Characterization and Recovery of Apricot Germplasm from an Old Stone Collection

C. Martín and J.I. Hormaza
Estación Experimental "La Mayora"
CSIC, 29760 Algarrobo-Costa, Málaga
Spain

M. Herrero
Dep. Pomología, Estación Experimental
"Aula Dei", CSIC, Apdo. 202/ 50080
Zaragoza
Spain

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Abstract

The recovery and conservation of traditional fruit tree cultivars is a necessity that faces a short window of opportunity due to accelerating cultivar erosion. Several efforts to preserve old material took place before the widespread use of molecular markers, but often that material has not been conserved in ex situ germplasm collections. Here we present a case in Spain where a thorough inventory of local fruit tree species was performed in the 1950s with detailed data of the origin of each genotype. However, from most of that material the only remnants are an old stone collection. We developed a protocol in apricot (*Prunus armeniaca*) to obtain DNA from maternal tissues of the stones of a sufficient quality to be amplified by PCR. Then we compared the results obtained with the profiles obtained from cultivars currently conserved in ex situ germplasm collections. The results highlight the lost and preserved variability and help to prioritize the recovery of particular cultivars. The approach used in this work provides information on genetic erosion that has taken place in fruit tree species, but could also be applied to other plant species.

INTRODUCTION

In the last decades the development of new technologies, the substitution of local varieties by foreign improved varieties or changes in cultural techniques, have resulted in an increasing erosion of germplasm resources that leads to the need of collecting endangered germplasm. In fact, conservation and use of plant genetic resources should be a priority in agricultural research (Tanksley and McCouch, 1997; Esquinas-Alcazar, 2005). However, this task is often hindered by the abundance of homonymies and synonymies in germplasm collections and the lack of information available on local germplasm erosion. In this work we present a case study with apricot (*Prunus armeniaca* L.) in Spain. A thorough variety inventory of different fruit tree species was performed in Spain in the 1950s with detailed data of the origin of each genotype (Herrero, 1964). However, from most of that material the only remnants are an old stone collection and most of the genotypes are no longer conserved in ex situ collections. Since two maternal tissues (the endocarp and the testa) are present in *Prunus* stones, it could be possible to extract DNA from them and predict the genetic composition of the maternal plant that originated the fruits. This could be a preliminary step to analyze genetic erosion during the last decades in different fruit tree species.

MATERIALS AND METHODS

A total of 45 apricot genotypes collected in the 1950s from different geographical areas in Spain and conserved in the form of stones at the E.E. Aula Dei in Zaragoza (Spain) were used in this study (Table 1). After trying different DNA extraction methods with both the testa and the endocarp (data not shown) we decided to use the protocol of Godoy and Jordano (2001) with some modifications. The maternal tissues were previously separated from the rest of the seed (endosperm and vestigial embryo) and treated with liquid nitrogen. DNA was extracted from 60 to 100 mg of testa and endocarp. Tissues were homogenized in 400 µl of extraction buffer (200 mM Tris-HCl

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pH 8.0; 70 mM EDTA; 2 mM NaCl; 20 mM sodium bisulfite) with a TissueLyser homogenizer (30 s; 30 Hz). After homogenization, 85 µl of sarkosyl was added and the sample was incubated at 65ºC for 30 min and centrifuged at 10,000 g for 20 min to remove insoluble material. In some cases this step had to be repeated for an additional 5 min. DNA was precipitated by the addition of 95 µl of 10 M ammonium acetate and 200 µl of isopropanol; the mixture was centrifuged for 20 min at 16,000 g. The pellet was washed with 70% ethanol, dried and resuspended in 100 µl MTE buffer (1M Tris-HCl pH 8.0; 0.5 M EDTA).

The extracted DNA was analyzed with 3 SSR loci developed in peach (Pcghms3, UDp96-001, UDp96-008) by Cipriani et al. (1999) and Sosinski et al. (2000) and proved to be transferable to apricot (Hormaza, 2002) and 9 loci developed in apricot (ssrPaCITA7, ssrPaCITA19, ssrPaCITA23, ssrPaCITA10, ssrPaCITA12, ssrPaCITA27, UDAp-414, UDAp-415, UDAp-420) by Lopes et al. (2002) and Messina et al. (2004) selected based on a higher number of alleles per locus and heterozigosity (Table 2).

A 15 µl reaction containing 16 mM $(NH_4)_2SO_4$, 67 mM Tris-Cl pH 8.8, 0.01% Tween20, 2 mM $MgCl_2$, 0.1 mM each dNTP, 0.4 µM each primer, 40 ng genomic DNA and 1 unit of BioTaq DNA polymerase (Bioline, London, UK) were used for amplification on an I-cycler (Bio-Rad Laboratories, Hercules, CA, USA) thermocycler using the temperature profiles described in Table 2 for each primer set after optimizing the annealing temperatures with gradient PCR. Forward primers were labeled with a fluorescent dye on the 5' end (Proligo, Paris, France). The PCR products were analyzed by capillary electrophoresis in a CEQ™ 8000 capillary DNA analysis system (Beckman Coulter, Fullerton, CA, USA). Samples were denatured at 90ºC during 120 s, injected at 2.0 kV 30 s and separated at 6.0 kV during 35 min.

RESULTS AND DISCUSSION

As a preliminary work, DNA was extracted and amplified from both testa and endocarp tissues. The results obtained showed a higher quality and repeatability of the amplifications with testa tissue (data not shown) and, consequently, all the experiments were performed using that tissue. All the loci tested produced polymorphic amplification fragments among the apricot testa tissue studied. Six of the samples analyzed ('Duro', 'Currot', 'Patriarca Temprano', 'Nancy', 'Patriarca' and 'Mayeros') presented null alleles for 4 or more loci and for two samples ('Galta Rocha' and 'Corbató') it was not possible to obtain repeatable amplification patterns for most of the loci analyzed. Consequently, repeatable and reliable amplification patterns could be obtained for 37 old genotypes. The age of the samples and the fact that the fruit stones had been conserved at room temperature could explain the amplification problems detected with some samples.

A high number of 11 rare alleles ($p \leq 0.05$) was obtained. However, only one allele was fixed ($p \geq 0.90$), in the UDp96-008 locus. Some genotype-specific alleles were also observed. The combination of the different amplification fragments obtained with the 12 SSR loci allowed us to distinguish 37 unique genetic profiles revealing several cases of homonymies but no synonymies were found in the material studied.

When the results obtained were compared with an ex situ apricot germplasm collection (Hormaza, 2002) only three of the genotypes analyzed in this work ('Luizet', 'Canino' and 'Gitano') have the same genotype profile suggesting that the rest of the genotypes are no longer conserved in the ex situ collection analyzed. These results support the use of the approach described in this work. In fact, two different 'Canino' accessions were conserved as stones and one of them proved to be the same as the genotype conserved ex situ. On the other hand, a homonymy was found between the genotype Paviot conserved in form of stones and the cultivar with the same name conserved ex situ, since the amplification profiles were different.

The results obtained in this work will be highly useful to help us to prioritize which apricot varieties should be collected in the future. The approach used can also be of great interest to study the loss of genetic diversity and the genetic erosion that has taken place in apricot and it could be applied to other plant species, provided that old

material is still preserved.

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Tables

Table 1. List of the cultivars studied that are conserved in form of stones.

Cultivars	
Acmé	Luizet
Amoscatelado	Mayeros
Antón	Moniquí
Blanco de Murcia 1	Moniquí Temprano
Blanco de Murcia 2	Nancy
Canino 1	Patriarca
Canino 2	Patriarca de Hueso Dulce
Carmelos	Patriarca Temprano
Corbató	Paviot
Currot	Perla
Damasco	Precoz de Boulbon
De Antón	Real Fino 1
De Confitar	Real Fino 2
De Hellín	Real Temprano 1
Duro	Real Temprano 2
Encarnado Fino	San Ambrosio
Galta Rocha	Santones
Galta Vermeya	Tapalahoja
Giletano 1	Temprano Colomer
Giletano 2	Temprano Gordo
Gitano	Toledo
Hatif Colomer	Velázquez
Hoja de Parra	

Table 2. Primers and PCR conditions used in this work.

Primers	Denaturation	Annealing	Elongation	N Cycles
Pcghms3	94°C 45 s	57°C 30 s	72°C 30 s	32
UDp96-001	94°C 45 s	57°C 45 s	72°C 45 s	35
UDp96-008	94°C 45 s	57°C 45 s	72°C 45 s	35
ssrPaCITA7	96°C 30 s	51°C 45 s	72°C 30 s	34
ssrPaCITA19	96°C 30 s	51°C 45 s	72°C 30 s	34
ssrPaCITA23	96°C 30 s	51°C 45 s	72°C 30 s	34
ssrPaCITA10	96°C 30 s	47°C 45 s	72°C 30 s	34
ssrPaCITA12	96°C 30 s	47°C 45 s	72°C 30 s	34
ssrPaCITA27	96°C 30 s	47°C 45 s	72°C 30 s	34
UDAp-414	94°C 30 s	56°C 45 s	72°C 30 s	27
UDAp-415	94°C 30 s	56°C 45 s	72°C 30 s	27
UDAp-420	94°C 30 s	56°C 45 s	72°C 30 s	27