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ORIGINAL ARTICLE

Assessment of genetic diversity of local Tunisian peach accessions [*Prunus persica* (L.) Batsch] using SSR markers

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Abstract

Key message **This work belongs to the Tunisian conservation strategies of local fruit tree resources. Results highlighted the important genetic richness of Tunisian peach as an unexplored source for peach future breeding.**

Abstract Tunisia is characterized by a rich genetic heritage of fruit trees. Nevertheless, local Tunisian accessions of different fruit crops face several threats that are causing a dramatic loss of some of these valuable landraces. This study was conducted to evaluate the genetic diversity and population structure of 23 Tunisian peach accessions using 27 microsatellite (SSR) loci. These Tunisian accessions were compared with accessions from America, mainland Spain and the island of La Palma (Canary Islands, Spain). A considerable genetic diversity was observed in Tunisian genotypes with allelic richness value of 0.47. The genetic richness was 0.37 in La Palma genotypes, 0.3 in mainland Spain and 0.38 in the American genotypes. Eight private alleles were obtained in the Tunisian genotypes, while six were observed in the mainland Spanish pool, five in the American pool and six in the La Palma pool. Structure analyses and similarity dendrogram based on SSRs were clearly consistent with a geographic structuring and highlighted the different introduction pathways of *Prunus persica* into Tunisia.

Keywords *Prunus persica*, · SSRs · Genetic diversity · Linkage disequilibrium · Germplasm dispersion

Introduction

The Rosaceae family is divided into four subfamilies (Rosoideae, Spiraeoideae, Maloideae and Amygdaloideae or Prunoideae) (Entani et al. 2003). The Prunoideae subfamily is characterized by trees that mostly produce a fruit type called a drupe: a soft, edible exocarp/mesocarp that surrounds a hard lignified endocarp (Abbott et al. 2007). *Prunus*, which includes stone fruits (such as peaches, plums, apricots, cherries or almonds), is a genus that encompasses approximately 250 species that share a small (250–300 Mbp) and highly syntenic genome with eight chromosomes (Aranzana et al. 2019). The deployment of molecular approaches during the pasthalf century has significantly advanced our understanding of important aspects of *Prunus* genetics (Aranzana et al. 2019). The public availability of a peach high-quality DNA sequence in 2010 (Zhang et al. 2012) and later those of other *Prunus* species has unlocked the *Prunus* genome, paving the way to build new knowledge for both basic and applied purposes (Aranzana et al. 2019).

An exception to the prevalent self-incompatibility in the genus *Prunus* is peach [*Prunus persica* (L.) Batsch; $2n = 16$], a fully self-compatible diploid species with no recent whole-genome duplication events (Abdallah et al. 2020). In addition, while polyploidy is common in the genus *Prunus*, the cultivated peach is diploid and has a chromosome number of $2n = 2x = 16$ (Hancock et al. 2008). The peach has a small

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genome, approximately 220 Mb, about twice that of Arabidopsis (Cao et al. 2012). *P. persica* has become a model fruit species for comparative and functional genomic studies in *Prunus*.

The cultivation of peaches dates back to ancient times due to the early domestication of this species. Consequently, studies on peach genetic diversity have become a requisite in the last few years for the efficient exploitation of genetic resources in breeding programs and the development of conservation strategies of genetic diversity (Pérez et al. 2020). For this purpose, numerous studies have been conducted utilizing different types of molecular markers due to the availability of whole-genome sequences.

Microsatellites, or simple sequence repeats (SSRs), play a relevant role for individual fingerprinting due to their relatively ease of use, their abundance in plant genomes as well as their co-dominant nature (Dettori et al. 2015). In peach, SSR markers have been used for parentage analysis (Yamamoto et al. 2003a,b), genetic diversity (Baránek et al. 2006; Maghuly et al. 2005; Marchese et al. 2005, 2006; Wünsch et al. 2006; Zeinalabedini et al. 2008) and genetic linkage mapping (Joobeur et al. 2000; Dettori et al. 2001; Aranzana et al. 2003; Sanchez-Pérez et al. 2006).

Tunisia has been a crossroad of several civilizations due of its strategic position and diversity of ecosystems and climatic conditions, and it is considered one of the richest Mediterranean countries in terms of plant genetic resources including landraces and wild relatives of different crops such as plums (Abdallah et al. 2019; Baraket et al. 2019), Citrus (Saddoud Debbabi et al. 2020), melon (Chikh-Rouhou et al.2021), pear (Ouni et al. 2020), date palm (Ben Abdallah et al. 2020) or pistachio (Guenni et al. 2016; Aouadi et al. 2019). Nevertheless, as in other regions of the world, genetic erosion is taken place in the country and, as an example, several local Tunisian varieties of different *Prunus* species have disappeared during the last years due to the lack of programs addressed to preserve local Tunisian germplasm (Nabli 2011; Abdallah et al. 2016; Baraket et al. 2019). Recently, studies were conducted aiming to preserve this valuable extant *Prunus* germplasm in Tunisia. As an example, Tunisian plums were phenotypically and genetically characterized (Baraket et al. 2019). In addition, self-incompatible genotypes and phenotypes related to Tunisian plums were also assessed and some *S*-genotypes, previously defined as rare genotypes and other identified as new genotypes, were reported (Abdallah et al. 2019). Tunisian plums were also characterized as highly tolerant to water deficit (Baraket et al. 2021). Several Tunisian peach accessions have been characterized phenotypically and some self-incompatible genotypes have been identified (Abdallah et al. 2020).

To obtain a comprehensive overview of the genetic diversity present in Tunisian peach accessions, 23 peach accessions were analyzed in this work using 27 SSR loci. These accessions were compared to previous results obtained with other genotypes from America, mainland Spain and the Canary Islands (Spain). Our specific aims were as follow: (i) use SSR analysis to draw inferences about Tunisian peach genetic diversity analyzing diversity parameters and Linkage disequilibrium; (ii) study the genetic relationships and the possible pathways of introduction of peach to Tunisia.

Material and methods

Plant material

In this work, 23 Tunisian peach accessions [*Prunus persica* ($2n = 2x = 16$)] were analyzed. These accessions represent local landraces and were sampled from different regions of Tunisiaas represented in Fig. 1.

To study their genetic relations with other peach varieties, peach DNA from 25 accessions maintained in La Palma (Canary Islands, Spain), 25 from mainland Spain and 25 from America (among them, five accessions originated from Bolivia) maintained at "CITA de Aragon" in Zaragoza, Spain were used (Pérez et al. 2020).

The samples analyzed were divided into four populations based on their geographic origins: "Tunisian samples", "La Palma samples", "mainland Spanish samples", and "American samples". Table 1 summarizes the information related to plant material.

DNA extraction and amplification

For Tunisian accessions, fresh young leaves were collected from each genotype in spring from isolated trees and dried in silica gel. Genomic DNA was extracted following a modified CTAB (Cetyltrimethyl ammonium bromide) method for *Prunus* (Hormaza 2002). Extracted DNA was quantified using a Nanodrop 1000 and diluted to 10 ng/µ*L*. For the other accessions, genomic DNA, previously extracted and maintained in a DNA bank, was used.

Twenty-seven SSR loci mapped in the eight linkage groups in the *Prunus*-TE-F2 linkage map (Joobeur et al. 1998; Aranzana et al. 2003; Dirlewanger et al. 2004; Howard et al. 2005; Sanchez-Pérez et al. 2010) were amplified (Table 2). PCR reactions were carried out in a volume of 15 µl, with 67 mM Tris–HClpH 8.8, 0.01% Tween-20, 16 mM $(NH_4)_2SO_4$, 20.3 mM $MgCl_2$, 0.1 mM of each dNTP, 0.3 µM of each primer, 20 ng of genomic DNA and 1 U of BioTaq™ DNA polymerase (Bioline, London, UK). PCR reactions were run in an I-cycler (Bio-Rad Laboratories, Hercules, CA, USA) thermocycler using the following temperature cycles: an initial step of 2 min at 94 °C, 35 cycles of 45 s at 94 °C, 45 s at 57 °C, 1 min



Fig. 1 Tunisian map representing the different origins of the peach accessions analyzed in this work. The different regions are marked in red

at 72 °C, and a final step of 5 min at 72 °C. The obtained fragments were analyzed by capillary electrophoresis with a Beckman Coulter GenomeLabGeXP™ capillary DNA analysis system. Forward primers were labeled with a fluorescent dye on the 5-end (Sigma-Aldrich, St. Louis, MO, USA). Samples were denaturalized at 90 °C for 120 s, injected at 2.0 kV for 30 s, and separated at 6.0 kV for 35 min.

Diversity analyses

To explore the genetic diversity of the studied cultivars, different diversity parameters of the SSR markers were calculated with Arlequin ver. 3.5 (Excoffier et al. 2005).The number of observed alleles (*A*o), the observed (*H*o) and expected (*H*e) heterozygosities and the power of discrimination (PD) of SSR loci were calculated. The presence of private alleles

Table 1 Identification of 98 peach accessions used in this work. Tunisian localities are represented in Fig. 1

Origin		Accession name	Origin	Accession name
Tunisia (TN)	Menzel bou Zelfa	Bargoug	Mainland Spain (SP)	Calante
	Tunis	BOUTABGAYA Tunis		Manolito
	Rafraf	BOUTABGAYA Rafraf		PARAGUAYO ALMUDI
	Gabes	BOUTABGAYA Gabes		PARAGUAYO J
	Menzel bou Zelfa	BOUTABGAYA Nabeul		Blanco del Mas
	Seliana	BOUTABGAYA Seliana		P. Almuna
	Sfax	BOUTABGAYA Sfax		Villa Mayor
	Djerba	BOUTABGAYA Djerba		Ambra
	Rafraf	MESKI		San Lorenzo
	Jendouba	AMBER		Calabacera
	Jendouba	KHARFI		Duraznillo
	Seliana	KHOUKH AHMER		Jeronimo
	Menzel bou Zelfa	BARGOU BOUNIMLI		Miraflores
	Sfax	BARGOU BOURAMLI		Montañana
	Seliana	BARGOU LIMAOUI		Maelle
	Tunis	BEDRI		Maruja
	Rafraf	Bedria		Generoso
	Jendouba	Rebiaa		Montes de Cierzo
	Sfax	ESSIFI		Sudanell
	Tunis	Porte greffe pêcher		Maluenda
	Rafraf	Chaaraoui		Fulla
	Gabes	TOUNSI		Zaragozano
	Rafraf	Bouchkara		Golden Zaragozano
				Jesca
				Campillo Rojo
American (AM)	USA	Large Lady	La Palma (LP)	Blanco Tardío
	USA	Primerose		Rojo
	USA	Spring White		AmarillloMelocotón
	USA	Large White		Blanco Mirollo
	USA	Ruby Rich		Mirollo
	USA	Garau		Blanca Palmera
	USA	Gamet Beauty		Colorado
	USA	Rich Lady		Las Caletas
	USA	Rich May		Mollar
	USA	Merrill Angels		Mollar Amarillo
	USA	Merrill Fransiscan		Blanco
	USA	Merrill Gemfree		Maracaton
	USA	Merrill Sundance		Monte Breña2
	USA	Flavor Top		Goressa
	USA	Halford		Blanco Rosado
	USA	Davis		Pelones
	USA	Andross		Amarillo
	USA	Sweet Cup		Paporrojo
	USA	Carolyn		AmarilloDulce
	USA	Carson		Blanco Dulce
	Bolivia	CASA		Negra Palmera
	Bolivia	GUMUCIO REYES		Venezolano
	Bolivia	OLICANTE AMARILLA		Blanco Palmera
	Bolivia	SOPACHUY		Morada

Table 1 (continued)

Origin	Accession name	Origin	Accession name
Bolivia	MAULSIVISO		MonteBreña 1

Table 2 Summary statistics of genetic variation at 27 SSR loci used in this work

SSR n°	Locus	LG	Genetic position (cM)*	A_o	A_e	H_o	H_e	f	PD
SSR1	Pchgms3	1	37.5	5	1.12	0.38	0.50	0.24	0.63
SSR2	BPPCT028	1	77.4	4	1.24	0.27	0.31	0.13	0.43
SSR3	CPPCT026	1	33.9	5	1.31	0.54	0.68	0.21	0.82
SSR4	CPPCT004	1	11.6	5	1.05	0.44	0.53	0.17	0.74
SSR5	CPPCT044	2	7.2	3	0.63	0.13	0.38	0.65	0.24
SSR6	CPSCT031	2	43.2	6	1.44	0.48	0.55	0.13	0.51
SSR7	BPPCT024	2	36.3	3	0.71	0.29	0.46	0.37	0.21
SSR8	UDP96-008	3	36.4	4	1.09	0.19	0.21	0.12	0.11
SSR9	BPPCT039	3	18	4	0.82	0.33	0.45	0.27	0.17
SSR10	BPPCT007	3	11.2	5	1.40	0.51	0.69	0.26	0.75
SSR11	UDP96-003	4	28.3	5	1.58	0.25	0.37	0.32	0.66
SSR12	CPPCT005	4	10.4	5	1.46	0.32	0.44	0.27	0.31
SSR13	BPPCT015	4	44	6	1.24	0.49	0.58	0.16	0.74
SSR14	CPPCT040	5	1.5	3	0.91	0.31	0.48	0.35	0.29
SSR15	BPPCT037	5	25.6	6	0.83	0.26	0.40	0.35	0.76
SSR16	CPPCT013	5	29.2	4	0.86	0.34	0.32	-0.06	0.31
SSR17	CPPCT030	6	86.5	5	1.01	0.43	0.57	0.25	0.45
SSR18	CPPCT008	6	8.7	2	0.12	0.31	0.43	0.30	0.15
SSR19	UDP98-412	6	72	6	1.46	0.34	0.32	-0.03	0.18
SSR20	UDP96-001	6	17.5	6	1.58	0.12	0.37	0.67	0.47
SSR21	CPPCT022	7	18.6	7	1.47	0.61	0.82	0.26	0.92
SSR22	CPPCT033	7	38.9	5	1.50	0.18	0.33	0.45	0.33
SSR23	CPPCT017	7	61.8	5	1.67	0.41	0.58	0.29	0.47
SSR24	CPPCT006	8	24.8	5	1.2	0.13	0.28	0.54	0.16
SSR25	UDP96-019	8	20.8	4	0.91	0.43	0.31	-0.38	0.28
SSR26	BPPCT006	8	14.1	9	2.43	0.52	0.74	0.30	0.84
SSR27	UDP98-409	8	44.5	6	1.09	0.49	0.60	0.18	0.66
	Mean	–		4.92	1.19	0.35	0.47	0.25	0.47

*Genetic Position of the SSR loci in centimorgan (cM) in the *Prunus*-TE-F2 linkage map (http://www.rosaceae.org/peach/genome) (Aranzana et al. 2003; Dirlewanger et al. 2004; Howard et al. 2005; Joobeur et al. 1998; Sanchez-Perez et al. 2010)

A_o mean number of observed alleles per locus

A_e mean number of Effective number of alleles

H_o observed heterozygosity

H_e expected heterozygosity

f Wright's fixation index

PD power of discrimination

was calculated using Genetic Data Analysis 'GDA' software (Lewis and Zaykin 2001).

Population differentiation analysis

To measure genetic differentiation between populations, we performed an analysis of molecular variance

(AMOVA); the pairwise *Fst* was analyzed using Arlequin v3.5 software (Excoffier et al. 2005).

Linkage disequilibrium analysis

Linkage disequilibrium (LD) was estimated using squared allele-frequency correlations (r^2) between each pair of alleles among the 27 SSR loci analyzed with PowerMarker 3.25, considering unphased genotype data (Li et al. 2013). Since the distribution of allele frequencies may have an effect on the extent of LD, rare alleles whose frequencies were less than 5%, were excluded prior to further LD analyses (Arunyawat et al. 2012). For each group, intra-chromosome LD was plotted against distance measured in centimorgan (cM). The overall decay of LD with genetic distance among the tested loci was evaluated by nonlinear regression using the average values for each subset of an equal number of pair comparisons covering adjacent intervals along distance. The threshold $r^2 = 0.1$ was used for decay distance estimation as suggested by Aranzana et al. (2010) and Li et al. (2013).

Phylogenetic analysis

Detected alleles with the SSR loci were scored as present (1) or absent (0). Genetic distances were calculated with Maximum Composite Likelihood (Tamura et al. 2013) in Popgene ver. 1.31 software, and then imported to Mega5 to construct a UPGMA tree. Numbers on major branches represent bootstrap support from 2000 replicates.

Genetic structure analysis

The model-based Bayesian clustering method implemented in the software package Structure v2.3.4 (Pritchard et al. 2000) was applied to infer the ancestral population structure. Independent replicate runs of Structure were carried out by setting the number of clusters (K) from 3 to 7. Each run consisted of a burn-in period of 100,000 steps followed by 1,000,000 Markov Chain Monte Carlo (MCMC) iterations, assuming an admixture model and correlated allele frequencies. For the choice of the most likely number of clusters (K) supported for our dataset, the plateau criterion proposed by Pritchard et al. (2000), and the 1 K method, described by Evanno et al. (2005) and implemented in Structure Harvester v.0.6.93 website (Earl and VonHoldt 2012) were used. To obtain optimal alignment of the independent runs, Clumpp v1.1 software (Jakobsson and Rosenberg 2007) was used with greedy algorithms, 10,000 random input orders and 10,000 repeats, to calculate the average pairwise similarity (H') of runs. Clumpp output was used directly as input for Distruct v1.1 (Rosenberg 2004) To graphically display the results. Accessions with probability of membership greater than 80% were assigned to corresponding clusters; otherwise they were considered as "admixed."

A phylogenetic tree was computed by Structure among the K Structure clusters by applying the neighbor joining algorithm to the matrix of allele-frequency divergence among clusters (net nucleotide distance). The tree was estimated using the program Neighbor by Mary Kuhner and John Yamato, implementing Saitou and Nei's (1987) "Neighbor Joining Method". The plot was produced using Drawtree by Felsenstein (2005). Both programs are distributed by Felsenstein as part of his Phylip phylogeny package.

Results

SSR polymorphism analysis

The 27 SSR loci analyzed amplified a total of 133 alleles, with an average of 4.92 alleles per locus (A_o). Thirty-two of them had frequencies lower than 5%, resulting in an average effective number of alleles (A_e) of 1.19 (Table 2). The observed heterozygosity (H_o) ranged between 0.12 (UDP96-001) and 0.61 (CPPCT022), with an average of 0.35, while the expected heterozygosity (H_e) ranged between 0.21 (UDP96-008) and 0.82 (CPPCT022), with an average of 0.47. Consequently, the fixation index (*f*) showed a mean of 0.25 for all loci. The PD of the tested SSR loci, ranged between 0.11 (UDP96-008) and 0.92 (CPPCT022), with an average of 0.47 (Table 2).

Patterns of diversity

The 23 Tunisian peach samples showed an important allelic richness (Table 3). A mean of 3.87 observed alleles per cultivar (A_o) was found (Table 3), where a mean of 1.66 effective alleles was obtained resulting in an average allelic richness of 0.47. The observed heterozygosity was higher than the expected in all cultivars with an average of 0.3 vs 0.38 respectively, yielding a positive value of *f* (0.21).

To compare the genetic diversity of Tunisian landraces with accessions from other origins, peach cultivars originated from America, mainland Spain and La Palma (Canary Islands) were used. In La Palma genotypes, a mean of 3.46 observed alleles per accession were identified with a mean of 0.63 effective alleles per cultivar resulting in a genetic richness of 0.37. The observed heterozygosity was higher than expected (0.48 vs 0.35) giving a positive value of *f* (0.13). The genotypes from mainland Spain amplified a mean of 3.21 observed alleles per accession with a mean of 1.78 effective alleles and a genetic richness of 0.3. The expected heterozygosity was also higher than the observed, with $f = 0.24$. The American genotypes showed an average of

Table 3 Summary of genetic diversity within the five peach groups analyzed in this group

Pool	N	A_o	A_e	Ar	H_o	H_e	f	PrA
Tunisia	23	3.87	1.66	0.47	0.3	0.38	0.21	8
La Palma	25	3.46	0.63	0.37	0.35	0.48	0.13	6
Mainland Spain	25	3.21	1.78	0.3	0.24	0.31	0.24	6
America	25	2.84	0.76	0.38	0.16	0.25	0.36	5

N: number of accessions

A_o:mean number of observed alleles per locus;

A_e: mean number of Effective number of alleles;

Ar: allelic richness

H_o: observed heterozygosity

H_e: expected heterozygosity

f: Wright's fixation index

PrA: private alleles

Table 4 Summary of LD parameters within each chromosomes in each subpopulation

Pool	Tunisia	America	Mainland Spain	La Palma
% of intra-chromosome comparisons with $r^2 > 0.1$	9%	16%	14%	11%
% of inter-chromosome comparisons with $r^2 > 0.1$	5%	12%	10%	7%

2.84 allele per accession with 0.76 effective alleles giving an average of 0.38 allelic richness and *f* value of 0.36 (Table 3).

Eight private alleles were obtained in the Tunisian genotypes, while six were observed in the mainland Spanish pool, five in the American pool and six in the La Palma pool (Table 3).

Linkage disequilibrium analysis

In Tunisian genotypes, 9% of intra-chromosome comparisons (within the same linkage group) showed significant LD (Table 4). However, the proportion of inter-chromosome comparisons in LD was 5%. The variation of pairs of alleles in LD for markers mapped in the same linkage group was plotted against genetic distance measured in centimorgans (cM).

The intra-chromosome LD was higher in the American pool (16%) than in mainland Spain pool (14%) and La Palma pool (11%) (Table 4).The proportions of inter-chromosome comparisons in LD were 12% in American genotypes, 10% in mainland Spain genotypes and 7% in La Palma genotypes (Table 4).

Figure 2 shows the LD variation with genetic distance measured in cM and showed different degrees of reduction in r^2 values due to differences in LD. In the Tunisian group, r^2 reached the significant 0.1 threshold at 19.5 cM. In mainland Spanish genotypes, the logarithmic curve representing the decay of LD with genetic distance intersected with the $r^2 = 1$ value at 10 cM, compared to 9 cM for American group, and 15 cM for La Palma group.

The variation of alleles in LD among the eight different linkage groups was evaluated. Figure 3 showed that the Tunisian gene pool had the lowest frequencies of alleles in LD among the eight linkage groups. In particular, these proportions were lower in LG3, LG4 and LG5.

As compared to other subpopulations, the highest proportions of alleles in LD were observed in American genotypes followed by mainland Spain and La Palma genotypes. Figure 3 showed also that the highest proportion of alleles in LD was observed at linkage group LG1.

Genetic relationship among the accessions

UPGMA dendrogram analysis

A dendrogram was constructed based on genetic distances between Tunisian accessions (Fig. 4). No clear groupings based on their geographic origin or phenotypic characteristics were found. The accession 'Porte Greffe Pêcher' (rootstock) seems genetically different and was clustered independently. Among the second group, three local Tunisian accessions ('Bargoug', 'Chaaraoui' and 'Bouchkara') were clearly separated from the remaining accessions. Among the rest of genotypes, some accessions sharing the same phenotypic characteristics, such as the accessions platycarpa 'Boutabgaya' and 'Bargou' sharing the characteristic of white flesh (mesocarp), clustered together. However, it was not the case of the accessions 'Bedri', 'Essifi' and 'Kharfi' that share the characteristic of yellow flesh.

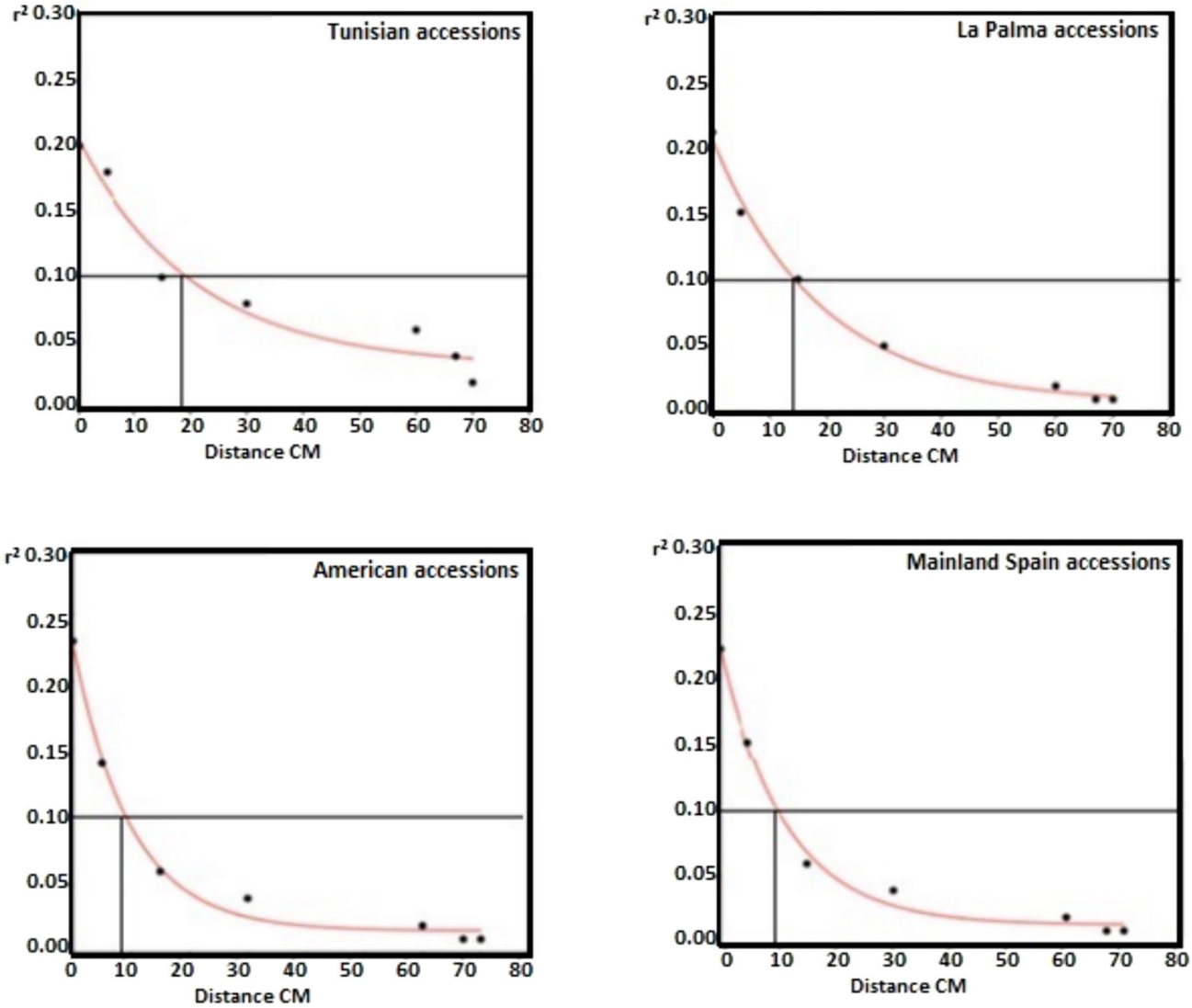


Fig. 2 LD decay plot in four peach groups. The correlation between the X and Y axis indicates the decay trend of the LD coefficient (r^2) with genetic distance in Centimorgans (Cm) within intra-chromosome. The horizontal line in each plot indicates $r^2 = 0.1$

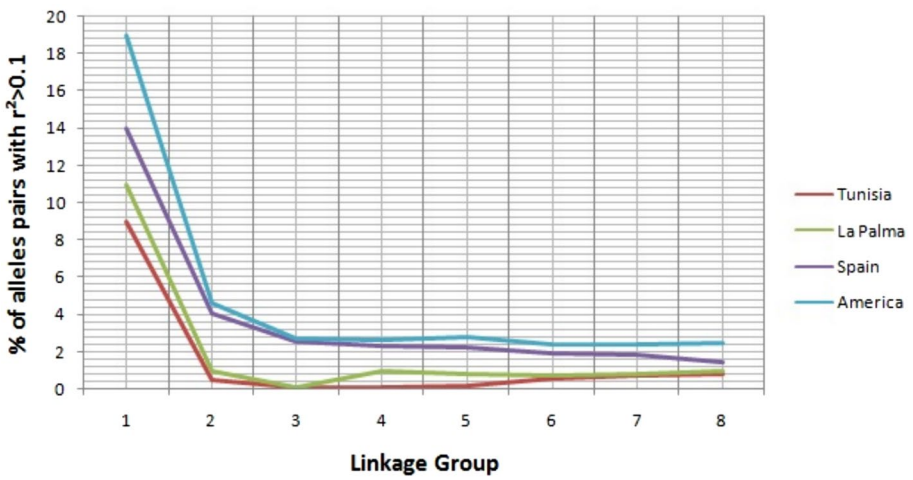


Fig. 3 Percentage of alleles with significant LD ($r^2 > 0.1$) at different linkage groups

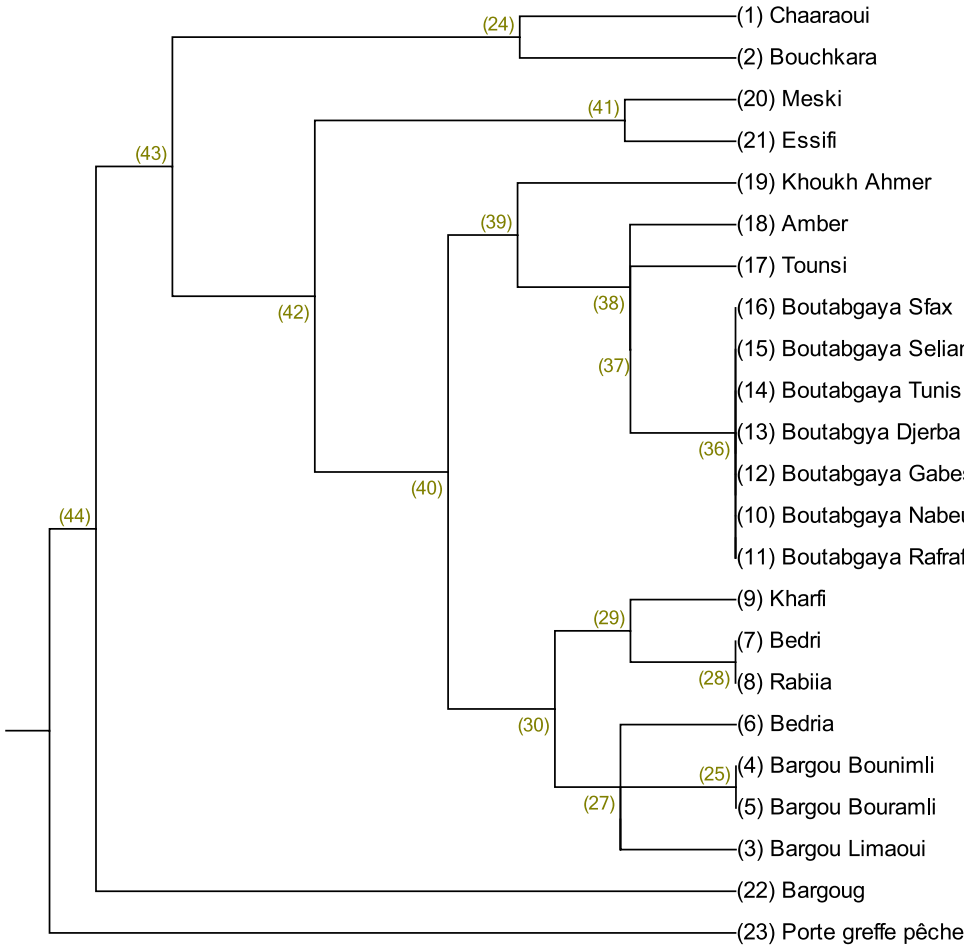


Fig. 4 UPGMA tree for the 23 Tunisian peach accessions. Bootstrap values are shown on the branches. SBL: 55.58

A second dendrogram was drawn using combined results of the 23 Tunisian accessions and 75 other genotypes from La Palma, mainland Spain and America (Fig. 5). A first group clustered accessions mainly from Bolivia (probably of Spanish origin and conserved in Spain) and three local Tunisian peaches ('Bargoug', 'Chaaraoui' and 'Bouchkara'). The remaining accessions were included into a second group and formed different clusters. In the upper part, accessions from America and Spain were clustered. Among this group, there was a cluster with samples of the platycarpa type. In a second cluster, samples mainly from La Palma and mainland Spain were observed. The last group clustered accessions from Tunisia, La Palma and mainland Spain (Fig. 5).

Structure analysis

The model-based Bayesian clustering approach implemented in Structure v. 2.3.4 (Pritchard et al. 2000) was used to investigate the genetic structure of peach cultivars according to the models with three (K = 3) to seven clusters (K = 7). The best population number identified by Evanno et al. (2005) was k = 4 (Fig. 6A).

According to this subdivision, a first cluster (red) included accessions from different origins and represents an admixed group. The main accessions gathered in this group are three local Tunisian samples ('Chaaraoui', 'Bouchkara' and 'Bargoug') and the samples belonging to Bolivia. A second group (green) gathered accessions mainly from Tunisia, La Palma and mainland Spain. A third group (blue) gathered accessions from America with the group of platycarpa 'Boutabgaya' from Tunisia. A fourth group (yellow) gathered the remaining accessions from La Palma and mainland Spain (Fig. 6A).

When a lower number of K was used, the mixed first cluster including the three local Tunisian accessions and those from Bolivia was combined with Spanish accessions (cluster in blue). Using higher K values, the admixed cluster remained unmodified, whereas they allowed disentangling internal pattern of subStructure of the Tunisian-La Palma-mainland Spain group. At K = 6, the accessions of flat peaches ('Boutabgaya') cluster separately from the USA group (green) (Fig. 6A).

A NJ tree was computed among the Structure clusters at K = 4, based on the allele-frequency divergence (Fig. 6B). The distances varied between 0.0853 (between clusters 3

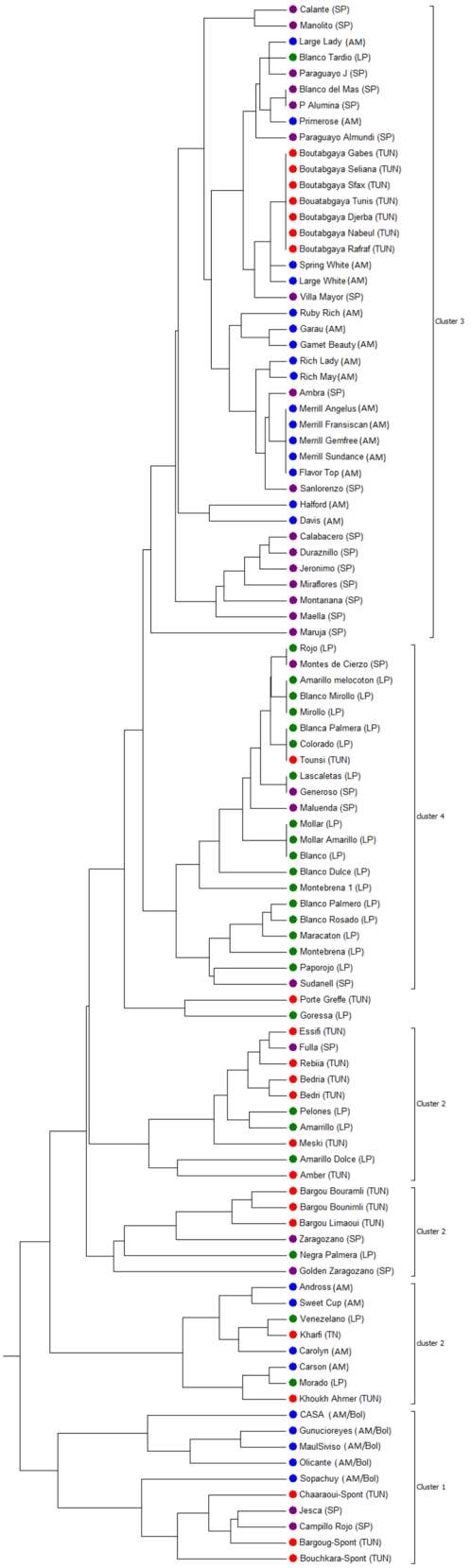


Fig. 5 UPGMA dendrogram of 98 peach accessions from different origins. *TUN* accessions from Tunisia, *SP* accessions from Spain, *AM* accessions from America, *AM/BOL* accessions from Bolivia and *LP* accessions from La Palma. *SBL*486.27. The colors and the clusters indicate the repartition inferred by **Structure** at k = 4

and 4) and 0.3479 (clusters 1 and 4). The representation showed that the first cluster was highly divergent from other clusters and seems genetically different. The second and third clusters were genetically close.

Population differentiation analysis

Differentiation parameters were calculated among the groups identified by Structure at K = 4 (Table 5).

The expected heterozygosity was calculated between individuals of the same cluster. The first admixture cluster (three local Tunisian cultivars and those from Bolivia) showed a value of 0.0547, the second cluster (Tunisia + LaPalma + mainland Spain) showed a value of 0.1889, the third cluster (mainly American) showed a value of 0.281 and the fourth one (remaining mainland Spain + La Palma) showed a value of 0.2940.

The Mean value of *F*st was calculated between individuals of the same cluster. The first cluster showed a value of 0.9050 (Nm = 0.27), the second cluster showed a value of 0.3961 (Nm = 0.375), the third cluster showed a value of 0.3659 (Nm = 0.42) and the fourth one showed a value of 0.2879 (Nm = 0.612).

The four groups obtained by Structure were used to calculate the Analysis of Molecular Variance (AMOVA). The AMOVA revealed that 63.48% of the total variation was detected within clusters, while the rest of variation (36.52%) was attributed among the clusters (Table 6). In addition, the overall *F*st was 0.3361 ($p < 0.05$) and the Nm was 0.507.

Discussion

This work aimed to evaluate the genetic diversity of local Tunisian peach accessions and explore their genetic relationships with accessions from other origins. For this purpose, we used 27 nuclear SSR microsatellite loci mapped on the eight peach-linkage groups.

Assessment of genetic diversity

Tunisian peach landraces harbor rich genetic diversity, highlighted by the presence of eight private alleles amplified at different loci. As in other crops, such as plum (Abdallah et al. 2016; 2019; Baraket et al. 2019, 2021) or melon (Chikh-Rouhou et al. 2021), the local Tunisian peach germplasm is a valuable gene pool repository for breeding

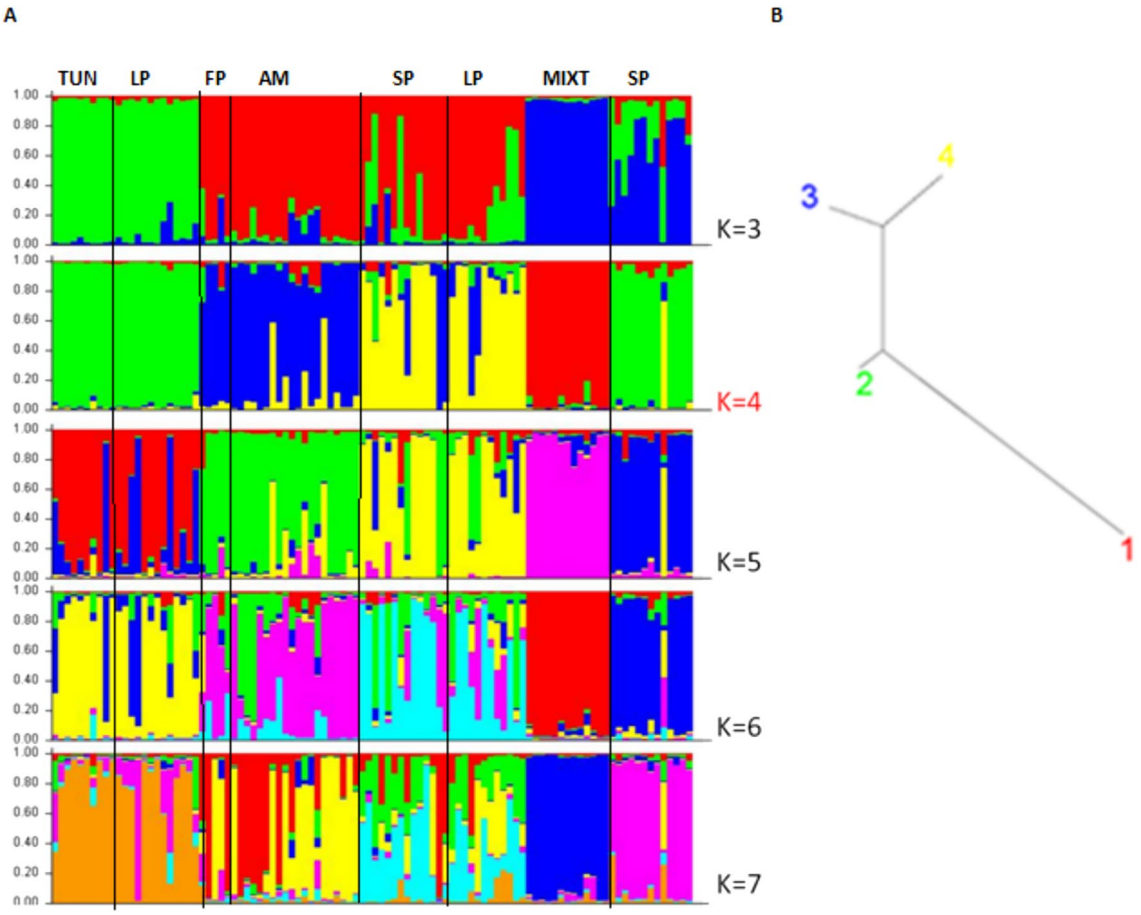


Fig. 6 **A** Population stratification based on Bayesian clustering approaches for K = 3 to K = 7 for 98 peach accessions of different origins. Different subpopulations are presented in different colors. **B** NJ tree among the clusters identified at K = 4. *TUN* Tunisian accessions, *LP* La Palma accessions, *FP* flat peach (Platycarpa), *AM* American accessions, *SP* Mainland Spain accessions, *MIXT* mixture group

Table 5 Population differentiation parameters among the clusters identified by Structure at K = 4

	Cluster 1	Cluster 2	Cluster 3	Cluster 4
H_e	0.054	0.188	0.281	0.294
*F*st	0.905	0.396	0.365	0.287
Nm	0.270	0.375	0.420	0.612

programs. Unfortunately, those local Tunisian peach accessions are on the risk of disappearance due to the absence of properly managed germplasm collections as it is the case with many other crops (Nabli 2011; Abdallah et al. 2016, 2019; Baraket et al. 2019, 2021).

The average number of alleles found in peach is lower than those in other *Prunus* species such as plums (Carrasco et al. 2012; Abdallah et al. 2019), almond (Fathi et al. 2008), sweet cherry (Wünsch and Hormaza 2004; Baris et al. 2017) or apricot (Gürcan et al. 2015). This is probably due to the self-fertile nature of peaches as compared to the outcrossing

Table 6 Analysis of molecular variance (AMOVA) based on the 27 SSR loci of 98 peach accessions among clusters identified by Structure at K = 4

Source of variation	d.f	Sum of square	Variance components	Fixation indices	Percentage of variation
Among clusters	4	333.629	0.60076 Va	*F*st: 0.3361	36.52
Within clusters	589	881.096	1.49592 Vb	*F*it: 0.25604	63.48
Total	593	1214.725	2.09668		

systems prevalent in most almond, apple, plum and sweet cherry varieties (Wiersma et al. 2001).

The highest values of genetic diversity were recorded in the accessions from La Palma followed from those from mainland Spain and America. Similar results were reported by Pérez et al. (2020). The lower levels of diversity observed in mainland Spanish and American accessions could be explained by a diversification related to adaptive characteristics and propagation by seeds from a limited number of original genotypes (Pérez et al. 2020). In fact, most of the European and American cultivars were obtained from breeding programs (Chen et al. 2007; Rojas et al. 2008) and were strongly adapted to the particular conditions of the region where they were developed (Razifard et al. 2020).

Linkage disequilibrium analysis

Low levels and rapid decreases of LD were observed in Tunisian genotypes. Populations with a broad genetic basis have lower levels of LD than narrow-based populations (Aranzana et al. 2010). Similar results have been found in landraces and wild accessions of peach (Li et al. 2013; Cao et al. 2012) as well in many other species such as wild French grapes (Barnaud et al. 2010) or sweet cherry (Arunyawat et al. 2012). Due to their genetic diversity and adaptation to local environmental conditions, landraces are considered as an important reservoir of new sources of alleles that could be exploited in breeding programs (Chikh-Rouhou et al. 2021). Of particular interest is that, this LD decay was observed at LG3, LG4 and LG5, suggesting that these linkage groups may carry more genes related to agronomic traits such as flesh texture and pit adherence M/m or F LG4; kernel (bitter/sweet) Sk /sk LG5; anthocyanin (normal/blood flesh) Bf /bf LG4; sweet fruit/normal fruit D /d LG5.

Population structure and relationships

*F*st values higher than 0.15 can be considered significant for differentiating populations (Frankham et al. 2002). Thus, significant divergence was found within each subpopulation according to the *F*st obtained by Structure. An overall *F*st value of 0.3361 indicated a significant genetic differentiation between populations. This aligns with the AMOVA results where the most of the total variation (63.48%) was found within clusters while only 36.52% of the total variation was found among clusters.

Both the NJ grouping and the population analysis in Structure showed that Tunisian peaches grouped with peach accessions from different origins. This could be due to the different introductions of peach to Tunisia. In fact, Tunisia has been a crossroad of several civilizations due of its strategic position in the Mediterranean and the diversity of its ecosystems and climatic conditions, and it is considered one of the richest Mediterranean countries in terms of plant genetic resources including landraces and wild relatives (Chikh-Rouhou et al. 2021). The ecogeographic history of domestication of different crops has been studied using microsatellite markers and model-based Bayesian clustering method; examples include peach (Yoon et al. 2006), sweet cherry (Mariette et al. 2016); apple (Urrestarazu et al. 2016) and olive (Besnard et al. 2013).

The clustering of some local Tunisian peach accessions ('Bouchkara', 'Bargou' and 'Chaaraoui') with genotypes from other origins (Bolivia) into a separate group (red for K = 4) may suggest that these accessions could have a different origin than the other Tunisian peach landraces. The low Nm value of this group (Nm = 0.27) indicates limited gene exchange among genotypes which led to a high genetic differentiation. Low Nm value suggests low genetic exchange or low gene flow. Given that the accessions from Bolivia were probably introduced from Spain, this grouping probably means that those Tunisian accessions could have also been introduced from Spain.

The closest relationship (Group 2, green) was identified between Tunisian, mainland Spanish and La Palma peach accessions. Although the Nm value was still low (0.375), it was higher than in the first group suggesting that peach was probably introduced to Tunisia (in larger sense to Northern Africa) from the European continent. In fact, the introduction of temperate fruits, especially of peach, to North Africa took place during the era of exploration and colonization in the sixteenth and seventeenth centuries by Europeans (Scorza and Sherman 1996). Because of its early introduction, peach is relatively well established in many highland areas.

The aggrupation of Tunisian flat peach or platicarpa ('Boutabgaya') with the American peach (group in blue at K = 4), may point out to an American origin of some Tunisian peaches, probably introduced recently. In addition, the higher Nm value in this group (Nm = 0.42), suggest higher genetic exchange or gene flow.

The close aggrupation, shown by the Bayesian analysis (yellow group at k = 4) and the NJ tree between genotypes from La Palma and mainland Spain, confirmed previous results found by Pérez et al. (2020) pointing out to the introduction of most of the Canary Island peach germplasm from mainland Spain. Thus, the gene flow between La Palma and mainland Spanish genotypes was important (Nm = 0.612).

Conclusion

The data reported here provide new information related to Tunisian peach genotypes as unexplored sources for marker-assisted breeding in peach. Thus, the available diversity

in the Tunisian landrace germplasm could be important tool to expand the allelic pool of cultivated peach through introgression.

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Author contribution statement **DA** conducted the experiments and statistical analyses, developed the genetic analyses, and wrote the manuscript. **GB** provided some plant material, and discussed and corrected the content. **VP** provided some plant material and discussed the results. **SBM** discussed the results. **AS-H** and **JH** provided experimental instructions, supervised the work, and assisted in writing the manuscript.

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Data availability statements Authors declare that all data are included within the manuscript and fully available without restriction.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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