Journal of Nuts 12(4) (2021) 279-291

Journal of Nuts

Journal homepage: ijnrs.damghaniau.ac.ir

ORIGINAL ARTICLE

Genetic Analysis of Early Growth Characteristics in a Pistachio (*Pistacia vera* L.) Population Raised under Greenhouse Conditions for use in Breeding Program

Hossein Dashti[1], Asghar Rahimi[1], Seyed Ahmad Hosseini[1], Shirin Davoodian[2], Mozhgan Gholizadeh Vazvani[*3]

[1]*Department of Genetics and Plant Production, Faculty of Agriculture, Vali-e-Asr University, Rafsanjan, Iran*

[2]*MS- Student of Plant Breeding, Factually of Agriculture, Vali-e-Asr University, Rafsanjan, Iran*

[3]*Ph.D. Student Plant Protection, Factually of Agriculture, Vali-e-Asr University, Rafsanjan, Iran*

ARTICLEINFO

Keywords:

Additive variance;
Dominance variance;
Genetic gain;
Genotypes;
North Carolina;
Vegetative growth

ABSTRACT

Pistachio is one of the important horticultural crops, comprising 6.4% of the agricultural exports of Iran. In this study, the genetics of some early growth traits of 40 pistachio genotypes was evaluated under a greenhouse setting. The 40 genotypes were created by crossing eight male trees with five female individuals using the North Carolina I mating design. The experimental design was a completely randomized design (CRD) with three replications established in the greenhouse. Seedlings were assessed for stem height, number of leaves, roots, and shoot dry weight. The results revealed significant variation for the phenotypic traits assessed among the male parents. Male M_6 had the lowest height among the genotypes. Males M_4 and M_2 had the highest and lowest number of leaves, respectively. The offspring of male M_7 had maximum shoot and root dry weight. The offspring of females within males were significantly different. Regarding all studied traits of hybrids plants derived from (male M_7×Fendoghi-48) had the highest height, leaf number and shoot dry weight. So M_7 was the best male for mating and selection for good early seedling growth in pistachio because genotypes with a taller stem had better vegetative growth which can be used for producing rootstocks. For all studied traits, the amount of additive variance was greater than dominance variance, and shoot dry weight had the highest narrow-sense heritability (0.518) and the most expected relative genetic gain with 10% of selection intensity, indicating that the selection can be used to improve these early growth traits in pistachio.

Introduction

Pistachio is one of the most important horticultural products in Iran (Sharifkhah *et al*., 2020; Norozi *et al*., 2019). The global export and production share of pistachios worldwide in 2019/2020 was 6.4%, and 31%, respectively (Statista, 2019/2020). However, the yield of pistachio in Iran (1300 kg/ha) is lower than in the main pistachio-producing countries (FAO, 2017). All Iranian edible pistachios belong to the species *Pistacia vera* L. The center of diversity of *P. vera* is northern Iran, southern Turkmenistan, and parts of Afghanistan (Abdullatif Shakhi *et al.,* 2019). In Iran, two pistachio species, *P. atlantica* (wild pistachio) and *P. khinjuk*, grow on a large scale in many parts of the Zagros and Alborz mountains (Pourreza *et al*., 2008; Ahmadi Afzadi *et al*., 2007).

There are about 11 wild species in the genus Pistacia, and their importance is significant as rootstock seed sources for cultivated *P. vera* and forest trees. Published information on the pistachio genome is limited (Motalebipour *et al*., 2016; Kafkas *et al.*,

[*]**Corresponding author**: Email address: mgholizadehvazvani@yahoo.com
Received: 5 December 2019; Received in revised form: 12 July 2021; Accepted: 20 November 2021
DOI: 10.22034/jon.2021.1884659.1074

2002). *P. atlantica* is used as a rootstock for edible pistachio trees.

Because pistachio trees are dioecious, and due to their cross-pollination, high genetic segregation occurs in seedlings. Therefore, seedlings differ in growth rate, nutrient absorption, resistance to drought stress, and salinity (Rezaei *et al*., 2019). Thus, non-uniformity in pistachio orchards has made orchard management difficult (Litz, 2005).

An investigation into the genetic variability and heritability of early growth traits in *P. atlantica* found that this species has a sufficient genetic variation to be used in breeding programs (Seyedi *et al*., 2012). Today, different methods are used to study genetic diversity in *Pistacia vera* using fruit and leaf morphology, biochemical traits, and the use of molecular markers of DNA (Hormaza *et al*., 1994; Ghareyazie *et al*., 1995; Pourkhaloee *et al*., 2017; Baghazadeh Daryaii *et al*., 2020). The use of morphological traits of plants, however, is one of the oldest classification tools (Caruso *et al*., 1998).

One of the most important factors affecting plant breeding is genetic diversity (Imani *et al*., 2022; Soltani *et al*., 2022). Therefore, the identification and estimation of genetic variation and its nature are very important for a successful breeding program (Vahdati *et al*., 2020). Not only is information about genetic diversity important, but knowledge of the traits and population sizes of plant germplasm also has importance for conservation, detection, and sustainable use in plant breeding programs, as well as other agriculture, uses (Khadivi, 2018; Shahghobadi *et al*., 2018).

Heritability and genetic gain are commonly used by plant breeders to estimate the accuracy of a selection index and for measuring the response to selection (Hajnajari *et al.* 2012; Hasheminasab and Mohammadtaghi Asad, 2017). The heritability of some phenological traits of pistachio, such as flowering and leafing date, was estimated based on half-sib family and parent-offspring regression analysis. The narrow-sense heritability of flowering date ranged from 0.68 to 0.88 and of leafing date from 0.60 to 0.75 (Chao *et al*., 2003).

The genetic study of phenological traits requires a long time (Fallahi *et al*., 2014). Chao *et al.* (1998) studied progenies from 78 different crosses from 1989 to 1996 and reported that heritability estimates for nut characters, tree size, and *Alternaria* late blight resistance ranged from 0.88 to 0.99. For planning a new orcharmarkerd, replacing unsuitable stocks (low growth rate) by developed rootstocks with rapid vegetative growth, as well as identifying suitable pollinator varieties are important objectives of breeding programs in pistachio cultivars.

The results of the study on genetic variation in *P. atlantica* showed that early growth traits (e.g., plant height and number of leaves) had the highest variation. The narrow-sense heritability of crown diameter, number of leaves, and branch length were moderate (0.53, 0.46, and 0.43, respectively) and of seedling height was high (0.71) (Seyedi *et al*., 2012).

In a research project on *Pistacia vera*, genetic analysis showed that the high magnitude of phenotypic and genotypic coefficients of variance along with broad-sense heritability and genetic gain were estimated in relative water protection, excised leaf water loss, relative water content, and relative water loss under drought stress conditions, indicating that the inheritance of these traits can be mainly controlled by additive gene effects followed by reflecting the possibility of effective selection in order to genetically improve these traits (Hasheminasab and Mohammadtaghi Asad, 2017; Safavi *et al*., 2011).

Kashanizadeh *et al* (2020) evaluated the effect of artificial pollination on pistachio trees in orchards. Their results indicated that artificial pollination had a significant effect on quantitative traits such as the number of flowers and fruits per cluster, reduced the percentage of blank fruits, and determined the best male tree for pollination. Therefore, the source of pollen grains is very important in the quantitative traits of pistachios.

Information about gene actions, additive or dominance variances, and heritability are basic requirements in an early vegetative traits breeding program in pistachio. Genetic information about the quantity and type of a gene's action in controlling vegetative traits in pistachios through controlled crosses in the sources is rare. Most of its wide genetic variability has not been exploited to solve production drawbacks. A limited crossing program was established in 1989/90 at IRTA- Mas Bové which produced about 2000 seedlings derived from 31 crosses, and this project provides information about the interest of some cross-combinations for two pistachio traits, including vigor and leafing date (Vargas *et al*., 1995). Regarding vigor, measured as trunk diameter of the seedlings, significant differences were found between families.

The results of 12 crosses showed that offspring of 'Mateur' × 'C' and 'Mateur' × 'M-502' were vigorous, while progenies derived from 'Larnaka' × 'Nazar', 'Larnaka' × 'M-502', 'Aegina' × 'Enk', and 'Larnaka' × 'M-38' were less vigorous. The cultivar 'Mateur', which is vigorous, gave usually vigorous seedlings, while 'Larnaka', which is less vigorous, produced seedlings of more reduced vigor.

The selection strategy in breeding programs is determined by the genetic diversity and function of the genes contributed in trait control (Brown and Aligari, 2008).

Most of the research that has been done into pistachio breeding has been the study of genetic diversity or evaluation of seedlings of different pistachio cultivars against salinity and plant nutrition in greenhouses, and has used seeds whose female parent only is known; no information about their male parents is available. Therefore, the results which are published in the literature are related to the reaction of genotypes whose maternal parent is known, while the genotype of the seeds of these cultivars changes every year. On the other hand, the selection of pistachio seedlings is made with no knowledge of gene action. If the action of genes in the growing power of pistachio seedlings (e.g., height, the weight of vegetative parts, number of leaves, and root weight) is not additive, or the additive share is less than the dominance effects, the selection from the progeny of controlled artificial crosses should be more successful in choosing the suitable and vigorous rootstock. If it is possible to reproduce through tissue culture, identifying the best seedlings from artificially controlled crosses is more successful. Therefore, the main purpose of this study was to determine the relative share of additive and dominance effects of genes in early growth characteristics to introduce the best pollinator and crosses to select the best seedlings for use as rootstock. Therefore, in this study, additive (σ^2A) and dominance (σ^2D) genetic variance, heritability, and genetic gain for some seedling growth traits in *P. vera* were estimated.

Materials and Methods

To obtain the required seeds, 8 male trees (source of pollen) and 40 female trees were randomly selected from the collection of the Pistachio Research Institute of Rafsanjan (Table 1). On February 5, 2016, at the beginning of the growing season before flowering, coinciding with the time of bud swelling, several branches from the male and female trees were covered with cloth bags to prevent the entry of unwanted pollens. During pollination, pollen from the male trees was collected, and every male was crossed with 5 females (except for one case which was crossed with 4 females) in the form of North Carolina design I (Kearsey and Pooni, 1996). A total of 39 crosses were obtained. Labels were attached to the clusters, and they were covered again with cloth bags. At the beginning of September, the seeds were collected and stored at 4ºC. Then, during the first half of March 2016, all crosses were grown in pots (20 cm diameter containing 4 kg sandy-loom soil with EC=2 ds m^{-1}) at a greenhouse at Vali-e-Asr University of Rafsanjan in a completely randomized design with 3 replications. The seeds of crosses were soaked in distilled water for 24 h and pretreated with 1% Captan (fungicide) solution, then placed in a germinator at 30ºC. Six germinated seeds were planted in each pot, and all pots were uniformly irrigated with tap water at intervals of 3-4 days (Banakar and Ranjbar, 2010). After three weeks, the number of plants was reduced by 2 plants per pot, and the average traits measured in the pots were considered for analysis. Three months after planting, all the shoots from each pot were cut at the soil surface. After counting the number of leaves and measuring stem height (cm), shoots were oven-dried at 70ºC for 48 h and weighed (gr) Then, the underground parts were carefully removed from the soil, washed and dried, and then weighed (gr).

Table 1. List of 40 female and 8 male's cultivars are used in this study from Pistachio research institute of Rafsanjan (station No.2), Iran.

Females	Ravar-3	Ravar-1	Ravar-2	Momtaz	Lahijani	Poostkhormaei	Javadaghaei	Vahedi
	Mosabadi	Hasani	Syrisi	Beheshtabadi	Shamsi	Sefid noogh	Amiri	Ghafori
	Qazvini	Eitaliaei	Khanjari	Momtaztajabadi	Lak-syrisi	Badamizarand	Khanjari-damghan	Herati
	Fendoghi-48	Jandaghi	Fendoghi-riz	Shahpasand	Ebrahimi	Gholamrezaei	Sabzpestah-noogh	Badami-nishkalaghi
	Ebrahimabadi	Akbari	Fendoghi-zoodras	Hasanzadah	Mohseni	Owhadi	Kalleh-ghoochi	Safaldini
Males	M_8	M_7	M_6	M_5	M_4	M_3	M_2	M_1

Statistical analysis to estimate genetic parameters

8 males and 40 female trees were randomly selected and each male tree was crossed with 5 female trees. 5 different females nested within each male (North Carolina I) and the statistical design was analyzed hierarchical nested design. The statistical model is. Yijk = μ + Si + D (i) j + e (ij) k, yijk is the phenotypic value of the k-th progeny of j-th female nested in i-th male, μ grand mean, Si is the effect of i-th male, Di (j) is the effect of j-th female nested in i-th male, and e (ij) k the random error term The analysis of variance of the design and the corresponding F-tests are shown in Table 2.

Table 2. Model of analysis of variance and MS components using for estimating genetic parameters.

S.O.V	df	MS	E (MS)	Covariance	F-ratio
male	m-1	M_1	$\sigma^2_e + r\,\sigma^2_{f/m} + rf\,\sigma^2_m$	$\sigma^2_e + r\,(cov_{Fs}\text{-}cov_{HS}) + rcov_{HS}$	M1/M2
male (female)	m (f-1)	M_2	$\sigma^2 e + r\,\sigma^2_{f/m}$	$\sigma^2_e + r\,(cov_{Fs} - cov_{HS})$	M2/M3
error	mf (r-1)	M_3	σ^2_e	σ^2_e	
total	mfr-1				

MS: Mean squares, E (MS). Expected mean square, σ^2m: male variance, r: number of replication: number of female within males, $\sigma^2_{f/m}$: female variance within males σ^2_e: error variance (environmental variance)

Genetic parameters were calculated using mathematical expectations of mean squares (shngh and Chaudhary, 2010: Kearsey and Pooni, 1996). Calculation of additive variance and dominance variance:

According to the random model of the analysis of variance table (Table 2), the equivalents of the covariance are: $\sigma^2_{f\,/\,m}$ = CovFS-CovHS, $\sigma^2_{f\,/\,m}$ = (M2-M3) / r and σ^2_m = COV HS, σ^2_m = (M1-M2) / rf.

Genetic translation and interpretation of relative covariance, when the inbreeding coefficient is zero (F = 0) and absence of epistasis (Singh and Chaudhary, 2010; Kearsey and Pooni, 1996):

COV (HS) = 1/4 σ^2_A, COV (FS) = 1/2 σ^2_A + 1/4 σ^2_D, So $\sigma^2_{f\,/\,m} = 1/2\,\sigma^2_A + 1/4\,\sigma^2_D - 1/4\,\sigma^2_A = 1/4\,\sigma^2_A + 1/4\,\sigma^2_D \rightarrow \sigma^2_D = 4\,(\sigma 2_{f\,/\,m} - \sigma^2_m)$.

Calculation of broad sense heritability (H) and narrow sense heritability (h^2):

$H = (\sigma^2_g / \sigma^2_p)\,100$ and genetic variance (σ^2_g) = $\sigma^2_A + \sigma^2_D$, $h^2 = (\sigma^2_A / \sigma^2_p)\,100$ and phenotypic variance (σ^2_p) = $\sigma^2_g + \sigma^2_e / r$, σ^2_e = M3.

Calculation of genetic variation coefficient (GCV) and genetic gain (G):

GCV = $(\sqrt{\sigma^2}\, g / \bar{Y}_{ooo})\,100$, $\bar{Y}_{ooo}$ = grandl means, G = $100\,(i * h^2 * \sigma_p) / \bar{Y}$ and i = 1.76 for k = 10%, i (selection intensity), k (selection percent) and σ_p, phenotypic standard deviation (Falconer, 1989).

Factor analysis was performed by the exploratory method through correlation matrix between traits and Varimax rotation for loading coefficients extraction. (Manly, 2000).

Mean comparisons of crosses were performed by LSD method. For the above statistical calculations, Minitab, Excel, and MSTAT-C soft wares were used.

Results

The results of ANOVA and estimation of genetic parameters are shown in tables 3 and 4. Analysis of variance showed a significant difference between males and females within males in all growth traits including stem height root and shoot dry weight and number of leaves (Table 3). Differences among males for stem height were significant ($P \leq 0.05$). M6 male had the lowest stem height, and males M_7, M_4 and M_1 had the greatest stem height and there was no significant difference among the other male's cultivars (Fig. 1). These results are in close agreement with those of Vargas *et al.,* (1995) regarding vigor and leafing date.

Table 3. Analysis of variance for the studied traits at seedling stage in *Pistacia vera*

		MS			
S.O.V	df	Root dry weight (gr)	Shoot dry weight (gr)	Number of leaves	Stem Height (cm)
male	7	3.41^{**}	23.03^{*}	128.9^{*}	199.09^{*}
male (female)	$(31)^{1}$	1.39^{**}	7.007^{*}	44.94^{***}	85.42^{**}
error	78	0.63	2.28	16.71	49.67
CV%		3.7	27.8	29.7	25.6

$^{*}, ^{**}, ^{***}$ significant at 0.05, 0.01 and 0.001, respectively

^{1}: One of the males is crossed with four females

Table 4. Genetic parameters, additive variance, dominance variance, narrow sense heritability, Broad sense heritability, Genetic variability (GCV) and genetic gain for the studied characters

Traits	mean	Genetic gain (%)	GCV (%)	Broad sense heritability (H)	Narrow sense heritability (h^2)	Dominance variance (σ^2_D)	Additive variance (σ^2_A)
Stem height	27.45	24.6	25.1	0.742	0.48	16.52	31.14
Number of leaves	13.72	44.6	44.7	0.87	0.532	14.62	23
Shoot dry weight	5.42	52.5	0.46	0.89	0.60	1.91	4.38
Root dry weight	2.77	31.6	36.3	0.82	0.45	0.45	0.55

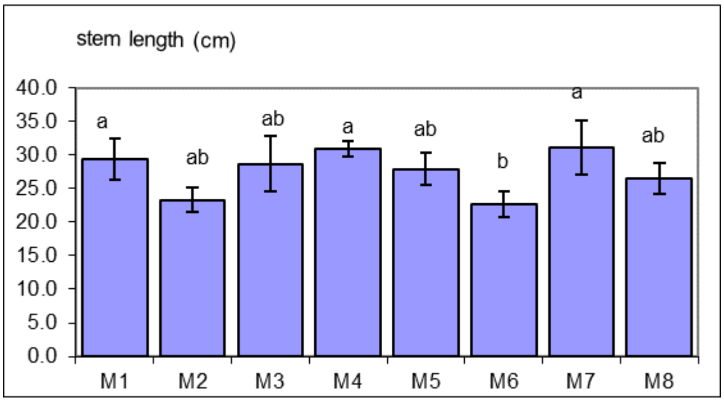

Fig. 1. Mean comparison among pistachio male cultivars for Seedlings stem length (cm).

A number of leaves were significantly different among male cultivars ($P \leq 0.05$). Males M_7 and M_4 had the highest number of leaves but had no significant difference with M_1, M_3, and M_8 cultivars. Also, males M_2, M_6 and M_5 had the least number of leaves (Fig. 2).

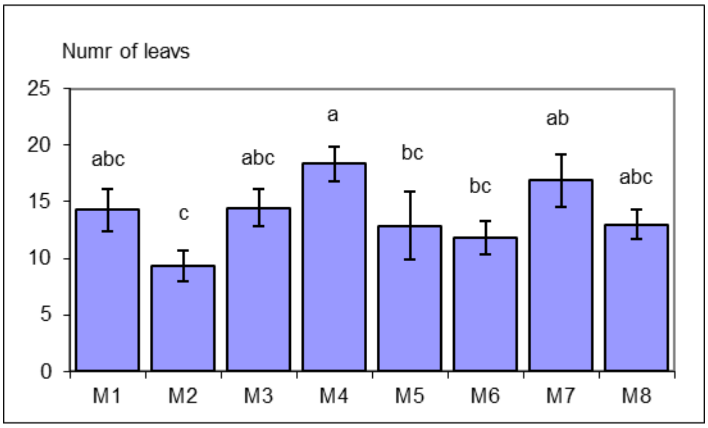

Fig. 2. Mean comparison among pistachio male cultivars for Number of leaves.

Males M_7 and M_4 had the highest shoot dry weight while the lowest one was observed in M_8 and M_1 (Fig. 3).

Also, significant differences were observed among male cultivars for root dry weight ($P \leq 0.01$), M_7 and M_6 had the highest and lowest root dry weight, respectively. The other males were not significantly different from M_7 (Fig. 4).

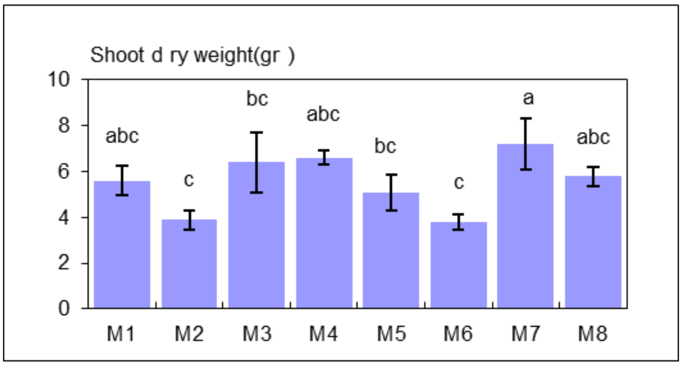

Fig. 3. Mean comparison among pistachio male cultivars for Shoot dry weight (gr).

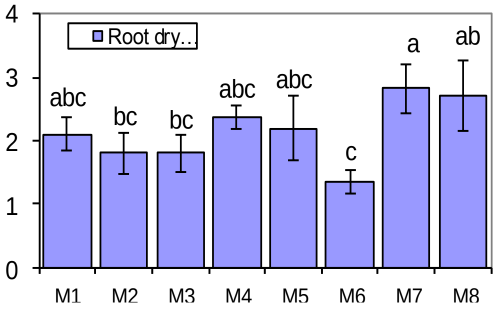

Fig. 4. Mean comparison among pistachio male cultivars for root dry weight (gr).

Estimated genetic parameters showed that the studied traits had high heritability and in general the incremental variance was greater than the dominance variance (Table 4). The population produced was very diverse in terms of the traits under study (Tables 3, 4 and 5). A significant positive correlation between heights, number of leaves, shoot dry weight and root dry weight were observed (Table 6).

Table 5. Mean comparison among crosses for the studied characters at 0.05 level of probability, by LSD method.

Male	Female	Traits			
		Number of leaves	Height (cm)	Shoot dry weight (g)	Root dry weight (g)
		mean	mean	mean	mean
M_1	Owhadi (F_1)*	19.66a-f	36.33abc	5.4bc	2.3c-f
	Syrsi (F_2)	13d-k	21.66b-g	5.63bc	2.17c-f
	Kalle-ghochi (F_3)	12e-k	22.33c-g	3.16bc	2.16c-f
	Gholamrezaei (F_4)	17.33b-h	35a-d	5.86bc	2.7b-f
	Badami zarand(F_5)	9.33h-k	31.66b-g	2.9bc	1.3f
M_2	Badami nishkalaghi (F_6)	11g-k	27b-g	4.26bc	2.8b-f
	Ravar-3 (F_7)	5k	27.33b-g	2.06c	1.53f
	Harati (F_8)	13d-k	22.66c-g	3.36bc	1.56f
	Mosabadi (F_9)	8f-k	21.66c-g	2.03c	1.2f
	Safaldini (F_{10})	9.66g-k	17.66f-g	2.63bc	2.16c-f
M_3	Sabzpestah noogh (F_{11})	16b-g	34a-e	6.1bc	2.53b-f
	Ravar-2 (F_{12})	14d-g	25.66c-g	3.43bc	1.16def
	Khanjari damghan(F_{13})	11g-k	22.33c-g	2.36c	1.5f
	Hassani (F_{14})	11.33f-k	19.33e-g	2.53bc	1.4f
	Early Fendoghi (F_{15})	20a-e	42ab	13.5a	9.7a
M_4	Ghafori(F_{16})	16b-g	31.33b-g	5.66bc	2.56b-f
	Behesht abadi (F_{17})	14d-g	26.66b-g	4.3bc	2.13b-f
	Sefid noogh(F_{18})	21a-d	30.33b-g	5.16bc	2.13def
	Amiry (F_{19})	22.66abc	33.33a-e	6bc	1.83def
	Shamsi(F_{20})	18a-g	32.66a-g	5.66bc	2.73b-f
M_5	Vahedi(F_{21})	14.33c-g	30.66b-g	4.13bc	2.53b-f
	Javadaghaei(F_{22})	9.33h-k	33a-f	4.23bc	2.26c-f
	Momtaz(F_{23})	8j-k	27.66b-g	3.3bc	2.06def
	Poostkhormaei(F_{24})	24ab	29b-g	6.85b	3.9bc
	Lahijani(F_{25})	8.66ijk	19.33efg	2.06c	1.16f
M_6	Momtaz tajabadi (F_{26})	17b-i	20.66b-g	2.96bc	1.73def
	Lak-syrisi(F_{27})	12e-k	28.66b-g	4.03bc	2.1def
	Ravar-1(F_{28})	11.66e-k	24.66c-g	2.6bc	1.26f
	Khanjari ravar (F_{29})	8.66ijk	17.33g	2.16c	1.23f
	Etaliaei (F_{30})	9.66g-k	21.66c-g	2.43bc	1.73def
M_7	Mohseni (F_{31})	12.33e-k	22.66c-g	2.8bc	1.66ef
	Fendoghi-48 (F_{32})	25.5a	46.5a	10.9a	2.7b-f
	Hassanzadeh (F_{33})	17.33b-h	29.33b-g	5.56bc	3.46bcd
	Ebrahimabadi (F_{34})	15.66b-j	26c-g	5.93bc	3.43b-e
	Akbari (F_{35})	13.66d-j	31b-j	5.83bc	22.23c-f
M_8	Ebrahimi (F_{36})	13d-k	28.33b-g	4.53bc	2.63b-f
	Shah pasand (F_{37})	12e-k	19efg	2.4c	1.56f
	Fendighi-riz (F_{38})	10g-k	28.66b-g	4.1bc	2.16b-f
	Jandaghi (F_{39})	17b-i	30b-g	3.83b	4.16b

Table 6. Correlation among the studied characters of pistachio seedlings

Traits	Stem height	Number of Leaves	Shoot dry weight
Number of leaves	0.62^{***}		
Shoot dry weight	0.87^{***}	0.75^{***}	
Root dry weight	0.52^{***}	0.48^{***}	0.72^{***}

***: Significant at 0.001 level of probability

Discussion

According to the results, in general, males M_7, M_4, and M_1 had a greater performance for all growth traits. It can be said that these males have higher general combining ability (GCA) than the other males, especially given that the additive variance is greater than the dominance variance (Table 4). Greater additive variance than dominance variance reflects the effectiveness of selection for improving these traits at the seedling stage in pistachio. For example, stem height additive variance (31.14) is greater than dominance variance, as broad and narrow sense heritability were in average level (0.74 and 0.48), it can be improved by selection (Table 4).

This is consistent with Seyedi *et al.*, (2012) study on wild pistachio (*P. atlantica*) which reported that genetic variation for plant height was higher than the other traits. They also reported that the expected relative genetic gain of height (by 10% selection intensity), which was also significantly high, shows there is a possibility of improvement through selection for height in wild pistachio trees.

In our study the narrow-sense heritability of height was 0.48, indicating that the additive gene effects are important in determining the trait. Narrow and broad-sense heritability, respectively; 0.53 and 0.87 for several leaves and 0.62 and 0.89 for shoot dry weight, and also had the most expected relative genetic gain by 10% selection intensity (44.8 and 69) representing an excellent response to selection (Table 4). So, selection will be effective to improve rootstock for these characteristics at the seedling stage in pistachio (Falconer, 1989).

On the other hand, although the additive variance of stem height, number of leaves, root dry weight and shoot dry weight was greater than dominance variance, but also the dominance variance was considerable, due to the high broad-sense heritability of these traits (74%, 87%, 89%, and 82%, respectively) (Table 4), suggesting that dominance variance is also effective in differentiating between hybrids. Some hybrids showed the highest performance of trait that it could be said caused by specific combining ability (SCA). Like cross M_7 × fendoghi-48 had the highest stem height (46.5 cm) and the highest number of leaves (25.5) and cross M_3 × Fendoghi-zoodras has the greatest root dry weight (9.7 gr), shoot dry weight (13.5gr) and number of leaves (Table 5). Therefore, we can select the best seedlings from the progeny of these crosses.

The correlation among different traits can be caused by genetic and environmental factors. For a breeder, both genetic and environmental correlations are important because they will determine the efficiency of the selection program. Thus, we may estimate the relative contribution of these factors to the observed correlation (Falconer, 1989).

Positive and significant correlation represents that increase in dry weight of roots caused an increase in the stem height, number of leaves, and shoot dry weight one reason for the existence of correlation among the traits can be linkage between genes controlling them as well as pleiotropic effects (effect of one gene on multiple traits) (Kearsey and Pooni, 1996).

In order to reduce the vegetative traits to limited variables, factor analysis was done on the base of means of different traits; three independent factors were extracted that explained 95% of data variance (Table 7).

Table 7. Factors and corresponding loading coefficients resulting from factor analysis based on means of studied traits for 39 crosses.

Traits	Factor 1	Factor 2	Factor 3
Stem height	0.91	-0.31	-0.24
Number of leaves	0.31	0.91	-0.23
Shoot dry weight	0.59	-0.56	-0.45
Root dry weight	0.24	-0.22	0.94
Explained variance	0.35	0.32	0.30

For the first factor, stem height and the shoot dry weight had the highest loading factor. For the second factor, number of leaf and in third one root dry weight showed the highest loading factor. By using Biplot scattering based on the first and second factors that explained 67% of the variance, crosses were grouped closely in four groups. This grouping of hybrid offspring based on two factors can be considered as grouping based on the hybrid performance power in the studied traits and perhaps this performance of hybrids can be compared to specific combining ability (SCA.) (Fig. 5). Crosses of $M_1 \times F_5$, $M_5 \times F_{22}$, $M_2 \times F_7$, $M_6 \times F_{27}$, $M_8 \times F_{38}$, $M_5 \times F_{23}$ and $M_5 \times F_{21}$ in respect to the traits that more effective in the first and second factors (height, shoot dry weight and number of leaves) have more similarity to each other and generally have higher shoot dry weight and stem height and have more specific combining ability for the two factors. The cross $M_7 \times F_{32}$ had the highest value for the first factor that shows the progeny of this cross had higher vegetative growth (stem height and shoot dry weight) from the other crosses, indicating M_7 and Fendoghi-48 (F_{32}) have high SCA for these traits (Fig. 5).

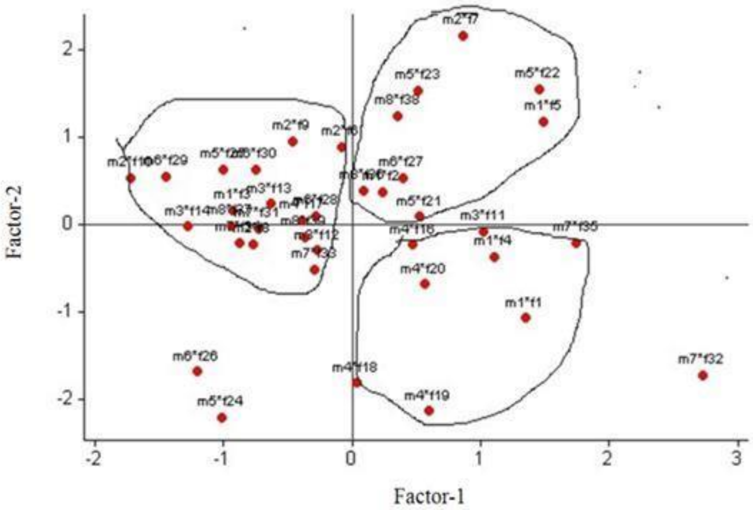

Fig. 5. Biplot and grouping of the crosses based on two first factors in factor analysis (Table 7). (The sign (fi, s) are the females sign based on Table 5).

Factor analysis based on the average vegetative traits for males showed that 96 percent of the variance was explained by two factors (Table 8). The first factor that explained 51% of the total variation has the highest loading on the root and shoots dry weight, and the second factor (44% of the total variation) has the highest factor loadings on the number of leaf and stem height.

Table 8. Factors and corresponding loading coefficients resulting from factor analysis based on means of studied traits for males.

Traits	Factor 1	Factor 2	Commonality
Stem Height	0.74	0.58	0.90
Number of leaves	0.94	0.29	0.97
Shoot dry weight	0.71	0.69	0.98
Root dry weight	0.32	0.94	0.99
Explained variance	0.51	0.44	0.96

As a result, from the scattering of the males based on these two factors can be stated that M_4 produces more stem and root dry weight in the offspring, M_8 produces more leaves and stem length in the offspring, and M_7 increases all traits in the offspring (Fig.6). Perhaps, in other words, it can be said based on these two factors (Fig. 6) general combining ability (GCA) of males is shown in this biplot. M_4 and M_7 had the highest effect or GCA in increasing vegetative growth of pistachio seedlings and M_7 was the best male in crosses to improve the growth characteristics. M_2, $M_{3,}$ and M_6 had the lowest GCA of the two factors, and M_8 had a high GCA on factor-2 or root and shoot dry weight (Fig. 6). In general, considerable variation was observed among studied traits in crosses of different trees.

Males used in hybridization, showed significant differences with each other, indicating a high genetic diversity among male trees. Also, large genetic differences were observed between females within males in terms of the growth characteristics of pistachio seedlings. By using genetic diversity among the studied vegetative traits of males, the best male can be selected under various environmental conditions. For example, in the circumstances of this experiment, M_7 was the best pollinator for mating and selection for good early seedling growth in pistachio, as others have stated, the pollen grain source is very important in the quantitative traits of pistachios (Kashanizadeh *et al*., 2020).

Stem height has shown substantial variation, so genotypes with a taller stem had better vegetative growth which can be used for producing rootstocks, however, the offspring of these crosses show segregation because of male and female trees are heterozygous and using them as rootstocks may not be the same in their growth power, but this segregation is usually expected to be less than the segregation of the UCB-1 that used as rootstock for *vera* nowadays, because UCB-1 is the result of a cross between two outbreeding, highly heterozygous species (cross between *Pistacia atlantica* (female) and *P. integerrima* (male) (Morgan *et al*., 1992) so they segregate for vegetative vigor. Commercial cultivars grafted onto UCB-1 seedlings grow varying greatly from tree to tree. However Clonal UCB-1 rootstocks are available, but some of these suffered from deleterious somaclonal variation that making them less desirable than seedling rootstocks. (Jacygrad *et al.*, 2020; Beede, 2017).

For plant height, the number of leaves, shoot and root dry weight; the gene action is often the additive type. Therefore, these traits can be improved by selection. A significant positive correlation between shoot growth and root dry weight show that seedlings with a better root growth will produce more leaves and taller stem as well. In Factor analysis, 3 factors were extracted explaining 95% of the variance in crosses, expressing there is a high correlation among traits.

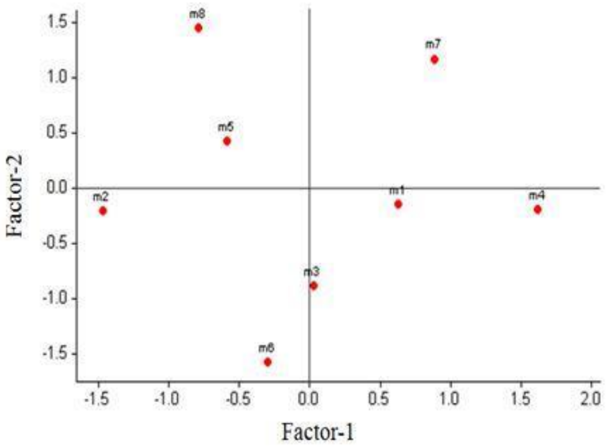

Fig. 6. Biplot analysis result and scattering the males based on the two first factors in factor analysis (Table 8).

Conclusions

The additive variance was greater than dominance variance in all studied vegetative traits and narrow-sense heritability was relatively high in seedling vegetative traits. This indicates that selection at the seedling stage is effective for improving early growth characteristics in pistachio, including stem length, number of leaves, and shoot dry weight. The results of this study showed that dominance variance is also significant in seedling vegetation traits, which indicates the importance of dominance variance in expressing the difference between hybrids, which is due to the specific combinability (SCA) of some crosses. M_7 × fandoghi-48 had the highest stem length and number of leaves and M_5 × fandoghi-zoodras had the highest root dry weight, number of leaves, and shoot dry weight. Therefore, selection can be done from within the offspring of these crosses to improve root growth. Factor analysis and biplot of crosses based on the first and second factors showed that the cross of M_7 $\times F_{32}$ has the highest Specific combinability. There was a great genetic variation among males (the source of pollen grains). Males M_7 and M_4 showed the highest general combinability (GCA) and finally M_7 was introduced as the best pollinator in this experiment.

Acknowledgements

The authors are grateful from the Vali -e- Asr University of Rafsanjan.

Conflict of interest

The authors declare that they do not have any conflict of interest.

References

Ahmadi Afzadi M, Tabatabaei BES, Mohammadi SA, Tajabadipur A (2007) Comparison of genetic diversity in species and cultivars of pistachio (*Pistacia* spp.) based on Amplified Fragment Length Polymorphism (AFLP) markers. Iranian Journal of Biotechnology. 3, 147-152.

Baghazadeh Daryaii L, Samsampour D, Bagheri A, Askari Seyahooei M, Raam M (2020) Molecular Characterization of Zhumeria majdea Iranian Germplasms Using ISSR Markers. International Journal of Horticultural Science and Technology. 7(4), 343-52.

Banakar MH, Ranjbar GH (2010) Evaluation of salt tolerance of pistachio cultivars at seedling stage. American-Eurasian Journal of Agricultural & Environmental Sciences. 9, 115-120.

Beede RH (2017) Pistachio canopy management and its effect on yield components. In: 8th Advanced in Pistachio Production, University of California, Davis.

Brown J, Caligari P (2008). An introduction to plant breeding. Blackwell Publishing Limited, Oxford, UK

Caruso T, Iannini C, Monastra F, Zakynthinos G, Rouskas D (1998) Genetic and phenotypic diversity in pistachio (*Pistacia vera* L.) germplasm collected in Mediterranean countries. Acta Horticulturae. 470, 168-178.

Chao CT, Parfitt DE, Ferguson L, Kallsen C, Maranto J (2003) Genetic analyses of phenological traits of pistachio (*Pistacia vera* L.). Euphytica. 129, 345-349.

Chao CT, Parfitt DE, Ferguson L, Kallsen C, Maranto J (1998) Breeding and genetics of pistachio: The California program. In: Second International Symposium on Pistachios and Almonds, ISHS, Davis (California, USA), 1997. Acta Horticulturae. 470, 152-161.

Fallahi E, Fallahi B, Vahdati K, Kiester MJ (2014) Phenological characteristics, yield, and fruit quality of yellow-fleshed nectarines under high desert conditions of the northwestern United States over five years. International Journal of Horticultural Science and Technology. 1(1), 21-29.

Falconer DS (1989) Introduction to Quantitative Genetics. Longman Scientific & Technical, New York. pp. 464.

FAO (2017) Food and agriculture organization of the United Nations. FAOSTAT database. http://faostat.fao.org.

Ghareyazie B, Huang N, Second G, Bennett J, Khush GS (1995) Classification of rice germplasm Analysis using AFLP and PCR-based RFLP. Theoretical and Applied Genetics. 91, 218-227.

Hajnajari H, Chashnidel B, Vahdati K, Ebrahimi M, Nabipour A, Fallahi E (2012) Heritability of morphological traits in apple early-ripening full-sib and half-sib offspring and its potential use for assisted selection. HortScience. 47(3),328–333.

Hasheminasab H, Assad MT (2017) Genetic and multivariate phenotypic analysis of some selection indices in pistachio (*Pistacia vera* L.) cultivar under drought stress condition. Genetika. 49(2), 705 – 715.

Hormaza JI, Dollo L, Polito VS (1994) Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulked segregant analysis. Theoretical and Applied Genetics. 89, 9-13.

Imani A, Amani G, Shamili M, Mousavi A, Hamed R, Rasouli M, José Martínez P (2021) Diversity and broad sense heritability of phenotypic characteristic in almond cultivars and genotypes. International Journal of Horticultural Science and Technology. 8(3),261-9.

Jacygrad E, Preece JE, Palmer WJ, Michelmore R, Golino D (2020) Phenotypic segregation of seedling UCB-1 hybrid pistachio rootstock. Trees. 34, 531–541.

Kafkas S, Kafkas E, Perl-Treves R (2002) Morphological diversity and a germplasm survey of three wild Pistacia species in Turkey. Genetic Resources and Crop Evolution. 49(3), 261-270.

Kashanizadeh S, Ghasemi M, Ghasemi S (2020) Effect of Artificial Pollination on Some Quantitative and Qualitative Traits of Three Pistachio Cultivars in Qazvin. Journal of Nuts. 11(1), 63-72.

Kearsey MJ, and Pooni HS (1996) The Genetical Analysis of Quantitative Traits. Chapman & Hall. pp. 381.

Khadivi A (2018) Assessment of genetic variability in pistachio (*Pistacia vera* L.) with nuclear SSR molecular markers. Erwerbs-Obstbau. 60, 289–294.

Litz RE (2005) Biotechnology of Fruit and Nut Crops. CABI publishing. pp. 768.

Manly FJ (2000) Multivariate statistic methods: A primer 2nd ed: Chapman & Hall. pp. 232

Morgan D, Epstein PL, Ferguson L (1992) Verticillium wilt resistance in pistachio rootstock cultivars: assays and an assessment of two interspecific hybrids. Plant Disease. 76, 310–313

Norozi M, ValizadehKaji B, Karimi R, Nikoogoftar Sedghi M (2019) Effects of foliar application of potassium and zinc on pistachio (*Pistacia vera* L.) fruit yield. International Journal of Horticultural Science and Technology. 6(1),113-23.

Pourkhaloee A, Khosh-Khui M, Arens P, Salehi H, Razi H, Niazi A, Afsharifar A, Van Tuyl J (2017) Genetic Diversity and Population Structure of Iranian tulips revealed by EST-SSR and NBS-LRR Markers. International Journal of Horticultural Science and Technology. 4(2),167-82.

Pourreza M, Shaw JD, Zangeneh H (2008) Sustainability of wild pistachio (*Pistachio atlantica* Desf.) in Zagros forests, Iran. Forest Ecology and Management. 72, 157-160.

Rezaei M, Hokmabadi H, Khadivi A, Safari-Khuzani A, Heidari P (2019) The selection of superior pistachio (*Pistacia vera* L.) genotypes among seedling trees originated from open-pollination. Scientia Horticultural. 251, 88-100.

Safavi SM, Farshadfar M, Kahrizi D, Safavi SA (2011) Genetic Variability of Some Morphological. American Journal of Scientific Research. (13), 113-117.

Seyedi N, Jalali SGH, Moghaddam M, Tabari M, Mohammadi SA (2012) Genetic variation and Inheritance of early growth characteristics in three wild pistachio populations. Journal of Plant Physiology and Breeding. 2, 35-42.

Shahghobadi H, Shabanian N, Rahmani MS, Khadivi A (2018) Genetic Characterization of *Pistacia atlantica* sub sp. kurdica from Northern Zagros Forestsin Iran. Trees. 33(2), 481-490.

Shakhi A, Arab MM, Brown PJ, Ferguson L, Akbari M (2019) Pistachio (*Pistacia* spp.) Breeding. In: Al-Khayri J., Jain S., Johnson D. (eds) Advances in Plant Breeding Strategies: Nut and Beverage Crops. Springer, Cham. https://doi.org/10.1007/978-3-030-23112-5_10.

Sharifkhah M, Bakhshi D, Pourghayoumi M, Abdi S, Hokmabadi H (2020) Effect of pollination time on yield and antioxidant properties of some pistachio cultivars. International Journal of Horticultural Science and Technology. 7(1), 51-8.

Singh RK, Chaudhary BD (2010) Biometrical Methods in Quantitative Genetic Analysis. New Dehli: Kalyani Publishers. pp, 318.

Soltani F, Shajari M, Mirbehbahani GS, Bihamta MR (2022) Assessment of Melon Genetic Diversity Based on Fruit Phenotypic Traits and Flowering Habits. International Journal of Horticultural Science and Technology. 9(1),97-116.

Statista (2020) https:/ /www. statista. com/ statistics/933042/global-pistachio-production -by- country

Vahdati K, Arab MM, Sarikhani S, Sadat Hosseini M, Leslie CA, Brown PJ (2019) Advances in Persian walnut (Juglans regia L.) breeding strategies. In: JM Al-Khayri, SM Jain and DV Johnson (eds.) Advances in plant breeding strategies, Vol 4. pp. 401-472. Nut and Beverage Crops. Springer, Switzerland.

Vargas FJ, Romero M, Clavé J, Batlle I (1995) First results of vigor and leafing in pistachio progenies. Acta Horticulturae. 419, 273-278.