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RESEARCH ARTICLE

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Comparative analysis of traditional and modern apricot breeding programs: A case of study with Spanish and Tunisian apricot breeding germplasm

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Abstract

Traditional plant breeding is based on the observation of variation and the selection of the best phenotypes, whereas modern breeding is characterised by the use of controlled mating and the selection of descendants using molecular markers. In this work, a comparative analysis of genetic diversity in a traditional (Tunisian) and a modern (Spanish) apricot breeding programme was performed at the phenotypic and molecular level using simple sequence repeat (SSR) markers. Seven phenotypic traits were evaluated in 42 Tunisian apricot accessions and 30 genotypes from the Spanish apricot programme. In addition, 20 SSR markers previously described as linked to specific phenotypic traits were assayed. Results showed that modern breeding using controlled crosses increases the size of the fruit. The fruit weight average observed in the Tunisian cultivars was of 20.15 g. In the case of traditional Spanish cultivars the average weight was 47.12 g, whereas the average weight of the other progenitors from France, USA and South Africa was 72.85 g. Finally, in the new releases from the CEBAS-CSIC breeding programme, the average weight was 72.82 g. In addition, modern bred cultivars incorporate desirable traits such as self-compatibility and firmness. Cluster and structural analysis based on SSR data clearly differentiates the genotypes according to their geographic origin and pedigree. Finally, results showed an association between some alleles of PaCITA7 and UDP96003 SSR markers with apricot fruit weight, one allele of UDAp407 marker with fruit firmness and one allele of UDP98406 marker with fruit ripening.

Additional key words: traditional breeding; modern breeding; molecular markers; SSRs; phenotype; association genetic.

Abbreviations used: CEBAS-CSIC (Centro de Edafología y Biología Aplicada del Segura-Consejo Superior de Investigaciones Científicas); FCA (factorial correspondence analysis); Fis (inbreeding coefficient); Fst (fixation index); GD (genetic distances); He (expected heterozygosity); Ho (observed heterozygosity); LG (linkage group); MAF (major allele frequency); MAS (marker assisted selection); NJ (neighbour joining); Nm (mean value of gene flow); nt (nucleotide); PIC (polymorphism information content); PPV (*Plum pox virus*); SSR (simple sequence repeat).

Authors' contributions: Performed the agronomical characterization of the Tunisian germplasm: MAB, LK, HB and NTF Performed the agronomical characterization of the Spanish germplasm: DR, MR. Analyzed the data: MAB and PMG.

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Introduction

Plant breeding programmes involve the application of different genetic techniques in order to obtain new varieties with improved productivity, fruit quality and resistance to existing diseases. Plant breeding is practiced by farmers and by professional plant breeders from different private and public institutions. Traditional breeding, based on the observation of phenotypic variation and the selection of the best phenotypes, was the first approach used around the world. Modern breeding, on the other hand, is characterised by the use of controlled mating and by the subsequent monitoring of the recombination obtained using molecular markers

(Breseghello & Coelho, 2013), although in many species breeding the marker assisted selection (MAS) protocols are still nor on development.

In the case of stone fruit (*Prunus*) species, breeding must address specific challenges arising from the nature of the tree, such as the extended juvenile period and a complex physiology. Traditional *Prunus* breeding based on selection and propagation of the best individuals open-pollinated have been practiced for thousands of years. Nowadays, these traditional methods continue to be the basis for breeding and production in different countries and for many species. In the 1920s, the University of California (USA) had the first modern breeding programme based on controlled pollinations in almond. Breeding programmes began using molecular markers in the early 1990s in characterisation, recombination monitoring and the selection of desirable traits (Martínez-Gómez *et al.*, 2003).

Apricot (*P. armeniaca* L.) breeding in Tunisia involves the application by farmers of traditional methods based on the observation of variation and the selection of the best phenotypes followed by propagation by seeds or grafting (Carraut & Crosse-Raynaud, 1950; Bourguiba *et al.*, 2010). Spanish apricot production, on the other hand, has traditionally been based on local cultivars. Nevertheless, a process of varietal renewal is now underway in Spain with the introduction of new varieties from modern breeding programmes such as the CEBAS-CSIC programme in Murcia. This breeding programme is based on the use of controlled crosses to obtain new populations with desirable traits. Furthermore, the selection for certain traits like self-compatibility (Badenes *et al.*, 2000) or sharka (*Plum pox virus*, PPV) resistance (Rubio *et al.*, 2014) is done on the basis of molecular markers, enabling researchers to select genotypes having those traits of interest in their progenies.

The objective of this work was to perform a comparative analysis of genetic diversity in Spanish and Tunisian apricot germplasm, from a traditional and a modern apricot breeding program respectively, at the phenotypic and molecular level using simple sequence repeat (SSR) markers.

Material and methods

Plant material

The plant material assayed consisted of 42 Tunisian apricot accessions, including 23 cultivars issued from traditional selections and propagated by grafting from the north (Ras Jbel and Testour), the centre (Kairouan), and the south (Gabes, Mareth), and 19 spontaneous accessions of 'Bargougs' (the most common apricot cultivar in the middle of Tunisia) propagated by seeds and issued from the Gafsa Oasis, Degache, Tozeur, Nefta and Midess (Fig. 1 & Table 1). In addition, 30 genotypes from the apricot breeding programme of CEBAS-CSIC in Murcia (Spain) were included in this study, including new releases, advanced selections and progenitors from different geographic areas (Table 2).

Phenotypic evaluation

The following seven agronomic traits of breeding interest were studied: flowering time evaluated every 1-2 days until 50% of the flowers were completely opened (F_{50}) from very early (before February 16) to very late (after March 12); ripening time considered when the fruit had suitable firmness and colour at the commercial maturity stage from early (before May 10) to very late (after June 20); flower compatibility evaluated by bagging as self-compatible when fruit set was presented or self-incompatible with null set (Burgos *et al.,* 1993); fruit weight measured in g, very light (< 20 g), medium (20-40 g), heavy (40-60 g), and very heavy (>80 g); flesh firmness from soft (less than 15 N) to firm (more than 30 N; and skin and flesh colour as determined by a Minolta Chroma Meter (CR-300, Minolta, Ramsey, USA). Pairwise standard genetic distances (GDs) were calculated between groups of cultivars based on the mean character difference of the assayed phenotypic traits constructing an unrooted Neighbour Joining (NJ) tree using DARwin software v.5.0 (Perrier & Jacquemoud-Collet, 2006). In addition, Factorial Correspondence Analysis (FCA) was carried out to provide a synthetic representation of the genetic variability of the studied accessions.

Molecular characterisation and association genetic studies

Total genomic DNA was isolated using an optimized variation of the procedure described by Doyle & Doyle (1987). Extracted apricot genomic DNA was PCR-amplified using 20 primer pairs flanking SSR sequences distributed among the whole genome and previously described as linked to specific phenotypic traits (Table 3). Amplified PCR products were separated by electrophoresis using 3% Metaphor® agarose (Biowittaker, Maine, USA) gel (1 X TBE buffer) stained with GelRed™ Nucleic Acid Gel Stain® (Biotium, Hatwad, CA, USA). A 1 Kb Plus DNA Ladder was used as molecular size standard. Polymorphic alleles were scored

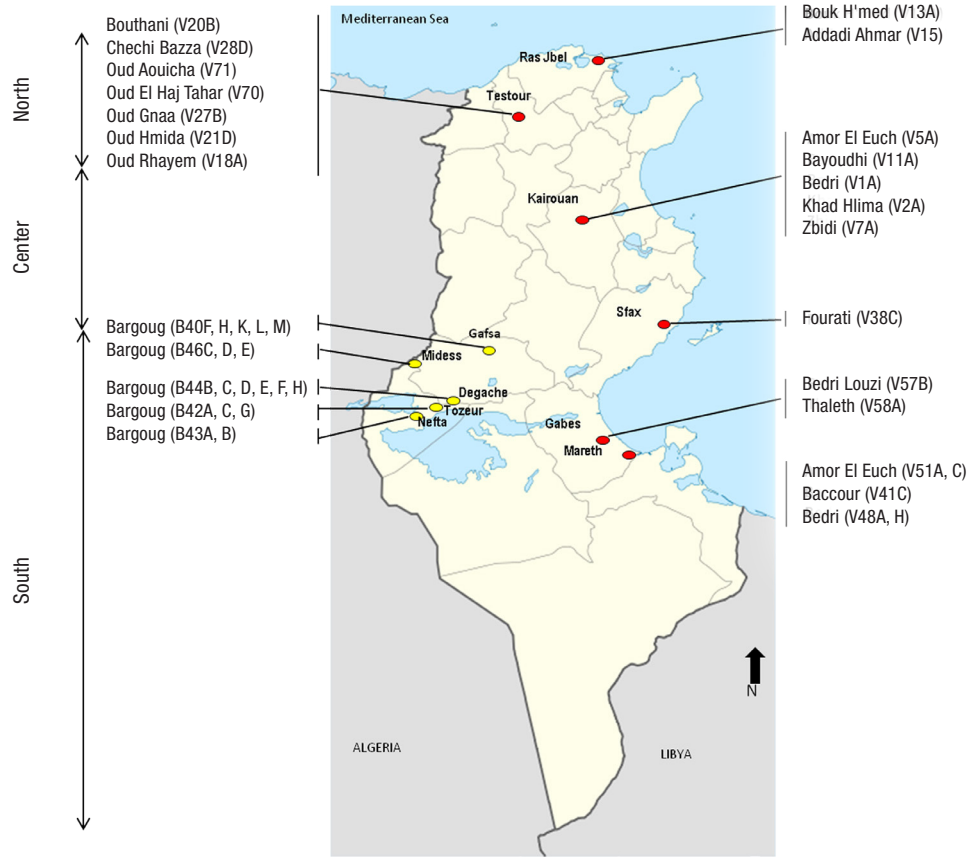

Figure 1. Location of apricot cultivars selected from the Tunisian breeding programme.

as present or absent (1/0). Band scoring was analysed using SYNGENE® GeneTools gel analysis software (Cambridge, UK). The SSR allelic data obtained were used to estimate diversity parameters including the total number of genotypes and alleles, major allele frequency and observed heterozygosity (Ho) as well as expected heterozygosity/gene diversity (He). For this purpose, the software GENETIX® 4.05 (Belkhir *et al.*, 2004) was used. Moreover, GENEPOP 4.0 software (Raymond & Rousset, 1995) was used to calculate Wright's F-statistics. Polymorphism information content (PIC), defined as the parameter for calculating the discriminating and informative power of SSR markers, was calculated for each SSR locus using PowerMarker 3.25 software (Liu & Muse, 2005). Genetic relationships among the defined apricot groups were assessed, genetic distances were calculated and a dendogram was constructed using unrooted NJ analysis with 1000 bootstraps over 20 SSR loci as implemented in DARwin software. Factorial Correspondence Analysis (FCA) was carried out to provide a synthetic representation of the genetic relationship based on molecular markers. SSR allele mining was also conducted on specific alleles associated with specific phenotypic trait expression.

Structure analysis

To infer the genetic structure of the apricot material, a model-based Bayesian clustering method implemented in the STRUCTURE 2.2 program (Pritchard *et al.*, 2000) based on SSR data was used. STRUCTURE was run using a model with admixture and correlated allele frequencies, with the assumed number of genetic clusters (K) ranging from 1 to 10, with 10 independent replicate runs for each K value. Statistical parameters based on the rate of change in the log probability of data between successive K values was calculated to confirm the exact estimation of the most likely number of K clusters using the Structure Harvester application (Earl & von Holdt, 2011).

Results

Phenotypic diversity and phenetic relationships

The study revealed the great phenotypic variability of the studied agronomic traits (flowering-time, ripening time, self-compatibility, fruit weight, flesh firmness,

Table 1. Apricot cultivars selected from the Tunisian breeding programme with their propagation mode and origin. The following seven agronomic traits were studied: self-compatibility, flowering and ripening time, fruit weight and firmness, and skin and flesh colour.

Cultivar	Propagation	Origin	Compatibility[1]	Flowering	Ripening	Weight	Firmness	Skin colour	Flesh colour
Addadi Ahmar V15	Grafting	Ras Jbel	S-C	Medium	Early	Medium	Medium Soft	Yellow Green	Yellow
Amor El Euch V5A	Grafting	Kairouan	S-I	Medium	Early	Medium	Firm	Light Orange	Orange
Amor El EuchV51A	Grafting	Mareth	S-I	Medium	Early	Medium	Medium Soft	Yellow Green	Yellow
Amor El Euch V51C	Grafting	Mareth	S-I	Medium	Early	Very Light	Medium Soft	Yellow Green	Yellow
Baccour V41C	Grafting	Mareth	S-I	Medium	Early	Very Light	Medium Soft	Yellow	White
Bargoug B40F	Seedling	Gafsa	S-I	Medium	Medium	Very Light	Medium Soft	Yellow Green	Yellow
Bargoug B40H	Seedling	Gafsa	S-I	Medium	Medium	Very Light	Soft	Yellow Green	Yellow
Bargoug B40K	Seedling	Gafsa	S-I	Medium	Medium	Very Light	Soft	Yellow Green	Yellow
Bargoug B40L	Seedling	Gafsa	S-I	Medium	Medium	Very Light	Soft	Yellow Green	Yellow
Bargoug B40M	Seedling	Gafsa	S-I	Medium	Medium	Very Light	Soft	Yellow Green	Yellow
Bargoug B43A	Seedling	Nefta	S-I	Medium	Early	Very Light	Soft	Yellow	White
Bargoug B43B	Seedling	Nefta	S-I	Medium	Early	Very Light	Soft	Yellow	White
Bargoug B42A	Seedling	Tozeur	S-I	Early	Early	Very Light	Medium Soft	Yellow	Yellow
Bargoug B42C	Seedling	Tozeur	S-I	Early	Early	Very Light	Medium Soft	Yellow	White
Bargoug B42G	Seedling	Tozeur	S-I	Early	Early	Light	Medium Soft	Light Orange	Light Orange
Bargoug B44B	Seedling	Degache	S-I	Early	Early	Very Light	Medium Soft	Yellow Green	Light Orange
Bargoug B44C	Seedling	Degache	S-I	Early	Early	Very Light	Medium Soft	Yellow Green	Yellow
Bargoug B44D	Seedling	Degache	S-I	Early	Early	Very Light	Medium Soft	Yellow Green	Light Orange
Bargoug B44E	Seedling	Degache	S-I	Early	Early	Very Light	Soft	Light Orange	Light Orange
Bargoug B44F	Seedling	Degache	S-I	Early	Early	Very Light	Soft	Yellow	Yellow
Bargoug B44H	Seedling	Degache	S-I	Early	Early	Very light	Soft	Yellow Green	White
Bargoug B46C	Seedling	Midess	S-I	Early	Early	Very Light	Soft	Yellow Green	Yellow
Bargoug B46D	Seedling	Midess	S-I	Early	Early	Very Light	Soft	Yellow Green	Yellow
Bargoug B46E	Seedling	Midess	S-I	Early	Early	Very Light	Medium Soft	Yellow Green	Light Orange
Bayoudhi V11A	Grafting	Kairouan	S-C	Very Late	Medium	Very Light	Medium Soft	White	White
Bedri V1A	Grafting	Kairouan	S-I	Early	Early	Very Light	Firm	Yellow	White
Bedri V48A	Grafting	Mareth	S-I	Early	Early	Very Light	Soft	Yellow	White
Bedri Thani V48G	Grafting	Gafsa	S-I	Early	Early	Very Light	Soft	Yellow	White
Bedri Louzi V57B	Grafting	Gabès	S-I	Medium	Early	Light	Soft	Yellow Green	Yellow
Bouk H'med V13A	Grafting	Ras Jbel	S-I	Medium	Medium	Light	Medium Soft	Yellow	White
Bouthani V20B	Grafting	Testour	S-C	Late	Early	Light	Firm	Yellow	White
Chéchi Bazza V28D	Grafting	Testour	S-I	Very Late	Late	Light	Soft	White	White
Fourati V38C	Grafting	Sfax	S-I	Medium	Early	Light	Soft	Yellow	White
Khad Hlima V2A	Grafting	Kairouan	S-I	Medium	Early	Light	Medium Soft	Yellow	White
Khad Hlima V2C	Grafting	Kairouan	S-I	Medium	Early	Light	Medium Soft	Yellow	White
Oud Aouicha V71	Grafting	Testour	S-I	Late	Medium	Medium	Medium Soft	Light Orange	Light Orange
Oud El Haj Tahar V70	Grafting	Testour	S-I	Very Late	Medium	Very Light	Soft	Yellow Green	Yellow
Oud Gnaa V27B	Grafting	Testour	S-I	Late	Medium	Medium	Medium Soft	Yellow	Light Orange
Oud H'mida V21D	Grafting	Testour	S-I	Late	Medium	Very Light	Firm	Yellow	Yellow
Oud Rhayem V18A	Grafting	Testour	S-C	Medium	Early	Medium	Medium Soft	Yellow Green	White
Thani V59B	Grafting	Gabes	S-I	Early	Early	Light	Soft	Yellow Green	Yellow
Zbidi V7A	Grafting	Kairouan	S-C	Medium	Medium	Light	Medium Soft	Yellow Green	Yellow

[1] S-C, self-compatible; S-I, self-incompatible

and skin and flesh colour) in Tunisian (Table 1) and Spanish (Table 2) apricot germplasm (Fig. 2). Regarding the self-incompatibility expressed by most Tunisian cultivars (90%) (Table 1), this trait has the advantage of maintaining genetic variability within populations even though it is a negative trait from the agronomic point of view. Self-compatibility is synonymous with higher production. In the case of the Spanish breeding programme, self-incompatibility is present in the North-American cultivars used as PPV resistance progenitors, although self-compatibility has been introduced in the new cultivars (Table 2).

All cultivars showed a wide range of flowering dates from early to very late. In the case of the traditional cultivars from Tunisia, early or late flowering were not key traits for selection (Table 1). In the case of the Spanish germplasm, traditional cultivars like 'Currot' were used in the breeding programme for introducing early flowering, although late flowering is present in the North-American cultivars used as PPV resistance

Table 2. Progenitors and new releases from the Spanish breeding programme at CEBAS-CSIC with pedigree, propagation mode and origin. The following seven agronomic traits were studied: self-compatibility, flowering and ripening time, fruit weight and firmness, and skin and flesh colour.

Cultivar	Pedigree[1]	Propagation	Origin	Compatibility[2]	Flowering	Ripening	Weight	Firmness	Skin colour	Flesh colour
Progenitors										
Búlida	Unknown	Grafting	Spain	S-C	Late	Medium	Medium	Medium Firm	Light Orange	Light Orange
Currot	Unknown	Grafting	Spain	S-C	Early	Early	Light	Medium Soft	White	White
Mauricio	Unknown	Grafting	Spain	S-C	Medium	Medium	Medium	Medium Soft	Yellow	Yellow
Bergeron	Unknown	Grafting	France	S-C	Very Late	Very Late	Heavy	Medium Firm	Light Orange	Orange
Goldrich	Sunglo × Perfection	Grafting	USA	S-I	Late	Late	Very Heavy	Firm	Orange	Orange
Orange Red	Lasg. Mashad × NJA2	Grafting	USA	S-I	Very Late	Medium	Medium	Firm	Orange	Orange
Palsteyn	Blenhein × Canino	Grafting	South Africa	S-C	Early	Medium	Heavy	Medium Firm	Light Orange	Orange
New releases										
Dorada	Bergeron × Moniquí	Grafting	Spain	S-C	Very Late	Very Late	Heavy	Medium Firm	Yellow	Yellow
Estrella	OR × Z211-18	Grafting	Spain	S-I	Medium	Medium	Very Heavy	Firm	Orange	Orange
Maravilla	OR × Z211-18	Grafting	Spain	S-I	Medium	Medium	Very Heavy	Firm	Light Orange	Light Orange
Mirlo Blanco	Rojo Pasión × BP	Grafting	Spain	S-C	Early	Early	Heavy	Medium Firm	Light Orange	Light Orange
Mirlo Naranja	Rojo Pasión × BP	Grafting	Spain	S-C	Early	Early	Heavy	Medium Firm	Orange	Light Orange
Mirlo Rojo	Rojo Pasión × BP	Grafting	Spain	S-C	Early	Early	Heavy	Firm	Light Orange	Light Orange
Murciana	OR × Currot	Grafting	Spain	S-C	Late	Medium	Heavy	Firm	Light Orange	Orange
Rojo Pasión	OR × Currot	Grafting	Spain	S-C	Medium	Medium	Heavy	Medium Soft	Light Orange	Light Orange
Rosa	OR × Palsteyn	Grafting	Spain	S-I	Medium	Medium	Very Heavy	Firm	Light Orange	Orange
Selene	Goldrich × A2564	Grafting	Spain	S-C	Late	Late	Heavy	Firm	Orange	Orange
Sublime	OR × Z211-18	Grafting	Spain	S-I	Medium	Medium	Very Heavy	Firm	Orange	Orange
Toñi	OR × Palsteyn	Grafting	Spain	S-C	Medium	Early	Very Heavy	Medium Soft	Light Orange	Orange
Valorange	OR × Currot	Grafting	Spain	S-C	Medium	Medium	Very Heavy	Firm	Orange	Orange
5-57	(OR × Currot) × BP	Grafting	Spain	S-I	Early	Early	Heavy	Medium Firm	Orange	Light Orange
7-41	Rojo Pasión × BP	Grafting	Spain	S-C	Early	Early	Heavy	Firm	Orange	Light Orange
8-50	OR × TB	Grafting	Spain	S-I	Very Late	Very Late	Heavy	Medium Firm	Orange	Orange
8-61	OR × TB	Grafting	Spain	S-C	Late	Late	Heavy	Medium Firm	Orange	Orange
9-5	OR × TB	Grafting	Spain	S-C	Very Late	Very Late	Heavy	Medium Firm	Orange	Orange
9-55	OR × TB	Grafting	Spain	S-I	Very Late	Late	Heavy	Medium Firm	Orange	Orange
10-18	OR × TB	Grafting	Spain	S-I	Very Late	Very Late	Very Heavy	Firm	Orange	Orange
10-20	OR × TB	Grafting	Spain	S-C	Very Late	Late	Heavy	Medium Firm	Orange	Orange
10-57	(Goldrich × TB) × OP	Grafting	Spain	S-I	Very Late	Very Late	Heavy	Medium Firm	Orange	Orange
11-1	(Goldrich × TB) × OP	Grafting	Spain	S-I	Very Late	Very Late	Heavy	Medium Firm	Orange	Orange

[1]OR, Orange Red; BP, Búlida Precoz; TB, Tardif de Bordaneil; OP, open pollination. [2]S-C, self-compatible; S-I, self-incompatible.

progenitors ('Orange Red', 'Goldrich'). In addition, most of the Tunisian apricot accessions showed early or medium ripening time (Table 1), whereas the Spanish genotypes showed a wide range of maturity dates, from early to very late (Table 2). In the Spanish breeding programme, both early and late ripening progenitors were used in the crosses in order to release new varieties covering the entire production time from the beginning of May to late June.

The fruit weight average (20.15 g) observed in the Tunisian cultivars was lower than that of the germplasm evaluated in Spain. In the case of traditional Spanish cultivars used as progenitors ('Currot', 'Mauricio', 'Búlida'), the average weight was 47.12 g, whereas the average weight of the other progenitors from France, USA and South Africa was 72.85 g. Finally, in the new releases from the CEBAS-CSIC breeding programme, the average weight was 72.82 g. Most of the Tunisian apricot cultivars showed soft or medium-soft fruits (Table 1). In the case of the Spanish collection, traditional cultivars used as progenitors were mainly characterised by medium-soft fruits. The North American cultivars used as progenitors in the Spanish breeding programmes, however, had firm fruit (Table 2). Most of the new releases resulting from the crosses performed at CEBAS-CSIC had medium-firm or firm fruits. On the other hand, most Tunisian apricot cultivars showed yellow or yellow green colouring in the

Table 3. Origin of the 20 SSR markers tested and relationships with the phenotypic traits. Linkage group (LG), genetic position in centimorgans (cM), physical location in the *Prunus* reference genome (Peach v1.0), trait linkage and references.

Marker	Species	Reference	LG	Position (cM)	Location Peach v1.0	Trait	References
BPPCT025	Peach	Dirlewanger *et al.* (2002)	6	56.4[1]	Scaffold_6:21129947	Skin colour	Salazar *et al.* (2013)
CPPCT022	Peach	Aranzana *et al.* (2002)	7	18.6[1]	Scaffold_7:10225365	Flowering time	Fan *et al.* (2010)
CPSCT005	Plum	Mnejja *et al.* (2004)	4	93.6[2]	Scaffold_4:29887942	Flowering time	Salazar *et al.* (2013)
						Ripening time	Salazar *et al.* (2013)
PaCITA7	Apricot	Lopes *et al.* (2002)	1	25.9[2]	Scaffold_1: --	Flowering time	Salazar *et al.* (2013)
						Fruit weight	Salazar *et al.* (2013)
pchcms5	Peach	Sosinski *et al.* (2000)	6	44.7[1]	Scaffold_6:19166407	Self-compatibility	Vilanova *et al.* (2003)
PGS.121	Apricot	Soriano *et al.* (2012)	1	23.9[2]	Scaffold_1:8078385	Flesh colour	Salazar *et al.* (2013)
UDA027	Almond	Testolin *et al.* (2004)	4	74.7[1]	Scaffold_4:18639695	Ripening time	Salazar *et al.* (2013)
UDAp404	Apricot	Messina *et al.* (2004)	4	9.3	Scaffold_4: --	Ripening time	Salazar *et al.* (2013)
UDAp407	Apricot	Messina *et al.* (2004)	7	41.3	Scaffold_7: --	Flowering time	Salazar *et al.* (2013)
UDAp439	Apricot	Messina *et al.* (2004)	4	49.6	Scaffold_4: --	Ripening time	Salazar *et al.* (2013)
UDAp449	Apricot	Messina *et al.* (2004)	3	42.5[2]	Scaffold_3: --	Skin colour	Ruiz *et al.* (2010)
UDAp460	Apricot	Messina *et al.* (2004)	7	28.4	Scaffold_7: --	Flowering time	Fan *et al.* (2010)
UDAp471	Apricot	Messina *et al.* (2004)	7	51.3[2]	Scaffold_7: --	Nut weight	Fernández *et al.* (2011)
						Ripening time	Fernández *et al.* (2011)
UDP96001	Peach	Cipriani *et al.* (1999)	6	17.5[1]	Scaffold_6:7040757	Skin colour	Verde *et al.* (2002)
UDP96003	Peach	Cipriani *et al.* (1999)	4	28.3[1]	Scaffold_4:8757450	Ripening time	Salazar *et al.* (2013)
						Fruit weight	Salazar *et al.* (2013)
UDP98021	Peach	Cipriani *et al.* (1999)	6	9.8[2]	Scaffold_6: --	Fruit weight	Eduardo *et al.* (2011)
						Skin colour	Eduardo *et al.* (2011)
UDP98025	Peach	Testolin *et al.* (2000)	2	9.6[1]	Scaffold_2:10872102	Fruit weight	Eduardo *et al.* (2011)
UDP98406	Peach	Testolin *et al.* (2000)	2	80.2[2]	Scaffold_1: --	Ripening time	Eduardo *et al.* (2011)
UDP98412	Peach	Testolin *et al.* (2000)	6	72.00[1]	Scaffold_6:24753353	Ripening time	Salazar *et al.* (2013)
UDP98416	Peach	Testolin *et al.* (2000)	6	10.6[2]	Scaffold_6: --	Ripening time	Verde *et al.* (2002)

[1]In *Prunus* reference Map Texas × Early Gold. [2]In the linkage map of the study

skin (Table 1). Regarding the genotypes from the Spanish collection, the cultivars used as progenitors are characterised by light orange or orange skin colour with the exception of 'Currot' (white) and 'Mauricio' (yellow). Most of the new releases included in this study resulting from the CEBAS-CSIC breeding program showed orange fruits (Table 2).

Finally, all Tunisian apricot cultivars showed white, yellow or light orange flesh colour with only one exception ('Amor El Euch V5A'), which showed orange flesh (Table 1). The traditional Spanish cultivars used as progenitors ('Currot', 'Mauricio' and 'Búlida') were characterised by white, yellow and light orange flesh, respectively, whereas the foreign cultivars used as progenitors in the Spanish breeding programmes produced orange fleshed fruits. Most of the new releases included in this study resulting from the crosses performed at CEBAS-CSIC had orange-fleshed fruits (Table 2).

Results of the cluster analysis revealed clear differences between the cultivars studied, which pooled into three main groups. Group I contained 16 accessions from the Spanish collection, including seven cultivars ('Currot', 'Goldrich', 'Mirlo Rojo', 'Valorange', 'Murciana', 'Selene', and 'Orange Red') and nine selections ('8-50', '11-1', '5-57', '9-5', '9-55', '10-18', '8-61', '10-2' and '7-41'). Group II contained 29 accessions, including 15 accessions from Tunisia (5 grafted and 10 propagated by seed) and the rest of the CEBAS-CSIC collection (14 accessions). This second group could be divided into three subgroups: II A (9 Tunisian accessions); II B (4 Tunisian and 4 Spanish accessions); and II C [10 Spanish and 2 Tunisian accessions ('Oud Aouicha V71' and 'Oud Gnaa V27B')]. The subgroups II B and II C contained accessions from the north and the centre of Tunisia, but none from the south. Finally, Group III included 27 accessions from Tunisia. This group could be divided into the following three subgroups: III A, which exclusively contained 'Bargougs' accessions [44 (B, C, D, E); 46 (C); and 42 (A, C, G)]; III B, which mainly included accessions from the south of Tunisia in addition to 'Oud El Haj Tahar V70' from Testour in the north (Fig. 1); and subgroup III C, which included all the remaining accessions from Tunisia (Fig. 3A).

In addition, the FCA analysis showed that the accessions studied were highly diverse in terms of phenotype. There were clear, closely-related groups of accessions, and these groupings were related to geographic origin: Group I is made up of Tunisian accessions and Group II consists of the CEBAS-CSIC collection. There were two main subgroups among the

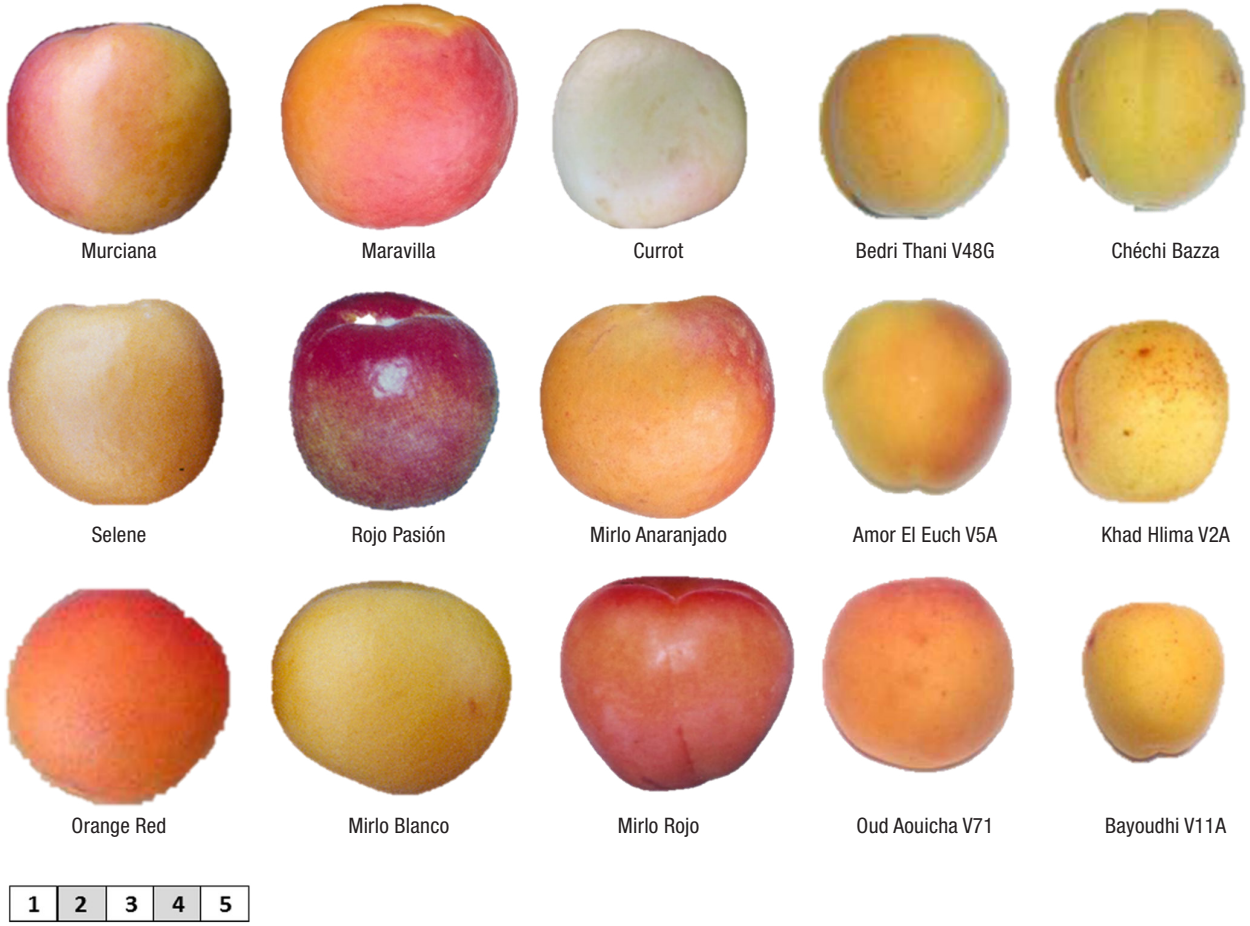

Figure 2. Fruits from some of the apricot cultivars and accessions evaluated. Scale bar of 5 cm.

Tunisian accessions: accessions from the north and the centre of Tunisia in the first subgroup (indicated in green and brown respectively in Fig. 3B), and accessions from the south and the oases of Tunisia in the second subgroup (indicated in blue). There was no clear grouping among varieties in the Spanish collection. FCA results also showed that apricot material in Tunisia was less diversified than the Spanish apricot material (in black in Fig. 3B).

Molecular diversity and association genetic studies

Amplification of SSR loci was successful with the 20 tested primers. Polymorphic bands were generated for all the primers used, with a total of 60 scored polymorphic bands. The number of presumed alleles revealed by the 20 SSRs ranged from two (BPPCT025, PaCITA7, pchcms5, UDA027, UDAp407, UDAp449, UDAp460, UDP96001, UDP98025 and UDP98416) to ten (PSG1.21), with a mean of three alleles per locus (Table 4). The average PIC value for the 20 loci was 0.410. The most polymorphic locus was PSG1.21 (0.833) and the least polymorphic was UDP98025 (0.040) (Table 4).

The 20 SSR markers assayed for genetic diversity studies generated a total of 110 genotypes with an average of 6 genotypes per marker. Mean expected heterozygosity (He) was about 0.48 with maximum He values recorded by SSR markers for PSG1.21 (0.851) and minimum He values recorded by SSR markers for UDP98025 (0.041). Observed heterozygosis (Ho) ranged from 0.000 (UDP96001) to 0.831 (UDAp471), with a mean value of 0.359. A significant heterozygosity deficit was observed for four loci (UDP96001, UDP98025, UDP98416 and UDAp449), however, for the rest of the SSR markers studied, no significant difference was found between the expected and the observed heterozygosity (Table 4). On the other hand, for the Wright's fixation index, the calculated Fst values ranged from 0.002 (UDA027) to 0.518 (UDAp407), with an average of 0.141. Fis values ranged from -0.22 (UDAp471) to 1 (UDP96001), with an average of 0.20 (Table 4).

Table 5 shows the SSR alleles obtained with a high degree of co-segregation within the phenotypes of the

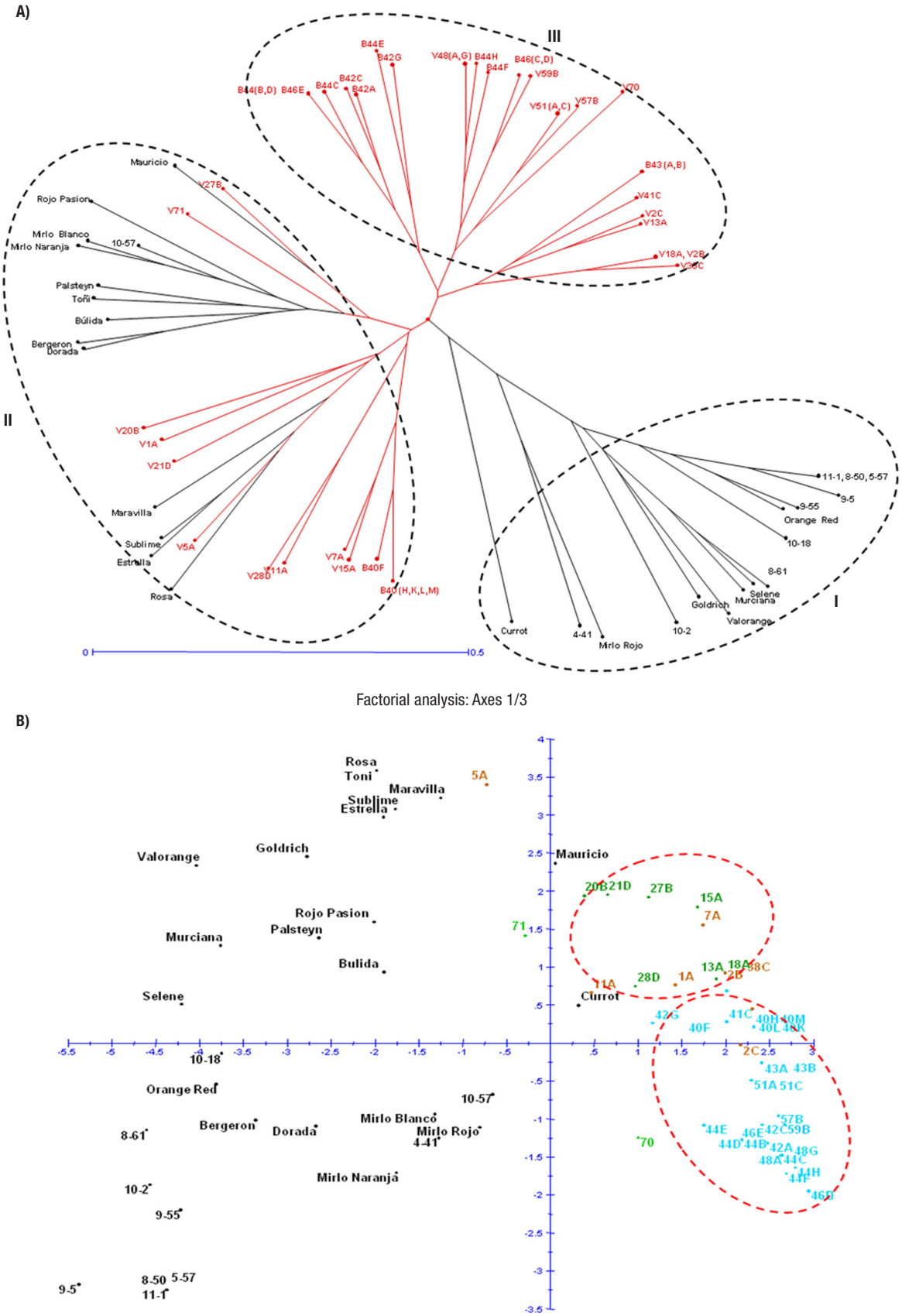

Figure 3. Unrooted NJ (Neighbour Joining) dendogram based on the mean character difference distances, Spanish apricot germplasm is indicated in black colour and Tunisian apricot germplasm in red (A). Genetic structure based on FCA analysis (B) among apricot cultivars evaluated using phenotypic traits; clearly identified subgroups of Tunisian apricot germplasm indicated in green, brown, blue; and Spanish accessions (black).

Table 4. Molecular diversity parameters of the SSR loci used in the apricot genetic analysis. Number of samples (N), number of samples that amplify (n), number of genotypes found (Genotypes), number of alleles amplified (Alleles), major allele frequency (MAF), expected heterozygosity (He), observed heterozygosity (Ho), polymorphism information content (PIC), fixation index (Fst), mean value of gene flow (Nm), and inbreeding coefficient (Fis).

Marker	N	n	Genotypes	Alleles	MAF	He	Ho	PIC	Fst	Nm	Fis
BPPCT025	72	67	3	2	0.619	0.471	0.433	0.360	0.005	48.77	0.09
CPPCT022	72	71	9	6	0.437	0.686	0.479	0.631	0.163	1.29	0.24
CPSCT005	72	72	6	3	0.451	0.646	0.472	0.573	0.252	0.74	0.15
PaCITA7	72	72	3	2	0.785	0.338	0.264	0.281	0.466	0.29	-0.11
pchcms5	72	69	3	2	0.681	0.434	0.261	0.340	0.021	11.71	0.41
PSG1.21	72	72	25	10	0.188	0.851	0.764	0.833	0.091	2.49	0.07
UDA027	72	72	3	2	0.715	0.407	0.403	0.324	0.002	103.92	0.02
UDAp404	72	70	6	3	0.721	0.440	0.257	0.398	0.214	0.92	0.34
UDAp407	72	71	3	2	0.521	0.499	0.338	0.375	0.518	0.23	-0.03
UDAp439	72	67	6	3	0.552	0.586	0.597	0.515	0.048	5.01	-0.04
UDAp449	72	72	2	2	0.972	0.054	0.056	0.053	0.013	19.59	-0.02
UDAp460	72	70	3	2	0.771	0.353	0.286	0.290	0.385	0.40	-0.05
UDAp471	72	71	9	4	0.458	0.684	0.831	0.633	0.022	11.06	-0.22
UDP96001	72	72	2	2	0.528	0.498	0.000	0.374	0.023	10.48	1.00
UDP96003	72	69	6	3	0.645	0.517	0.449	0.459	0.124	1.77	0.08
UDP98021	72	72	6	3	0.639	0.508	0.458	0.438	0.073	3.17	0.07
UDP98025	72	72	2	2	0.979	0.041	0.042	0.040	0.046	5.15	-0.04
UDP98406	72	71	5	3	0.493	0.571	0.451	0.479	0.054	4.40	0.20
UDP98412	72	72	5	3	0.479	0.556	0.292	0.457	0.049	4.89	0.47
UDP98416	72	71	3	2	0.634	0.464	0.056	0.356	0.014	18.13	0.88
Mean	72	71	6	3	0.613	0.480	0.359	0.410	0.141	1.53	0.20

different apricot genotypes assayed. Only UDP98406 (Eduardo *et al.*, 2011) showed a good degree of linkage with ripening time. A total of 86% of the germplasm studied with the allele 118 had an early or a medium ripening date and only 14% had a late or very late ripening date.

Only the two markers showed good linkage with fruit weight. When the genotypes were separated into two groups of fruit weight (> or < 60 g), we could observe that the allele 434 (PaCITA7) was present in all genotypes with fruits weighing less than 60 g (a total of 25 genotypes). The alleles 391 (PaCITA7) and 114 (UDP96003) were present in most genotypes with fruits weighing over 60 g (Table 5). The presence of the allele of 449 nucleotides (nt) from the marker UDAp407 has been correlated with fruit firmness (Table 5). About 90% of accessions with the allele 449 present were firm or medium-firm, and only 6% were soft.

Molecular heterozygosity, relationships and structure analysis

The genetic heterozygosity of apricot cultivars evaluated using SSR markers ranged from 0.050 ('Bedri V48A') to 0.600 ('9-5' and 'Valorange'), with an average value of 0.39 (Table 6). The group of traditional cultivars from Tunisia and Spain showed lower genetic heterozygosity, which was independent of the propagation mode. The genetic heterozygosity ranged between 0.050 (Tunisian cultivar 'BedriV48A') and 0.650 (CEBAS-CSIC selections '7-41' and '8-50'), with an average value of 0.55 for all the studied genotypes (Table 6).

The 72 genotypes were clearly classified into three major groups based on their molecular characterisation using SSRs, A, B and C (Fig. 4). Group A contained 27 Spanish and 5 Tunisian genotypes; group B mainly

Table 5. SSR alleles obtained with the highest level of co-segregation within phenotypes of the different apricot genotypes assayed

Marker	SSR allele (nt) [1]	Percentage of a given phenotype	
		Ripening time	
		Late/very late	Early/medium
UDP98406	118	14	86
		Fruit weight	
		Small (<60 g)	Big (>60 g)
PaCITA7	391	6	94
	434	100	0
UDP96003	114	6	94
		Fruit firmness	
		Firm/medium	Soft
UDAp407	449	90	6

[1] Band scoring was analysed using SYNGENE® GeneTools gel analysis software.

Table 6. Genetic heterozygosity (Ho) observed in the different apricot genotypes assayed from the Tunisian and Spanish (CEBAS-CSIC) breeding programmes using 20 SSR markers.

Genotype	Ho	Genotype	Ho	Genotype	Ho
Cultivars selected from the Tunisian breeding programme					
Addadi Ahmar V15	0.368	Bargoug B42G	0.250	Bedri Louzi V57B	0.150
Amor El Euch V5A	0.368	Bargoug B44B	0.400	Bouk H'med V13A	0.350
Amor El EuchV51A	0.400	Bargoug B44C	0.368	Bouthani V20B	0.350
Amor El Euch V51C	0.350	Bargoug B44D	0.250	Chéchi Bazza V28D	0.400
Baccour V41C	0.316	Bargoug B44E	0.350	Fourati V38C	0.350
Bargoug B40F	0.316	Bargoug B44F	0.316	Khad Hlima V2A	0.211
Bargoug B40H	0.389	Bargoug B44H	0.300	Khad Hlima V2C	0.222
Bargoug B40K	0.400	Bargoug B46C	0.400	Oud Aouicha V71	0.200
Bargoug B40L	0.200	Bargoug B46D	0.350	Oud El Haj Tahar V70	0.300
Bargoug B40M	0.250	Bargoug B46E	0.300	Oud Gnaa V27B	0.350
Bargoug B43A	0.200	Bayoudhi V11A	0.250	Oud H'mida V21D	0.100
Bargoug B43B	0.350	Bedri V1A	0.053	Oud Rhayem V18A	0.400
Bargoug B42A	0.263	Bedri V48A	0.050	Thani V59B	0.158
Bargoug B42C	0.450	Bedri Thani V48G	0.053	Zbidi V7A	0.222
Mean	0.286				
Spanish progenitors from the Spanish breeding programme (CEBAS-CSIC)					
Búlida	0.250	Currot	0.368	Mauricio	0.300
Mean	0.306				
Foreign progenitors from the Spanish breeding programme (CEBAS-CSIC)					
Bergeron	0.421	Goldrich	0.550	Orange Red	0.300
Palsteyn	0.550	Mean	0.455		
New releases from the Spanish breeding programme (CEBAS-CSIC)					
Dorada	0.474	Rosa	0.500	8-61	0.474
Estrella	0.474	Selene	0.400	9-5	0.600
Maravilla	0.450	Sublime	0.450	9-55	0.500
Mirlo Blanco	0.526	Toñi	0.450	10-18	0.450
Mirlo Naranja	0.450	Valorange	0.600	10-20	0.550
Mirlo Rojo	0.400	5-57	0.526	10-57	0.350
Murciana	0.450	7-41	0.650	11-1	0.250
Rojo Pasión	0.500	8-50	0.650	Mean	0.550

contained Tunisian genotypes; and group C contained nearly all the remaining Tunisian genotypes in addition to three of the traditional Spanish cultivars. Group A could be divided into two subgroups, with the first subgroup containing all the Spanish genotypes. The second subgroup included five 'Bargougs' propagated by seeds and originating from the oasis of Gafsa in the south-west of Tunisia. In Group B, the distribution of Tunisian cultivars in secondary subgroups was related to geographic origin, so that genotypes from the north and the south clustered together and 'Bargougs' clustered separately. Group C included the rest of the Tunisian cultivars and three traditional cultivars from Spain ('Currot', 'Búlida' and 'Mauricio'), which were related to the cultivars 'Baccour V41C' and 'Amor El Euch V51C' from the south of Tunisia as well as to one accession of 'Bargoug B46D' from the oasis of Midess.

Finally, using model-based Bayesian clustering, the estimated log probability of the data (ln Pr (X|K)), given the assumed number of ancestral populations K, was highest at K=2. As defined by Evanno *et al.* (2005), an *ad hoc* quantity based on the second order rate of change of the likelihood function with respect to K (ΔK) showed that the best model is at K=2 (Figure 5). According to the model at K=2, apricot genotypes were assigned to two genetically different clusters identified by STRUCTURE analysis: cluster 1 grouped together all the Tunisian cultivars (42 cultivars) with three accessions from Spain ('Currot', 'Búlida' and 'Mauricio'). The second cluster was made up of the Spanish collection (27 cultivars). The model under K=3 classified the 72 apricot genotypes into the following three clusters: the first cluster included 27 Spanish cultivars; the second cluster included six cultivars from Tunisia; and the third cluster included three cultivars from the Spanish breeding programme ('Currot', 'Búlida' and 'Mauricio') and 36 Tunisian cultivars (Fig. 5).

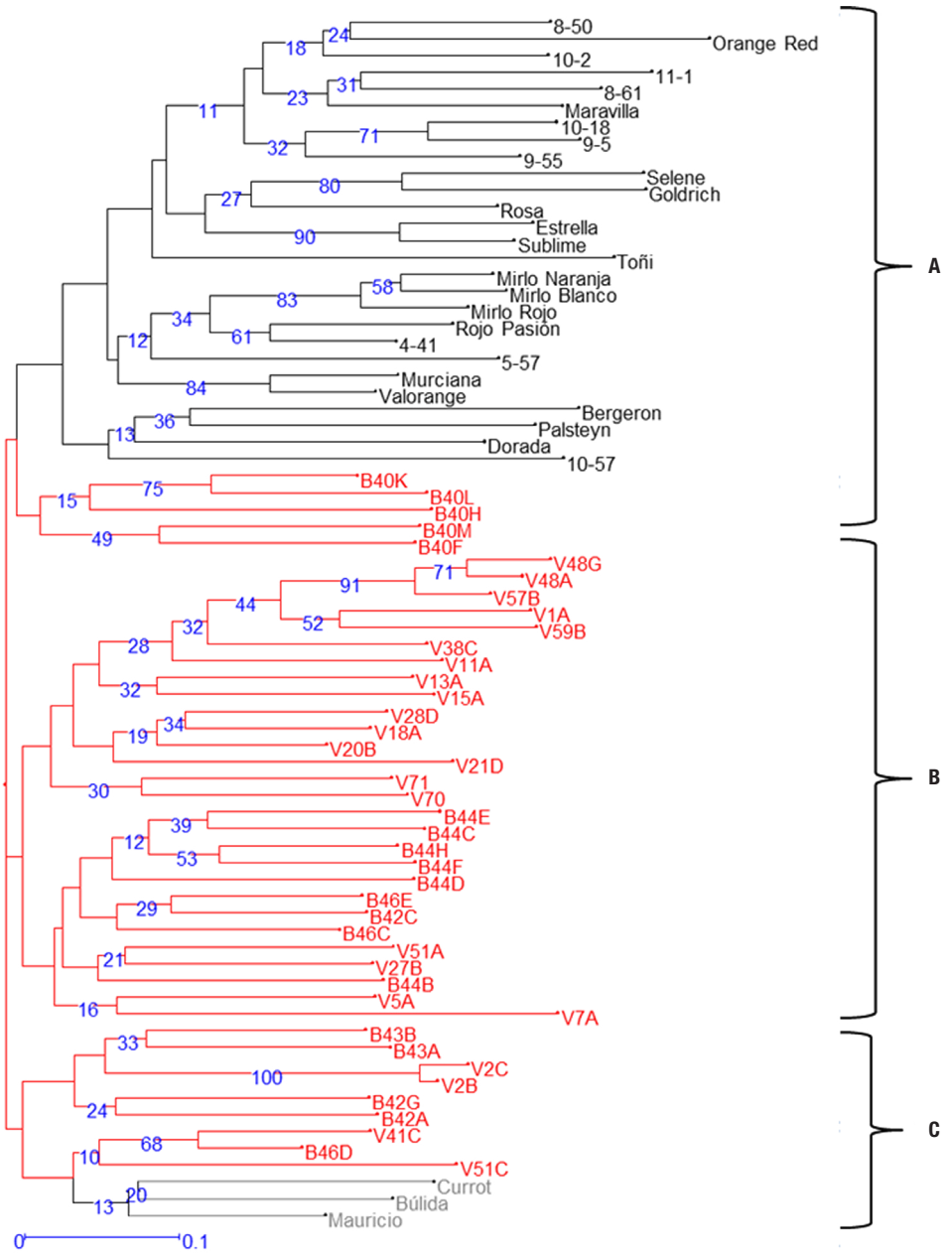

Figure 4. Dendogram obtained by NJ (Neighbour Joining) cluster analysis among 72 apricot genotypes evaluated using 20 SSR markers. Capital letters indicate the main cluster observed. In black Spanish germplasm and in red Tunisian germplasm. Numbers below branches represent bootstrapping values (1,000 replicates).

Discussion

Phenotypic diversity and phenetic relationships

The majority of the traditional apricot cultivars assayed (both Spanish and Tunisian) were identified as self-incompatible. These results, however, contrast with evaluations of Moroccan apricot germplasm. In fact, Kodad *et al.* (2013) identified 65% of Moroccan apricot cultivars as self-compatible. Taking into account the fact that self-compatibility is not widely distributed in the traditional Tunisian cultivars, it seems that this trait has been selected for propagation in new cultivation areas in Tunisia, as occurred in Spain in the past. In addition, this will be one of the most important trait in the design of new crosses always with self-compatible cultivars in order to incorporate this trait. The high percentage of self-compatibility found in the Moroccan apricot cultivars can support the theory of positive selection for self-compatibility from apricot cultivars originating from Tunisia.

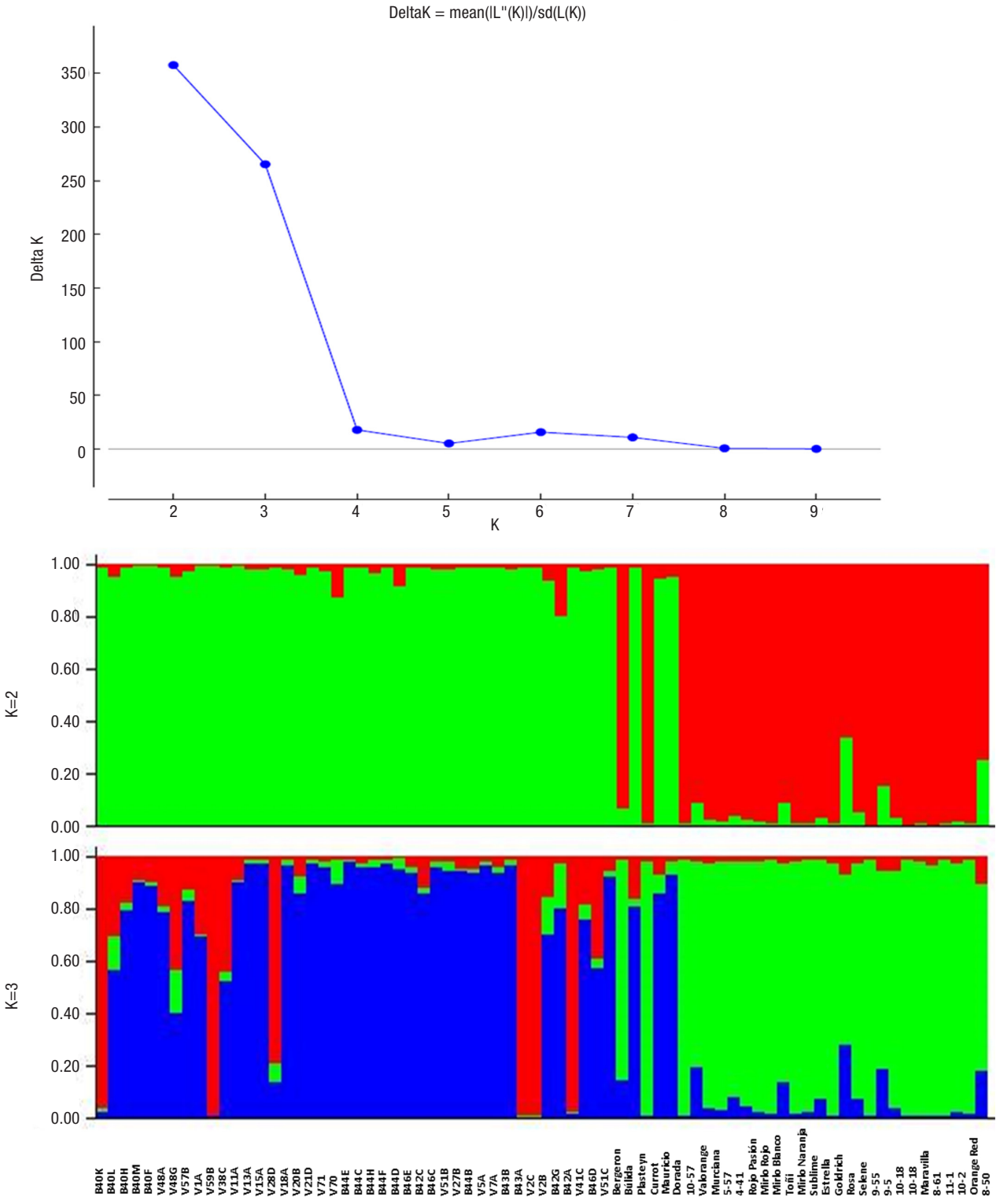

Figure 5. Model-based Bayesian clustering among apricot cultivars evaluated. Similar colour indicate similar cluster for the assayed genotype.

Most of the Tunisian accessions were characterised by early or medium flowering and ripening time, very light fruit weight, soft or medium-soft fruit and yellow colouring. These features are common to the Tunisian germplasm, indicating a high level of similarity in terms of geographic and genetic origin. Furthermore, these results also indicate the absence of crosses with foreign varieties that would have introduced greater genetic and phenotypic variability. By contrast, the genotypes evaluated from the CEBAS-CSIC breeding

programme showed high phenotypic diversity for all traits studied. The joint use of traditional Spanish cultivars and foreign cultivars with distinct characteristics in Spain has led to a high level of phenotypic diversity in the new apricot releases.

With regard to phenetic relationships, factorial analysis showed that the accessions studied are highly diverse and that the groups obtained are related to their geographic origin. For the Tunisian accessions, there are two main groups: the first group with accessions from the north and the centre of Tunisia and the second group with accessions from the south and the oases. Similar results were obtained by Krichen *et al.* (2014) in morphological studies and by Bourguiba *et al.* (2012), who confirmed this structure of Tunisian apricots with the Bayesian method using STRUCTURE software (Pritchard *et al.*, 2000). Regarding the genotypes evaluated from the Spanish breeding programme, results showed a high level of diversity linked to the different genetic origins of the genotypes.

Molecular diversity and association genetic studies

Molecular results indicate the effectiveness of the SSR markers used to detect molecular polymorphism. All of the 20 SSR markers used in the assayed 72 apricot genotypes were polymorphic and revealed a total of 60 alleles and 110 genotypes. These values are lower than the 152 alleles and 262 genotypes reported by Bourguiba *et al.* (2010) using 32 SSR primers for the characterisation of 47 Tunisian apricot cultivars. Krichen *et al.* (2006) revealed 103 alleles and 155 genotypes assaying 54 Tunisian cultivars with 24 SSR markers. Nevertheless, our values are higher than the 135 alleles and 80 genotypes revealed from 82 apricot accessions reported by Bourguiba *et al.* (2010) using 24 SSRs with an average of 4.75 alleles per locus. Sánchez-Pérez *et al.* (2005) obtained a mean value of 3.9 alleles per locus for Spanish apricot using 17 polymorphic SSRs.

The expected heterozygosity of all apricot accessions was similar to the results reported by Romero *et al.* (2003) in previous studies with peach SSRs on apricot (0.46). However, higher results (0.64) were reported by Zhebentyayeva *et al.* (2003). Markers with PIC values higher than 0.5 were efficient in discriminating among genotypes and were extremely useful in detecting polymorphism (De Woody *et al.*, 1995). In our study, PSG1.21, with a PIC value of 0.83, was the most polymorphic locus, and UDP98025, with a PIC value of 0.040, was the least polymorphic locus. Similar values have been reported by Bourguiba *et al.* (2010) identifying CPSCT039 as the most polymorphic primer with a PIC value of 0.79 and AMPA119 as the least polymorphic primer.

In the present study, the 20 SSRs used for genetic diversity studies generated a total of 110 genotypes with an average of 6 genotypes per marker. The mean value of gene flow (Nm) is estimated from average Fst values. This shows that Fst values (0.141 on average) in this study are higher than those calculated by Bourguiba *et al.* (2010). Such values prove that there has been little genetic exchange, which may be explained by historical events and the relative proximity of the geographic origins of the accessions studied in Tunisia. The results obtained in our study confirm that SSR markers are suitable for the characterisation of apricot germplasm. Our results, also show the great genetic diversity of the apricot cultivars studied.

In accordance with our results UDP96003 and PaCITA7 SSR markers have been previously described as being linked to fruit weight in apricot by Salazar *et al.* (2013). The SSR markers CPPCT022, UDAp460, CPPCT005 and PaCITA7 have been described as being related to flowering time in different species including peach (Fan *et al.*, 2010) and apricot (Salazar *et al.*, 2013), however no such relations were observed in this study.

Observed association between some alleles of PaCITA7 and UDP96003 SSR markers with the fruit weight of apricot genotypes and one allele of UDAp407 marker with the fruit firmness indicate the possibility of using electrophoresis in Metaphor® agarose for distinguishing alleles of special interest. In addition, these results suggest that these markers would perform well in a potential selection even when we know that this selection could be an artefact. These results will provide efficient MAS strategies for selection of big fruit weigh, early ripening and firm new genotypes originated in the modern apricot breeding programs from controlled crosses.

The presence of the 238 nt allele from the marker PGS1.21 is a necessary although insufficient condition for showing resistance to *Plum pox virus* (PPV, sharka) (Rubio *et al.*, 2014). The presence of this allele in the CEBAS-CSIC collection was previously determined by Rubio *et al.* (2014) in the study mentioned above. Only three Tunisian cultivars among the 42 studied showed the corresponding allele, indicating the scarce possibilities of finding cultivars resistant to sharka among the Tunisian genotypes. These results allow us to foresee an evaluation for PPV resistance among the three genotypes showing the allele of interest in controlled conditions (Rubio *et al.*, 2014).

Molecular heterozygosity, relationships and structure analysis

Our results revealed the lesser genetic diversity in the traditional Tunisian and Spanish apricot germplasm in comparison with other European and North American germplasm. The genetic heterozygosity levels of descendants of crosses between Spanish and North American cultivars confirm the value of the use of North American germplasm in European breeding programmes in terms of enlarging the genetic variability of local cultivars as indicated by Sánchez-Pérez *et al.* (2006). The use of cultivars from North America greatly increases the level of heterozygosity in the Spanish apricot breeding programmes. The strategy of using North American cultivars was to introduce new traits like firmness, orange colouring and PPV resistance into traditional Spanish cultivars. The release of highly successful cultivars like 'Rojo Pasión' (Egea *et al.,* 2004), 'Mirlo Blanco', 'Mirlo Naranja' and 'Mirlo Rojo' (Egea *et al.*, 2010) in Spain confirm the efficacy of this crossing strategy (*i.e.,* selecting genotypes which combine both genetic backgrounds).

Genetic distance analysis of the SSRs assayed also confirmed the different origin of several traditional Spanish cultivars ('Currot', 'Búlida' and 'Mauricio') introduced in Spain from the North of Africa in comparison with the rest of the assayed cultivars introduced in Europe, North America or South Africa directly from the Middle East (Bourguiba *et al.*, 2012; Kodad *et al.*, 2013). In addition, the five 'Bargougs' from the Gafsa oasis that placed outside of the Tunisian group could be due to the introduction of French cultivars in this area and subsequent cross pollination with local apricots.

The correlation between the clustering based on SSRs and the agronomic data was very low. Cluster analysis based on SSR data clearly differentiated the genotypes according to their origin and pedigree, whereas cluster analysis based on agronomic data differentiated some genotypes according to their phenotypic characterisation. These results agree with previous findings showed by Maghuly *et al.* (2005). Results also indicate the effectiveness of the SSR markers used to analyse the structure of the studied populations or the germplasm group. The genetic structure identified by STRUCTURE allowed us to distinguish 2 clusters: Tunisian germplasm with 3 accessions from Spain and the rest of the Spanish collection. These results once again confirm the FCA and NJ tree results and the findings provided by Bourguiba *et al.* (2010).

Breeding perspectives

The obtained results showed that modern breeding using controlled crosses increases the efficiency of the obtained new releases size. The new varieties present bigger fruits. The average observed in the Tunisian cultivars was of 20.15 g in contrast with the average weight of 72.82 g of the new releases from the CEBAS-CSIC breeding programme. In addition, modern bred cultivars incorporate traits such as self-compatibility, desirable fruit colour and firmness.

On the other hand, observed association between some alleles of PaCITA7 and UDP96003 SSR markers with apricot fruit weight, one allele of UDAp407 marker with fruit firmness and one allele of UDP98406 marker with fruit ripening indicate the possibility of using electrophoresis in Metaphor® agarose for distinguishing alleles of special interest. These results will provide efficient MAS strategies for selection of big fruit weigh, firm and early ripening new genotypes originated in the modern apricot breeding programs from controlled crosses.

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