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ARTICLE

Phenotypic and genotypic variation in Iranian Pistachios

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Abstract As Iran is one of the richest pistachio germplasms a few studies have been conducted on different sexes of pistachio trees, in areas where this crop emerged. To this end, 40 male and female Iranian pistachio genotypes from Feizabad region, Khorasan, Iran; were evaluated using morphological characters and randomly amplified polymorphic DNA (RAPD) markers. For morphological assessments, 54 variables were considered to investigate similarities between and among the studied genotypes. Morphological data indicated relative superiority in some female genotypes (such as Sefid 1, Sefid Sabuni 2, Garmesiah, and Ghermezdorosht Z) regarding characters such as halfcrackedness, the percentages of protein and fat content. 115 polymorphic bands were recorded with 92.83% average polymorphism among all primers. The total resolving power (Rp) of the primers was 74.54. The range of genetic similarity varied from about 0.31 to about 0.70. Genotypes were segregated into eight groups at the similarity limit of 0.41. Results of present investigation could be helpful for strategic decisions for maintaining Iranian pistachio genotypes.

1. Introduction

The Pistachio, *Pistacia vera* L. which belongs to Anacardiaceae is a deciduous, dioecious and wind-pollinated tree species [22,10]. It is referred to as the "green gold tree" due to its high economic and high nutritional value [3]. Its origin is still undefined, but the majority of researchers believe that it most likely originated from the Middle East [6,17,7]. Number of pistachio cultivars and genotypes indicate that Iran is one of the richest resources of pistachio in the world [20,17]. Although Iran has the greatest cultivation area of pistachio in the world, it is clear, that for breeding of promising pistachio cultivars using this germplasms, an appropriate characterization and discrimination of the pistachio cultivars are indispensable. To date, different types of markers such as morphological, biochemical and molecular have been used for genetic variation analysis in pistachio. Different types of DNA markers have been studied for genetic diversity evaluation in pistachio, such as RFLPs [4], AFLPs [1,18,15], ISSR [11] and RAPD. Among them, RAPD developed by

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Williams et al. [9] has been commonly used for genetic variability assessment in pistachio [21,8,19]. All in all, among this vast range of studies genetic diversity of some Iranian Pistachios is not fully investigated by molecular markers. Hence, the present study sought to investigate a wide range of Iranian pistachio genotypes using morphological and RAPD markers and determine the associations between these markers.

2. Materials and methods

2.1. Plant materials and DNA extraction

The young leaves of 40 male (15 genotypes) and female (25 genotypes) pistachio genotypes were gathered from Khorasan, Iran (Table 1). DNA extraction was carried out using [13] with minor modifications. The DNA concentration and quality were estimated electrophoretically and spectrophotometrically, respectively.

2.2. Morphological evaluation

The morphological characters of the samples were determined on the basis of the pistachio descriptor. To this end, 55 morphological variables (44 quantitative and 11 qualitative) were recorded during 2 years as described in Tables 2 and 3.

2.3. Molecular evaluation

Initially, a total of 60 RAPD primers were applied for PCR amplification. Of these, 15 RAPD primers which produced discernible and reproducible bands were selected for amplification (Table 4). RAPD amplification was carried out according to Williams et al. [9] with minor modifications in thin-walled microcentrifuge tubes by thermocycler (iCycler, Bio Rad Co., USA).

Reaction was performed in a final volume of 25 µl, containing 2.5 µl of 10× PCR buffer (20 mM of Tris–HCl, pH = 8.4, 50 mM KCl), 1.75 mM of $MgCl_2$, 0.2 mM of each dNTPs, 0.2 µM of each primer, 10 ng of genomic DNA, and 1 unit of Taq DNA polymerase. The amplification conditions for the RAPD markers were an initial step of 3 min at 94 °C followed by 40 cycles of 1 min at 94 °C for denaturing 45 s at 37.5 °C for annealing, 2 min at 72 °C for extension and a final extension of 5 min at 72 °C. Amplified PCR products were electrophoresed through 1.2% (w v^{-1}) agarose gels using

Table 2 Descriptive statistics for quantitative morphological traits among 15 male pistachio genotypes during 2 years of the study.

No.	Character	Min	Max	Mean	CV	Unit
1	Tree canopy	4.63	28.63	16.09	51.67	m
2	Tree diameter	27.67	96.33	63.26	39.17	cm
3	Yearly growth of trees	8.17	32.33	14.09	42.33	cm
4	Number of buds	5	12.33	8.75	25.51	No.
5	Density of buds	1.09	3.34	1.76	39.13	No.
6	Length of multiple buds	0.73	1.13	0.915	10.29	cm
7	Width of multiple buds	0.5	0.7	0.615	9.13	cm
8	Thickness of multiple buds	0.3	0.63	0.462	18.62	cm
9	Number of leaflets	2.67	4.67	3.22	16.89	No.
10	Length of leaves	10	16.17	12.06	13.01	cm
11	Width of leaves	10.83	19.5	15.04	16.08	cm
12	Length of petioles	3.33	7.83	4.67	24.95	cm
13	Length of the terminal leaf	6.5	13.17	9.85	17	cm
14	Width of the terminal leaf	4.67	8.5	6.33	17.59	cm
15	Length of inflorescence	1.6	4.63	3.31	25.54	cm
16	Number of racemules per flower	11.67	20	15.46	13.69	No.
17	Pollen germination	44	87	61	0.24	%

m = meter, cm = centimeter, No. = number, % = percent.

Table 1 Forty male and female studied genotypes.

No.	Sex				
	Female			Male	
	Genotype	No.		No.	Genotype
1	Badami sefid1	16	Genotype16	1	Genotype1
2	Badami sefid2	17	Genotype17	2	Genotype2
3	Badami sefid3	18	Genotype18	3	Genotype3
4	Sefid sabuni1	19	Genotype19	4	Genotype4
5	Sefid sabuni2	20	Genotype20	5	Genotype5
6	Badamighermez	21	Genotype21	6	Genotype6
7	Ghermezzodrasdorosht	22	Genotype22	7	Genotype7
8	Germe riz1	23	Genotype23	8	Genotype8
9	Germe riz2	24	Genotype24	9	Genotype9
10	Germesiah	25	Genotype25	10	Genotype10
11	Germezodras			11	Genotype11
12	Akbari			12	Genotype12
13	Ohadi			13	Genotype13
14	Momtaz			14	Genotype14
15	Kale ghochi			15	Genotype15