

Identification of a New Phenotype (Siah Barg) of Pistachio (*Pistacia vera* L.) with Shiny-blackish Green Leaves Using RAPD Assay

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ABSTRACT

New male phenotype of pistachio called Siah Barg is different from normal type in color of leaves and flowers. Morphological characteristics were evaluated by using descriptors for pistachio. Results showed that the newly emerging leaves are light red that gradually change into blackish green. The color of flower anther at opening time is blackish green in contrast to the other phenotypes and cultivars that produce yellow and reddish yellow flowers. This phenotype has moderate growth vigor and semi-upright habit. Flowering time of this phenotype is simultaneous with moderate flowering cultivars. Siah Barg genetic identification using 11 arbitrary primers revealed 46 polymorphic fragments, which varied from 1 - 10. RAPD revealed that genetic characteristics of Siah Barg are very closely related to commercial Ohadi cultivar.

Key Words: New phenotype; *Pistacia vera*; Morphology; Genetic characteristic; RAPD

INTRODUCTION

The genus *Pistacia* is a member of Anacardiaceae and consists of at least eleven species (Zohary, 1952). *P. vera* has edible nuts and is commercially important. There are two wild *Pistacia* species in Iran; *P. atlantica* sub sp. *mutica* and *P. khinjuk*. These species are used mainly as rootstock for *P. vera* and rarely for oil extraction in some countries (Kafkas, 2002b). Iran is one of the centers of pistachio genetic diversity and has many unique wild and cultivated pistachio accessions. Greater than 100 pistachio accessions are identified so far and it is expected that there are many cultivars, which are not identified. Tajabadi (1997) studied relationships among pistachio cultivars based on 113 qualitative and quantitative characters. Kafkas (2002a) studied relationships among pistachio species via morphological traits.

The development of Randomly Amplified Polymorphic DNA (RAPD) technique (Williams *et al.*, 1990) provided vast information on cultivar identification and genetic relationship evaluation of fruit trees (Besnard *et al.*, 2001; Wunsch & Hormaza, 2002). Little information is available concerning the genetic relationship and characterization of *P. vera* genotypes (Kafkas *et al.*, 2002a, b & 2006; Katsiotis *et al.*, 2003; Golan-Goldrith *et al.*, 2004). Genetic relationships of pistachios were studied based on isozyme (Rovira, 1995; Aalami, 1996). Mirzaei (2005) studied genetic relationships of 20 varieties of pistachio along with two wild genotypes; *P. mutica* and *P. vera* var. *sarakhs* based on RAPD markers. Katsiotis *et al.* (2003) and Golan-Goldrith *et al.* (2004) developed AFLP

and RAPD markers for assessing genetic relationships among pistachio species. Kerman variety of pistachio was used for identification and isolation of micro satellite markers (Ahmad *et al.*, 2003).

The Siah Barg is a phenotype of *P. vera*, which is distinct from other pistachio phenotypes. The objectives of this study were to characterize this phenotype with morphological traits and RAPD markers and to determine relatedness of this phenotype with most commercial pistachio cultivars and wild *P. vera* (*Sarakhs* variety) in Iran.

MATERIALS AND METHODS

Morphological characteristics were evaluated by using descriptors for pistachio (IPGRI Publication, 1997) and developed descriptors for Iranian pistachio genotypes (Tajabadi, 1997). Qualitative and quantitative characters divided to 4 sections, including: tree, flower, leaf and flower bud and these characters measured in this phenotype.

RAPD assay. Genomic DNA was extracted from leaf tissue following the CTAB method of Hormaza *et al.* (1998) and DNA purification was made. Qualitative and quantitative measurement of DNA was made by the electrophoresis agarose gel (1.5%) method and spectrophotometric measurements. Extracted DNA was diluted to 25 ng/μL and used for PCR amplification. Based on the former study (Mirzaei, 2003) amplification reactions were done using 11 arbitrary decamer primers (Operon Technology) as described by Williams *et al.* (1990) with minor

modification. Amplification reactions were performed in volumes of 25 µL containing 10 mM tris-HCl pH 8.3, 50 mM KCl, 2 mM MgCl₂, 200 µM of each dNTP (Roche), 0.4 µM primer (Operon technology), 0.75 units of taq DNA polymerase (Roche) and 50 ng of genomic DNA overlaid with mineral oil. DNA amplification was carried out in an Eppendorf mastercycler gradient thermal cycler programmed for 50 cycles of 40s at 94°C, 70s at 48°C and 120s at 72°C, with final elongation step at 72°C for 10 min. The PCR products were stored at 4°C prior to analysis. Amplification products were separated on 1.2% agarose gel in TBE buffer and detected by staining with ethidium bromide. Siah Barg relationship was compared with two commercial cultivars (Ohadi & Akbari) and Sarakhs variety (Table I).

Based on polymorphic fragments a similarity matrix was generated using the Jaccard coefficient (Riaz *et al.*, 2003). A dendrogram was constructed based on the similarity data by applying un-weighted pair group method with arithmetic average (UPGMA), cluster analysis using the NT SYS-pc software.

RESULTS

Description. Siah Barg phenotype is male producing shiny blackish green leaves quite different from ordinary pistachio leaves known as Siah Barg (Figs. 1a & c). The apical and newly grown leaves are light red that gradually change to blackish green. The color of flower anther at the time of opening is blackish green in contrast to other phenotypes and cultivars that produce yellow and reddish yellow flowers. Flowering time of this phenotype is simultaneous with moderate flowering cultivars. The bark color of current season's growth is blackish brown (Fig. 1b), while the older shoots and main trunk of Siah Barg is whitish. The bark color of current season's growth is light brown, while that of other cultivars and phenotypes is light gray.

This phenotype has a moderately vigorous with a semi up-right growth habit. The leaf length and width was 14.3 and 13 cm, respectively. The length and width of apical leaflets were 10.5 and 6 cm, while the leaf area approximated 77 cm² and apical leaflet area 33 cm². The ratio of apical leaflet area to lateral leaflet was nearly 2 but this ratio is 1 to 1.5 in other cultivars (Fig. 1a).

Fingerprinting analysis. The RAPD technique was used to characterize the Siah Barg phenotype and to determine its relatedness with two major Iranian commercial pistachio cultivars and sarakhs variety (a wild *P. vera* variety). Amplification using 11 arbitrary primers revealed 46 polymorphic fragments. The number of polymorphic bands scored for each primer varied from 1 - 10. RAPD fingerprinting patterns of pistachio genotypes using primers: OPB10, OPB17, MG11, MG16, AJ05 and AJ20 (Figs. 2 & 4). Among 46 polymorphic bands, OPB10 - 1180, OPB17 - 740, MG16 - 780 AJ05 - 2700 bands were specific in Siah Barg. AJ05 - 580 was also specific in fingerprinting

Table I. *Pistacia vera* genotype included in this study

Number	Genotype
1	Barg Siah
2	Akbari
3	Ohadi
4	Sarakhs
M	Marker III (Roche)
M ₁	Marker (Ladder 1 kb)

Fig. 1a. Leaf color of the new phenotype (Siah Barg) as compared with other pistachio phenotypes, (b) Wood color of the new phenotype (Siah Barg) as compared with other pistachio phenotypes, (c) Branch color of the new phenotype (Siah Barg) as compared with other pistachio phenotypes

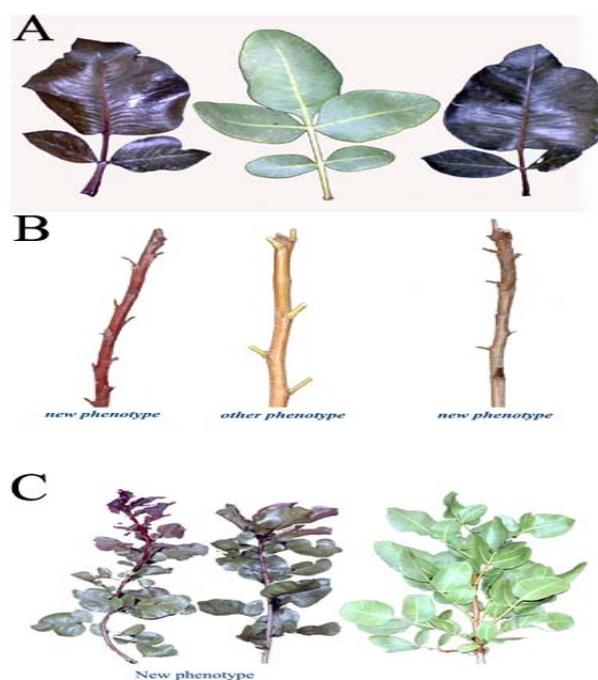
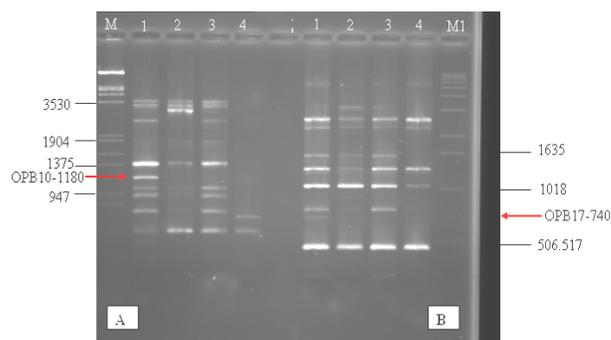


Fig. 2. RAPD pattern obtained from the four genotypes with the primer OPB10 (a) and OPB17 (b) Genotype numbers correspond to those in Table I



analysis, because of its absence in Siah Barg and its presence in the other genotypes (Figs. 2 & 4). Analysis of

Fig. 3. RAPD pattern obtained from the four genotypes with the primer MG11 (c) and MG16 (d) Genotype numbers correspond to those in Table 1

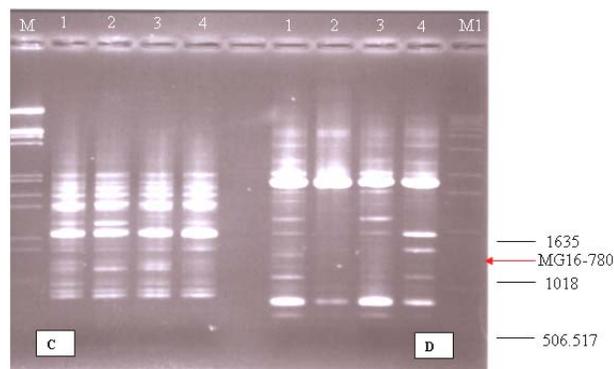
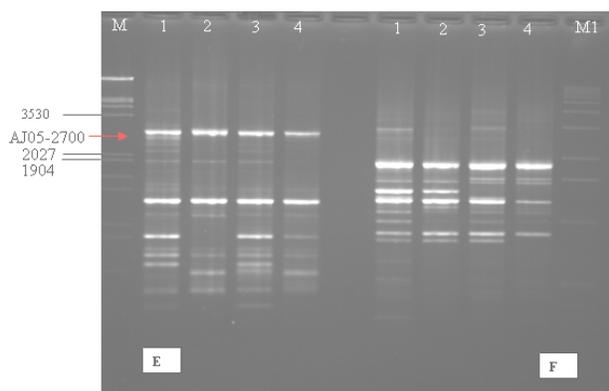


Fig. 4. RAPD pattern obtained from the four genotypes with the primer AJ05 (e) and AJ20, (f) Genotype numbers correspond to those in Table 1



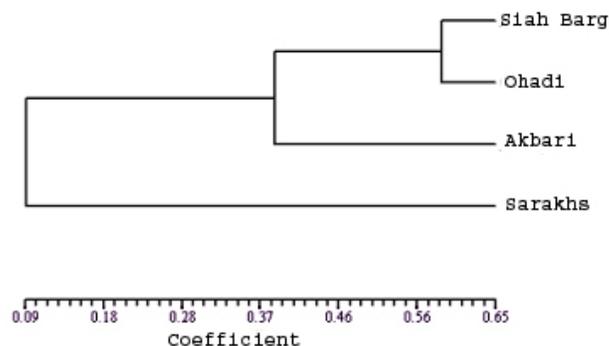
the data using cluster analysis clearly showed that the Siah Barg genotype was genetically more related to Ohadi cultivar with level similarity of 0.59 rather than the other genotypes and also wild pistachio (Sarakhs variety) (Fig. 5).

DISCUSSION

Siah Barg is male phenotype and produces shiny blackish green leaves. Leaf dimensions are larger than ordinary pistachio leaves. This phenotype has a moderately vigorous growth. Because of moderate flowering time, this phenotype can pollinate with moderate flowering cultivars such as the Ohadi cultivar.

With RAPD markers we can find cultivar-specific marker and a unique molecular fingerprinting for each cultivar (Dollo *et al.*, 1995). In combination with phenotypic information, the data from RAPD markers could be used to characterize new phenotype. The results obtained in this study showed that RAPD technique can be used to study of genetic diversity among pistachio cultivars. This is of potential importance in pistachio breeding as utilization of marker-assisted selection (MAS) depends upon the degree of polymorphism within the germplasm to be exploited

Fig. 5. Cluster dendrogram of pistachio accessions using UPGMA method



(Caruso *et al.*, 1998; Kafkas *et al.*, 2001). MAS can be important in pistachio, because pistachio has a long generation time. In addition specific bands of Siah Barg genotype can be clearly discriminated with a few well chosen primers including OPB10, AJ05, AJ20 and MG16. The attractive and unique color of the leaves of this phenotype and also outstanding characteristics of the Pistachio genus such as resistance and adaptation to desert conditions (low air & soil humidity, high temperature, salty water & soil) suggest that this phenotype may be important in landscaping of cities and suburbs.

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