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Genetic structure in Tunisian apricot, *Prunus armeniaca* L., populations propagated by grafting: a signature of bottleneck effects and ancient propagation by seedlings

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Abstract

In order to give insights into the origin and historical selection process of Tunisian apricot propagated by grafting, 31 cultivars from three areas presenting contrasting ecological conditions – Kairouan, Testour and Ras Jbel were compared to cultivars from Europe, North America, North Africa, Turkey, Iran and China, using 234 AFLP markers. The phenetic analysis allowed to distinguish 5 clusters, the four previously defined groups: – 'diversification', 'geographically adaptable', 'continental European' and 'Mediterranean' – groups and the Tunisian one. The partitioning of genetic diversity within and between cultivar groups assessed according to the Bayesian approach and assuming Hardy–Weinberg equilibrium, showed a loss of 21.81–38.49% of genetic diversity in Tunisian apricot compared to Mediterranean and diversification groups, respectively. Genetic variation occurred within Tunisian subgroups rather than among (F_{ST} = 0.060) evidencing a narrow genetic pool. Mediterranean and Tunisian groups were the least differentiated. Comparing them, 24 AFLP fragments discriminated the Mediterranean group from the Tunisian group but most of them where also shared by the other groups. Strongly differentiated gene pool and low genetic diversity are probably the result of bottleneck effects linked to the occurrence of propagation by seedlings rather than by grafting during the introduction periods in the North and the Centre of Tunisia. This study points at the propagation by seedlings as an important factor which should be taken into account to understand the evolution of apricot in South Mediterranean areas.

Introduction

Loss of genetic diversity is one of the major consequences of plant domestication. Indeed limited initial sampling from the crop's wild relatives is followed by human selection (Frankel et al. 1995). The magnitude of this loss depends on how domestication proceeded, the reproductive system and whether the traits under selection are controlled by few or many genes (Wang et al. 1999). For instance, we can expect limited loss of genetic diversity as measured using neutral DNA markers when several domestication events have occurred in different locations in an outcrossing species. Further, genetic diversity can be maintained when the plant has been domesticated in contrasted

ecological areas and by gene exchange with the crop's wild relatives. Such evolutionary scenarios are valid for outcrossing species for which cultivated and wild populations are sympatric. Conversely, loss of genetic diversity is expected for introduced long lived fruit species like apricot because of bottleneck effects and human selective pressures.

The common apricot, *Prunus armeniaca* L., was most probably primarily domesticated in China (mountains of north-eastern, central and western China) where forests of wild apricot are found (Bailey and Hough 1975; Vavilov 1992; Faust et al. 1998). The first domesticated forms would then have been diffused through central Asia and the Irano-Caucasian area which are considered as a secondary centre of diversification (Vavilov 1992). Apricot was introduced into the Mediterranean basin through two major routes; the first is through the Near East to whom the Irano-Caucasian group is refered as defined by (Kostina 1969) and the second is through Central Europe (Faust et al. 1998). Despite the lack of representative apricot cultivars from the centre of origin, (Hagen et al. 2002) identified four cultivar groups based on the genetic information, the agronomic likeness and geographic origins of the cultivars. These groups called 'diversification', 'geographically adaptable', 'continental Europe' and 'Mediterranean Basin' displayed a gradient of decreasing genetic diversity of cultivars from the East to the South-west. These results which are in agreement with the history of apricot diffusion from its centre of origin, evidence the large genetic erosion for introduced long lived species.

Compared to European and North Mediterranean cultivars which are exclusively propagated by grafting, apricot in North Africa is also propagated by seedlings and particularly in areas where traditional cultivation methods are still in use. For instance, in South Morocco, apricot is exclusively propagated by seedlings in oasis agrosystems and local populations called 'mech-mech' are adapted to arid conditions (Barbeau and Bouami 1980). These adaptations probably result from both direct human selection and indirect selection linked to ecological conditions during numerous reproductive events. However, the origin and selection process since apricot introduction in these areas are not yet clarified.

In the present study, we focused on Tunisian germplasm as one of the extreme diffusion zones of apricot from its centre of origin. According to the hypothesis proposed by (Kostina 1969) and by (Bailey and Hough 1975), Tunisian apricot belongs to the Irano-Caucasian group, which was introduced by Arabic people from the Near East. Its cultivation extended to oasis areas where trees are at present propagated by grafting but also by seedlings. This latter probably corresponds to apricot cultivars originally brought by land to South Tunisia. In North Tunisia apricot is probably originated from the Spanish germplasm and would have been introduced by the Arabs arriving from Andalusia during the sixteenth century (Valdeyron and Crossa-Raynaud 1950). But the introduction of apricot via the sea has probably also occurred in the North and in the South as well (Carraut and Crossa-Raynaud 1974).

We analysed Tunisian local apricot using AFLP markers in comparison to cultivars from Europe, North America, North Africa, Turkey, Iran and China which have been studied by (Hagen et al. 2002). The principal aim of this paper is to clarify the genetic relationships between Tunisian apricot and the four groups identified in the previous study (Hagen et al. 2002). The second aim is to study the genetic structure in Tunisian apricot and to give a first insight on the origin and historical selection process. Our results were interpreted with regard to the historical events and to the hypothesis proposed on apricot introduction in Tunisia (Bailey and Hough 1975; Vavilov 1992; Faust et al. 1998). We also discuss the use of seedlings vs. grafting as the ancient method of apricot propagation in these areas.

Materials and methods

Plant material

A total of 31 Tunisian local apricot cultivars from three areas, Kairouan, Ras Jbel and Testour, were studied (Figure 1). Based on the information provided by local farmers and morphological characterisation, these apricot cultivars were recognised as traditional cultivars specific to the three areas (Krichen 2001). Ras Jbel and Testour are a northern costal and a northern mountainous areas, respectively. They present semi-arid

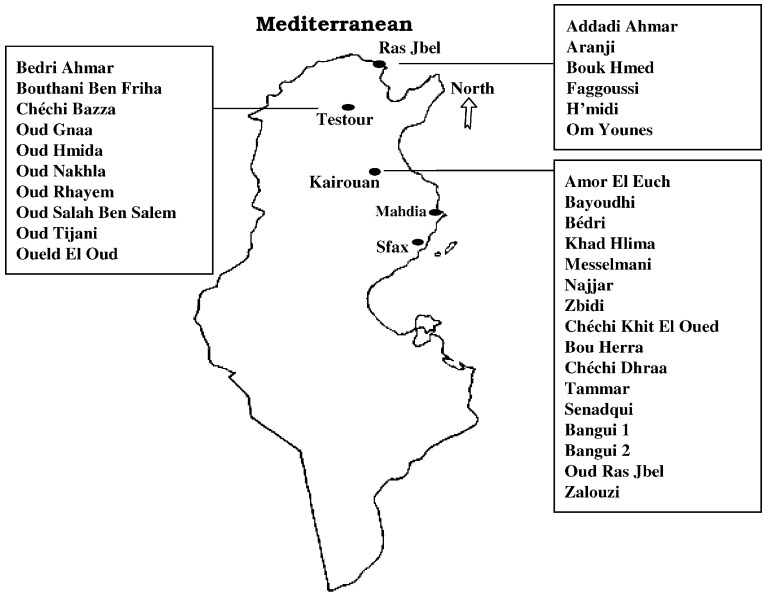

Figure 1. Location of Tunisian apricot cultivars according to three areas: Kairouan, Ras Jbel and Testour. The cultivars Zalouzi from Sfax and the cultivars Oud Ras Jbel as well as two accessions of the cultivar Bangui from Mahdia are belonging to the Kairouan group.

bioclimatic conditions and are ancient and traditional areas for local apricot. In contrast, Kairouan is a plain in the east centre under arid conditions and corresponds to a recent area for apricot cultivation with different origins including local and foreign cultivars. The cultivars 'Bangui 1', 'Bangui 2' and 'Oud Ras Jebl' from Mahdia and the cultivar 'Zalouzi' from Sfax are considered originated from the Kairouan area. In order to study Tunisian apricot in comparison to cultivars from Europe, North America, North Africa, Turkey, Iran and China, genetic data obtained on the 51 previously studied accessions (Hagen et al. 2002) plus Polonais and Taddeo cultivars from the Montfavet INRA collection were used.

Molecular analysis

DNA from young leaves of the 31 Tunisian local apricot cultivars was extracted following (Bernatzky and Tanksley 1986) and (Lefevre et al. 1993). AFLP analysis was conducted using the same *Eco*RI- *Mse*I primer combinations as used by (Hagen et al. 2002): E32-M36, E33-M40, E35-M35, E38-M43 and E46-M40. AFLP fragments scored were identified independently by two persons on each autoradiogram and compared to those scored in the previous one by (Hagen et al. 2002). The same terminology was used: each of the AFLP markers is identified by the corresponding *Eco*RI–*Mse*I primer combination and its mobility compared to a standard. Compared to the previous data matrix comprising 187 AFLP markers, 47 additional markers were added in the present study. Indeed, despite polymorphism, these 47 markers had not been scored in the previous study because of the faintness of the bands. In order to verify the reproducibility of amplified fragments, DNA extractions were performed on a subset of 3 cultivars chosen in each group with similar results. In addition, 22 AFLP markers corresponding to 3 of the 5 primer combinations used in this study have been previousely mapped in apricot, on a subset of a mapping population (Lambert et al. 2004), showing their inheritence and their locus specificity.

Data analysis

Phenetic analysis of AFLP data was used as described in the previous study (Hagen et al. 2002). Based on the binary AFLP matrix, 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).

Once cultivar groups were defined according to genetic data or area of origin, statistics of genetic variation within and between groups were computed using the software AFLP-SURV version 1.0 (Vekemans 2002). Because AFLP data were scored as dominant markers, allelic frequencies at AFLP loci were estimated from the observed frequencies of fragments, using the Bayesian approach proposed by (Zhivotovsky 1999) for diploid species and assuming Hardy–Weinberg equilibrium. A non-uniform prior distribution of allelic frequencies was assumed with its parameters derived from the observed distribution of fragment frequencies among loci (Zhivotovsky 1999). This procedure has been shown to produce unbiased estimates of allelic frequencies for dominant markers (Krauss 2000). Estimated allelic frequencies were then used as input for the analysis of genetic diversity within and between defined cultivar groups according to the method proposed by (Lynch and Milligan 1994).

Parameters of genetic diversity within cultivar group were reported: the number and proportion of polymorphic loci at 5% level (loci with the frequency of the marker allele comprised between 0.05 and 0.95), the genetic diversity Hj which is an estimate of the average heterozygosity expected under Hardy–Weinberg equilibrium and its standard error estimated according to the variance due to the sampling of individuals and the variance due to the sampling of loci. Parameters of the partitioning of genetic diversity within and among cultivar groups were reported for each analysis level: the overall loci diversity (H_T), the average loci diversity within cultivar group (H_S), the estimated Wright fixation index interpreted as the proportion of genetic differentiation among cultivar groups (F_{ST}). The significance of the genetic differentiation between cultivar groups was tested by comparison of the observed F_{ST} with a distribution of F_{ST} under the hypothesis of no genetic structure, obtained using 1000 random permutations of cultivars among groups. A neighbour-joining tree was computed based on a matrix of pairwise F_{ST} estimated between cultivar groups and a thousand bootstraps were performed over AFLP loci using AFLP-SURV and the Phylip software package (Felsenstein 1995).

Results

Genetic relationships between cultivars

The analysis was performed using a total of 234 AFLP markers. The 83 cultivars plus *P. holocericea* had unique profiles and could be distinguished from each other. Phenetic analysis based on similar part of the total MCA variance, 54.58% in (Hagen et al. 2002) and 55.83% in the present study, allowed to distinguish clearly 5 clusters: the four groups defined in the previous study: D = 'diversification group', C = 'geographically adaptable group', B = 'continental European group' and A = 'Mediterranean basin group' (Hagen et al. 2002) and the Tunisian group (Figure 2). Cultivars analysed in the previous study were assigned to the same clusters, as expected, with the exception of 'Oranzeno Krasnyj' and 'Précoce de Tyrinthe' previously classified in D and C groups which came out in this new analysis within the 'Mediterranean basin group', and 'Harcot' which is moved from group C to group D. Tunisian cultivars were clearly separated from the other groups but the cluster also included 'Bergeron A114' which was classified in the B group and three additional Mediterranean cultivars: 'G1 2121 a4', 'Polonais' and 'Sceara' (Figure 2). No clear structure within the Tunisian group was observed according to the area of origin.

Genetic diversity within cultivar groups

Based on phenetic analysis or area of origin, we defined 7 cultivar groups: the 'diversification group' with 'Oranzeno Krasnyj', 'geographically adaptable group' with 'Harcot' and 'Précoce de Tyrinthe', the 'continental European group' with 'Bergeron A114', the 'Mediterranean group' with 'G1 2121 a4', 'Polonais' and 'Screara', 'Kairouan', 'Testour' and 'Ras Jbel' groups as mentioned in Figure 1. The highest proportion of polymorphic markers (74.9%) was observed in group D, and the lowest in Tunisian group (43.8%; Table 1). Despite limited sample sizes, groups C, B and A

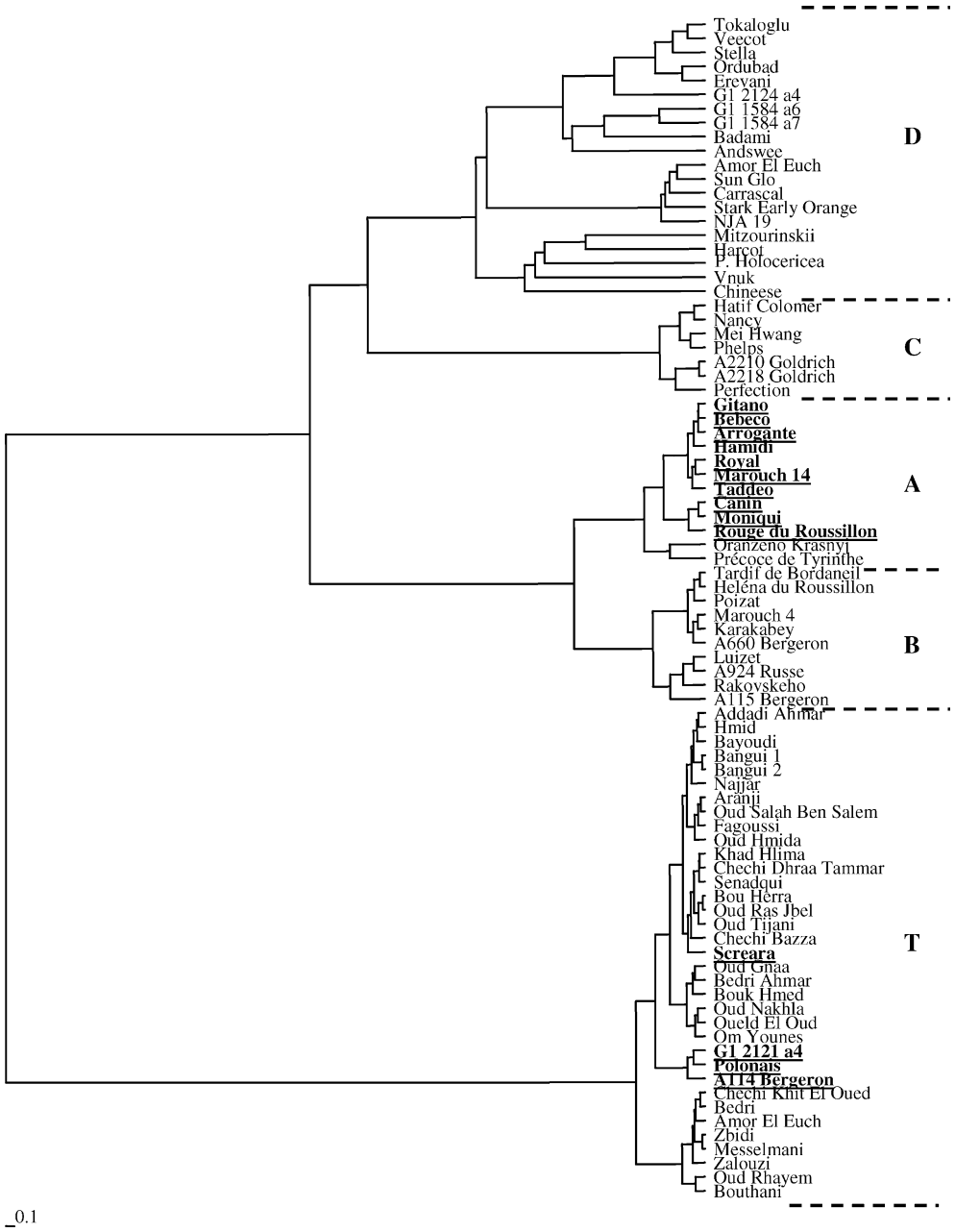

Figure 2. Genetic relationships among apricot cultivars based on Euclidean distances constructed by Ward algorithm comprising the twelve first axes of multiple coordinate analysis explaining 55.83% of the total variance. The clusters are referred as A = Mediterranean group, B = Continental European group, C = Geographically adaptable group, D = Diversification group and T = Tunisian group. Underlined and bold type mean cultivars belonging to Mediterranean group.

presented intermediate proportions of polymorphic markers ranging from 45.5 to 54% (Table 1). However, only group D is characterised by numerous specific markers (31.2%), whereas a few specific markers were observed in the other groups ranging from 0.4 to 8%. Genetic diversity within the diversification group was 0.239 and decreased to 0.147 in the Tunisian group. There was a gradient of decreasing genetic diversity in the following sequence: D, C, A, B and Tunisian groups

Table 1. Genetic diversity within cultivar group.

Cultivar group	Sample size	Number of polymorphic loci	Proportion of polymorphic loci (in%)	Number and (%) bands unique to group	Genetic diversity Hj	S. E. (Hj)
Diversification (D)	21	176	74.9	73 (31.2)	0.239	0.0105
Geographically adaptable (C)	8	127	54	8 (3.4)	0.198	0.0124
Continental Europe (B)	11	120	51.1	2 (0.8)	0.184	0.0127
Mediterranean (A)	13	107	45.5	4 (1.7)	0.188	0.0131
Tunisian (T)	31	107	43.8	1 (0.4)	0.147	0.0117
Kairouan (Tunisian)	15	100	42.6	0	0.149	0.0121
Testour (Tunisian)	10	105	44.7	1 (0.4)	0.143	0.0113
Ras Jbel (Tunisian)	6	90	38.3	0	0.140	0.0117

(Table 1). Compared to the diversification and Mediterranean groups, Tunisian apricot displayed a loss of 38.49 and 21.81% of genetic diversity, respectively.

Despite of the limited sample size, cultivars from the Testour area displaid the highest proportion of polymorphic markers within the Tunisian group and presented a specific marker. However, they were less diversified than the cultivars from Kairouan (Hj = 0.149; Table 1).

Genetic differentiation among cultivar groups

The partitioning of genetic diversity within and between cultivar groups is given in Table 2. The amount of genetic differentiation among all the groups is about 16.3%, but decreases to 9.7% when Mediterranean and Tunisian cultivars are compared. Genetic variation in Tunisian apricot occurs within groups rather than among (F_{ST} = 0.060) showing that Kairouan, Testour and Ras Jbel groups are more closely related to each other than to the D, C, B and A groups. These results are illustrated by the Neighbour joining dendrogram based on pairwise F_{ST} values (Figure 3). Distinction of all the 7 groups is supported by bootstrap values ranging from 72.3 to 100%. Tunisian groups are clearly differentiated from the other ones and the branch separating these two gene pools is supported by a 100% bootstrap value (Figure 3).

Among the D, C, B and A groups, the Mediterranean one is the least differentiated from Tunisian apricot. These two gene pools are differentiated by 24 AFLP bands which are absent in Tunisian apricot and one band which is specific to Tunisian apricot (Table 3). Among the 24 AFLP markers, 4 are specific to the Mediterranean group whereas 16 are shared by all eco-geographic groups.

Discussion

Except for the cultivars 'Oranzeno Krasnyj', 'Précoce de Tyrinthe' and 'Harcot' which were found misclassified, our results confirmed the finding of the previous study (Hagen et al. 2002). Using the phenetic analysis, we distinguished clearly the four clusters: D = 'diversification group', C = 'geographically adaptable group', B = 'continental European group' and A = 'Mediterranean basin group'. Genetic differentiation between each pair of groups was significant as attested by the high bootstrap values. Moreover, we showed a

Table 2. Genetic structure within diversification (D), geographically adaptable (C), continental European (B), Mediterranean (A) and Tunisian groups.

Groups (*n*)	Number of polymorphic loci	Overall loci diversity H_T	Average loci diversity within group H_S	F_{ST}
A, B, C, D, and Tunisian groups (7)[a]	234	0.212	0.177	0.163***
Mediterranean and Tunisian groups (4)[a]	134	0.295	0.266	0.097***
Tunisian groups (3)[a]	105	0.328	0.309	0.060***

***Significant $p < 10^{-3}$.

[a]Number of cultivar groups compared.

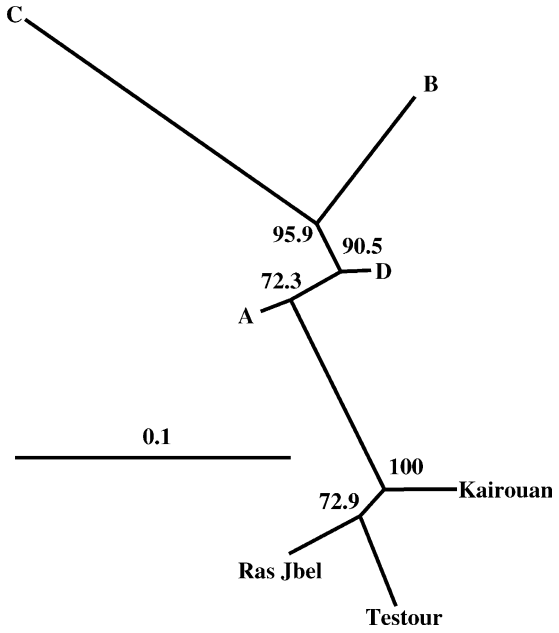

Figure 3. Genetic relationships among cultivar groups illustrated by Neighbour joining based on pairwise F_{ST} values. The numbers of bootstraps are given at each branch.

gradient of decreasing genetic diversity from D to A and B groups. Mediterranean and continental European groups had a similar genetic diversity value. Our results support the D group, mostly originated from Asia and Irano-Caucasian areas, as being the origin and diversification gene pool of studied apricot. With 74.9% polymorphic loci, this group is the most diversified one supporting the zone from central Asia to Irano-Caucasus as being the area of origin as proposed by (Kostina 1969) and (Vavilov 1992). On the other hand, the D group comprises one third of the specific AFLP bands and the remaining markers are shared by the other groups indicating limited direct introductions of germplasm from East to West including continental European and Mediterranean groups. These results fit well with the domestication and selection process model proposed by previous authors (Kostina 1969; Bailey and Hough, 1975; Vavilov 1992; Faust et al. 1998) and supported by (Hagen et al. 2002).

Beyond the confirmation of the previous study (Hagen et al. 2002), we show that the Tunisian apricot is a distinct group presenting a narrow genetic basis and discriminating markers mostly shared by the four groups D, C, B and A. Indeed, the phenetic analysis based on the Ward algorithm

Table 3. Frequencies of the discriminating AFLP fragments between the Mediterranean (A) and the Tunisian cultivars. The other groups are: diversification (D), geographically adaptable (C), continental European (B). Underlined and bold type mean specific marker.

AFLP fragment	Cultivar group				
	A	Tunisian cultivars	D	C	B
E46-M40-31.5	0.31	0.00	0.24	0.88	0.64
E46-M40-141	0.38	0.00	0.14	0.25	0.18
E46-M40-307	0.77	0.00	0.67	0.88	0.91
E35-M35-126	0.77	0.00	0.10	0.25	0.64
E35-M35-168.5	**0.08**	0.00	0.00	0.00	0.00
E35-M35-237	0.46	0.00	0.00	0.00	0.09
E35-M35-241	**0.08**	0.00	0.00	0.00	0.00
E38-M43-91	0.46	0.00	0.19	0.13	0.73
E38-M43-123	0.69	0.00	0.57	0.50	0.91
E38-M43-147	0.31	0.00	0.19	0.00	0.36
E38-M43-169	0.00	**0.20**	0.00	0.00	0.00
E33-M40-66	0.77	0.00	0.67	0.88	0.91
E33-M40-149	0.54	0.00	0.38	0.50	0.55
E33-M40-191	0.62	0.00	0.43	0.25	0.09
E33-M40-211	0.08	0.00	0.24	0.00	0.00
E33-M40-258	0.54	0.00	0.43	0.50	0.73
E33-M40-265	**0.31**	0.00	0.00	0.00	0.00
E33-M40-340	0.08	0.00	0.05	0.00	0.00
E32-M36-54	**0.31**	0.00	0.00	0.00	0.00
E32-M36-57	0.08	0.00	0.05	0.13	0.27
E32-M36-94	0.46	0.00	0.24	0.50	0.36
E32-M36-121	0.62	0.00	0.10	0.13	0.45
E32-M36-164	0.38	0.00	0.33	0.25	0.64
E32-M36-213	0.15	0.00	0.14	0.13	0.36
E32-M36-269	0.69	0.00	0.48	0.63	0.73

and the Euclidean distance of MCA coordinates showed a clear distinct cluster with close genetic relationships between Tunisian cultivars. Further, the partitioning of genetic diversity within and between cultivar groups showed a loss of 21.81–38.49% of genetic diversity in Tunisian apricot with a variation occurring within Kairouan, Testour and Ras Jbel groups rather than among groups indicating a narrow gene pool.

What insights do these results give us into Tunisian apricot origin and its historical selection process? High differentiation and low genetic diversity fit with a bottleneck model followed by numerous successive reproductive events. Hence the most likely scenario is that the Tunisian apricot is the result of few introduced genotypes which have been propagated by numerous seedling events. However, this scenario is not in agreement with apricot culture in Northern and Central Tunisia since cultivars are propagated exclusively

by grafting. If we consider that grafting was the only mean of propagation used since the Arabic's people arrival from the Near East ten centuries ago and more recently from Andalusia during the sixteenth century, we would have expected 1) that Tunisian apricot was closely related to the D group (Irano-Caucasian) or to the Mediterranean group including Spanish cultivars, and 2) that genetic diversity within Tunisian apricot would have been similar to the one observed within the Mediterranean group. However, we obtained opposite results compared to what we might expected under propagation by grafting. In fact, a strongly differentiated gene pool with closely related genotypes is probably the signature of bottleneck events followed by genetic drift over numerous reproductive events. It seems likely that propagation by seedling during apricot introduction in the North and Centre of Tunisia was more frequent than grafting as it is the case today in oasis areas from Tunisia to Morocco (Barbeau and El Bouami 1980). In South Europe, two intermediate situations are encountered when apricot seedling is used as rootstock. The first one stated in Campania (Italy) is based on the plantation of an apricot seedling issued from the local population in orchad and its grafting at his own place with the expected cultivar. If the grafting didn't succeed the tree is maintained or removed according to its agronomic performances (Scaramuzzi 1961). The second one stated in Greece is based on a multiplication in nursery by grafting onto a 'Bebeco' apricot seedling (the main apricot cultivar in Greece) and the plantation of the whole material issued from the nursery whatever the success in grafting.

Despite of historical events suggesting that Andalusian germplasm is at the origin of the gene pool of apricot in the North and Centre of Tunisia (Valdeyron and Crossa-Raynaud 1950), our results do not allow to draw clear conclusions about the origin of apricot in Tunisia. Among the four groups, the least differentiated gene pools from Tunisian apricot are the Mediterranean and surprisingly the diversification groups (see Figure 3). Moreover, the Mediterranean and Tunisian groups have only one specific AFLP marker among 234 polymorphic loci and are they are distinguished by 25 markers among which 16 are shared with the other groups including diversification one. Because of the nuclear DNA polymorphism and the dominant feature of the markers, genetic information obtained by AFLP analysis is probably of limited value to provide insights on Tunisian apricot origin especially as most of AFLP markers are shared by all groups. Cytoplasmic polymorphism is relatively low and maternally inherited in most angiosperms allowing to study seed-mediated dispersal and hence to clarify diffusion routes of apricot (Domolin-Lapègue et al. 1997; Muller et al. 2001). Until clarifying the origin of Tunisian apricot by analysing more local cultivars and particularly those in oasis areas using cytoplasmic and nuclear DNA polymorphism, our study points at an important concern for understanding the evolution of non indigenous species in Mediterranean areas: bottleneck effect and increase in number of genotypes due to numerous successive reproductive events.

Focusing on Tunisian apricot, we showed that genetic diversity is low but structured according to the three areas: Kairouan, Ras jbel and Testour, as attested by a significant pairwise genetic differentiation (see Figure 3). These results are in agreement with the scenario based on few introduced genotypes in each area and the propagation of apricot by numerous seedling events. However, the low level of genetic differentiation and the lack of specific AFLP markers suggest that cultivars from Kairouan, Ras Jbel and Testour originate from the same gene pool. These three areas displays contrasting ecological conditions: the costal area of Ras Jbel and the mountainous area of Testour are characterised by semiarid bioclimatic conditions while Kairouan is a plain zone under arid conditions (Krichen 2001). Cultivars from Kairouan and Ras Jbel are early blooming while those from Testour are semi late to late flowering. Cultivars from Kairouan have white to yellow fruit flesh, while cultivars from Testour and Ras Jbel have diversified colour fruit flesh (Krichen 2001). Apricot propagation by numerous seedling events under contrasting ecological conditions had led probably to select adaptive traits like date flowering suggesting original genetic resources which should be preserved for conservation and for breeding schemes.

Our study showed the strong distinct gene pool of Tunisian apricot structured according to the location of origin with adaptive traits probably due to human selection and ecological constraints. We analysed only a part of Tunisian germplasm located in areas displaying progressive plantation

of modern orchards and cultivars which are propagated exclusively by grafting with no local or hybrid cultivars. In the oasis areas of south Tunisia, the propagation of local apricot by seedling is still the common practice suggesting the possible occurrence of original genetic resources especially if the presumed Irano-Caucasus gene pool origin is confirmed (Kostina 1969; Faust et al. 1998; Valdeyron and Crossa-Raynaud 1950). Studying these local apricot using nuclear and cytoplasmic DNA polymorphism would allow us to clarify the origin of Tunisian germplasm and to identify adaptive traits related to the excepted genetic structure. Beyond clarifying the genetic structure of Tunisian apricot, this study points at the necessity to study local populations in oasis areas in order to understand the evolution of non indigenous species in Mediterranean areas.

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