Characterization of Iranian Hazelnut (*Corylus avellana* L.) Cultivars Using Microsatellite Markers

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Abstract

Catkins from twenty-three hazelnut accessions were sampled from a collection field at the SPII Horticultural Research Station at Kamal-Abad (Karaj, Iran). The accessions included sixteen cultivars of the Iranian germplasm and seven internationally known cultivars. DNA was amplified using dye fluorescent labeled primers and polymorphism detected using a semi automated ABI-PRISM 377 sequencer. Allele number of loci ranged from 6 to 12, and the heterozygosity level ranged from 0.59 to 0.86. A dendrogram was elaborated that divided the genotypes into three main clusters. The first cluster grouped together nine cultivars, five of which showed identical genetic profiles. The second cluster grouped four Iranian cultivars. The third cluster included the six foreign cultivars and three Iranian accessions. The 'Dobooseh' cultivar was set apart from all the other genotypes in the dendrogram and had several unique alleles. Results showed a good genetic variability within the Iranian germplasm and indicated the possible presence of cases of synonymy that should be further investigated.

INTRODUCTION

A pre-requisite for improving overall plant characteristics is an understanding of the structure of the germplasm collection, which in turn will allow systemic sampling of the germplasm for breeding and conservation purposes. DNA markers have been used to quantify the genetic diversity and determine phenetic relationships in several plant species.

SSRs (Simple Sequence Repeats) are short stretches of DNA consisting of tandemly repeated units and are usually very polymorphic due to the high level of variation in the number of repeats (Gianfranceschi et al., 1998). The use of multi-allelic, co-dominant markers for analysis of heterozygous allogamous species, such as most nut and fruit trees, is extremely useful, since it allows individuals to be uniquely genotyped (Powell et al., 1996). This is particularly important for cultivar identification and for enhancing the genetic characterization of germplasm collections and natural populations, which permits effective identification and characterization of plant genetic resources (Gianfranceschi et al.,1998).

RAPD markers were used in *Corylus* to characterize rootstocks (Radicati et al., 1996), identify cultivars grown in Campania (Galderisi et al., 1999), characterize 'Tonda Gentile delle Langhe' (Valentini et al., 2001) and study the phylogenetic relationships between 18 cultivars of commercial interest (Miaja et al., 2001). SSR markers have recently been isolated in hazelnut (Botta et al., 2002; Bassil et al., 2003; Bassil et al., 2004) and were used in this work for the DNA-typing of Iranian cultivars.

MATERIALS AND METHODS

Plant Materials

Catkins from twenty-three hazelnut accessions were sampled from a field collection at the SPII Horticultural Research Station (Seed and Plant Improvement Institute) at Kamal-Abad (Karaj, Iran). The accessions included sixteen genotypes of the Iranian germplasm and seven internationally known cultivars.

DNA Extraction and PCR Amplification

DNA was extracted following the method of Thomas et al. (1993) with some minor modifications. PCR amplification of 9 SSR loci (Botta et al., 2002) was carried out using a reaction mixture (20 µl) containing 50 ng DNA, 0.5 U Taq-DNA polymerase (AmpliTaq Gold polymerase, Applied Biosystems, USA), 2 µl 10x PCR buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2 mM $MgCl_2$, 200 µM dNTPs and 0.5 µM of each primer. PCR amplification was carried out with a MJ research PTC100 thermal cycler and consisted of an initial step of 9' at 95°C, followed by 28 cycles of 30 sec at 95°C, 45 sec at 55°C and 90 sec at 72°C; a final extension step was then performed for 45' at 72°C. DNA was amplified using dye fluorescent labeled primers and polymorphism was detected using a semi automated ABI Prism 377 apparatus (Applied Biosystems, USA).

Data Analysis

DNA data obtained at nine polymorphic microsatellite loci were analysed using Identity 4.0 software (Wagner et al., 2004), and distances between genotypes were calculated (as 1-proportion shared alleles) using Microsat (Minch, 1997) and Mega2 (Kumar et al., 2001) software.

RESULTS AND DISCUSSION

DNA was successfully amplified at all nine loci. The number of allele per locus ranged from 6 (CaT-C504) to 12 (CaT-B508 and CaT-B107) with an average of 9.2 alleles/locus (Table 1). The amount of expected heterozygosity ranged between 0.59 and 0.86 with a mean of 0.78. The total probability of identity was $2{,}28e^{-011}$ (Paetkau et al., 1995).

Cluster analysis (Fig. 1) produced a dendrogram that grouped cultivars into three main clusters. The first comprised nine cultivars, including 4 Iranian varieties and the foreign cultivar 'Longue d'Espagne' that showed identical profiles; the SSR profile of a true-to-type 'Longue d'Espagne' was compared with the genotype of the individual sampled in Iran and found to be different. This confirmed the mislabelling of this cultivar in the Iranian collection.

The second cluster grouped together 4 Iranian genotypes. From their names, it was decided that they were probably from the area of Ghare Bagh, near the border between Iran and Azerbaijan. This is a region where hazelnut has been grown for a very long time.

The third cluster grouped together six internationally known cultivars ('Segorbe', 'Cosford', 'Daviana', 'Merveille de Bollwiller', 'Negret' and 'Tonda Gentile delle Langhe'), which had been imported into Iran over 30 years ago, and three Iranian cultivars. Two of latter ('Tabari Rood' and 'Khandan') proved to be synonymous varieties. The identity of the 6 international cultivars was checked with the database available at University of Torino: cultivars 'Segorbe', 'Cosford', 'Negret' and 'Tonda Gentile delle Langhe' resulted true-to-type, while the identity of the 'Daviana' and 'Merveille de Bollwiller' plants sampled in Iran was not confirmed.

Finally, the Iranian cultivar 'Dobooseh' was set apart, located in one branch of the cluster showed many taxon-specific alleles and a unique SSR profile making it separately.

CONCLUSIONS

SSR markers proved to be a powerful tool for distinguishing genotypes. They were able to detect mislabelled cultivars in the collection and provided data for drawing a

dendrogram depicting interesting relationships between Iranian cultivars. This is the first report on the genetic structures of cultivars grown in Iran and further genetic, morphologic, and agronomic, studies should provide interesting information for breeding and germplasm conservation.

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Tables

Table 1. Characteristics of the loci studied on the cultivars of the Iranian collection.

Locus	Number of alleles	Heterozygosity expected	Heterozygosity observed	Frequency of null alleles	Probability of identity
CAT-B106	9	0.79	0.91	- 0.068	0.067
CAT-B107	12	0.83	0.83	0.002	0.048
CAT-B501	10	0.83	0.91	- 0.045	0.049
CAT-B503	8	0.83	0.91	- 0.044	0.051
CAT-B504	10	0.86	0.96	- 0.053	0.036
CAT-B505	8	0.82	0.96	- 0.072	0.052
CAT-B507	8	0.75	0.78	- 0.018	0.087
CAT-B508	12	0.76	0.69	0.036	0.089
CAT-C504	6	0.59	0.61	- 0.010	0.194
MEAN	9.2	0.78	0.84	TOTAL	$2.28e^{-011}$

Figures

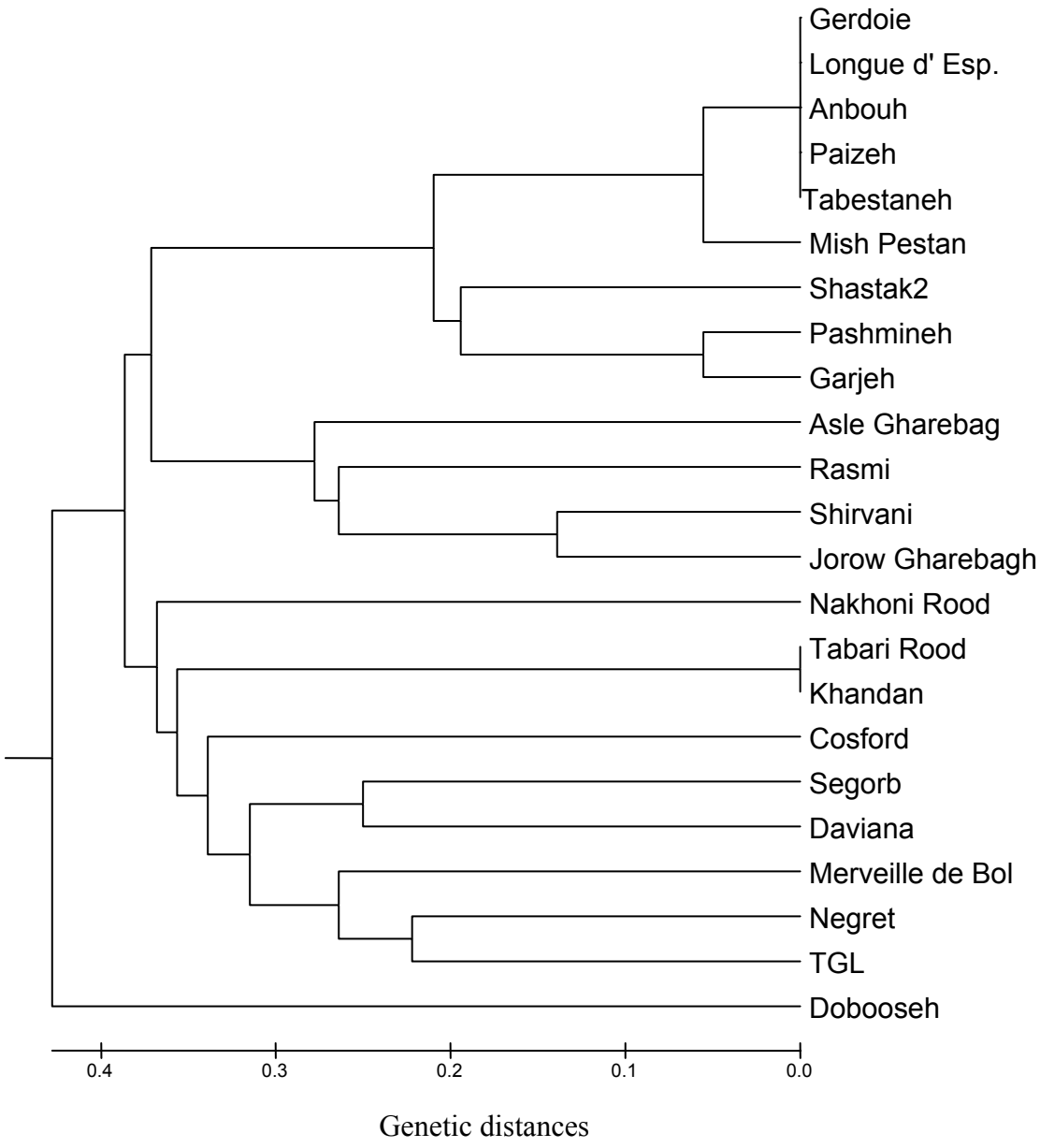

Fig. 1. Dendrogram generated by cluster analysis using the UPGMA method, representing the genetic distances between 23 hazelnut cultivars.