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Genetic diversity in apricot revealed by AFLP markers: species and cultivar comparisons

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Abstract The genetic diversity of apricot (Prunus arme*niaca*; 2n = 16) was studied using AFLP markers. Forty seven apricot cultivars were selected from the following geographic regions: Europe, North America, North Africa, Turkey, Iran and China. Five EcoRI-MseI AFLP primer combinations revealed 416 legible bands, of which 379 were polymorphic markers. A similarity matrix was prepared using the simple matching coefficient of similarity. A UPGMA dendrogram demonstrated a gradient of decreasing genetic diversity of varieties from the former USSR to Southern Europe. This is coherent with the historical dissemination of apricot from its center of origin in Asia. The American cultivars were intermediate demonstrating a different genetic base than the European and/or Mediterranean cultivars. Euclidean distances from the first ten Factorial Component Analysis coordinate axes were used to generate a tree using the Ward algorithm. The results of these analyses were evaluated based on the known geographic origins and agronomic characteristics of the cultivars studied. Four cultivar groups were identified: Diversification, Geographically Adaptable, Continental Europe and Mediterranean Basin. To evaluate the relationship of the common apricot with some closely related species, one or two accessions of the following related species or sub-species from within the section Armeniaca were included in the analysis: Prunus armeniaca var. ansu, Prunus mume, Prunus brigantiaca, Prunus dasycarpa, and Prunus holosericea. A Neighbour Joining dendrogram was made

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U.R. Génétique et Amélioration des Plantes, Bat. 33, INRA-ENSA Montpellier, 2 Place Viala, F-34060 Montpellier Cedex1, France using the similarity matrix. The *P. holosericea* accession fell well within the cultivar group, thus supporting its classification as a variant of *P. armeniaca*. The *P. armeniaca* var. *ansu* accession was sister to the common apricot cluster with a bootstrap value of 96%. *P. mume* was farther removed. *P. brigantiaca* was the most-distant from the common apricots. *P. dasycarpa* was intermediate between *P. brigantiaca* and *P. mume*, in accord with its plum-apricot hybrid origin. The results have a direct application for the selection of new breeding progenitors.

Keywords AFLP · Genetic diversity · Apricot · *Prunus* · *Rosaceae*

Introduction

Apricot belongs to the *Rosaceae* family, subfamily *Prunoideae*, genus *Prunus* L., subgenus *Prunophora* (Neck.) *Focke*. The section *Armeniaca* presently comprises five species (Rheder 1940): *Prunus armeniaca* (known as the common apricot), *Prunus mume* Sieb. and Zucc. (Japanese apricot), *Prunus brigantiaca* Vill. (Alpine plum), *Prunus dasycarpa* Ehrh. (black apricot), and *Prunus holosericea* Batal. (Tibetan apricot). Some separate species were re-classified as botanical varieties of *P. armeniaca*: *P. armeniaca* var. *sibirica* L. (the Siberian apricot), *P. armeniaca* var. *mandshurica* (Maxim) Koehne (Manchurian apricot), and *P. armeniaca* var. *anus* Komar (Ansu apricot) (Bailey 1927). Apricot is diploid (2n=16) and has a small genome size (5.9×10^8 bp/2n) (Arumuganathan and Earle 1991).

Wild apricot trees are found throughout the mountains of the temperate region of Asia (Kostina 1969). Kostina classified apricots into four ecogeographical groups: the Central Asian group, the Dzhungar-Zailij group, the Irano-Caucasian group, and the European group. Vavilov (1992) proposed three centers of origin for apricot: the Chinese Center (mountains of northeastern, central and western China), the Central Asian Center (mountains of Tien-Shan, Hindu Kush to Kashmir), and the Near-Eastern Center [mountains west of the Caspian Sea including the Caucasus (C.E.I.) and mountains of Georgia, Azerbaidjan, Armenia, Turkey and Northern Iran], the latter being a secondary center of diversification (Vavilov 1992). Bailey and Hough (1975) suggested a North Chinese group (Siberian apricot and Manchurian apricot) and an East Chinese group (Ansu apricot).

Apricot was introduced into France through two different routes reflecting two major historical importations: the first being brought by the Arabs through Armenia or North Africa to Southern France in the year 1000, and the second 440 years later for Northern-adapted varieties coming from Hungary and Central Europe (for reviews see Mehlenbacher et al. 1990, and Faust et al. 1998). Because of this, Northern and Southern European cultivars are both cultivated in France, and the French collection appeared well-suited to study the genetic diversity of European apricot cultivars. Cultivars of the European group have been difficult to sub-group morphologically and are thought to have a narrow genetic base (Bailey and Hough 1975; Byrne and Littleton 1989).

Molecular markers have proven very useful to study genetic diversity. The AFLP technique allows one to study many loci and generates highly reproducible markers which are also considered to be locus-specific within a species (Rouppe van der Voort et al. 1997b; Waugh et al. 1997). The AFLP technique has proven its validity and reproducibility (Lin and Kuo 1995; Rouppe van der Voort et al. 1997a; Okano et al. 1998). AFLP markers have recently been used to study genetic diversity at the species level in many plants (Hill et al. 1996; Maughan et al. 1996; Kardolus et al. 1998; Angiolillo et al. 1999; Mace et al. 1999) and at the varietal level (Pakniyat et al. 1997; Singh et al. 1999; Lashermes et al. 2000; Virk et al. 2000).

Previous diversity studies on the common apricot have used isozymes (Byrne and Littleton 1989; Badenes et al. 1996), RFLP markers (de Vicente et al. 1998) and microsatellites (Hormaza 2001). These studies included only the common apricot (P. armeniaca) of primarily European and North American geographic origins. Isozymes and RFLP markers have the advantage of being locus specific, but the number of polymorphic markers available is limited. Hormaza (2001) recently used heterologous Prunus species' microsatellites to study genetic diversity of the common apricot. Hormaza's analysis revealed a North American node and a European node divided into French and Spanish nodes. De Vicente et al. (1998) used 18 polymorphic almond RFLP markers which gave a well-defined Spanish node and several nodes holding mixed North American, French, Italian and Greek cultivars. Byrne and Littleton (1989) studied isozymes on European, Central Asian, North Chinese apricots and their hybrids; however, no dendrogram was produced as few cultivars were identified uniquely. Badenes et al. (1996) using isozymes were able to group the North American cultivars, the Tunisian cultivars and the Spanish-Europeans together, according to like-genotypes.

These studies allowed important conclusions to be made about the genetic diversity of apricot: (1) the European and, more markedly, the Spanish germplasm has reduced genetic diversity and reduced heterozygosity, and that (2) the current North American varieties should no longer be considered as derived primarily from European breeding stock. However, they were not able to address the genetic diversity of apricot on a larger germplasm base because of a lack of representative accessions from more diverse origins (Badenes et al. 1996; de Vicente et al. 1998; Hormaza 2001) or a lack of informative markers (Byrne and Littleton 1989).

Our study addresses two objectives: to compare subspecies or closely related species to the common apricot and to compare cultivars from more-varied origins. To address these objectives, we chose, from the INRA apricot collection, one or two accessions of the closely related species *P. armeniaca* var. ansu, *P. mume*, *P. brig*antiaca, *P. dasycarpa* and *P. holosericea*, and 47 common apricots (*P. armeniaca*) from the following countries: France, USA, Spain, former U.S.S.R, Canada, Tunisia, Morocco, Turkey, Greece, Iran, China and the former Czechoslovakia, and we used a high-throughput marker system to study many loci. The results on apricot genetic diversity will be discussed based on the geographic origins and agronomic characteristics of the cultivars, and on their implications for apricot breeding.

Materials and methods

A total of 50 apricot accessions representing 47 cultivars, one *P. armeniaca* var. *ansu*, one *P. mume*, one *P. brigantiaca*, two *P. dasycarpa* (A880 Marhula Cierna, and D14) and one *P. holosericea* from the Montfavet, INRA collection, were studied. DNA was extracted as per Bernatzky and Tanksley (1986) and/or Lefort and Douglas (1999).

AFLP was carried out essentially as per Vos et al. (1995) and detailed in Saliba-Colombani et al. (2000). The *Eco*RI-*Mse*I primer combinations used were E32-M36, E33-M40, E35-M35, E38-M43 and E46-M40. Autoradiographs were read independently by two people, and bands which had conflicting data between the two readings were eliminated from the analysis.

Data analysis

AFLP polymorphic bands were scored as present (1) or absent (0) on autoradiographs. Two genotypes lacking a band of a certain size were considered to carry the same allele at that locus. Based on a binary matrix with no missing data, we have estimated similarity among all genotypes according to the simple matching coefficient of similarity (Sokal and Sneath 1963). The unweighted pairgroup method with arithmetic averages (UPGMA; Benzecri 1973) and Neighbor Joining algorithms (Saitou and Nei 1987) were used to construct dendrograms. Based on the binary matrix and using defined groups of cultivars, the similarity between the apricot species and the groups of cultivars were assessed using the simple matching coefficient of similarity and Neighbor Joining algorithms. In order to evaluate the robustness of the genetic relationships between the different groups, a bootstrap analysis with 1,000 replicates was performed.

A multiple correspondance analysis (MCA) was performed using the SAS Corresp procedure (SAS Institute 1994). Euclidian distances were calculated on a MCA coordinate matrix for all genotype pairs, and a Ward's minimum variance algorithm was used to construct a dendrogram (Ward 1963). The principle of this algorithm is to cluster genotypes or groups at each step by keeping a maximum value of the ratio intergroup sum of squares/total sum of squares (Saporta 1990; Lebart et al. 1997). A distance/similarity matrix and cluster algorithms were performed with the clustering calculator program developed by John Brzustowski (http://www.biology. ualberta.ca/jbrzusto/cluster.ph). Dendrograms were prepared using Treeview software, version 6.1 (Page 1996).

Results

AFLP markers

Four hundred and fifty bands were read in total. An average of 122.9 bands were read for each accession (range =105 bands, Marouch No. 14, to 152, P. dasycarpa D14). The two independant blind readings identified the most problematic bands (e.g. multiple bands which were very close, and very faint bands). In the present study, 34 out of 450 (7.5%) such bands were eliminated from the data analysis. Of the resulting 416 bands, 37 (8.9%) were present in all 56 accessions (common apricots plus related species). The 379 polymorphic markers remaining were either found only in the common apricots and not in the related species (90 bands), were recorded in the related species but not found in the cultivars (159 bands), were found in both the common apricots and related species (97 bands) or were present in all of the common apricots and variably present in the related species (33) bands). There were 187 polymorphic bands among the 50 cultivar accessions plus P. holosericea. Thus, half of the polymorphic markers (50.6 %) were contributed by the five related-species.

Species comparison

The relationships between the different *Prunus* species closely related to *P. armeniaca* were analyzed using both Jaccard and Simple Matching Coeficient Similarities. The results were similar, and Fig. 1 presents the dendrogram based on the Simple Matching method. *P. holosericeea* fell within the *P. armeniaca* cultivar cluster. *P. armeniaca* var. *ansu*, *P. mume*, *P. dasycarpa* and *P. brigantiaca* were removed from the common apricot cluster via one unique branch. The farthest removed was *P. brigantiaca*. The two *P. dasycarpa*, known to be an apricot by plum hybrid, were found intermediate between *P. brigantiaca* and *P. mume*.

Relationship between cultivars

All of the cultivars had unique AFLP profiles and could be distinguished from each other. The *P. armeniaca* cultivars along with the *P. holosericea* were analyzed separately (Fig. 2). A Ward algorithm was used for clustering based on the Euclidiean distances generated from



Fig. 1 Dendrogram of apricot cultivars and related species constructed by Neighbour Joining based on Simple Matching distances; based on 379 polymorphic markers. The names of the cultivars are not given next to their branches. (Please refer to Fig. 2 for between-cultivar comparisons.) The scale bar represents simple matching distance

the first ten axes of a multiple correspondence analysis. Four clusters (A, B, C and D) were found (Fig. 2a). A UPGMA analysis based on the individual markers is presented in Fig. 2b. Four major nodes were generally conserved between the two trees (A, B, C and D). However, there were some accessions which were classed differently. For example, the three Bergeron accessions were clustered in the Ward dendrogram, but Bergeron A114 did not share the same node with the other two Bergeron accessions in the UPGMA analysis.

Table 1 presents the number of polymorphic markers and the maximum genetic distances within each apricot group as shown in Fig. 2. The maximum distances showed a gradient of decreasing genetic diversity from the D group ($D_{max} = 0.1654$) to the A group ($D_{max} =$ 0.0815). The number of polymorphic markers within the five groups differed (Table 1). The D group had the most polymorphic markers (165). The A group had the fewest polymorphic markers (64).

Relationship between species and cultivar groups

In order to evaluate the robustness of the four groups, we performed a bootstrap analysis which minimizes the variance between cultivars in each group by treating each group as a population (Fig. 3). The bootstrap values indicated that the common apricots grouped together (bootstrap = 99%), and that *P. armeniaca* var. *ansu* was a

Fig. 2 A Dendrogram of apricot cultivars based on Euclidean distances constructed by Ward comprising the ten first axes of a multiple coordinate analysis explaining 54.58% of the total variance. The scale bar represents the Euclidean distance. B Dendrogram of apricot cultivars constructed by UPGMA based on Simple Matching, based on 169 polymorphic markers. The groups are highlighted based on their geographical origins and agronomic characteristics. The scale bar represents simple matching distance. Country of origins (Della Strada et al. 1989) were abbreviated: *Can* = Canada; Cz = former Czechoslovakia; Fr = France; Gr = Greece; Mor = Morocco; Sp = Spain; Sy = Syria; Tun = Tunisia; Tur = Turkey; USSR = formerUSSR



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 Table 1
 Number of markers and maximum genetic distance for each of the four *P. armeniaca* groups as shown in Fig. 2a

Group	Number of	Number and	D _{max} within
	polymorphic	(%) bands	group (simple
	bands within	unique to	matching
	a group	a group	coefficient)
A	64 80	5 (7.8)	0.0815
C	74	8 (10.8)	0.1031
D	165	56 (33.9)	0.1654
All groups	187		0.1654



Fig. 3 Bootstrap re-sampling analysis based on phenotypic and geographical origins of cultivars grouped as in Fig. 2a and related species, based on Simple Matching distances; 1,000 replications were done. The percentage of trees containing the members to the right of the nodes are given above or to the left of each branch. The scale bar represents simple matching distance

sister to the common apricot groups (bootstrap = 96%). However, the cultivar groups were not significantly resolved (Fig. 3).

Discussion

Species comparison

In comparison to the related species, the common apricots were clustered suggesting a common genetic basis. Our results support the Holosericea apricot as being a common apricot rather than a separate species because it shared the same node as the cultivars. The genetic relationships between the related species were founded by high values of the bootstrap analysis. P. mume, P. dasycarpa and P. brigantiaca were removed from the common apricot cluster. Uematsu et al. (1991) demonstrated that the chloroplast DNA restriction pattern of P. mume was different from that of *P. armeniaca*. Shimada et al. (1994) studied the genetic relationship among P. mume cultivars based on RAPD markers and found that all groups were distinct from the Bungo ume group (apricotmume hybrids). Recently, a molecular phylogenetic analysis based on the ITS sequence of nuclear ribosomal DNA placed *P. mume* closer to *P. domestica* than to the P. armeniaca var. mandshurica analyzed (Lee and Wen 2001). Our results also indicate that P. mume is well differentiated from the common apricot. The farthest removed from the common apricots is P. brigantiaca (Fig. 1). It has several morphological differences from the common apricot such as its prune-like fruit, dark bark and inflorescence type. Takeda et al. (1998) using RAPD markers found P. brigantiaca farthest removed from ansu and the common apricot varieties, which is concordant with our results. The P. armeniaca var. ansu was grouped with the common apricots in this analysis with a bootstrap of 96% (Figs. 1 and 3). Indeed, in the analysis done by Takeda et al. (1998) on 35 accessions, the P. armeniaca var. ansu apricots plus the Japanese P. armeniaca cultivars formed a sister clade to the Western apricot group. The two P. dasycarpa, known to be an apricot-plum hybrid, are intermediate between P. brigantiaca and P. mume (Figs. 1 and 3). Likewise, Takeda et al. (1998) found Ren-xing, a supposed P. dasycarpa, intermediate between P. brigantiaca and P. mume. The results of our present study, which was focused primarily on P. armeniaca, are in full agreement with these studies which were focused primarily on P. mume or P. armeniaca var. ansu.

Apricot genetic diversity

The cultivar analysis was based on 187 polymorphic AFLP markers which should be sufficiently large to cover the small genome of apricot, presuming a relatively even distribution over the genome. For AFLPs used in our study, the enzyme EcoRI was chosen as it is relatively insensitive to methylation differences, and EcoRI-*MseI* markers have been shown not to cluster to a large extent in peach (Dirlewanger et al. 1998). The four groups (Fig. 2b) were examined based on the agronomic likeness and geographic origins of the cultivars. The D node includes 4 out of 7 of the accessions from the former USSR and cultivars having heavy chill requirements (e.g. Russian seedlings G1 A1584 a7 and G1 A1584 a16, Badami and Oranzeno-Krasnyj). They are late flowering (e.g. Badami, Stark Early Orange, and Russian seedlings G1 A1584 a7 and G1 A1584 a16). This group is characterised by a large variability for most of the agronomical traits including fruit quality (i.e. Badami with pure white fruit flesh) and pest and disease resistance (e.g. Stark Early Orange sharka resistance, NJA 19-ACLR tolerance). The C group (Fig. 2) includes some related cultivars (see section below on cultivar geneologies). They have few adaptation problems and are grown over a large geographic area (e.g. Colomer, Goldrich, Perfection and Precoce de Tyrinthe). Perfection represents one of two principal genetic resources for the geographic adaptability trait. The B group has 5 out of the 10 French cultivars, with the Czech cultivar Rakovskeho and the former USSR cultivar A. Russe. It groups most of the cultivars from North Europe. These cultivars are characterized by yellow fruit flesh and average to high chilling requirements. The A group holds accessions from Southern France, Spain, Morocco, Tunisia, Greece, Spain, Turkey and Iran. These have average to low chill requirements, and early blooming cultivars such as Bebeco, Hamidi, Moniqui, Rouge du Roussillon and Screara. Taking into account the phenetic analysis, the geographic origin and agronomic characters, we will refer to these groups as A = Mediterranean Basin group, B = Continental European group, C = Geographically Adaptable group and D = Diversification group.

The maximum genetic distances clearly show that the genetic variation present in the Diversification group ($D_{max} = 0.1654$) is greater than in the other groups, reflecting the richness of the material in this area (Table 1). The European genetic base (Mediterranean Basin and Continental Europe) is much narrower as demonstrated by the lower percentages of unique markers. This has been previously suggested by various authors (Bailey and Hough 1975; Byrne and Littleton 1989; de Vicente et al. 1998). Also, it is likely that these cultivars share a common genetic base and are interrelated, which makes classification based on genetic distances difficult (Kraft et al. 2000). The low bootstrap values for the nodes separating the common apricot groups would also be indicative of their being closely related and/or interrelated.

There is a gradient of decreasing genetic diversity of cultivars from East to South-West. For example, cultivars from the former USSR are found in the Diversification and Continental European groups which have higher genetic distances. In contrast, the Spanish cultivars are found almost exclusively in the Mediterranean Basin group. This is primarily due to limited introductions of germplasm (bottlenecks) by man from East to West but may also be due to the method of propagation of apricots. In much of Asia and even in Iran and Turkey, agroforestry with natural seedlings is still practiced and may have preserved genetic diversity. In Europe, on the other hand, clonal production through grafting has been practiced since the 1600s with seedlings used primarily for selection purposes.

The Mediterranean Group includes cultivars from both Spain and North Africa (Fig. 2), and likewise Badenes et al. (1996) found the isozyme *Got-1.1* allele common to Southern European and North African accessions. This would support the hypothesis that Spanish cultivars were derived from North African genotypes brought by the Arabs (Crossa-Raynaud 1961; Egea et al. 1988).

It has been suggested by previous authors (Crossa-Raynaud 1960; Guerriero 1982; Vavilov 1992) that the Near-Eastern Center is a secondary diversification zone. In our study, the Irano-Caucasion accessions included Erevani, Hamidi and Amor Leuch, and the Central Asian cultivars included Badami and Oranzeno Krasnyj. While the small intermediate node of Fig. 2b which groups the Iranian and Armenian cultivars Ordubad, Erevani and Andswee might appear to support this hypothesis, this secondary Armenia/Iran diversification group was not confirmed in the Ward dendrogram (Fig. 2a). Thus, further studies including *P. armeniaca* var. *mandshurica* and *P. armeniaca* var. *sibirica* germplasm are needed to test the relationships between the Central Asian group,

the Irano-Caucasion group, and the Northern and Eastern Chinese groups.

Known cultivar genealogies

There are some cultivars whose parentage is known. Screara was obtained from the cross Rouge du Roussillon \times Delmas = Canino and the three are very close on the tree in the Mediterranean Basin group. Helena du Roussillon is derived from the cross Bergeron × Rouge de Rivesaltes (a seedling of Rouge du Roussillon). Harcot is derived from the cross [(Geneva \times Naramata) \times Morden 604 × (Phelps × Perfection), and Harcot, Phelps and Perfection are in the Geographically Adaptable group. Likewise, the parents of Goldrich are Sun Glo × Perfection, and Goldrich and Perfection share a common node in the Geographically Adaptable group. Royal is a seedling of Nancy, and they are widely separated on the dendrogram; but Royal was obtained at the Jardin du Luxembourg in Paris so it is possible that the pollen donor was distantly related. There are several cultivars which fall within different groups which are surprising (i.e. Amor Leuch, a Tunisan cultivar which falls in the Diversification group and not in the Mediterranean group); Carrascal, which is a Spanish cultivar and was found to be closer to Bebeco or to Moniqui in previous studies (Badenes et al. 1996; de Vicente et al. 1998; Hormaza 2001) while we found it removed from other Spanish cultivars; and the great similarity of Veecot (Canada) and Tokaloglu (Turkey). However, their genealogies are unknown, and it is also possible that some errors within our collection have occurred.

Implications for apricot breeding

In order to obtain a commercial variety in only one generation and to maintain the primary characteristics of European or Mediterranean apricots, the number of characters to recombine must be kept to a minimum, thereby restraining the choice of parents to the Mediterranean and Continental European groups. For example, we have had good success using Bergeron as a breeding progenitor. Also, most of the apricot breeding programs in Europe have been based on the use of local cultivars (Audergon 1995). The number of cultivar registrations attest to their efficiency. Hybridization within a group has been the preferred strategy and has issued forth a number of cultivars. However, to attain certain breeding goals, particularly for disease resistance and novel fruit quality traits, the use of germplasm from different groups and diversification zones will be necessary. Currently, European programs are enlarging their genetic variability by using American cultivars of mostly complex hybrid origins as breeding genitors.

Ecogeographic adaptability has been sought in the past by using Canino (Mediterranean Basin group) or Perfection (Geographically Adaptable group) as breeding progenitors, two sources of geographic adaptability. Canino might readily be used to confer geographic adaptability to the Southern European and Mediterranean groups. However, Canino and the Mediterranean group has a low level of variability. Thus, Perfection and Goldrich could be used to enhance the variability in addition to conferring geographic adaptability. The genetic determinism(s) of the adaptability of Canino and Perfection are unknown but merit further studies to determine if they are distinct or related.

Wild apricots or the closely related species could also be a source of variation useful for plant breeders because within the genus interspecific crosses are usually feasible and efficient in terms of selection pressure. For example, selection for adaptation extremes could be initiated using P. mume for southern/warm regions or P. armeniaca var. ansu for humid regions (Bailey and Hough 1975; Faust et al. 1989). As a consequence, preservation of, and studies evaluating, the disease resistance and fruit quality characteristics of germplasm collections (Badenes et al. 1998; Gurrieri et al. 2001) are essential to future breeding programs. Molecular genotyping of the accessions will aid not only the classification and management of apricot collections but can also be a tool to identify traitlinked markers and potentially promising parental genotypes (Pakniyat et al. 1997). In addition, our selection breeding program at INRA is based principally on controlled pollination between selected parental progenitors in a diallel design. We have chosen as progenitors representatives from each of the major groups reported in this paper (e.g. Stark Early Orange, Goldrich Bergeron and Moniqui among others). We believe this will allow the combination of some major characteristics of each ecogeographical group and thus enrich our breeding program.

We have studied the genetic diversity present in part of our apricot collection. This was a prerequisite for good germplasm conservation and will be useful to improve current plant breeding schemes. This work reinforces the necessity to exchange and include more Asian accessions in addition to closely related species and variants of apricot in European collections, which would better represent the richness in morphological and genetic diversity of apricot.

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