

Genetic Characterization of Chestnut Accessions from ‘Centro Regionale di Castanicoltura’ (Cuneo, Italy)

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Abstract

The Dipartimento di Colture Arboree at Torino, with the financial support of three public partners (Regione Piemonte, Ente Gestione Parchi e Riserve Cuneesi, and Comunità Montana ‘Valli Gesso, Vermengna e Pesio’), established in 2005 the ‘Centro Regionale di Castanicoltura’ located in Chiusa Pesio (Cuneo province, northwestern Italy). A germplasm collection field of chestnut genetic diversity was realized. It includes the main local and national cultivars, together with several European varieties from Portugal, Spain, France, and Switzerland and accessions from U.S.A., China, and Japan. A set of 7 SSR loci was used to fingerprint 36 accessions, in order to start the genetic characterization of all plants in collection. The results obtained showed that microsatellite loci detected considerable polymorphism and thus particularly suitable for DNA-typing of chestnut cultivars. Genetic relationships among the accessions were investigated by UPGMA cluster analysis.

INTRODUCTION

The general interest for genetic resources is based on the opportunities offered by their utilization (Maxet et al., 2002). Genetic resources not only provide the required raw material for sustainable genetic improvement of crops, but offer a unique gene combination, as naturally occurring co-adapted gene constellations, to ensure adaptability and productivity. Some trees cultivations are also agro-ecosystems of good environmental and landscape quality. Therefore, their conservation is of paramount importance to achieve sustainable production and food security for future generations (Kassar and Lasserre, 2004). This is reflected in the objectives of the Convention on Biological Biodiversity (CDB) and the FAO International Treaty on Plant Genetic Resources for Food and Agriculture (PGRFA). The inherent level of genetic diversity represented within many of our food and fiber crops is often quite limited. As the genetic base of many commercially important crops, especially long-lived perennial tree crops, has generally become narrow, comprising only a limited number of cultivars grown as monoculture over vast areas, they are highly vulnerable to the rapid spread of insects and pests. Plant breeders are therefore dependent on the diverse genes to continue developing new cultivars to keep ahead of such calamities. However the conservation of agro-diversity is of critical importance, because of the direct benefits to humanity that can rise from its exploitation in improving agricultural crops as well as the potential for developing new medicinal and other products. The management of genetic resources is

based on conservation, evaluation, and utilization with the conservation function played by *ex-situ* genebanks (Berthaud, 1997).

In this contest, the Dipartimento di Colture Arboree at Torino, with the financial support of three public partners (Regione Piemonte, Ente Gestione Parchi e Riserve Cuneesi, and Comunità Montana 'Valli Gesso, Vermenagna e Pesio'), established in 2005 the 'Centro Regionale di Castanicoltura' located in Chiusa Pesio (Cuneo province, northwestern Italy). The main activities of this institute concern applied research studies on the local chestnut germplasm, plant propagation techniques, genetic and phytosanitary monitoring, optimization of cultural practices, and training. Moreover, a germplasm collection field of chestnut genetic diversity was realized. It includes the main local and national cultivars, together with several European varieties from Portugal, Spain, France, and Switzerland and accessions from U.S.A., China, and Japan.

The objective of this study was to test a fingerprinting set of 7 SSR (simple sequence repeat) loci for differentiating among chestnut accessions and to start the genetic characterization of all plants in collection.

MATERIALS AND METHODS

Leaves from 36 chestnut accessions were sampled in the collection field of the "Centro Regionale di Castanicoltura" located at Chiusa Pesio (Cuneo province, northwestern Italy). DNA was extracted from 0.2 g of young leaves using the protocol described by Thomas et al. (1993).

Samples were analysed at 7 SSR loci isolated in *Castanea sativa* Mill. and characterized by Marinoni et al. (2003): CsCAT-1, CsCAT-8, CsCAT-14, CsCAT-15, CsCAT-16, CsCAT-17, and CsCAT-41. Loci were chosen based on the ease of allele scoring, multiplexing ability, and their linkage group assignment (Barreneche et al., 2004). PCR reactions were carried out using a mixture (20 μ l) consisting of 2 μ l 10x buffer (100 mM Tris-HCl, pH 8.3, 500 mM KCl), 2 mM MgCl₂, 200 μ M dNTPs, 0,5 μ M of each primer, 0,5 U of Taq-DNA polymerase (AmpliTaq Gold polymerase, Applied Biosystems) and 50 ng of DNA. All SSR amplifications were performed using the following temperature profiles: 9 min of denaturation at 95°C, then 28 cycles of 30 sec of denaturation at 95°C, 45 sec of annealing at 50°C, and 90 sec of extension at 72°C; and a final 30 min extension step at 72°C. Amplified fragments were analysed on a capillary ABI-PRISM 3130 Genetic Analyzer sequencer (Applied Biosystems). Results of the run were processed with Genemapper v.4.0 software and allele sizes (in base pairs, bp) were estimated using a GeneScan-500 LIZ size standard (Applied Biosystems).

Data obtained at 7 SSR loci for 36 accessions cultivar were processed using the software Identity 1.0 (Wagner and Sefc, 1999) to calculate: number of alleles, number of observed heterozygotes, expected heterozygosity (Nei, 1973), frequency of null alleles (Brookfield, 1996), and probability of identity (Paetkau et al., 1995). Genetic relationships among the accessions were investigated by UPGMA cluster analysis. Genetic distances (1000 bootstraps) were computed as $D = [1 - (\text{proportion of shared alleles})]$ by Microsat (Minch, 1997). Cluster analysis was performed using the Neighbor software in the Phylip v.3.5c package (Felsenstein, 1989) and a dendrogram was constructed by Tree View program (Page, 1996).

RESULTS AND DISCUSSION

The 7 SSR loci analysed identified 27 unique genotypes among 36 accessions and showed a good polymorphism level (Table 1). The number of alleles totaled 59 and

ranged from 6 to 11 per locus, with an average of 8.4. The loci with high numbers of alleles were CsCAT1 (10 alleles) and CsCAT41 (11 alleles). Expected heterozygosity (He) averaged 0.77 and ranged from 0.65 (for CsCAT15) to 0.85 (for CsCAT17), while observed heterozygosity (Ho) averaged 0.89 and ranged from 0.73 (for CsCAT16) to 1.00 (for CsCAT17). The estimated frequency of null alleles (r) was a positive value for locus CsCAT 16 (0.019). The total probability of identity at all loci was 2.99×10^{-8} , thus cultivars with identical genotypes were considered synonyms.

The results obtained in our set of accessions showed that microsatellite loci detected considerable polymorphism and confirmed that these markers are suitable for fingerprinting chestnut cultivars. The polymorphism and discriminant power of each locus were evaluated on the basis of number of alleles, expected and observed heterozygosity (Table 1). The mean values of these parameters were comparable to obtained in chestnut by Marinoni et al. (2003) and Gobbin et al. (2007). All loci analysed are highly polymorphic and thus particularly suitable for DNA typing of chestnut cultivars.

Cluster analysis performed for 36 accessions produced an UPGMA dendrogram depicting the genetic relationships within the studied accessions (Fig. 1). 'Gabiana' and 'Travisò' accessions were grouped in cluster I. Almost all accessions named 'Marrone' clustered with most Italian cultivars in the large cluster II. The interesting data observed was the existence of a unique genetic profile among these 'Marrone' accessions that are cultivated in different Italian regions. Finally, all Euro-Japanese hybrids analysed, except 'Bouche de Bétizac', were placed in group III together with some *C. sativa* accessions.

CONCLUSIONS

The initial SSR fingerprinting of 36 chestnut accessions from 'Centro Regionale di Castanicoltura' confirms the usefulness of SSR markers in the management of germplasm collections. Moreover, the set of 7 SSR marker used in this work showed a high level of polymorphism and it will be used to establish base marker patterns for each chestnut accessions in the collection field. Thus, a SSR database of the molecular fingerprints will be available through the Dipartimento di Colture Arboree website as world-wide resource.

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Tables

Table 1 – Characteristics of 7 SSR loci in 27 chestnut unique genotypes. Na: number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; r: frequency of null allele; LG: linkage group (Barreneche et al., 2004).

Locus	Allele size range (bp)	Na	He	Ho	r	LG
CsCAT-1	186-222	10	0.79	0.92	- 0.077	8
CsCAT-8	178-227	8	0.77	0.97	- 0.112	6
CsCAT-14	133-162	6	0.71	0.92	- 0.123	2
CsCAT-15	121-159	7	0.65	0.84	- 0.116	8
CsCAT-16	118-150	8	0.77	0.73	0.019	6
CsCAT-17	130-161	9	0.85	1.00	- 0.081	2
CsCAT-41	198-235	11	0.83	0.87	- 0.024	8

Figures

Fig. 1 - UPGMA dendrogram of 36 chestnut accessions based on alleles at 7 SSR loci.



