)	LW (cm)	LP (cm)	LA (mm^2)	LL/LW	TH (cm)	CD (cm)	TH/CD	SD (mm)	GV (3-11)	BD (3-9)	BA (1-9)	GH (1-5)	YBD (3-7)	YBA (1-9)	LD (day)
Population 1	7.79^{a}	2.62^{a}	2.28^{a}	1688.87^{a}	3.01^{b}	172.127^{b}	88.127^{ab}	2.11^{a}	28.59^{a}	6.36^{b}	4.98^{b}	6.12^{a}	3.02^{a}	4.97^{b}	5.3^{c}	6.17^{c}
Population 2	7.59^{ab}	2.59^{a}	1.71^{b}	1708.17^{a}	2.95^{b}	182.38^{a}	93.63^{a}	2.053^{a}	32.33^{a}	6.98^{a}	5.16^{b}	5.77^{b}	2.92^{a}	5.26^{a}	6.08^{b}	8.68^{a}
Population 3	7.56^{b}	2.45^{b}	1.61^{c}	1524.67^{b}	3.14^{a}	171.89^{b}	87.78^{b}	2.057^{a}	31.14^{a}	7.13^{a}	5.61^{a}	4.93^{c}	2.88^{a}	4.89^{b}	6.8^{a}	7.22^{b}

*: Similar letters in each column indicate no significant difference in Duncans Multiple Range Test. Population 1: Hybrid seedlings from crossing Sefid cultivar as male parent and Shahroud 16 cultivar as female parent. Population 2: Hybrid seedlings from crossing Shahroud 12 cultivar as male parent and Shahroud 16 cultivar as female parent. Population 3: Hybrid seedlings from crossing Marcona cultivar as male parent and Shahroud 12 cultivar as female parent.

Descriptive Statistics of Traits

The characteristics of measured traits, including minimum, maximum, mean, standard deviation and coefficient of variation for each trait in the progeny of different populations are presented in Table 4. The coefficient of variation indicates that there were variations in progeny for each of the traits. Traits with a high coefficient of variation have a more comprehensive range of trait quantity, which has provided more choice for that trait in progeny. For example, the high diversity coefficient for the leafing date trait indicates the range of variation and high diversity of this trait (59.70%) among the hybrids (Table 4). Leafing date is one of the critical traits in the preliminary evaluation of progeny concerning late-flowering traits (Dicenta et al., 2005).

There was a 33-day range among seedlings for the leafing date. The lowest coefficient of variation was related to leaf length (11.94%). Although populations were not significantly different in terms of growth habit trait (Table 3), the coefficient of variation of this trait among hybrid seedlings within populations (24.87%) indicated a relatively good diversity among seedlings (Table 4). According to the results, the heritability of the tree growth habit (branching pattern) was reported to be relatively low, so the diversity of this trait would not be very different among populations (Socias I Company et al., 2005). Perhaps one of the reasons that this trait was not significantly different among populations was due to its low diversity or lack of significant parental differences in this trait. In this respect, low diversity of tree growth habit (growth type) was reported in 55 apricot cultivars, with eight cultivars having upright growth type and the others having intermediate growth type (Badenes et al., 1998).

The results for the mean, minimum, and maximum numerical values and coefficient of variation for traits measured in the studied populations are shown in Table 5, respectively. According to the results, the minimum and maximum leaf length (5.20 and 10.18 cm) and minimum and maximum seedling height (65 and 279 cm) were obtained from progenies of the 'Sefied' × 'Shahrood 16' cross, which indicates a higher diversity for these traits in the progenies of this cross than the progenies of the other crosses. The previous study reported a significant difference in seedling height of ten cherry stands in the fifth year of growth between 65 and 270 cm (Ghanji Moghadam and Khalighi, 2006). Progeny of 'Shahrood 12'× 'Shahrood 16' cross had maximum leaf area traits compared to the other populations. The minimum and maximum leaf length to leaf ratio (L / W) and population growth traits were obtained from a cross between 'Marcona' and 'Shahrood 12', respectively. This trait showed more variation in this population than in the other populations. The highest canopy width was for the Second Population ('Shahroud 12'× 'Shahrood 16'), while the lowest was for the First Population ('Sefied' × 'Shahroud 16').

Different populations showed similar diversity in seedling growth types. The earliest leafing date seedling was from the First Population and the latest was from the Third Population. The late leafing date progenies were in 'Shahrood 12'×'Shahrood 16' and 'Shahrood 12' × Marcona. 'Shahroud 12' and 'Shahrood 16' cultivars are late flowering and very late flowering, respectively. Considering the relationship between late-leafing date and late flowering (Dicenta et al., 2005), it seems that the progenies of the cross between the 'Shahrood 12' and 'Shahrood 16' cultivars whose both parents have a late-leafing date trait are more likely to show late-flowering traits and to achieve late-flowering progenies in this population than other populations. In this regard, previous research has shown that crossing between the two parents with the late-flowering trait is much more effective to achieve late-flowering cultivars than the other crosses (Dicenta et al., 2005; Sánchez-Pérez et al., 2007).

Table 4. Descriptive statistics of studied traits in all hybrid seedlings

Trait	Abrivation	Minimum	Maximum	Mean	Standard deviation	CV
Leaf Length	LL	5.2	10.18	7.65	0.91	11.94
Leaf Width	LW	1.58	8.4	2.56	0.51	20.11
Leaf Petiole Length	LPL	0.86	3.62	1.87	0.44	23.49
Leaf Area	LA	770.11	3081.91	1640.57	401.57	24
Leaf Length/Leaf Width Ratio	LL/LW	1	4.72	3.04	0.38	12.68
Tree Height	TH	65	279	175.47	34.79	19.83
Canopy Diameter (Canopy width)	(CW)CD	27	198	89.85	24.91	27.72
Tree Height/Canopy Diameter	TH/CD	0.56	5.71	2.08	0.63	30.29
Trunk Diameter (Stem Diameter)	TD	13.9	51.2	30.69	6.19	20.18
Growth Vigor	GV	3	11	7	1.68	23.89
Branch Density	BD	3	9	5	1.52	30.4
Branch Angle	BA	1	9	5	1.42	28.4
Growth Habit	GH	1	5	3	0.73	24.33
One-year Branch Diameter	YBD	3	7	5	1.29	25.8
One-year Branch Anthocyanin	YBA	1	9	7	1.37	19
Leafing Date*	LD	1	33	7.37	4.4	59.7

Frequency of Traits

Some of the measured traits showed a high variation among progenies and progeny frequency varied for different traits. The frequency of traits for vigor and growth habit, shoot density, shoot angle and leafing date in progenies are shown in Figs. 1 - 5, respectively. According to Fig. 1, progenies with moderate growth vigor (code 7) had the highest frequency (45.1%) among the three population compositions. Subsequently, weak growth vigor (code 5) with 25.1% frequency and strength of growth vigor (code 9) with 24.4% frequency were in the following ranks (Fig. 1).

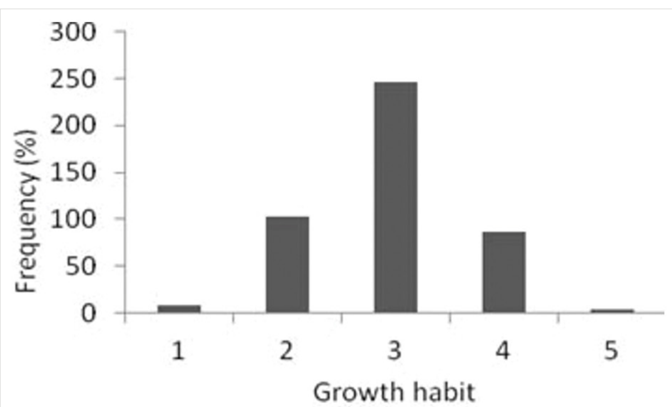


Figure 1. Frequency of growth habit in progenies of studied populations

The highest frequency of growth habit was in the widespread state, with 54.9% (Fig. 2). The highest frequency of shoot density in the progenies was observed in the moderate state with 44% and dense state with 32.7% (Fig. 3).

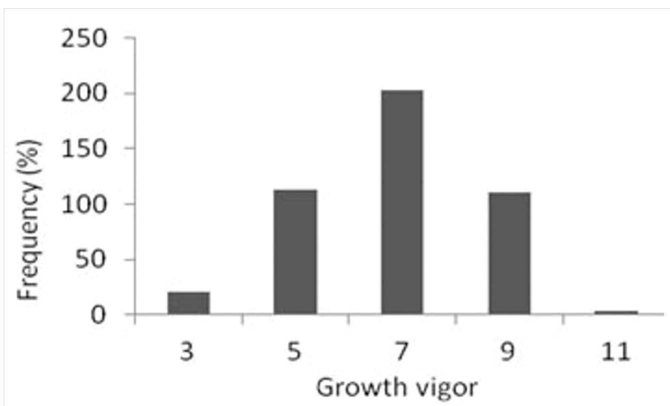


Figure 2. Frequency of growth vigor in progenies of studied populations

The percentage of branch angle in progeny was 42.9% in the open state (between 45° and 60°) and 42.9% in a very open state (Fig. 4).

Table 5. Mean, minimum and maximum values of measured traits in populations from controlled crossing of almond cultivars

		Measured traits															
		LL	LW	LP	LA	LL/LW	TH	CD	TH/CD	SD	GV	BD	BA	GH	YBD	YBA	LD
efid×Shahroud16	Mean	7.79	2.62	2.28	1688.87	3.01	172.13	88.13	2.12	28.59	6.36	4.99	6.12	3.02	4.97	5.31	6.17
	Minimum	5.2	1.72	1.22	831.2	1.18	65	27	1.04	13.9	3	3	1	1	3	1	1
	Maximum	10.18	7.4	3.62	3049.54	3.9	279	149	5.71	50.99	9	9	9	4	7	9	21
(Shahroud12×Shahroud16)	Mean	7.6	2.59	1.72	1708.17	2.96	182.38	93.63	2.05	32.33	6.99	5.16	5.77	2.93	5.27	6.08	8.68
	Minimum	5.22	1.74	0.98	888.16	2.17	108	46	0.56	16	3	3	3	1	3	3	3
	Maximum	9.92	3.56	2.46	3081.91	3.88	261	198	3.75	48.2	9	7	9	4	7	9	29
(Marcona×Shahroud12)	Mean	7.56	2.46	1.61	1524.67	3.15	171.89	87.79	2.06	31.15	7.13	7.61	4.93	2.88	4.89	6.8	7.22
	Minimum	5.62	1.58	0.86	770.11	1	87	35	1	18.8	3	3	3	1	3	3	3
	Maximum	9.62	8.4	2.66	2673.5	4.72	275	165	4.66	51.2	11	7	7	5	7	9	33
Total	Mean	7.65	2.56	1.87	1640.57	3.04	175.47	89.85	2.08	30.69	6.83	5.25	5.61	2.94	5.04	6.06	7.36
	Minimum	5.2	1.58	0.86	770.11	1	65	27	0.56	13.9	3	3	1	1	3	1	1
	Maximum	10.18	8.4	3.62	3081.91	4.72	279	198	5.71	51.2	11	9	9	5	7	9	33

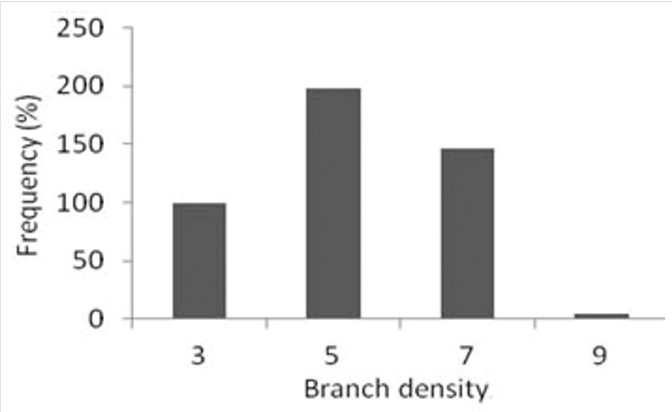


Figure 3. Frequency of branch density in progenies of studied populations

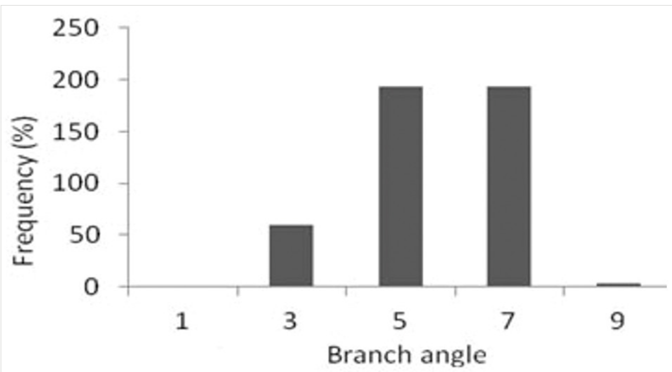


Figure 4. Frequency of branch angle in progenies of studied populations

This indicates that most of the progenies had a broad canopy, consistent with the growth habit results with a widespread abundance of 54.9% (Fig. 1). The frequency of leafing late varied between progenies from 0.2% to 25.8% and the highest frequency was related to the time of leafing late with eight days after the basal time (date of first leafing). The highest frequency was between 3 and 10 days after the date of the first leafing (Fig. 5).

According to the results, the coefficient of variation for the growth habit was about 25% and for the leafing date about 60% (Table 4), which indicates a higher variation of the leafing date trait among the progenies. The frequency of some traits, including the growth habit among the progenies, had a relatively normal distribution (Fig. 2). In contrast, the abundance of the late trait was not a function of a normal distribution (Fig. 5). This difference was probably due to the non-significant differences between parents in growth habit (Tables 3 and 4), so the progenies also had a normal distribution. However, the parental flowering date in the used populations was different. Because the leafing date has a significant positive correlation with the flowering date (Dicenta et al., 2005; Vargas and Romero, 2001), the frequency of this trait was not uniform in progenies of three populations.

The variation of leafing date in the First Population derived from an early parent ('Sefied' as the male parent) and a late-flowering parent ('Shahrood 16' as the female parent), was greater than in the other populations. The progenies of this population varied from very early to late in leafing date traits,whereas the frequency of late leafing date was more significant in the Second Population, which was the result of a cross between a late-flowering parent ('Shahrood 12' as the male parent) and a very late-flowering parentage ('Shahrood 16' as the female parent). These results confirmed the correlation between the flowering date and the leafing date, which is consistent with previously reported results (Dicenta et al., 2005; Vargas and Romero, 2001).

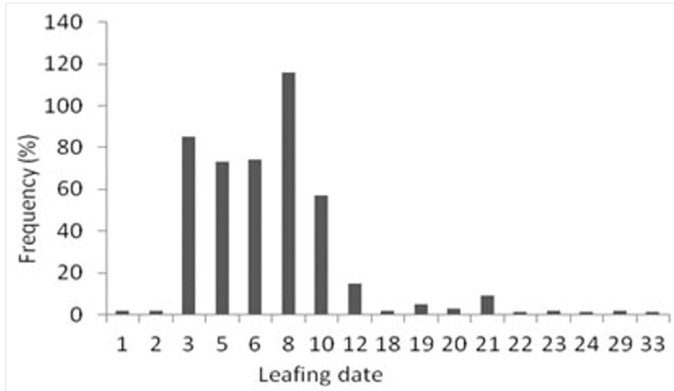


Figure 5. Frequency of leafing date in progenies of the studied populations

Based on previous results, the heritability of the tree growth habit trait is relatively low, so the diversity of this trait will not be very different between populations from controlled crosses (Socias I Company, 1988). Perhaps one of the reasons for a non-significant difference among populations for this trait (Tables 3 and 4) was due to the low heritability and diversity of this trait and or non-significant differences in this trait between their parents. In this respect, low diversity of tree growth habit was reported in 55 apricot cultivars, so that eight cultivars having upright (direct) growth habit and other cultivars had intermediate growth habit (Badenes et al., 1998).

Because of the association between late leafing date and late flowering (Dicenta et al., 2005), it seems that the progenies of the cross between the 'Shahrood 12' and 'Shahrood 16' cultivars if both parents have a late-leafing date trait, are more likely to show late-flowering traits and to achieve late-flowering progenies in this population than other populations. In this regard, previous research has shown that crossing between the two parents with the late-flowering trait is much more effective to achieve late-flowering cultivars than the other crosses (Dicenta et al., 2005; Sánchez-Pérez et al., 2007).

Correlation Traits

Simple correlation coefficients of traits measured in the 450 almond hybrids are presented in Table 6. Correlation traits are used to investigate and establish a logical and significant relationship among traits. Creating relationships among several traits can provide the pathway for traits that are difficult to measure. Based on the results, the correlation of the studied traits was significant in most cases, and some cases showed high correlations. For example, there was a significant positive correlation between trunk diameter with branch density and growth vigor with the thickness of one-year branches (Table 6). According to the results, the correlation values among traits in this study were classified into three groups:

Table 6. Cofficient of simple correlation for measured traits in 450 seddlings of almond hybrid

	LL	LW	LP	LA	LL/LW	TH	CD	TH/CD	SD	GV	BD	BA	GH	YBD	YBA	LD
LL	1															
LW	0.496**	1														
LP	0.488**	0.271**	1													
LA	0.8**	0.665**	0.414**	1												
LL/LW	0.197**	-0.639**	0.033	-0.269**	1											
TH	0.112*	-0.02	0.126**	0.051	0.107*	1										
CD	-0.03	-0.132**	0.041	-0.111*	0.182**	0.352**	1									
TH/CD	0.059	0.102*	0.065	0.123**	-0.130**	0.286**	-0.712**	1								
SD	0.195**	0.007	0.025	0.109*	0.175**	0.521**	0.491**	-0.183**	1							
GV	0.136**	-0.04	0.014	0.02	0.182**	0.757**	0.404**	0.063	0.61**	1						
BD	-0.06	-0.138**	-0.08	-0.164**	0.203**	0.141**	0.436**	-0.368**	0.477**	0.255**	1					
BA	0.061	0.011	0.293**	0.002	0.096*	0.146**	0.44**	-0.343**	0.194**	0.093*	0.197**	1				
GH	-0.02	-0.08	0.110*	-0.106*	0.167**	-0.02	0.535**	-0.538**	0.203**	0.07	0.252**	0.618**	1			
YBD	0.264**	0.107*	0.173**	0.245**	0.048	0.473**	0.15**	0.134**	0.382**	0.462**	-0.05	0.088	0.012	1		
YBA	0.13**	-0.05	-0.334**	-0.116*	0.005	0.122**	0.024	0.041	0.076	0.113*	0.114*	-0.178**	-0.06	-0.04	1	
LD	-0.116	0.047	-0.275**	-0.02	-0.184**	-0.253**	-0.141**	-0.03	-.0142**	-0.222**	0.098*	-0.202**	-0.131**	-0.135**	0.152**	1

Note: * and ** significant in level 5% and 1%

1. The correlation over 70%: there was a positive correlation over 70% (r = 0.76) between leaf area with leaf length (r = 0.80) and growth vigor with seedling height, and there was a negative correlation above 70%(r = -0.712) between canopy width with width to length of canopy (CW/CL) ratio (Table 6).
2. The correlation 70-50%: there was a positive correlation (70-50%) between leaf area with leaf width (r = 0.665) and trunk diameter with seedling height (r = 0.552) and between vigor growth with trunk diameter (r = 0.610) and seed height (r = 0.535) and between growth habit and branch angle (r = 0.618). A positive correlation was between leaf width with length to width leaf (LL/WL) ratio (r = - 0.639) and between canopy width with width to length of canopy (CW/CL) ratio (Table 6).
3. Correlation below 50%: other traits showed a significant correlation of less than 50% (Table 6).

In this regard, a significant correlation was observed among traits in almond hybrids. Trunk diameter had a significant correlation with growth vigor and growth habit. Also, the growth habit had a significant correlation with the number of branches at 1% level and the growth habit with the growth, branching, and branch length at 5% level. Branch length was positively correlated with trunk diameter and branch size at 1% level and trunk diameter at 1% level with branch size and 5% level (Salim Pour et al., 2013) Also, these researchers reported that canopy density was highly correlated with branching density, so that canopy density had a positive correlation with the number of branches at 1% level and with branch size at 5% level (Salim Pour et al., 2013).

In the previous report there was stated a positive significant correlation between seedling height with growth vigor and trunk diameter in plum genotypes (Aran et al., 2011). There was also a significant positive correlation between seedling height with trunk diameter and growth vigor in mahaleb (*Prunus mahaleb* L.) (Ganji Moghadam and Khalighi, 2006). These results were in agreement with the results of this study. The strong correlation between leaf length with width and petiole length, as well as the correlation of leaf traits with seedling height and trunk diameter in apple hybrids (Farokhzad et al., 2011; Gharaghani, 2008), are also in agreement with the results of this study.

In another study, Rezaei et al. (2007) reported a significant correlation between height and mean shoot length in 101 walnut seedlings. The correlation between leaf area and seedling height has been reported previously (Prista et al., 2003), which is inconsistent with the results of the correlation table in this study. In the present study, it seems that the direct effect of leaf area on trunk diameter is more than seedling height.

According to the results, there was a high correlation between crown density and branching (70%) in seedlings, so that seedlings with higher branching had higher canopy density. Also, there was a correlation between growth vigor and trunk diameter (70%), where high correlation could play an essential role in the early selection of superior genotypes. The lowest correlation was found between growth strength and canopy density, indicating that the higher the growth power, the lower the canopy density (Salim Pour et al., 2013).

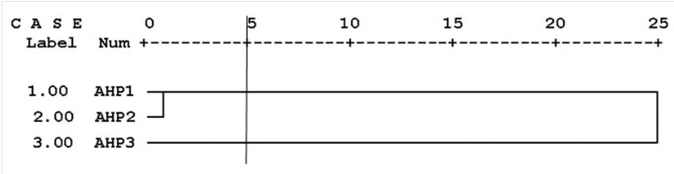
There was a positive relationship between leaf characteristics such as leaf length, width and leaf area with almond tree growth, so that the tree with more growth had longer leaves. There was also a negative relationship between leaf length to width ratio with tree growth vigor, length and diameter of trunk tree, and branching of almond genotypes (Nikoumanesh et al., 2011). In almond breeding programs, we firstly must recognize the relationships between traits and their correlations. The correlation between traits helps to select important traits indirectly, facilitating and speeding up breeding programs (Vargas and Romero, 2001). This correlation also helps to select important traits indirectly and this will facilitate and speed up breeding programs (Vargas and Romero, 2001).

The high correlation between traits in juvenile with maturity phase is easy to measure and is of great value in the almond breeding program as it can lead to removing the large numbers of unfavorable progenies in the early selection that will save time and cost. Among these, there is a correlation between late leafing in progenies with late flowering in mature trees (Imani, 2010; Dicenta et al., 2005).

The traits such as growth vigor, growth habit and leafing date are helpful in the early selection of progenies so that at the end of the first year progenies with poor growth ability and progenies with poor growth habit can be evaluated and removed from progeny evaluation programs (Vargas et al., 2005). Since there is a positive correlation between the date of leafing and flowering, the initial selection of progenies can be carried out in the nursery before transferring them to evaluation plots. If breeding program aims to access late flowering, only the progenies with leafing date trait should be transferred in evaluation plots and the late-flowering trait should be examined in these progenies. With the initial selection of progenies, maintenance costs will reduce due to the reduced land and labor needed for fewer progenies. This early selection of progenies will lead to efficiency and cost-effectiveness in almond breeding programs (Vargas et al., 2005). Early selection for late flowering cultivars based on seed germination indices and leafing time of progenies was studied by Dicenta et al.(2005) and they reported a correlation between these indices with late flowering. They reported that if the parents with different flowering time (early and late flowering) which had a high diversity within the population progenies were chosen, the leaf date index could be used with high confidence to select late-flowering progenies in early stages of almond breeding programs.

Cluster Analysis

Grouping cultivars, genotypes, or populations based on many traits can be a reliable way of determining their similarities or genetic differences. In this study, the clustering of populations was performed using Ward's cluster analysis. Based on the results (Fig. 6), the studied populations were categorized into two groups at 25 Euclidean distance. In the first group, there was First Population (AHP1) from crossing 'Sefied' (male parent) and 'Shahrood 16' (female parent) and Second Population (AHP2) from crossing 'Shahrood 12' (male parent) and 'Shahrood 16' (female parent) but in the second group, there was Third Population (AHP3) from the cross between 'Marcona' (male parent) and 'Shahrood 12' (female parent).



[AHP1 = (Shahroud16 × Sefied); AHP2 = (Shahroud16 × Shahroud12); AHP3 = (Shahroud12 × Marcona)]

Figure 6. Grouping of studied populations based on all measured traits by the Ward method

Generally, populations showed significant differences in leaf length, petiole length, branch angle, one-year branch diameter, one-year branch anthocyanin and leafing date. The First (AHP1) and Second (AHP2) Populations had the same maternal parent ('Shahrood 16') and showed high similarity in the grouping. The progenies of one (AHP1) and two (AHP2) populations were more similar in length, width and leaf area, canopy width, trunk diameter, shoot density and growth habit. These populations were not statistically significant (Table 3). In contrast, the Third Population (AHP3) in leaf length, width, and surface area, petiole length, canopy width, branch density, branch angle, one-year branch anthocyanin and leafing date showed significant differences from the First (AHP1) and Second (AHP2) Populations (Table 3).

In a study based on vegetative traits, 259 plum seedlings were grouped by the UDPGMA method and it was reported that seedlings were classified into two main groups at a 114.4 distance. Seedling height, type of growth, number of suckers, an average branch length and the number of thorns were the most essential traits in grouping seedlings (Aran et al., 2011). In this regard, Rezaei et al. (2007) reported that 101 walnut seedlings were categorized according to their growth vigor into three groups: strong growth, moderate growth, and slow growth.

Garcia Gusano et al. (2009) reported that the flowering time of the male parent (pollen donor) had little effect on the chilling requirement and the germination time of almond seedling, and their chilling requirement in this state was affected by the flowering time of the female parent (pollen recipient).

Cluster analysis using the Ward method at five Euclidean distances was able to classify the progenies with the same parents in one group. According to these results, the same female parent between the First (AHP1) and Second (AHP2) Populations played a more decisive role in the inheritance of traits and population grouping than the male parent. This conclusion can be used in corrective planning for specific purposes. These results were confirmed by previous reports (Farokhzad et al., 2011; Gharaghani, 2008; Naghshin, 2007) based on the influence of female parents (pollen recipients) on the grouping of studied populations.

Conclusion

The results of this study showed that the three studied populations were significantly different in measured traits. Traits in progenies were strongly influenced by the parental type and the role of female parents was greater than the role of male parents. Considering the significant correlations among most of the traits, it seems that the traits highly correlated with each other can be used in the evaluation of large population od progenies, thus saving considerable time and cost in breeding programs.

The results showed that traits such as leafing date with a higher coefficient of variation (59.70%) had higher variation among studied populations so that the extra leafing date was found among the progenies from crossing between two cultivars 'Shahrood 12' and 'Shahrood 16', with both parents having late-flowering traits. Since this trait is strongly correlated with the flowering date (Ghanji Moghadam and Khalighi, 2006; Dicenta et al., 2005; Vargas et al., 2005), it is predicted that the expression of late-flowering trait in progenies is higher and thus the probability of reaching the late flowering in this population is higher than in two other populations.

CRediT Authorship Contribution Statement

Asghar Mousavi: Project administration, investigatin and validation. **Maryam Tatari:** Formal analysis, writing- original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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