Genetic relationships in apricot (*Prunus armeniaca* L.) using SSR markers and their implications for breeding

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Abstract

In this paper we evaluated the genetic diversity among apricot cultivars of different origins using SSR markers. Thirty-one apricot cultivars were evaluated, including one Japanese plum and one apricot x plum hybrid as outgroup. Eleven primer pairs developed in peach were assayed. Seven primer pairs (64% of the assayed markers) were amplified successfully and resulted in 44 polymorphic bands, ranging from 2 (UDP96008) to 8 (UDP98406 and UDP98411) alleles. The observed heterozygosity ranged from 0.09 (UDP96008) to 0.88 (UDP96003). There is a high degree of homology for the SSR *loci* between peach and apricot and transportability of these markers among *Prunus*. According to genetic distances obtained within the apricot group, four clusters were identified: Western European; North American; one cluster including 'Bebeco', 'Tirynthos' and 'Ninfa' and finally another cluster including other North American cultivars. Results show that the cultivars of the American genotypes share a common genetic background. Most of the evaluated cultivars that belong to the European eco-geographical group present a common genetic base, which suggests that apricot breeding has been mainly based on the hybridization and selection of European genotypes. The information generated through the application of SSR on this apricot germplasm bank would be useful for breeders who want to enrich their genetic base and introduce new genes on their background.

Key words: *Prunus*, apricot, SSR markers, cluster analysis, phylogenetic relations.

Introduction

The apricot is a member of the Rosaceae, section Armeniaca, which includes five different species: *P. armeniaca*, *P. mume* Sieb. and Zucc., *P. brigantiaca* Vill., *P. dasycarpa* Ehrh. and *P. holosericea* Batal. Furthermore, other genotypes have been classified as botanical varieties of *P. armeniaca*: *P. armeniaca* var. *sibirica* L., *P. armeniaca* var. *mandshurica* (Maxim) Koehne and *P. armeniaca* var. *ansu* Komar [1].

On the other hand, the cultivated apricot can be also classified into six main ecogeographical groups: Central Asian, Dzhungar-Zailij, Irano-Caucasian, European, East Chinese and North Chinese. The Central Asian group is the oldest and is a more diverse group and includes mostly self-incompatible and small-fruited genotypes with high chilling requirement. The Iran-Caucasian group produces larger fruits and also most of them are self-incompatible with low chilling requirement. The European group, which includes cultivars from Europe, North America, South Africa and Australia, is the youngest and shows a narrow genetic diversity, probably originating from a few Asian ancestors. In general, this group is self-compatible, more precocious and exhibits lower chilling requirement [2].

The apricot is cultivated in temperate and subtropical regions and occupies the third place in the world among the stone fruits of economic importance after peach and plum [3]. China, Turkey, Iran, central Asia, Europe and North America are the zones where apricot production is concentrated [4]. In this context, possibilities for crop extension in Europe and other countries are limited by two main factors: the lack of adapted varieties and the spread of *plum pox virus* (Sharka) [5]. In order to overcome these problems, many breeding programs have focused on these breeding objectives [6, 7]. The development of new genotypes requires fast and reliable techniques to study the genetic diversity in germplasm and to identify and protect newly released genotypes [8].

Molecular markers have become a very useful tool for these purposes. RFLP and RAPD have been used to assess genetic variability of apricot germplasm [9]; nevertheless, SSRs have shown better results based on advantages such as high level of polymorphism, general codominance, abundance in plant genome and high reproducibility that allows reliable exchange and standardization of protocols [10]. SSR markers have been widely used in studies of molecular characterization and genetic diversity of different geographical groups of apricot [4, 8, 11].

In order to estimate genetic diversity among apricot cultivars based on DNA polymorphism, several SSR markers were used to evaluate an apricot (*P. armeniaca*) germplasm collection located at the University of Chile in Santiago de Chile (Chile).

Material and Methods

Plant material: In this assay, thirty-one apricot cultivars were evaluated, including one Japanese plum (*P. salicina*) (Red Beaut) and one apricot x plum hybrid (Flavor rich) as outgroup. These cultivars were maintained as part of the germplasm base of the breeding program carried out by the Universidad de Chile (Santiago de Chile, Chile).

DNA extraction: Approximately 0.1 g of young and fresh leaves was used for total genomic DNA extraction [12]. In order to estimate DNA purity and concentration of DNA in the extracts, UV absorbance was measured using a spectrophotometer (Shimadzu UV-1601, Tokyo, Japan). Optical density was recorded at 260 and 280 nm and relationships $A_{260}/A_{280}>1.8 \pm 0.1$ were considered useful [12].

PCR amplification and product electrophoresis: PCR was carried out in a volume of 25 µL containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1 mM of each dNTP, 0.2 µM of each primer, 20 ng genomic DNA and 1 U Taq Polymerase (Invitrogen, Maryland, EEUU). Eleven primer pairs developed for peach [13, 14] were assayed (Table 1). Thermal cycling was performed using a PTC-100 thermocycler (MJ Research Inc., Waltham, USA) and the amplification was conducted following the thermal profile: initial denaturation at 95ºC for 3 min; 35 cycles at 94ºC for 1 min, 57ºC for 1 min and 72ºC for 1 min and a final extension at 72ºC for 10 min [15]. SSR products were separated using polyacrylamide gel electrophoresis (6% polyacrylamide, 7M urea) [16] in 1 X TBE buffer (50 mM Tris, 50 mM boric acid, 1 mM EDTA, pH 8.0) at 3,000 V, stained by silver nitrate.

Data analysis: The presence (1) or absence (0) of amplified fragments was recorded for each cultivar. These data were analyzed with "PC-Ntsys" version 2.02 (Exeter Software, Setauket, US) using the formula derived from the Dice similarity index. Similarity data was processed by UPGMA (Unweighted Pair Group Method with Arithmetic averages) and dendrograms of clustering cultivars studied were constructed [17]. The expected heterozygosity (He) for each locus was calculated according to $He = 1-\Sigma p_i^2$, where "pi" is the frequency of the i^{th} allele. The observed heterozygosity (Ho) was calculated as the number of heterozygous genotypes divided by the total number of genotypes evaluated. For this purpose, Genepop DOS Version 3.3 was used.

Results and Discussion

Molecular characterization and genetic diversity: Seven of the eleven primer pairs analyzed amplified successfully and resulted in 44 polymorphic bands, ranging from 2 (UDP96008) to 8 (UDP98406 and UDP98411) alleles per *locus* with a mean value of 6.1 (Table 2), higher than previously observed values 3.9 [8, 11]. This difference could be explained because we used different SSR and apricot germplasm studied in this case is more heterogeneous than collections studied before; in fact we compared traditional Italian and Spanish cultivars with South African genotypes and new Californian releases among others. The use of polyacrylamide gel for differentiating PCR fragments allowed a higher degree of accuracy, compared with the results with Metaphor® agarose [8, 11]. For example, a difference of 2 bp between 'Orange Red' and 'Búlida' was observed by the amplification of UDP98411, while Sánchez-Pérez *et al*. [8] failed to discriminate both cultivars using the same marker. This lower discrimination capacity of Metaphor® agarose for evaluating genetic diversity was previously reported in almond [18].

SSR used in this study showed a high performance considering the expected heterozygosity index (0.62), confirming the reliable use of SSR markers for genotype identification and genetic diversity studies. The observed heterozygosity ranged from 0.09 (UDP96008) to 0.88 (UDP96003), with an average of 0.62 (Table 2). This average value is very close to 0.63 obtained before [2] and higher than 0.32 obtained by Romero *et al*. [4], both in apricot.

PCR products in some genotypes showed a double pattern (Fig. 1), explained by the addition of dATP at the ends by the DNA polymerase, generating threads of the same DNA segment with different electrophoretic mobility. Those genotypes that showed only one fragment were considered as homozygous for that given *locus*, since it is not possible to detect the presence of null alleles without a segregation analysis. The design of new primers that are able to identify alternative sequences close to SSR could help to amplify lost alleles and would confirm the presence of null alleles [19].

Table 1. SSR markers assayed and sizes of PCR products obtained in the evaluation of genetic diversity in the apricot germplasm collection studied.

Locus code	Primer sequence (5' – 3')	Length (bp)	Reference
UDP96001	AGTTTGATTTTCTGATGCATCC TGCCATAAGGACCGGTATGT	120	Cipriani *et al*. [13]
UDP96003	TTGCTCAAAAGTGTCGTTGC ACACGTAGTGCAACACTGGC	143	Cipriani *et al*. [13]
UDP96005	GTAACGCTCGCTACCACAAA CACCCAGCTCATACACCTCA	155	Cipriani *et al*. [13]
UDP96008	TTGTACACACCCTCAGCCTG TGCTGAGGTTCAGGTGAGTG	165	Cipriani *et al*. [13]
UDP96018	TTCTAATCTGGGCTATGGCG GAAGTTCACATTTACGACAGGG	253	Cipriani *et al*. [13]
UDP97402	TCCCATAACCAAAAAAAACACC TGGAGAAGGGTGGGTACTTG	136	Testolin *et al*. [14]
UDP98405	ACGTGATGAACTGACACCCA GAGTCTTTGCTCTGCCATCC	104	Testolin *et al*. [14]
UDP98406	TCGGAAACTGGTAGTATGAACAGA ATGGGTCGTATGCACAGTCA	101	Testolin *et al*. [14]
UDP98409	GCTGATGGGTTTTATGGTTTTC CGGACTCTTATCCTCTATCAACA	129	Testolin *et al*. [14]
UDP98411	AAGCCATCCACTCAGCACTC CCAAAAACCAAAACCAAAGG	150	Testolin *et al*. [14]
UDP98412	AGGGAAAGTTTCTGCTGCAC GCTGAAGACGACGATGATGA	100	Testolin *et al*. [14]

Since SSR markers utilized were developed for 'Redhaven' peach [14], there is a high degree of homology for the SSR *loci* between peach and apricot and transportability of these markers among *Prunus* species is possible, as observed before [13, 14]. This homology among different species could explain the low level of barriers for intercrossing *Prunus* genotypes, which is useful for breeding purposes [8].

Genetic relationships and implications for apricot breeding: The similarity matrix obtained from the analysis of 7 SSR markers and 44 alleles resulted in a dendrogram that groups all apricot genotypes in the same cluster, widely separated from the plum and the hybrid genotypes that clustered in isolated groups. According to genetic distances obtained within the apricot group, four clusters were identified and were named: Western European (1.1); North American (1.2); Cluster 1.3 including 'Bebeco', 'Tirynthos' and 'Ninfa' and Cluster 1.4, including other North American cultivars (Fig. 2).

The western European cluster includes the French 'Luizet' the Italians 'Pisana' and 'Perla' and the Spanish 'Búlida', which is closely related to 'Haggith'. This association is not easily explained, considering that 'Haggith' is an old and North American genotype with high chilling requirement, which is used mainly as rootstock and lacks remarkable pomological traits. Most of North American cultivars were consistently assigned to a unique Cluster (1.2), which also includes the Italians 'Pelese di Giovanniello', 'San Castrese' and 'Reale di Imola' and the Spanish 'Canino Tardío' (Fig. 2).

The western European or the North American groups grouped together correlated with the classification by Romero and co-workers [4], who studied the genetic diversity of different geographical groups of apricots using SSR. According to the results obtained in this work, a very close association among Western Europe and North American genotypes was observed, suggesting a common genetic background for these cultivars. Furthermore, Badenes *et al*.[20] indicate that North American cultivars originated by hybridization between European and Asian genotypes. In the case of the Californian 'Perfection', it has been widely utilized in breeding programs and has a common genetic background shared by 'Helena', 'Castlebrite', 'Modesto', 'Tilton' and 'Pinkcot', explaining the close relationship among them (Fig. 2).

Cultivars Royal-Blenheim and Patterson show low genetic distance, in which case both are traditional Californian genotypes widely cultivated for industrial purpose, so they should share common ancestrors, even if their pedigree is unknown. They cannot be improved so that they could share common ancestors, even when their pedigree is unknown. However, this closer relationship was also evidenced before in a study of six North American genotypes assessed by SSR [21]. Cluster 1.3 includes the Greeks 'Bebeco', 'Tirynthos' and 'Ninfa'. The last is a rather new and successful Italian cultivar, which originated from a crossing between 'Oardy' and 'Tirynthos', so this association is supported by a strong genetic base. The North American cultivars not included in Cluster 1.2 were grouped together in other cluster (1.4): Robada, Orange Red, Goldcot and Lorna. These cultivars have been recently developed by breeding programs in California.

Table 2. SSR markers successfully amplified, number of polymorphic alleles detected, and variability parameters assessed in the apricot germplasm collection studied.

Locus	Allele	He	Ho
UDP96003	6 (a-f)	0.7380	0.8750
UDP96005	6 (a-f)	0.7123	0.3030
UDP96008	2 (a-b)	0.0881	0.0909
UDP98406	8 (a-h)	0.7331	0.7941
UDP98409	7 (a-g)	0.6611	0.7647
UDP98411	8 (a-h)	0.6041	0.6969
UDP98412	7 (a-g)	0.8149	0.7813

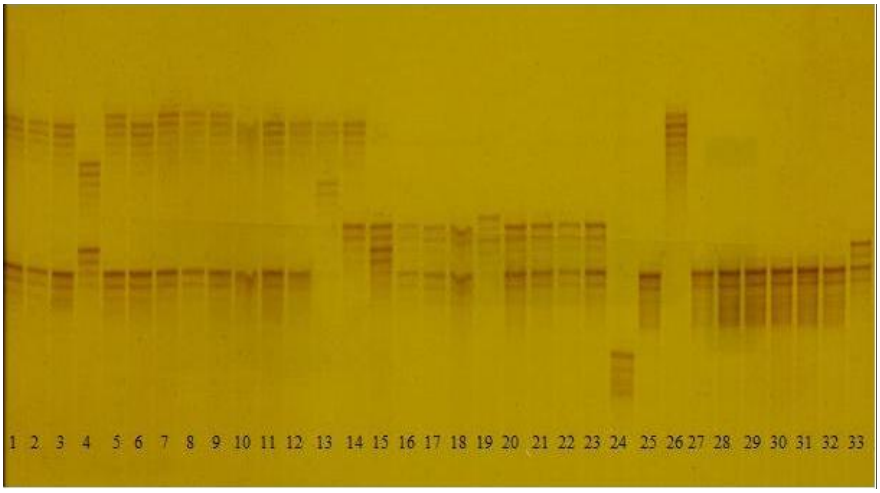

Figure 1. Polyacrylamide gel showing the fragments of apricot cultivars amplified by UDP98409 SSR marker. From left to right: 1 Helena, 2 Pelese di Giovanniello, 3 Bebeco, 4 Búlida, 5 Canino Tardío, 6 Reale di Imola, 7 Pisana, 8 Farmingdale, 9 Veecot, 10 Grandir, 11 Palsteyn, 12 FR 32-4, 13 Robada, 14 Orange Red, 15 Perla, 16 San Castrese, 17 Perfection, 18 Laycot, 19 Red Beaut, 20 Royal-Blemheim, 21 Goldcot, 22 Patterson, 23 Harcot, 24 Flavor Rich, 25 Ninfa, 26 Luizet, 27 Haggith, 28 Pinkcot, 29 Tilton, 30 Castlebrite, 31 Lorna, 32 Goldrich and 33 Tirynthos.

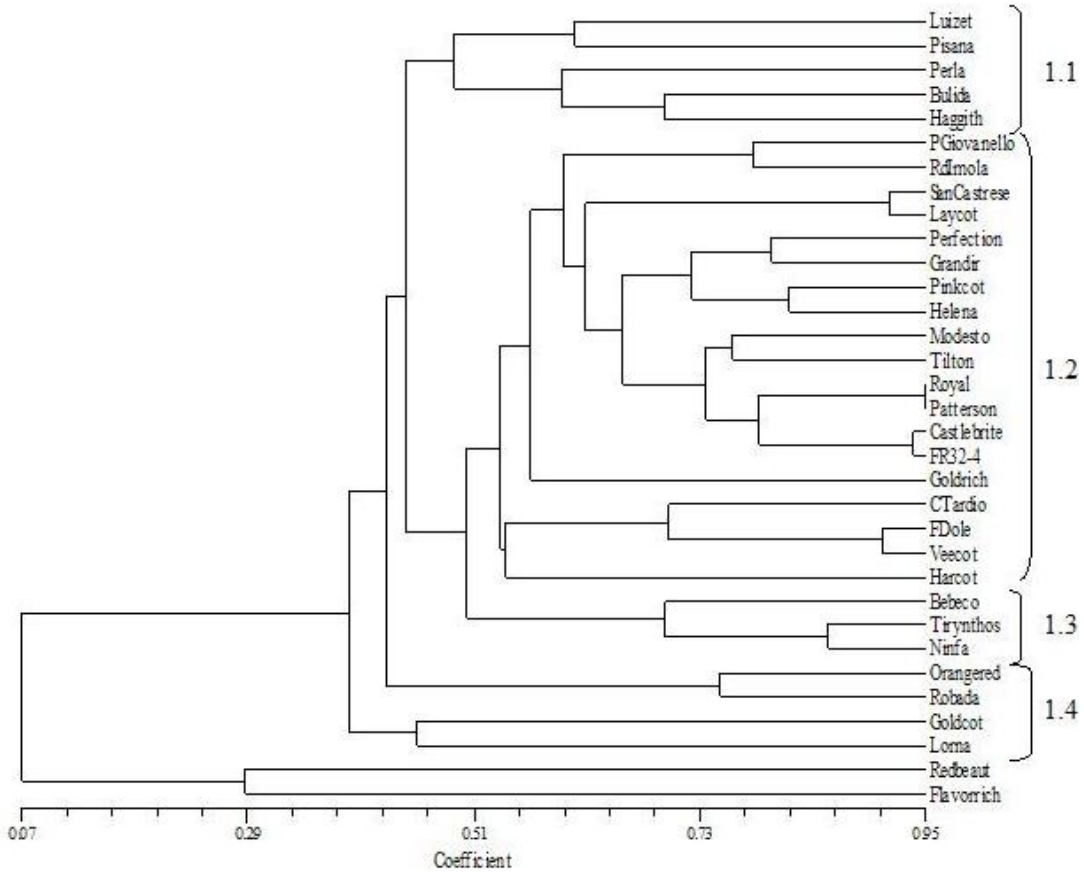

Figure 2. Dendrogram of 33 genotypes of an apricot germplasm collection based on the results of a UPGMA cluster analysis using the data obtained from 7 SSR markers. Red Beaut Japanese plum and the hybrid Flavor Rich were assayed as outgroup.

The majority of the apricot genotypes included in this study, segregated within clusters that are genetically and geographically consistent; nevertheless, there are some groups, which included cultivars from different origins, possibly explained by a trend of free exchange of germplasm among breeding programs around the world, suggesting a common and mixed genetic background for most of the modern apricot cultivars. Furthermore, only little information is currently available about the precise pedigree of cultivars; therefore it is difficult to establish genetic relationships based on genetic origin.

Most of the evaluated cultivars that belong to the European ecogeographical group showed a common genetic base, suggesting that apricot breeding has been mainly based on the hybridization and selection of varieties within the European group. The enrichment of the genetic germplasm [22] of current breeding programs should be encouraged, since there is always a demand for fruits with distinct fruit shapes, colors, aroma and flavors.

Acknowledgements

This research has been supported by the project "CHILECOT: Apricot Quality" D03–I-1070. Authors gratefully acknowledge the support of Dr. Pedro Martínez-Gómez in the final revision of the manuscript.

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