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Molecular analysis of native cultivars of sweet cherry in Southern Italy

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Abstract

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Campania region has a long tradition of autochthonous cultivated sweet cherries, which constitute a very rich germplasm resource. This biodiversity is highly valued for flavour, aroma and pulp texture. The interested cultivars are well known and sold in regional and local markets, but rarely outside. Genetic studies and phenotypic classifications are useful tools to increase our knowledge of such cherry cultivars and to disseminate their particular characteristics outside the region. The aim of the present study was the molecular characterization of ten autochthonous cultivars of *Prunus avium* using 30 RAPD markers. Among all, U17 was useful for fingerprinting eight out of ten cultivars. On cvs Del Monte, Della Recca, Pagliaccio, Montenero, Nera Dura, Mulegnana Nera, Passaguai and Malizia, unique molecular profiles were obtained. Furthermore, it was possible to distinguish between two most important cultivars on the Campanian market (cvs Del Monte and Della Recca) with HAP18 marker. The results obtained in this study confirmed the power of RAPD markers to easily analyse genetic diversity and to find new molecular profiles in a very short time. Moreover, confidential bands, characteristics of Campania native cultivars, can be used for genotype identification.

Keywords: *Prunus avium*; RAPD; genetic diversity; fingerprinting; biodiversity

The native sweet cherry, *Prunus avium* L., is one of the most highly-prized fruit crops in Italy. Campania region has an interesting and rich germplasm of this fruit. Indeed, cherry growers in Campania have an increasing interest in exploiting and preserving local cultivars. This rich genetic resource is defined by particular fruit characteristics, like flavour, aroma and pulp texture, which are appreciated by local and national consumers. To enhance our knowledge about these cultivars, diversity studies with phenotypic or/and genetic characterization are needed. The first straightforward criterion to measure diversity is based on phenotypic analysis for traits of interest. Despite its limitations, elaborate and time-consuming, this method has been used for long time to identify genotypes and to design breeding schemes. The counterpart of phenotyping is genotyping. DNA-based molecular markers can overcome many of the limitations of phenotypic-based tools and provide a more direct measure of genetic diversity. Molecular markers development and use in *Prunus* species has been more active in peach (*P. persica*) than cherry (*P. avium*) due to its relatively short juvenile period and commercial importance (Struss et al. 2003). Among the narrow amount of molecular techniques already available in cherry, randomly amplified polymorphic DNA (RAPD) markers have proven to be a reliable marker system for genetic fingerprinting and also in determining the genetic relationships among germplasm collections. RAPD have the advantage of being simple, able to detect relatively small amounts of genetic variation and do not need prior information on the genome

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(Yu et al. 2012). These technique has been already used successfully to reveal genetic variations both in cherry (Gerlach et al. 1997; Hormaza 1999; Downey, Iezzoni 2000; Wuensch, Hormaza 2002) and other crops, as grapevine (Ulanovsky et al. 2002; Kocsis et al. 2005; Karataş, Ağaoğlu 2010), blueberry (Rowland, Levi 1994), peach (Downey, Iezzoni 2000), apricot (Di Vaio et al. 2010) and *Brassica oleracea* (Dos Santos et al. 1994). The aim of this study was to carry out genetic profiling of native Campania sweet cherry cultivars, using RAPD molecular markers, to study genetic variation and find variety-specific markers.

MATERIAL AND METHODS

The study was carried out in a plot containing a regional collection of sweet cherry cultivars at the Improsta experimental station in Salerno province (40°37'01"N, 15°03'23"E). A group of 10 representative cultivars, originating from Campania was chosen from the collection. The cultivars chosen are among those of the greatest economic and agronomic interest (Table 1). The collection was planted in 2004 and all the cultivars were grafted onto rootstock Maxma Delbard 14. The cherry trees were trained to be short vase-shaped, with a spacing of 5 m between rows and 3 m between trees. Samples of leaves were collected randomly for each cultivar.

Thirty RAPD markers were selected for analysis (Table 2). Genomic DNA for each sample was extracted from approximately 100 mg of leaves according to Doyle and Doyle (1990). The DNA quality and quantity was checked on agarose gel at 0.7% (w/v). Quantification was carried out by comparing to the standard λ Hind III, using the Quantity One Gel Doc software (Bio-Rad, Hercules, USA). PCR reactions were conducted in a volume of 25 µl containing 20 ng of genomic DNA, 3.5mM $MgCl_2$, 1X Buffer, 0.2 mM dNTP, 2µM primers and 1U Taq polymerase (Promega, Madison, USA). The PCR cycle was performed with 1 cycle at 94°C for 5 min, 8 cycles at 94°C for 4 min, 38°C for 4 min and 72°C for 4 min and 40 cycles at 94°C for 30 s, 38°C for 1 min and 72°C for 2 min; the final extension was at 72°C for 5 minutes. The reactions were set up in a thermocycler Gene Amp^{TM} PCR System 2400 (Perkin Elmer, Waltham, USA). The fragments obtained were separated by electrophoresis on 1.5% agarose gel (w/v). Each product was visualized on gel with 100 bp molecular marker (Promega) to calculate the bands sizes. The electrophoretic patterns were analysed using Quantity One Gel Doc software (Bio-Rad). The results were confirmed reproducing the experiments from three to five times on three biological replicates.

RESULTS AND DISCUSSION

Among all the oligonucleotides used for RAPD analysis, U17 showed the highest discriminating power amplifying a different molecular pattern for each cultivar, especially for cvs Del Monte, Della Recca, Pagliaccio, Montenero, Nera Dura, Mulegnana Nera, Passaguai and Malizia. The U17 elec-

Table 1. Results of sweet cherry cultivars analyses (Campania production area)

Cultivar	Skin colour	Pulp colour	Ripening time
Bertiello	deep red	red	June 5
Cornaiola	red	red	June 10
Del Monte	light yellow-red	light yellow	May 31
Della Recca	light yellow-red	light yellow	June 7
Malizia	dark red	red	June 1
Montenero	deep red	light red	May 27
Mulegnana nera	dark red	red	June 3
Nera Dura	dark red	dark red	June 5
Pagliaccio	dark red	red	June 3
Passaguai	deep red	light red	May 15

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Table 2. RAPD primers used in molecular analysis for each marker and sequence

Primer name	Sequence 5'→3'	Primer name	Sequence 5'→3'
HAP 17	AAG CTT ACC AGG T	V 10	GTC GCT CAG A
HAP 18	AAG CTT AGA GGC A	V 18	TTC ACC CAC C
HAP 19	AAG CTT ATC GCT C	V 19	TGG GAA CGG T
HAP 20	AAG CTT GTT GTG C	NQ 3	CCA TTC ACC G
HAP 21	AAG CTT TCT CTG G	NQ 9	GCT TAC CAC C
HAP 22	AAG CTT TTG ATC C	U 17	CTG CCA CGA T
HAP 23	AAG CTT GGC TAT G	AO 2	GTC GAG CCG T
HAP 24	AAG CTT CAC TAG C	NO 6	ACA CGC AGA G
M 28	CCG GCC TTA A	112	CAA GTG TTC G
M 32	GGG GCC TTA A	117	TCT GGC GAT T
M 33	CCG GCT GGA A	126	TCG TGC TTT C
M 43	AAA ACC GGG C	127	CGA CGG TCT A
M 57	TTC CCC GAG G	1247	TCC CCG AGA A
M 58	TTC CCG GAG C	1254	AAC CGA CGC C
M 59	TTC CGG GTG C	1283	ACC CCT AGC G

trophoresis pattern of these cultivars is shown in Fig. 1. In only two cultivars, U17 showed a similar electrophoresis pattern, cvs Della Recca and Del Monte. Those cultivars can be easily distinguished using HAP17 and HAP18 markers, which showed a completely different pattern between the two cultivars. HAP17 amplified a lower number of bands in cv. Della Recca than in cv. Del Monte (Fig. 2), whereas HAP18 showed a higher number of bands in cv. Della Recca than in cv. Del Monte (Fig. 3).

For diversity analysis, the measurement of genetic similarity or difference among plant species using genotype-specific markers can provide important information in crop conservation and varietal development (Romero et al. 2009). Furthermore, such information is also useful for accessions, biotypes and cultivars characterising in plant germplasm collections and taxonomic studies (Kalia et al. 2011). The present study was developed in this context, focusing on the value and preservation of

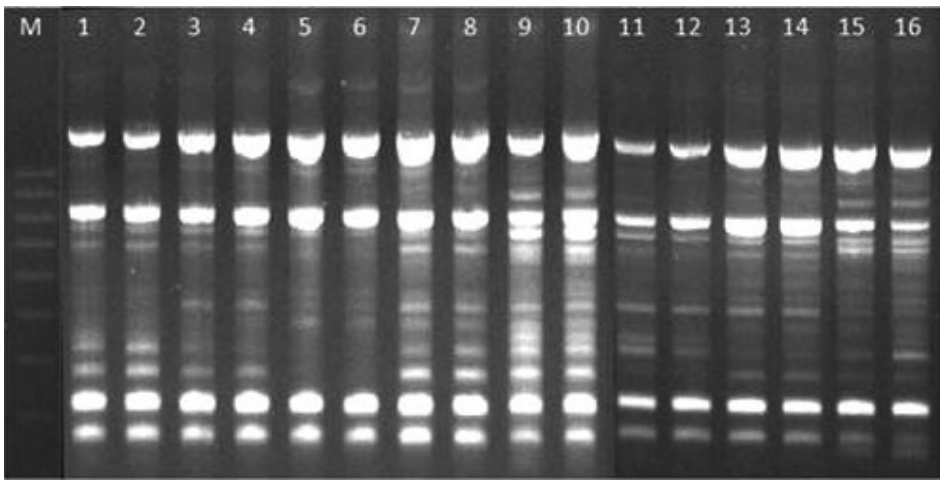

Fig. 1. RAPD amplification profile obtained with the oligonucleotide U17

M – molecular marker; 1, 2 – cv. Del Monte; 3, 4 – cv. Della Recca; 5, 6 – cv. Pagliaccio; 7, 8 – cv. Montenero; 9, 10 – cv. Nera Dura; 11, 12 – cv. Mulegnana Nera; 13, 14 – cv. Passaguai; 15, 16 – cv. Malizia

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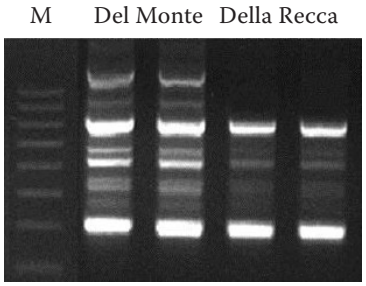

Fig. 2. Fingerprinting of two varieties of cherry with the oligonucleotide HAP18
M – molecular marker

Campania's typical cherry cultivars using a simple and effective technique, namely RAPD markers. These molecular markers are swift and easy to use, since they do not require sequence knowledge and can produce abundant polymorphic fragments. RAPD markers were successfully used in cherry (Cai et al. 2007; Ercisli et al. 2008) and other plant species, like lemon (Iannelli et al. 1998), apple (Koller et al. 1993), peach (Testolin et al. 2000) and apricot (Mariniello et al. 2002). This technique consists in a Polymerase Chain Reaction (PCR) performed on genomic template with a single oligonucleotide (10–13 pairs of bases), able to pair so many times on the template. This marker allowed us to obtain a unique molecular profile in Campania cherry cultivars. Indeed, complex patterns were obtained in all genotypes, especially in the native cultivars, Della Recca and Del Monte. Identification of genotype-specific profiles is important to discover and increase the value of native cultivars which are grown in small areas. By enhancing our knowledge of such cultivars, they can become more marketable on a national basis and compete with other cultivars for their flavour and authenticity. In particular two cultivars, Della Recca and Del Monte are listed among Campania's typical products and are awaiting Protected Geographical Indication certification. The organoleptic characteristics of these two cultivars are comparable to the most popular cherry cultivar in Italy, Duracine, matching the consumer's demand. In this research it was proved that the RAPD technique can be applied to identify cherry cultivars and for early screening of specific traits for selection, providing a theoretical basis to identify cherries with the same or similar names. This result is in line with previous studies on cherry and other fruit species (Stockinger et al. 1996; Gerlach, Stosser 1998; Luo et al. 2001; Huang et al. 2003).

In conclusion, the findings resulted in the characterisation of the molecular basis of cultivars belonging to the large sweet cherry collection in Campania, making a contribution to the protection and enhancement of local product quality.

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