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RESEARCH ARTICLE

# Genetic Diversity assessment of Male and Female Pistachio Genotypes Based on ISSR Markers

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**ABSTRACT:** Genetic study of pistachio, especially male genotypes due to the effects of pollen on nut quality and quantity and next generation characterizations, helps to improve its management and breeding program. In the present study, the genetic diversity among 20 male and 36 female pistachio genotypes was investigated using ISSR marker. In total, 178 DNA fragments were proliferated using 12 primers out of which 169 fragments were polymorphic. The average polymorphism information content (PIC) varied from 16% to 35%. Pistachio genotypes were classified into five main categories by cluster analysis. The highest similarity was among the 'Poostkhormayee'and 'Momtaz' cultivars (78%) and the lowest genetic similarity was among 'Ravar3' and 'Ghazvini' with K40 genotype (25%). K38 male genotype had the lowest genetic similarity with female cultivars. Thus, it can be introduced as appropriate pollinizer for other studied cultivars. The results of analysis of molecular variance showed that variability among male and female populations (8%) was lower than the variation within the populations (92%). Based on achieved results, ISSR marker recognized as a powerful tool to study the genetic variation among male and female pistachio genotypes.

**Keywords:** Pistachio, Molecular marker, Cluster analysis, Molecular variance, Genetic diversity

## INTRODUCTION

Assessing the genetic diversity in the population is the prerequisite to start and develop plant breeding projects. *Pistacia vera* is considered as a commercial species of *Pistacia* genus [20]. In Iran, Pistachio export is in the second place in terms of non-oil exports and in the first place among horticultural crops [6].

Iran is considered as one of the main centers for genetic diversity of pistachio including a high diversity of female varieties and male genotypes [15]. Understanding plant genetic diversity is necessary for optimal use of the products, leading to special attention to biodiversity by breeders and researchers [17]. Studying the genetic diversity of pistachio helps to improve the management and cultivation. Identification and accurate assessment of Iranian pistachios is done to design appropriate improvement programs and ensures the provision of improved cultivars is essential [18].

Todays, researchers increasingly use molecular methods to study genetic variation [2, 8-10, 14, 21]. Molecular markers for studying DNA can help to recognize and

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identify a specific breed or variety, evaluating relationships in a particular line or between line and different collections and identifying and locating genes controlling desirable attributes in a genotype and also can improve the management and use of genetic resources. Molecular markers are valuable to identify genetic similarities or differences, and identify duplicate samples [18]. Several studies have been conducted on pistachios using molecular methods [2, 8]. Pazouki et al. [22] analysed the genetic diversity and relationships among *Pistacia* species and cultivars using SSR markers. Their study showed that Iranian cultivars have a different genetic background from foreign cultivars. The ISSR markers are dominant, reproducible, and fast, which can be used for genetic studies of individuals who have high genetic relationship [23]. In the first study using ISSR indicator to check the genetic diversity of Iranian pistachio cultivars, they found that the markers have high efficiency for determining variation between those cultivars [19]. In another study in order to determine genetic variation and detection of polymorphic DNA in pistachio, ISSR and AFLP markers were produced replicable bands and ISSR was preferred to RAPD, especially when financial capital and technical expertise are limited [13]. The ISSR needs lower volumes of DNA and it is much less expensive and more available than RFLP and AFLP markers which are more expensive and time-consuming [29]. Since, there is not much information about genetic identities of male cultivars and genotypes in Iran, therefore the identification of genetic diversity can solve a lot of problems regarding to pistachio pollination [16]. Afshari et al. [1] studied the effect of pollen type on three pistachio cultivars ('Kalehghoochi', 'Ohadi' and 'Ahmadaghaee'), it was found that pollen type can affect nitrogen, phosphor, potassium, iron, and boron contents of the kernel of pistachio, also According to their study total fruit weight and blankness were affected by pollen type. It has been shown that by using protein marker, male genotypes of pistachio has more polymorphisms compared to the female cultivar [4]. In present study, we have investigated the genetic variation among female cultivars and male genotypes and determined the genetic similarity and distance between male and female cultivars. Since, several studies indicated that the ISSR marker is a fast method and has high potential to produce polymorphism segments, therefore, we used this technique for DNA fingerprinting and determining the genetic relationship between female cultivars and male genotypes.

## MATERIALS AND METHODS

### Plant samples

20 male and 36 female Pistachio trees were selected from the pistachio collection of Rafsanjani Pistachio Research Institute. Leaves were collected and transferred to laboratory for the future experiments. The list of female cultivars and male genotypes is presented in Table 1.

### DNA extraction

Genomic DNA was extracted from leaf samples using CTAB method with slight modifications [5]. The extracted buffer contained Tris-HCL100 mM, NaCl 1.4 M, EDTA 20 mM, CTAB 2%, PVP 2%, $Na_2S_2O_5$ 0.1% and $\beta$-mercaptoethanol 0.2%. The quantity and quality of the extracted DNA was determined using spectroscopic methods and 1% agarose gel electrophoresis.

### Polymerase chain reaction (PCR)

20 ISSR primers were used for PCR optimization out of which only 12 primers showed good reproducing bands and were used to continue the experiment (Table 1). Amplification of DNA was performed using PCR reaction using Thermal Cycler Bio-Rad, C1000 tm in the final volume of 20 µl including 10 µl PCR 2X Master mix (Cinnagen), DNA template (50 ng) and 1 µl primer (10 pmol). PCR steps were as follow: 1) initial denaturing; 5 minutes at 94°C, 2) amplification; 35 cycles as denaturation for 30 s at 94°C, primer annealing at the optimized temperature for each primer for one minute and extension for two minutes at 72°C, and 3) final extension at 72°C for 5 minutes. The amplified PCR products were electrophoresed using 1.7% agarose gel. The amplified segments were stained using Red Safe (Cinnagen) and gel images were taken by Gel Documentation system.

### Data analysis

The amplified bands were scored as one and zero, according to the presence and absence of the band, using Totallab TL120 software. The similarity matrix and tree diagram were obtained using NTSYS 2.02e software based on Jaccard's coefficient using UPGMA (Unweighted Pair Group Method with Arithmetic) method. The marker index [23], polymorphism information content [28] and resolution power [24] were calculated for each primer and finally were compared together. Principal Coordinates Analysis (PCOA) to investigate genetic variation between males and female

populations was done using GenAlEx 6.503 software and the results of this analysis were compared with the results of cluster analysis and then, analysis of molecular variance (AMOVA) was performed to determine the diversity between and within male and female pistachio populations.

**Table 1.** Names of cultivars and genotypes used in this study

| Code | Genotype/ cultivar | Sex | Code | Genotype/ cultivar | Sex |
|---|---|---|---|---|---|
| 1 | K34 | M | 29 | Shasti | F |
| 2 | K37 | M | 30 | Beheshtabadi | F |
| 3 | N1 | M | 31 | Momtaztagabadi | F |
| 4 | K38 | M | 32 | Sirizi | F |
| 5 | K41 | M | 33 | Phandoghireez | F |
| 6 | R25 | M | 34 | Jandaghi | F |
| 7 | R27 | M | 35 | Sabzpestehnoogh | F |
| 8 | K33 | M | 36 | Harati | F |
| 9 | K39 | M | 37 | Kalehghoochi | F |
| 10 | K40 | M | 38 | Khanjaridamaghani | F |
| 11 | R26 | M | 39 | Ghafoori | F |
| 12 | R24 | M | 40 | Lacksiri | F |
| 13 | R23 | M | 41 | Ghazvini | F |
| 14 | N4 | M | 42 | Seifadini | F |
| 15 | N12 | M | 43 | Mohseni | F |
| 16 | N10 | M | 44 | Moosaabadi | F |
| 17 | N5 | M | 45 | Ahmadaghaee | F |
| 18 | R19 | M | 46 | Khangariravar | F |
| 19 | R20 | M | 47 | Amiri | F |
| 20 | N3 | M | 48 | Bayaz | F |
| 21 | Ravar1 | F | 49 | Ebrahimi | F |
| 22 | Poostkhormayee | F | 50 | Sephidpestehnoogh | F |
| 23 | Momtaz | F | 51 | Akbari | F |
| 24 | Javadaghayee | F | 52 | Shahpasand | F |
| 25 | P-ghafoori | F | 53 | Italiaee | F |
| 26 | Ravar3 | F | 54 | Phandoghi48 | F |
| 27 | Badaminishkalaghi | F | 55 | Badamiravar | F |
| 28 | Vahedi | F | 56 | Badamizerand | F |

M: male, F: female

## RESULTS AND DISCUSSION

In order to investigate the primers efficiency, 20 ISSR primers were used in PCR and 12 primers were selected based on the amplification and reproducibility of primers to proceed the experiment. In total, 178 amplified loci were obtained with 12 primers for 56 genotypes. The most amplified loci were related to P1 and P12 primers with 18 amplified sites and the less amplified loci were related to P9 primer with 10 amplified loci (Table 2). The average amplified loci for each primer was 15 loci. Among the amplified places, 169 loci showed polymorphism. The lowest and highest number was related to the primers that produced the lowest (P9) and the highest (P1 and P12) loci, respectively. The average number of polymorphic loci for each primer was 14 loci. Taghizad et al. [26] studied 19 genotypes of pistachio by using 10 ISSR primers, overall, 114 polymorphic loci were achieved out of which 73 loci showed polymorphism (64%) and a primer with repeated AC unit could produce the highest percentage of polymorphism. In the present study, also, the P1 primer with repeated AC unit produced 94% of polymorphism; therefore, it can be concluded that primers with AC repeated units can be used as a suitable primer for such experiments. In other study on pistachio, Kafkas et al. [13] observed 73 polymorphic locations (46.2%) of the 156 produced locations when they used 20 ISSR primers. Norozi et al. [19] in an experiment on 31 pistachios genotypes, 13 polymorphic loci (46.42%) observed from 28 produced loci. In our study, polymorphic percentage was higher than the reports of Kafkas et al. [13] and Norozi et al. [19] which might be due to the larger number of subjects or different sequences of primers used in the experiments. Fares et al. [7] in their study on 15 genotypes of Tunisia pistachio using 13 ISSR markers found 26 polymorphic sites. The most polymorphic location was produced by the primer with AG repeated unit, similar to our study, $(AG)_8G$ primer was 100% polymorphism.

The average polymorphism information content (PIC) was varied from the least of 16% for P9 primer to the highest of 35% for P5 primer. P12 and P1 Primers had marker index (MI) and the coefficient of resolution (Rb) higher than the other primers (Table 2). In Taghizad et al. [26] study the polymorphism information content (85% to 91%) and marker index were higher than our results. The higher MI and PIC in previous studies might be due to difference in primer sequences. According to the results

**Table2.** List and sequencing of ISSR primers, Number of bands, the number of polymorphic bands, Percent polymorphism, Polymorphism information content (PIC), Marker Index (MI) and Resolution Power (RP) used for ISSR primers in this study.

| Code | Sequences | Tm ˚(C) | No. of bands | No. of polymorphic bands | Percent of polymorphism (%) | (PIC) | (MI) | (RP) |
|---|---|---|---|---|---|---|---|---|
| P1 | $(AC)_8$YG | 53.9 | 18 | 17 | 94 | 0.3 | 4.82 | 8.39 |
| P2 | $(CA)_7$AG | 48.2 | 14 | 14 | 100 | 0.19 | 2.66 | 4.03 |
| P3 | $(GA)_8$YC | 53.9 | 16 | 14 | 87 | 0.23 | 2.82 | 5.57 |
| P4 | $(GA)_7$GYC | 52.4 | 16 | 15 | 93 | 0.31 | 4.36 | 7.35 |
| P5 | $(AG)_8$YT | 51.6 | 15 | 14 | 93 | 0.35 | 4.57 | 7.82 |
| P6 | $(GT)_8$YC | 53.9 | 16 | 15 | 93 | 0.28 | 3.94 | 6.64 |
| P7 | $(CA)_8$RC | 53.9 | 12 | 11 | 91 | 0.26 | 2.62 | 4.67 |
| P8 | $(AG)_8$G | 52.4 | 14 | 14 | 100 | 0.33 | 4.62 | 7.17 |
| P9 | $(AG)_8$T | 50 | 10 | 9 | 90 | 0.16 | 1.3 | 2.17 |
| P10 | $(GA)_8$A | 50 | 16 | 15 | 93 | 0.31 | 4.36 | 7.46 |
| P11 | $(CA)_8$T | 50 | 13 | 13 | 100 | 0.3 | 3.9 | 6.14 |
| P12 | $(GA)_8$TT | 51.6 | 18 | 18 | 100 | 0.31 | 5.58 | 8.25 |
| Total | - | - | 178 | 169 | - | - | - | - |

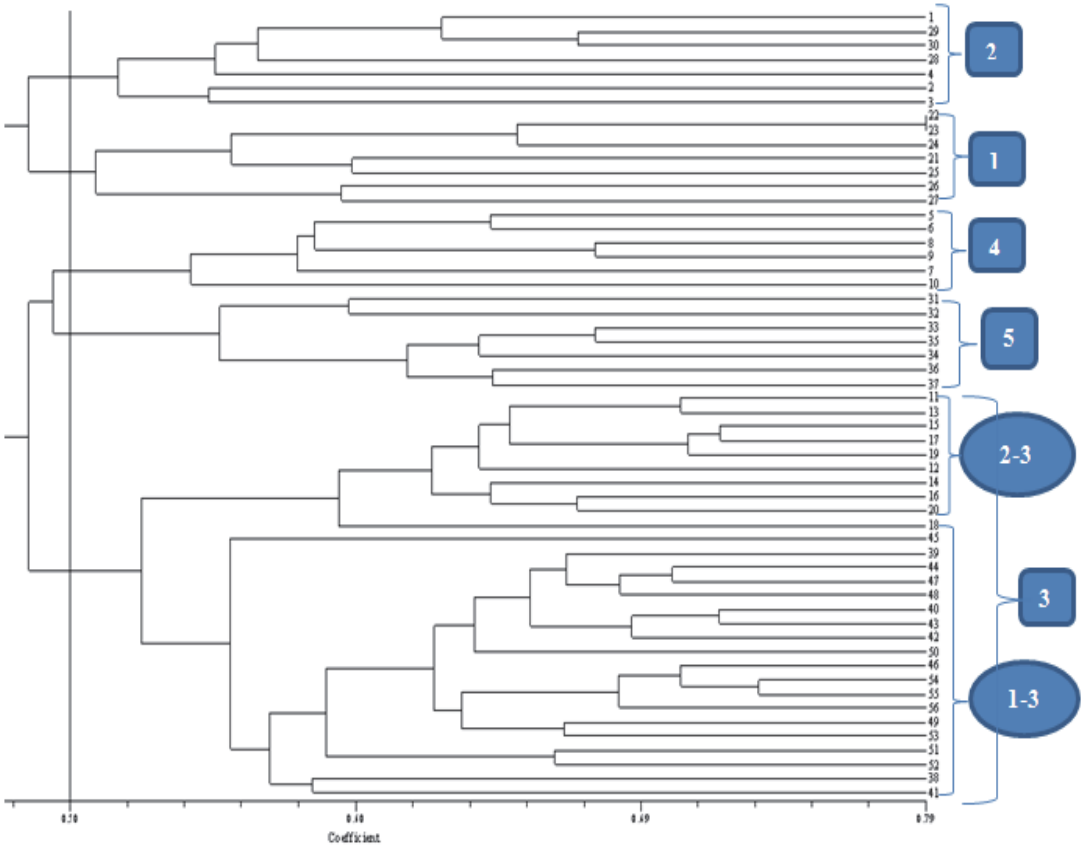


**Figure 1.** Dendrogram of 56 genotypes and cultivars of male and female pistachio based on 12 ISSR primers using UPGMA method. (Branch numbers are codes in table 1).

of similarity matrix, the most genetic similarity was between 'Poostkhormayee' and 'Momtaz' cultivars with 0.78% similarity and the lowest genetic similarity was between 'Ravar3' with 'Ghazvini' cultivar and K40 genotype with 0.25% similarity. In a morphological study reported that 'Poostkhormayee' and 'Momtaz' cultivars were similar related to leaf and flower traits [25] that confirmed our molecular results related to high genetic

similarity of this two cultivars. Results showed that 'Ravar3' and 'Ghazvini' had a high genetic distance confirming previous studies. It is reported that 'Ravar3' and 'Ghazvini' cultivars were different related traits to morphological such as branching, vigor, growth habit, flowering, bud shape, leaf color, and nut dry weight [25]. The male genotype K38 had the lowest genetic similarity with the female cultivars; it can be introduced as suitable pollinizer for mentioned female cultivars with simultaneous flowering.

The cluster graph was obtained by UPGMA method. As shown in Figure 1, the cluster graphs divided the genotypes into five main groups in 50% similarities: The first group consisted only female cultivars, unlike Norozi et al. [19] as well as Taghizad et al. [26] classifications 'Javadaghayee' and 'Seifadini' cultivars were not in a same group and also against Vezvai et al. [27] results 'Momtaz' and 'Kalehghoochi' cultivars were not in a same group.

The second group was a mixture of male and female cultivars divided into two subgroups; the first subgroup included only the male genotypes K37 and N1, and the second sub group was a combination of male genotypes (K34 and K38) and female cultivars ('Shasti', 'Beheshtabadi' and 'Vahedi').

The third group included the male genotypes and female cultivars and divided into two subgroups. The first subgroup included only the female cultivars and the second subgroup included only male genotypes. According to the Taghizad et al. [26] results, 'Ghazvini' with 'Sephidpestehnoogh' were belonged to one group, 'Akbari', 'Seifadini' and 'Ahmadaghaee' were in one group, and 'Mohseni', 'Amiri' and 'Khanjaridamaghani' cultivars were also together in another group. These results were similar to our results, but these cultivars were in different groups with other cultivars available in the third group of our cluster graph. The results of Hajirezayi et al. [12] showed that 'Badamizerand' and 'Badamiravar' were in a group that was consistent with our results and since both cultivars are considered as Almond cultivars belonging to one group is reasonable. Also, based on our division, 'Lacksiri', 'Sephidpestehnoogh', 'Mohseni' and 'Amiri' cultivars belonged to one group, but in their study, 'Shasti', 'Sirizi' and 'Khangariravar' cultivars were grouped together, that according to our results, each cultivar belonged to different groups. The results of Hagizadeh et al. [11] showed that 'Ebrahimi' and 'Ghazvini' were in a group that was consistent with our results, but unlike our results, 'Jandaghi', 'Ebrahimi' and 'Ghazvini', belong to one group. Genotypes N12, N5, N4, N10 and N3 were in a group with origin of Naserieh (in Kerman), so belonging to one group seems reasonable, but genotype N1 is separated from the rest and can be seen in the first group, might be due to different paternal parent in mentioned Genotype.

The fourth group was composed only of male genotypes including male K41, R25, K33, K39, R27 and K40 genotypes, which indicate similar maternal parent in these genotypes with different origin.

Only female cultivars were observed in the fifth group including 'Momtaztagabadi', 'Sirizi', 'Phandoghireez', 'Sabzpestehnoogh', 'Jandaghi', 'Harati' and 'Kalehghoochi' cultivars. Unlike our results, 'Kalehghoochi' and 'Seifadini' cultivars were in one group based on the results by Taghizad et al. [26]. However, those two cultivars are different in morphological characters including tree's growth habit, apical dominance, flowering, full bloom stage, flowering length, shape of flower buds, number of leaflets, green skin weight, nut dry weight, green hull texture and nut shape [25]. Therefore, categorizing these two cultivars in two separate groups is reasonable. However, different conclusions about the same genotypes in different studies can be due to different markers and primers.

In this study, some genotypes with same maternal parent were placed in different groups, which could be due to the influence of paternal parent and high heterozygosity in pistachios populations. In order to study the genetic variation between male and female populations, principal component analysis (PCOA) was used. Since the first two components justify a higher percentage of changes compared to other components, therefore interpreting the results based on the first two components, able to explain the variation in the studied population was performed (Figure 2). According to results, male and female pistachio genotypes were placed in biplot environment in four areas. At the positive area of the first component and positive area of the second component was a combination of female cultivars and male genotypes. At the positive area of the first component and negative area of the second component a combination of female cultivars and male genotypes was observed. At the negative area of the first component and the negative area of the second component there were only female cultivars and at the negative area of the first component and at the positive area of the second component only male genotypes were observed. Since the distribution of genotypes in the biplot

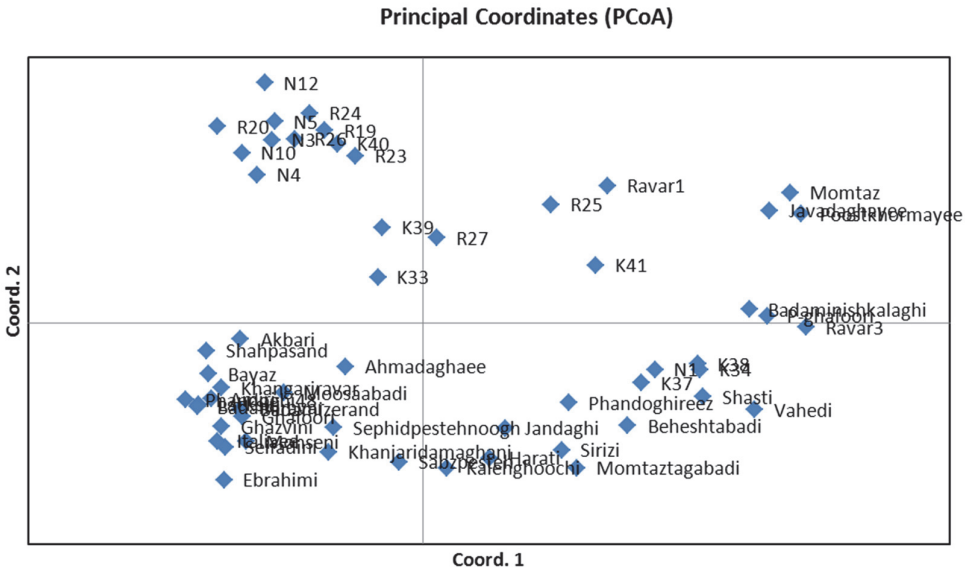


**Figure 2.** Biplot two-dimensional graph based on the first and two components (PC1 and PC2) To study the genetic diversity of studied male and female pistachio populations.

is due to the genetic variation among genotypes, so genotypes accumulate in one area of the plot represents the genetic similarity of the genotypes.

The results of analysis of principal component using covariance matrix showed that out of 26% of the variance was between the two main components, 17.28% was related to the first component and 8.25% was related to the second component. The results of Hajirezayi et al. [12] using RAPD marker on the pistachio showed that 'Kalehghoochi', 'Momtaz' and 'Akbari' cultivars were placed together in one area, but in our results, each cultivar was in a separate area. The difference might be related to the use of different used markers. In the Arjmandghahestani et al. [3], study using the ISSR marker, 'Badamiravar' and 'Amiri' cultivars were in in a region that was consistent with our results, but in our study, unlike their results, 'Sirizi' cultivar was in another area that might be due to different used primers in the two studies.

Analysis of variance was performed to understand the relationships within and between the populations. The results of analysis of molecular variance using GenAlExe 6.503 software showed that out of the total observed variances, 8% was related to the variation between population and 92% was related to the variation within the populations (Figure 3). Therefore, in total, proportion of variation between male and female populations was lower than diversity within the populations, suggesting the male and female pistachio samples used in this study have genetic similarity with possibility of same maternal parent. Low variation among populations could be due to low genetic diversity in the primary parents' population, also primary parents probably were selected within an area or two areas close together. The inbreeding of these genotypes caused serious loss of diversity among populations. Summary results of molecular variance of the data obtained by ISSR marker is provided in the Table 3. The percentage of polymorphic in the male population was 76.97%, and in the female population was 88.5% that suggesting the higher numbers of polymorphism of female cultivars can be due to more female cultivars compared to the male genotypes (Table 4).

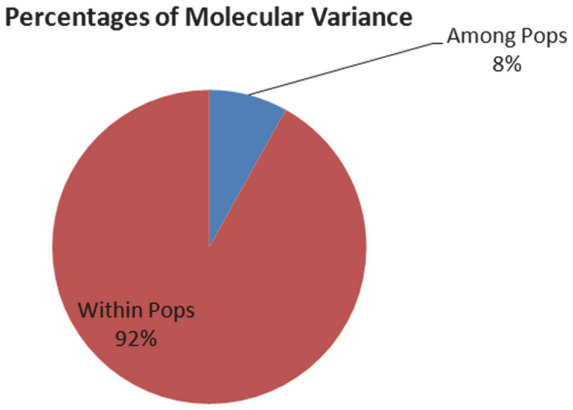


**Figure 3.** Percentage of genetic diversity between and within male and female pistachio populations in this study.

**Table 3.** Analysis of molecular variance using ISSR marker between and within male and female Pistachio populations.

| Sources of variance | DF | SS | MS | Est. Var. | % Var. |
|---|---|---|---|---|---|
| Between populations | 1 | 81.45 | 81.450 | 2.198 | 8% |
| Within populations | 54 | 1345.80 | 24.922 | 24.922 | 92% |
| Total | 55 | 1427.25 | - | 27.121 | 100% |

DF: Degrees of freedom, SS: sum of squares, MS: Mean square, Est.Var: Calculated variance for within and between populations, % Var: Percent of the variance of each source to the total variance.

**Table 4.** Polymorphic percentage of male and female populations

| Populations | Polymorphic percentage |
|---|---|
| Male | 76.97% |
| Female | 88.20% |
| Mean | 82.58% |
| SE | 5.62% |

SE: standard error

## CONCLUSION

In this study, we compared the percentage of genetic similarity between male and female cultivars and we found that genotype K38 has the lowest genetic similarity within female cultivars and it can be used as an appropriate pollinizer for these cultivars. The high genetic variation in pistachio populations indicates it's high heterozygous. Also, results showed that the proportion of variation between male and female populations was lower than within the population diversity. This suggests that male and female pistachio samples used in this study have high genetic similarity which can be due to the same female parent. The results showed that ISSR markers can be used as an effective tool to detect and characterize the genetic diversity of pistachio and could be used to explain the male genotypes suitable for mating with the female cultivars and new cultivars with better specification of the characteristics of their parents. In additional, we found that ISSR marker have high performance to estimate polymorphism. The high marker index indicates high polymorphism and the higher number of amplified segments per primers in the population. P12 and P1 primers can be useful for testing pistachio fingerprinting, since they have higher MI and Rb than the other primers.

## REFERENCES

[1] Afshari, H., Talaei, A.R. and Sadeghi, GH. R. 2008. A study of some of the commponents in the pistachia nut and the effect of pollen grains on quantitive and qualitative traits of them. J Hortic Sci, 2: 13-24.

[2] Ahmad, R., Ferguson, L. and Southwick, S.M. 2005. Molecular marker analyses of pistachio rootstocks by Simple Sequence Repeats and Sequence-Related Amplified Polymorphism. J Hortic Sci Biotech, 80: 382-386.

[3] Argmand Ghahestani, R., Tavassolian, I. and Mohammadi Nejad, Gh. Evaluation of genetic diversity in 25 Iranian pistachio genotypes using ISSR markers. 2016. Jo Agri Biotech, 7: 1-17.

[4] Barone, E., Di Marco, L., Marra, F.P. and Sidari, M. 1996. Isozymes and canonical discriminant analysis to identify pistachio (*Pistacia vera* L.) germplasm. Hort Science, 31: 134-138.

[5] Doyle, J.J. and Doyle, J.L. 1987. A rapid isolation procedure for small quantities of fresh leaf tissue. Phytochem Bull 19: 11–15.

[6] Esmaeilipur, A. 2005. Characteristics and traits of some Iranian cultivars of pistachio. Pistachio Research Institute Publishers, Rafsanjan, Iran.

[7] Fares, K., Guasmi, F., Touil, L., Triki, T. and Ferchini, A. 2009. Genetic diversity of Pistachio tree using intersimple sequence repeat markers (ISSR) supported by morphological and chemical markers. Biotechnology, 8: 24-34.

[8] Golan-Goldhirsh, A., Barazani, O., Wang, Z.S., Khadka, D.K., Saunders, J.A., Kustiukovsky, V. and Rowland, L.J. 2004. Genetic relationships among Mediterranean *Pistacia* species evaluated by RAPD and AFLP markers. Plant Syst Evol, 246: 9-18.

[9] Goodarzi, F., Darvishzadeh, R. and Hassani, A. 2015. Genetic analysis of castor (*Ricinus communis* L.) using ISSR markers. J Plant Mol Breed, 3(1):18-34.

[10] Haghpanah, M., Kazemitabar, S-K., Hashemi, S-H. and Alavi, S-M. 2016. Comparision of ISSR and AFLP markers in assessing genetic diversity among Nettle (*Uritica dioica* L.) populations. J Plant Mol Breed, 4(1): 10-16.

[11] Hagizadehhosseinabadi, M., Karimi, H.R., Dashti, H., Shamshiri, M.H. and Tagabadipour, A. 2013. Assessment of genetic diversity among some male and female pistachio (*pistacia vera* L. genotypes using RAPD marker. Journal of Applied Crop Breeding, 1: 23-32.

[12] ajirezayi, M., Baghizadeh, A., Javadi, GH. and Sadeghizadeh M. 2009. Genetic diversity assessment of a few numbers of pistachio cultivars in Kerman province

based on RAPD markers. Iranian Journal of Biology. 462-469.

[13] Kafkas, S., Ozkan, H., Acar, I., Atli, H.S., Koyuncu, S.A.K., Acar, B.E., Atli, I. and Koyuncu, H.S. 2006. Detecting DNA polymorphism and genetic diversity in a wide pistachio germplasm: comparison of AFLP, ISSR, and RAPD markers. J Am Soc Hortic Sci, 131(4): 62-74.

[14] Malekzadeh, Kh., Mohsenifard, E. Jalalzadeh Moghaddam Shahri, B. and Farsi, M. 2014. Identification and strain-typing of button mushroom using ISSR, ITS and IGS markers. MGJ, 3(38): 343-352.

[15] Mardi, M. 2007. Analysis of genetic diversity in Iranian wild and cultivated pistachio genotypes using morphological traits molecular markers (AFLPs and SSRs). agris.fao.org.

[16] Martinez, E. and Herreco, M. 1994. Male performance in pistachio (*Pistacia vera* L.). J Hortic Sci, 69: 1117-1122.

[17] Mirzaei, S., Bahar, M. and Sharif Nabi, B. 2005. Genetic diversity among Iranian pistachio cultivars using RAPD molecular marker. Third Biotechnology Congress of Islamic Republic of Iran, Mashhad.

[18] Moghbeli, M., Rashidi, M.H., Afshari, H and shushtari, M. B. 2012. Genetic diversity assessment Among the main cultivares of pistachio in Damghan based on RAPD markers. National Conference on Environment and Plant Production. University of Semnan, Semnan, Iran.

[19] Norozi, S.H., Baghizadeh, M. and Jalali Javaran, M. 2009. The genetic diversity of Iranian pistachio (*Pistacia vera*L.) cultivars revealed by ISSR markers. Biol Di Con, 2: 50-56.

[20] Padulosi, S., Van Mele, P., Caruso, T., Kaska, N. and Barone, E. 1998. IPRIG's initiatives for the promotion of better. conservation and use of Pistacia spp. genetic resources. Acta Hortic, 470: 138–142.

[21] Panahi, B., Afzal, R., Ghorbanzadeh Neghab, M., Mahmoodnia, M. and Paymard, B. 2013. Relationship among AFLP, RAPD marker diversity and Agromorphological traits in safflower (*Carthamus tinctorius* L.). PBioSci, 3(1): 90-99.

[22] Pazouki, L., Mardi, M., Salehi Shanjani, P., Hagidimitriou, M., Pirseyedi, S.M., Naghavi, M.R., Avanzato, D., Vendramin, E., Kafkas, S., Ghareyazie, B., Ghaffari, M.R. and Khayam Nekoui, S.m. 2010. Genetic diversity and relationships among *Pistacia* species and cultivars. Conserv Genet, 11:311–318.

[23] Powell, W., Morgante, M., Andre C., Hanafey, M., Vogel J., Tingey S. and Rafalski, A. 1996. The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol Breeding, 2: 225-238.

[24] Prevost, A. And Wilkinson, M. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet, 98: 107.

[25] Tagabadi, A. 1998. Identification some of Pistachio cultivarse. M.Sc Thesis, Tehran University, Tehran, Iran.

[26] Taghizad, A., Ahmadi, J., Haddad, R. and Zarrabi, M. 2011. studying genetic diversity in Iranian pistachio cultivars based on someof RAPD markers. J Hortic Sci, 4: 453-460.

[27] Vezvai, A., Vahdati, K. and Tajabadi pour, A. 2003. Evaluation guide for almond, walnuts and pistachios trees. Khaniran Press, Tehran, 145.

[28] Weising, K., Nybon, H., Wolff, K. And Kahl, G. 2005. Applications of DNA fingerprinting in plant sciences. In: Taylor and Francis Group (Ed.), DNA fingerprinting in plants. Principles, methods and applications. CRC press boca ration, London, New York, Singapore, Chapter 6, 235-276.

[29] Williams, J.G.K., Kubelik, A.R., Levak, K.J., Rafalski, J.A. and Tingey, S.V. 1990. DNA polymorphism amplification by arbitrary primers are useful as genetic markers. Nucleic Acids Res, 18: 6531-6535.

# بررسی تنوع ژنتیکی ژنوتیپ‌های نر و ماده پسته با استفاده از نشانگر ISSR

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## چکیده

مطالعه ژنتیکی پسته به‌خصوص ژنوتیپ‌های نر، با توجه به اثراتی که دانه گرده بر کیفیت و کمیت میوه و خصوصیات نسل بعدی دارد، به بهبود مدیریت و برنامه‌های به نژادی آن کمک می‌کند. در این پژوهش تنوع ژنتیکی۲۰ ژنوتیپ نر و ۳۶ رقم ماده پسته با استفاده از نشانگر ISSR مورد بررسی قرار گرفت. با استفاده از ۱۲ آغازگر، در مجموع ۱۷۸ قطعه DNA تکثیر و امتیازبندی شد که در این میان ۱۶۹ قطعه چندشکلی نشان دادند. میانگین محتوای چندشکلی اطلاعات برای آغازگرهای مورد استفاده از ۱۶ تا ۳۵ درصد متغیر بود. بر اساس تجزیه خوشه‌ای، ژنوتیپ‌های پسته در پنج گروه اصلی قرار گرفتند. بیش‌ترین شباهت ژنتیکی بین ارقام پوست خرمایی و ممتاز با ۰/۷۸ درصد تشابه و کم‌ترین شباهت ژنتیکی، بین رقم راور ۳ با رقم قزوینی و ژنوتیپ K۴۰ با ۰/۲۵ درصد تشابه بود؛ ژنوتیپ نر K۳۸ کم‌ترین تشابه ژنتیکی را با ارقام ماده داشت، بنابراین به عنوان گرده‌دهنده مناسب برای ارقام مورد مطالعه می‌تواند معرفی شود. نتایج آنالیز واریانس مولکولی نشان‌داد که تنوع بین جمعیت‌های نر و ماده (۸ درصد) از تنوع داخل جمعیت‌ها (۹۲ درصد) کمتر بود. براساس نتایج مطالعه حاضر نشانگر ISSR به‌عنوان ابزاری قدرتمند برای بررسی تنوع ژنتیکی بین ژنوتیپ‌های نر و ماده شناخته شد.

**کلمات کلیدی:** پسته، نشانگر مولکولی، تجزیه خوشه‌ای، واریانس مولکولی