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Genetic Variation and Inheritance of Early Growth Characteristics in Three Wild Pistachio Populations

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Abstract

Pistacia atlantica is the most important tree species for the economy of many rural areas in west of Iran, but no effort has been made for the genetic improvement of this species. The aim of this investigation was to study the genetic variation and inheritance of early growth traits in *P. atlantica*. For this purpose, three wild pistachio populations comprising 60 randomly selected adult trees from northwest of Iran were sampled and half-sib seeds were sown in a forest nursery using randomized complete block design with three replications. During the growing season, plant height, collar diameter, number of leaves and sprout length were measured on the seedlings in 2009 and 2010. Analysis of variance based on split plot in time was carried out for the two-year data and squared phenotypic coefficient of variation (PCV^2) were calculated for the above mentioned traits. Furthermore, additive genetic variance, narrow sense heritability and expected genetic gain were estimated among the half-sib families under study. In addition, phenotypic correlation coefficients of the traits from 30 two-year old seedlings with several characters of mother plants were calculated. Analysis of variance showed significant differences among trees for all of the seedling traits indicating the existence of enough genetic diversity within this species that can be utilized in breeding programs. Plant height and number of leaves had the highest PCV^2 among the measured traits. Narrow sense heritability estimates were moderate for collar diameter (0.53), number of leaves (0.46) and sprout length (0.43) and high for plant height (0.71). Considerable genetic gain was also expected for plant height. In addition, plant height of half-sib progenies was significantly correlated with tree height, stem diameter and maximum seed diameter of mother plants. Therefore, plant height could be regarded as a reliable early selection index because of its high heritability and genetic gain and also, significant correlation with the characteristics of parental trees.

Keywords: Early selection, Genetic gain, Heritability, *Pistacia atlantica*, Progeny test

Introduction

The genus *Pistacia* is a member of the *Anacardiaceae* family and consists of at least 11 species (Kafkas and Perl-Treves 2001; Kafkas *et al*. 2001; Golan-Goldhirsh *et al*. 2004). Three species of the genus *Pistacia*, *P. vera* L., *P. atlantica* Desf. and *P. khinjuk* Stocks, grow naturally in Iran (Ahmadi Afzadi *et al*. 2007). *Pistacia atlantica* Desf. (wild pistachio), locally known as “Baneh”, is economically the most important species for rurals in the forest areas of the Zagros mountains in Iran (Pourreza *et al*. 2008). It is grown with almond, oak and other forest trees in alpine areas, foothills and at different altitudes between 600-3000 m. The resin of wild pistachio, called Saqez, is used for a variety of industrial and traditional purposes, including food and medicine (Pourreza *et al*. 2008). *P. atlantica* is also used as a rootstock for the edible pistachio tree, *P. vera* L., (Kafkas and Perl-Tereves 2001; Kafkas *et al*. 2001; Ranjbar Fordoei *et al*. 2002; Barazani *et al*. 2004; Ahmadi Afzadi *et al*. 2007). Adaptation of *Pistacia* trees to harsh desert conditions and their longevity make them ideal candidates for reforestation in arid zones (Golan-Goldhirsh *et al*. 2004). In spite of its various uses, no efforts for the genetic improvement of this species have been made (Golan-Goldhirsh *et al*. 2004; Javanshah *et al*. 2006).

Knowlegde about the genetic diversity within *P. atlantica* is useful in establishing management strategies for conservation of this species and improving its production potential. Najowa *et al.* (2009) reported wide genetic diversity among *P. atlantica* genotypes of Suweida province in Syria. Kafkas and Perl-Treves (2001) investigated the inter- and intra-specific variability among the wild *Pistacia* genotypes from Turkey on the basis of 30 phenotypic characters and found that 24 characters were polymorphic among and within species. Furthermore, Kafkas *et al.* (2002) characterized the phenotypic and morphologic diversity within the important wild *Pistacia* species of Turkey, including *P. atlantica*, at the inter and intra-specific level, using a larger number of qualitative and quantitative traits and found high diversity at both levels. In order to study the morphological variability within and among *P. atlantica* Desf. populations of Algeria, Belhadj *et al*. (2008) undertook a comparative analysis of morphological characters of leaves and fruits, as well as micromorphlogical aspects of leaves, in eight wild populations grown under different climatic conditions. Analysis of variance for the morphological traits under study showed significant diversity within and among the populations. Pazouki *et al.* (2010) studied the genetic variability within *P. atlantica* and *P. vera* from Iran by microsatellite markers. However, they indicated that variability within wild pistacia species was considerably lower than the cultivated species. In addition, Salehi Shanjani *et al.* (2009) analyzed amplified fragment length polymorphisms in a total of 216 pistachio accessions which included seven populations from three wild species (*P. vera, P. khinjuk, P. atlantica* subsp. kurdica). The lowest amount of polymorphism was observed within *P. atlantica* subsp. kurdica which indicated strong genetic erosion of this species in Iran due to over-exploitation and consequently severe decline in habitat.

Selection of the best provenance of desired species for a given site or region is necessary for achieving maximum productivity in plantation forestry, agroforestry and future breeding work (Dhanai *et al*. 2003). Moreover, detecting the best genotype in such populations is breeding art, which is performed through various methods (Chaturvedi and Pandey 2004). Progeny tests have been established on multiple sites to evaluate the genetic merits of the selected trees (Gulcu and Celik 2009).

Heritability estimates have been found to be useful in revealing the relative value of selection based on phenotypic expression of different characters (Safavi *et al.* 2011). High estimates of heritability indicate that selection for the characters of study will be effective because of being less influenced by the environment. Based on our knowledge, no studies are available in the literature about the inheritance of morphological characters in wild pistachio. Therefore, the aims of this study were to estimate genetic variation, heritability and genetic advance for some growth characters of *P. atlantica* Desf., using half-sib progenies obtained from mother plants of three different locations.

Materials and Methods

Progeny test data

In September 2008, seeds were collected from 60 randomly selected trees of wild Pistacio (*P. atlantica* var. *kurdica*) from three natural populations in northwest of Iran. The fruit and resin of wild pistachio are important economical sources for rural people in these areas. Trees under study were mostly located in natural forest stands and were at least 100 meters apart to decrease the possibility of being the components of the same parent (Ginwal *et al*. 2005). Furthermore, the distances between selected populations were more than 200 km. The sampling areas ranged from 44° 35′ to 46° 42′ E and 36° 04′ to 38° 06′ N (Table 1 and Figure 1). Some characteristics of the mother trees such as height, stem diameter, maximum seed diameter, minimum seed diameter and seed weight were reported to estimate the phenotypic correlation coefficient of characters of these trees with traits of their half-sib progenies.

Figure 1. Location map of three *Pistacia atlantica* populations in Iran that were sampled for this study

Table 1. Description of selected wild pistachio populations under study

Population	1	2	3
Name	Salmas	Shahindezh	Baneh
Latitude	38˚ 06′ N	36˚ 04′ N	36˚ 30′ N
Longitude	44˚ 35′ E	45˚ 40′ E	46˚ 42′ E
Altitude (m)	1900	1310	1700
Rainfall (mm)	531.75	708	618.7
Mean temp, ºC (min-max)	2.7-15.3	8.6-18.6	3.7-17.76
Average relative humidity (%)	57	44	54

Thirty seeds from each mother plants were sown in plastic pots in the Javanshir forest nursery in December 2008, which is located at 36˚39′ N, 45˚08′ E and 1387 meters above sea level. The resulting half-sib families were evaluated in a randomized complete block design with three replications. Ten seeds from each mother tree were randomly allocated in the experimental plots. Collar diameter (cm), seedling height (cm), number of leaves and length of sprout (cm) were recorded in the first and second growing seasons.

Data analysis

To test the statistical differences among the populations and progenies nested within the populations, analysis of variance was carried out on the data with the GLM procedure of SAS software, based on the recorded characteristics. A linear random model was used for the combination of the two-year data based on split plot in time. The statistical model and its components were as follow:

$$Y_{ijn} = \mu + R_j + S_n + G_i(S) + R_jS_n + R_jG_i(S) + \gamma_k + \gamma_kS_n + \gamma_kG_i(S) + R_j\gamma_k + R_j\gamma_kS_n + R_j\gamma_kG_i(S) + \varepsilon_{ijnk}$$

$$\boldsymbol{Y_{ijk}} = m + \boldsymbol{R_j} + \boldsymbol{G_i} + \boldsymbol{R_jG_i} + g_k + g_k\boldsymbol{G_i} + \boldsymbol{R_j}g_k + e_{ijk}$$

Where $\boldsymbol{Y_{ijn}}$ = the phenotypic value of the progeny of the i-th mother tree nested within the n-th population in the j-th block; m = the grand mean; $\boldsymbol{R_j}$ = the effect of j-th block; $\boldsymbol{S_n}$ = the effect of n-th population; $\boldsymbol{G_i(S)}$ = the effect of i-th progeny nested within the n-th population; $\boldsymbol{R_jS_n}$ = the interaction effect of the j-th block and n-th population; $\boldsymbol{R_jG_i(S)}$ = the interaction effect of the j-th block and i-th progeny nested within the n-th population; g_k = the effect of k-th year; $g_k\boldsymbol{S_n}$ = the interaction effect of k-th year and n-th population; $g_k\boldsymbol{G_i(S)}$ = the interaction effect of k-th year and i-th progeny nested within the n-th population; $\boldsymbol{R_j}g_k$ = the interaction effect of the j-th block and k-th year; $\boldsymbol{R_j}g_k\boldsymbol{S_n}$ = the interaction effect of the j-th block, k-th year and n-th population; $\boldsymbol{R_j}g_k\boldsymbol{G_i(S)}$ = the interaction effect of the j-th block, k-th year and i-th progeny nested within the n-th population; e_{ijnk} = the random error.

In addition, an analysis of variance was performed on all progenies, disregarding the populations, to estimate the components of variance and narrow sense heritability of the characters under study. The model was as follows:

Since the number of surviving seedlings was different in the plots and several experimental units were lost before data recording, the data were not balanced and thus were analyzed by GLM procedure of SAS.

Progenies of each mother tree were assumed as half-sib families and additive genetic variance (s_A^2) was estimated as $s_A^2 = \boldsymbol{4}s_f^2$ where s_f^2 is the among-family genetic variance (Falconer and Mackay 1996). Narrow sense heritability ($\boldsymbol{h_n^2}$) among families, on the entry mean basis, was estimated as follows (Falconer and Mackay 1996):

$$h_N^2 = \frac{s_f^2}{s_f^2 + \frac{s_e^2}{n} + \frac{s_{gy}^2}{y}}$$

Where, s_e^2 is variance due to the random error and s_{gy}^2 is variance of interaction of genotype with year and $\boldsymbol{y}$ and $\boldsymbol{n}$ are the number of years and half-sib progenies, respectively.

Percent of genetic gain among families was also calculated using the following formula (Kown and Torrie 1964):

$$G = \frac{K \times h_n^2 \times \sigma_{ph}}{\bar{x}} \times 100$$

Where, K is the standardized selection differential, s_{ph} = is the square root of phenotypic variance and $\bar{x}$ is the grand mean for each trait.

Phenotypic correlation coefficients between all pairs of the studied traits were determined by the following equation (Kown and Torrie 1964):

$$rp = \frac{M_{ij}}{(M_{ii} \times M_{jj})^{1/2}}$$

Where rp is phenotypic correlation coefficient, M_{ij} is the mean products, M_{ii} and M_{jj} are mean squares for traits i and j, respectively.

Results and Discussion

Results of the analysis of variance over two years for the studied characters are shown in Table 2. Significant differences were obtained among half-sib progenies within the populations for all of the characters under study. There were no significant differences among the populations for the measured characters. As was expected, significant differences were observed between years for all growth characters. However, the only significant genotype by year interaction was that of population*year for collar diameter indicating that differences among populations were not similar in two years for this trait. Differences between the trees based on the studied characters recorded on their progenies would imply the possibility of selection for investigated traits in breeding programs (Sebbenn *et al*. 2003; Lesser and Parker 2004). Furthermore, a large within-population variation provides the buffering capacity to cope with the change in environmental conditions. Based on the results, genetic variation for plant height was more than other traits. Squared phenotypic coefficient of variation was also high for plant height (51.1%) and number of leaves (20.21%). These results reflect superiority of selection for plant height in the early growth stages (Table 3).

Table 2. Analysis of variance of the three populations of *Pistacia atlantica* over two years for the characters under study

Source of variation	df	Mean squares			
		Collar diameter	Plant height	Number of leaves	Sprout length
Replication	2	23.24	567.73	193.27	26.29^{**}
Population	2	8.20	480.68	147.77	8.44
Tree (Population)	56	4.87^{**}	251.31^{**}	124.59^{**}	6.62^{**}
Rep*Population	4	2.64	37.01	86.33^{*}	1.46
Rep*Tree (Population)	72	2.57^{**}	80.11^{**}	72.41^{**}	4.39^{**}
Year	1	125.74^{**}	13776.00^{**}	11135.00^{**}	454.50^{**}
Year*Population	2	3.81^{**}	12.04	1.67	6.93
Year*Tree (Population)	56	0.96	28.22	5.04	1.59
Rep*Year	2	3.23^{**}	110.90^{**}	48.06^{**}	5.13
Rep*Year*Population	4	0.20	8.44	1.39	1.56
Rep*Year*Tree (Population)	72	0.51	18.24	12.00	1.74
Error	1259	1.23	33.53	23.82	1.50

** Significant at 0.01 probability level, respectively

Table 3. Grand mean and squared phenotypic coefficient of variation (PCV^2) for seedling characteristics of *Pistacia atlantica* half-sib families

Trait	Mean	PCV^2 (%)
Collar diameter (mm)	5.70	4.03
Plant height (cm)	21.52	51.1
Number of leaves (Number)	28.70	20.21
Sprout length (cm)	6.18	5.01

Estimates of additive genetic variance, narrow sense heritability and expected genetic gain from selection are presented in Table 4. Collar diameter, number of leaves and sprout length had moderate heritability. Their expected genetic gains were not also high. On the other hand, narrow sense heritability of plant height was relatively high (0.71). It means that additive gene effects are important in determining this character (Ajmal *et al.* 2009). However, there are some possibilities of pollination within small groups of related trees giving room for a certain degree of full-sib mating, ending to over-estimation of the narrow sense heritability. In this case, as Baliuckas *et al*. (2005) argued, the estimates must be regarded as upper limits heritability. The expected genetic gain of plant height (with 10% selection intensity) was also substantially high which indicated again the possibility of improving wild pistachio genotypes for early plant growth. Very little data have been published regarding genetic structure of wild pistachio tree (Pedersen *et al.* 2007).

Table 4. Estimates of additive genetic variance, narrow sense heritability and genetic gain among half-sib families of *Pistacia atlantica*

	Collar diameter	Plant height	Number of leaves	Sprout length
Additive genetic variance	7.76	0.12	2.70	3.10
Narrow sense heritability	0.53	0.71	0.46	0.43
Genetic gain (%)	8.65	26.6	6.66	6.78

Based on the half-sib progenies evaluated, genotypes number 103, 109 and 125 from Salmas population, number 204, 206, 209, 215 and 221 from Shahindezh population and number 301, 303, 306, 309, 312, 313, 315 and 322 from Baneh population were regarded as good general combiners for seed collection. Among these, entries 204, 309 and 312 were the most promising genotypes to be used in the genetic improvement programs of wild pistachio (data not shown).

Phenotypic correlation coefficients of seedling traits from half-sib progenies with the characteristics of the mother trees are presented in Table 5. Collar diameter showed positive significant correlation with all of the characters of mother tree, indicating that trees with larger stem diameter, taller height, larger and heavier seeds had progenies with larger collar diameter. Also, taller seedlings belonged to taller mother trees with larger stem diameter and maximum seed diameter. Sprout length of the half-sib progenies were also significantly correlated with the tree height, stem diameter and minimum seed diameter of the mother trees. On the other hand, leaf number showed no significant phenotypic correlation coefficient with the characteristics of mother tree. The largest positive phenotypic correlation was found between collar diameter of the half-sib progenies and seed weight of the mother trees (r = 0.42). Knowledge of relationship between vegetative characters of wild pistachio tree would be essential to select effectively superior mother plants for developing seed orchard of the species by which fast growing seedlings could be produced. Phenotypic correlation coefficients are mainly used for indirect selection in plant breeding programs (Chaturvedi and Pandey 2004; Blada and Popescu 2007). Therefore, significant positive phenotypic correlation of seedling plant height with mother tree height, as well as high heritability and large genetic gain, suggest that seedling height may be regarded as the potential predictor of the tree height in wild pistachio. However, the correlation coefficients were not high and, therefore, more data are needed to make decisive conclusions about these relationships.

Table 5. Phenotypic correlation coefficient between seedling traits and characteristics of mother trees in *Pistacia atlantica*

Traits of progeny	Traits of mother tree				
	Tree height	Stem diameter	Maximum seed diameter	Minimum seed diameter	Seed weight
Collar diameter	0.328*	0.262*	0.35**	0.292*	0.424**
Plant height	0.377**	0.319*	0.314*	0.187	0.267
Number of leaves	0.16	0.095	0.208	0.185	0.132
Sprout length	0.317*	0.267*	0.129	0.257*	0.138

*'** Significant at 0.05 and 0.01 probability levels, respectively.

Conclusion

Progeny test on three populations of *Pistacia atlantica* revealed useful information for selection at the early growth stage. Large squared phenotypic coefficient of variation, heritability estimates and expected genetic gain for seedling height and its significant phenotypic correlation coefficient with the height of mother tree indicated the possibility of utilizing existing genetic variation for this character in *P. atlantica* species.

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