Development of a Microsatellite Database for Identification of Olive (*Olea europaea* L.) Cultivars in Mendoza, Argentina

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Keywords: *Olea europaea*, SSR, cultivar characterization, fingerprinting

Abstract

Olives were introduced in Argentina from Europe over 400 years ago. Several cultivars are grown in Mendoza province and problems exist on misclassification of plants in orchards and nurseries. This work was aimed to develop a cost effective protocol for fingerprinting olive germplasm using microsatellites (SSRs) and generate a database in order to help in the identification of olives trees in Mendoza. Four highly informative polymorphic SSRs (DCA3, DCA9, DCA11 and DCA16) were selected to fingerprint olive cultivars. A protocol using a single dye-labeled forward common primer with the M13 (-21) sequence was used in combination with the reverse specific SSR primers. PCR amplifications were multiplexed and resolved in automated capillary genetic analyzer equipment. Profiles of the cultivars and a list of alleles with the sizes obtained by capillary electrophoresis are provided. Several genetic parameters were calculated to characterize the microsatellite loci. A database of SSR markers for eleven of the more distributed olive cultivars in Mendoza was developed.

INTRODUCTION

Approximately 2,000 olive (*Olea europaea* L.) cultivars are known in the world (Fontanazza, 1993). In Argentina olives were introduced more than 400 years ago by the Spanish settlers and during the 20th century by the Italian and Spanish immigrants. Most of the plants introduced maintained the original denomination; however, local nurseries select trees for cuttings from old orchards where the identification records were incomplete or unreliable, leading to confusion and misclassification of plant material (Cavagnaro and Masuelli, 2002). Several microsatellites (Simple Sequence Repeats or SSRs) were developed for olives characterization and became popular markers used for identification of olive cultivars (Rallo et al., 2000; Carriero et al., 2002). A consensus list of SSR primers was proposed by Baldoni et al. (2009) based on several parameters like reproducibility, information content and discrimination capacity. Fluorescence-base detection of multiplexed SSR patterns with automated DNA sequencer and the employment of tailed primers reduce costs and make the analysis more efficient (Vaughan and Lloyd, 2004). The goal of this work was to use available SSR markers for development of a cost-effective protocol for olive cultivar fingerprinting and a SSR database of the more important olive cultivars growing in Mendoza, Argentina.

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MATERIALS AND METHODS

DNA from eleven cultivars grown in Mendoza was isolated from young leaves following the method of Dellaporta (1983). The identities of the clones were determined by the comparison of the SSR patterns with reference samples from the World Olive Germplasm Bank of Cordoba-Spain (WOGB). Reverse primers of four SSR markers (DCA3, DCA9, DCA11 and DCA16) were used in combination with a forward M13-tailing primer labeled with FAM or NED fluorochromes. A single PCR reaction was performed with three primers: the tailed forward primer, the reverse primer and the M13-dye labeled primer, according with Boutin-Ganache et al. (2001). Allele analysis was carried out on an automatic capillary sequencer ABI 3130 Genetic Analyzer (Applied Biosystems/HITACHI) using the internal standard GeneScan 400 HD-Rox (Fig. 1). To assess the level of polymorphism of the SSRs the Nei's (1978) unbiased heterozygosity (He) of each microsatellite was calculated.

RESULTS AND DISCUSSION

The analysis of four SSR markers from 21 accessions of 11 cultivars (Table 1) grown in Mendoza, Argentina, showed abundant allelic variation and high genetic diversity values. These results are consistent with previous studies reporting high diversity levels of olive cultivars (Baldoni et al., 2006; Belaj et al., 2010). The number of alleles per microsatellite locus ranged from 7 to 10 and the four loci were highly informative (PIC>0.5), ranging from 0.81 in the locus DCA3 to 0.71 in the loci DCA11 and DCA16 (Table 2). Therefore, the makers assayed are a valid tool for discriminating among the olive cultivars distributed in Mendoza. The expected (He) and the observed heterozygosity (Ho) per locus ranged from 0.76 to 0.85, with an average of 0.79, and from 0.5 to 0.86, with a mean value of 0.74, respectively (Table 2). Similar values were reported by Belaj et al. (2010) for cultivated and wild olive populations in Spain. The loci DCA9 and DCA16 showed higher He values than Ho, indicating a degree of inbreeding for these loci. On the other hand, for DCA3 and DCA11 there were more heterozygous than expected (Ho>He) indicative of high level of outbreeding for these loci. As was pointed out by Baldoni et al. (2008) the excess of heterozygosity could be explained by the accumulation of mutations in plants propagated clonally, leading to divergence within individuals. Finally, in Table 3 a database with alleles amplified by the four SSR loci to identify the 11 cultivars growing in Mendoza is reported. The high discrimination power of the SSR markers used in combination with the cost-effective protocol of automated genotyping with tailed primers will allow to reduce the costs and make more efficient the fingerprinting of olive cultivars.

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Tables

Table 1. List of the 11 cultivars used for the development of the SRR database.

Cultivar	Number of clones	Origin	Use
Arauco	1	Argentina	Oil/table
Arbequina	2	Spain	Oil
Coratina	1	Italy	Oil
Empeltre	1	Spain	Oil
Farga	1	Spain	Oil
Frantoio	2	Italy	Oil
Hojiblanca	3	Spain	Oil
Changlot Real	1	Spain	Oil
Manzanilla	5	Spain	Oil/table
Picual	3	Spain	Oil
Leccino	1	Italy	Oil

Table 2. Size ranges (bp), number of alleles (N), observed (Ho) and expected heterozygosity (He) and polymorphic information content (PIC) for four SSR markers in 21 olive clones.

Locus	Size range	N	Ho	He	PIC
DCA3	130-252	10	0.86	0.85	0.81
DCA9	172-207	8	0.74	0.77	0.72
DCA11	157-183	8	0.86	0.76	0.71
DCA16	151-175	7	0.5	0.77	0.71
Mean	-	8.25	0.74	0.79	0.74

Table 3. Allelic profiles of the 11 cultivars analyzed with four SSR markers.

Cultivar[1]	DCA3	DCA9	DCA11	DCA16
Arauco	237-247	184-193	145-183	123-125
Arbequina	230-241	184-205	145-183	123-148
Coratina	237-241	182-193	134-175	151-151
Empeltre	241-241	184-205	145-183	156-156
Farga	235-241	172-186	134-183	151-151
Frantoio	235-241	182-205	134-183	150-150
Hojiblanca	237-250	205-205	145-165	156-175
Changlot Real	243-252	184-205	165-183	156-156
Manzanilla	243-250	205-205	145-165	156-175
Picual	237-247	184-191	145-181	156-156
Leccino	130-130	172-193	145-157	123-148

[1] The allelic profile of 'Arbequina', 'Frantoio', 'Hojiblanca', 'Manzanilla' and 'Picual' correspond to the sample from World Olive Germplasm Bank of Cordoba-Spain (WOGB).

Figures

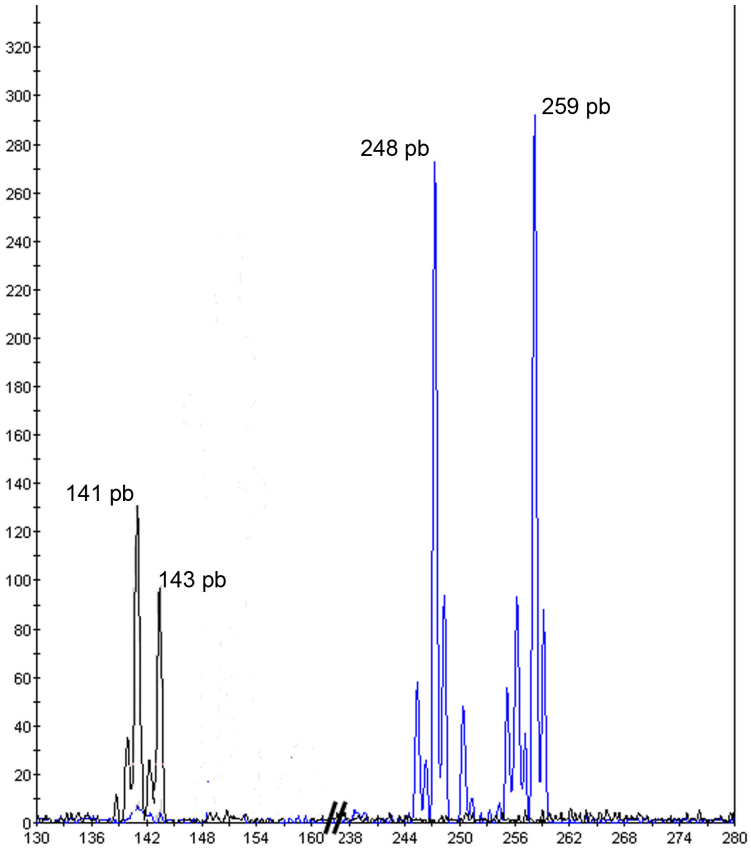

Fig. 1. SSR reactions with primers DCA16 and DCA3, the tails used were labeled with NED and FAM fluorochromes, respectively. The reactions were mixed and resolved together in an automatic capillary sequencer ABI 3130 Genetic Analyzer. The alleles length include the 18 bp of the labeled tail.