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Genetic diversity and structure in Tunisian wild cherries and cultivated sweet cherries revealed by SSR markers

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ABSTRACT. Wild cherries are concentrated at high altitudes in northern Tunisia and cultivated sweet cherries are well adapted to low altitudes. This study aims to compare local wild and cultivated sweet cherries *(Prunus avium L.)* in the northern Tunisia. To elucidate genetic variation of 21 cultivated sweet cherries (one landrace and 20 foreigner cultivars) and 4 local wild sweet cherries, we used 16 SSR (Simple Sequence Repeat) loci markers. Results showed a significant reduction of genetic diversity parameters in the cultivated germplasms compared to wild populations. The average number of alleles per locus was 8.188 alleles per locus for the 21 cultivated sweet cherries and 3.68 alleles per locus for the 4 wild sweet cherries. The average value of genetic differentiation of population (Fst) over all loci revealed a low genetic differentiation (Fst = 0.04) between Tunisian wild and cultivated sweet cherry groups. The UPGMA (Unweighted Pair-Group Method Analysis) dendrogram and genetic population structure showed that Tunisian wild sweet cherries presented high genetic variability since they belong to different clusters and consequently could be considered as a potential source of germoplasm to be exploited in sweet cherry improvement.

Key words: Wild cherries; Landrace; Dendrogram; Population structure.

INTRODUCTION

Sweet cherry (*Prunus avium L.*) is an important tree crop, which grows both wild and cultivated species. The diversity of plant genome is affected by human intervention. Firstly, traits convenient for human use, such as development of organs used by man, or adaptation to new environments, have been selected, resulting in selection signatures at specific loci. Secondly a bottleneck affecting the genome [1]. Human domestication of plant is the modification of a wild species to create a new form of plant altered to meet human needs [2].

Analysis of genetic diversity and population structure of the existing population is required for the purpose of conservation and reintroduction of rare and endangered species [3]. Knowledge of genetic diversity within population and among populations is important for conservation management, especially for identifying genetically unique structural units within species and determining populations that need protection [4]. Exploring cherry genetic diversity is crucial in order to create well adapted new cultivars to climate change or invasion of new pathogens like "*Drosophila suzukii*" [5].

Accurate estimates of genetic diversity are particularly useful for optimization of sampling strategies and for conservation and management of the genetic diversity [6]. Since the mid-1990s, Prunus species (especially peach, sweet cherry and plum) have been characterized molecularly [7]. Despite the worldwide cultivation of fruit trees, few studies have analyzed the genetic domestication and breeding history of the Prunus species [1]. In Tunisia, wild and cultivated sweet cherry, as compared to other fruit species, exhibit high genetic variability that has not yet been well explored and exploited. Therefore, these germoplasms have not been characterized using molecular methods. Their relatedness and genetic constitution remain unknown. In the present study, 25 accessions of local wild sweet cherries and cultivated sweet cherries (landrace "Bouargoub" and foreigner cultivars from different geographical origin) were investigated for the first time in Tunisia. Sixteen Simple Sequence Repeat (SSR) markers were used in order to detect the genetic diversity, and to clarify the relatedness of 25 accessions of sweet cherry. The identification of three new cultivated accessions, planted in northern Tunisia, was also studied.

MATERIALS AND METHODS

Plant material

Four Tunisian wild sweet cherries and 21 cultivated sweet cherries (20 foreigner cultivars and one landrace) were analyzed to assess sweet cherries (*Prunus avium* L.) diversity. This gene pool originated from different breeding zones in Tunisia. The origin of foreigner cultivars was more evident since they were obtained from various breeding programs (Italy, French, Hungry, Canada and Germany) (Table 1).

Table 1. Origin, breeding zones and Parentage of the 25 accessions of sweet cherries in northern Tunisia

				Parentage		
	Accessions	**Origin**	**Breeding zone**	**Mother**	**Father**	**Reference**
Cultivated sweet cherries	Napoleon	Germany	Makthar			Shuster (2017)
	Van	Canada	Makthar	Empress eugenie	Open pollinated	
	Moreau	French	Makthar			
	Sunburst	Canada	Makthar	Van	Stella	
	Stella	Canada	Makthar	Lambert	JI 2420(emperor francisxnapoleonX-rayed pollen)	
	Burlat	French	Makthar	Selected by leonard burlat, from Rhone valley		
	Adriana	Italy	Tunis	ISF 123	Mora di Cazzano	

	Carmen	Hungary	Tunis	Yellow Dragan	H203 (Germersdorfer xopen pollinated)	
	Ferrovia	Italy	Tunis	Local cultivar, similar to bella di pistoia, Bar region	Stella compact	
	New star	Canada	Tunis	Van	Stella	
	Early bigi	French	Tunis			
	Vera	Hungary	Tunis	Ljana (Trusenszkaya 6)	Van	
	Sweet early	Italy	Tunis	Burlat	Sunburst	
	Early star	Italy	Tunis	Burlat	Stella compact	
	Samba	Canada	Tunis	Stella 35Ax open pollinated	Stella 16A-7	
	Black star	Italy	Tunis	Lapins	Burlat	
	Grace star	Italy	Tunis	Burlat	Open pollinated	
	Unknown-Makthar 1	Unknown	Makthar			
	Unknown-Makthar 2	Unknown	Makthar			
	Unknown-Makthar 3	Unknown	Makthar			
	Bouargoub	Tunisia	Makthar	Landrace		
Wild cherries	Wild local Ain-Draham 1	Tunisia	Ain-Draham	Wild		
	Wild local Ain-Draham 2	Tunisia	Ain-Draham	Wild		
	Wild local Beja1	Tunisia	Beja	Wild		
	Wild local bBeja 2	Tunisia	Beja	Wild		

Choice of markers and molecular genotyping

Each accession was genotyped for 16 SSRs (Simple Sequence Repeats). These SSRs markers were given in Table S1.Total genomic DNA was extracted from young leaf from a single tree for each genotype using the CTAB method [8] with minor modifications. DNA purity and concentration were checked on 1%(w/v) agarose gels by using 1.0X TBA buffer. PCR was carried out in a volume of 25 µL including 50 ng template DNA, 1X PCR buffer, 1.5 mm MgCl2, 0.2 mM of each dNTP, 1.67 Pmol of each SSR primer (forward and reverse), and 1.5 U Taq DNA polymerase (Sigma, USA) using the following temperature: 95°C for 5 min, then 35 cycles of (94°C for 45 s, 57°C for 45 s and72°C for 45 s) finishing with 72°C for 8 min. PCR products were separated by electrophoresis in 1% agarose gels.

Data analysis

Population genetics and linkage disequilibrium analyses were carried out using the Genealex6.5 software. UPGMA dendrogam was generated using DARwin software. The model based program STRUCTURE v2.3.4 [9] was used to infer the population structure of the 25 accessions.

Expression trend of bta-miR-33a in BMECs

In the present study, 16 loci in a range of 25 cherry genotypes were assayed (Table S1). The number of observed alleles per locus ranged from 6 to 11 with an average of 8.18 alleles per locus in cultivated sweet cherries. However, this parameter varied from 1 to 6 alleles per locus in local wild cherries. A moderate level of polymorphism was assayed in local wild cherries, giving an expected number of alleles per locus ranging from 1 (EMPAS10) to 5.33 (EPDU3392).

This latter was the most polymorphic among 16 loci, with the highest effective number of alleles. In the present study, the expected heterozygosity was 0.69 and 0.71 for local wild cherries and cultivated sweet cherries respectively. Observed and expected heterozygosity were higher in the local wild cherry group, than in the cultivated sweet cherries for the primers CPSCT034

(Ho=1) and EPDU3392 (He=0.813) respectively. To assess the markers' discriminatory potential, we evaluated the Shannon index for the 16 loci. This index value was 2.172 for the most informative locus (BPPCT040) in cultivated sweet cherries and 1.733 for the most informative locus (EPDU3392) in local wild cherries (Table 2).

Local wild sweet cherries were characterized by 11 specific alleles in the range of 9 SSR primers (Table 3). Size in base pairs (bp) ranged from 302bp to 142bp in the primer EPPB4230 and EMPaS02 respectively. On the other side, local wild sweet cherries possess in common with landrace, 2 specific alleles for the two primers EPPB4230 and CPSCTO22 by allele base pairs 302bp and 268bp respectively.

The Wright inbreeding coefficient (Fis) was computed according to [10]. Over all loci, the average Fst (the Genetic Differentiation of Populations) value was 0.04. This parameter ranged from 0.010 (EPPB4230) to 0.067 (EPDU3392) (Table 4). In the same way the Fst was calculated and compared between every two groups. Wild-landrace (Fst=0.207), wild-foreigner (Fst=0.04) and landrace-foreigner (Fst=0.18) (Table S2).

Gene flow between the wild and cultivated sweet cherry groups estimated as the number of migrants using private alleles after correction for sample size [11]. In this study, gene flow was very high (Nm = 6).

The UPGMA dendrogram confirmed the presence of differentiation between breeding zones, although only in rare cases it was possible to observe a structuring linked to their geographical location. The 25 accessions were grouped into four clusters (GI-IV) (Figure 1).

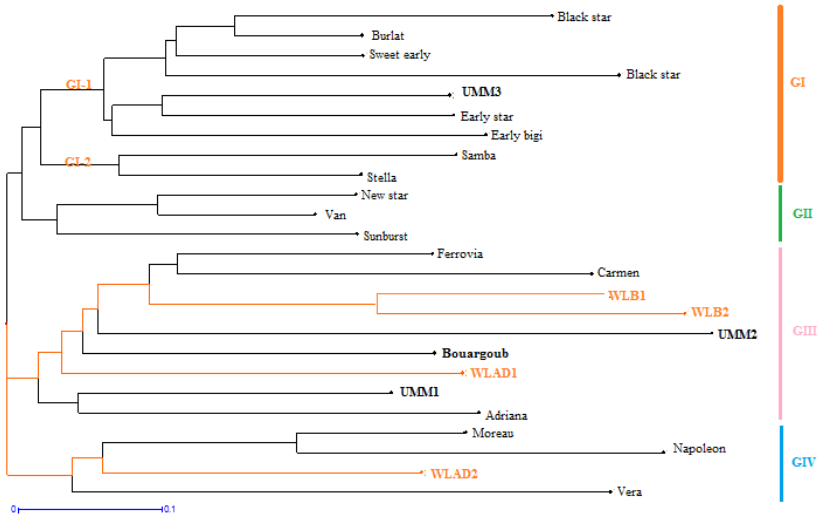

Figure 1. Unweighted Pair-Group Method Analysis (UPGMA) dendrogram of 5 breeding zones of *Prunus avium* in northern Tunisia based on 16 SSR markers.

RESULTS

In the present study, 16 loci in a range of 25 cherry genotypes were assayed (Table S1). The number of observed alleles per locus ranged from 6 to 11 with an average of 8.18 alleles per locus in

cultivated sweet cherries. However, this parameter varied from 1 to 6 alleles per locus in local wild cherries. A moderate level of polymorphism was assayed in local wild cherries, giving an expected number of alleles per locus ranging from 1 (EMPAS10) to 5.33 (EPDU3392). This latter was the most polymorphic among 16 loci, with the highest effective number of alleles. In the present study, the expected heterozygosity was 0.69 and 0.71 for local wild cherries and cultivated sweet cherries respectively. Observed and expected heterozygosity were higher in the local wild cherry group, than in the cultivated sweet cherries for the primers CPSCT034 (Ho=1) and EPDU3392 (He=0.813) respectively. To assess the markers' discriminatory potential, we evaluated the Shannon index for the 16 loci. This index value was 2.172 for the most informative locus (BPPCT040) in cultivated sweet cherries and 1.733 for the most informative locus (EPDU3392) in local wild cherries (Table 2).

Table 2. Genetic diversity parameters calculated for 16 SSR markers in Tunisian wild cherry and cultivated sweet cherry genetic pools

Markers	Na		Ne		I		Ho		He	
	Cultivated	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated	Wild	Cultivated	Wild
EMPA002	7.000	2.000	2.940	1.600	1.339	0.562	0.857	0.500	0.660	0.375
CPSCT034	11.000	5.000	4.846	4.571	1.859	1.560	0.857	1.000	0.794	0.781
pchgms49	7.000	2.000	2.901	1.600	1.422	0.562	0.429	0.500	0.655	0.375
Udp98-022	9.000	3.000	3.920	2.462	1.672	0.974	0.810	0.250	0.745	0.594
CPSCT022	9.000	4.000	3.991	4.000	1.668	1.386	0.714	0.750	0.749	0.750
EPDU3392	11.000	6.000	4.768	5.333	1.892	1.733	0.714	0.500	0.790	0.813
EPPCU3090	9.000	4.000	3.571	3.556	1.546	1.321	0.952	0.750	0.720	0.719
EMPAS10	7.000	1.000	2.130	1.000	1.174	0.000	0.476	0.000	0.531	0.000
BPPCT040	11.000	5.000	7.412	4.000	2.172	1.494	0.762	0.750	0.865	0.750
EMPA026	5.000	3.000	2.765	2.909	1.184	1.082	0.714	0.750	0.638	0.656
EMPaS14	6.000	4.000	3.207	2.286	1.395	1.074	0.762	0.500	0.688	0.563
BPPCT034	9.000	3.000	4.388	2.667	1.757	1.040	0.619	0.000	0.772	0.625
EMPaS02	8.000	5.000	4.546	3.200	1.745	1.386	0.619	0.750	0.780	0.688
CPSCT038	6.000	3.000	2.485	2.667	1.227	1.040	0.476	0.500	0.598	0.625
BPPCT037	10.000	5.000	4.410	3.200	1.817	1.386	0.524	0.500	0.773	0.688
EPPB4230	6.000	4.000	3.802	3.556	1.473	1.321	0.571	0.250	0.737	0.719
Mean	8.180	3.680	3.880	3.038	1.584	1.120	0.607	0.516	0.718	0.694
Mean of two groups	6		3.48		1.37		0.59		0.67	

Na: Number of different alleles ; Ne: Number of effective alleles ; I: Shannon's Information Index ; Ho: Observed heterozygosity ; He: expected heterozygosity.

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Table 3 . Private detected allele in wild local cherries (A) and common allele among local wild cherries (*) and landrace (**), size in base pairs (bp) and relative frequency

	Private detected allele (A)		Common allele	
Markers	**Size (bp)**	**Frequency**	**Size (bp)**	**Frequency**
pchgms49	167	0,25		
CPSCT022	268	0,25	268	0,125*
				0,500**
EPDU3392	186	0,125		
EMPAS10	178	0,125		
	180	0,125		
EMPA026	227	0,25		
BPPCT034	198	0,125		
EMPaS02	142	0,125		
BPPCT037	152	0,125		
EPPB4230	290	0,125	302	0,125*
	302	0,375		1**

Size in base pairs (bp) ranged from 302bp to 142bp in the primer EPPB4230 and EMPaS02 respectively. On the other side, local wild sweet cherries possess in common with landrace, 2 specific alleles for the two primers EPPB4230 and CPSCTO22 by allele base pairs 302bp and 268bp respectively.

The Wright inbreeding coefficient (Fis) was computed according to Weir BS et al. [10]. Over all loci, the average Fst (the Genetic Differentiation of Populations) value was 0.04. This parameter ranged from 0.010 (EPPB4230) to 0.067 (EPDU3392) (Table 4). In the same way the Fst was calculated and compared between every two groups. Wild-landrace (Fst=0.207), wild-foreigner (Fst=0.04) and landrace-foreigner (Fst=0.18) (Table S2).
Gene flow between the wild and cultivated sweet cherry groups estimated as the number of migrants using private alleles after correction for sample size [11]. In this study, gene flow was very high (Nm=6).

Table 4. Inbreeding coefficient (Fis) and the pairwise (Fst) values in the whole 25 cherry accessions.

Locus	**Fis**	**Fst**
EMPAOO2	-0,311	0,050
CPSCT034	-0,179	0,027
pchgms49	0,099	0,052
Udp98-022	0,209	0,011
CPSCT022	0,023	0,053
EPDU3392	0,242	0,067
EPPCU3090	-0,183	0,011
EMPAS10	0,225	0,033

BPPCT040	0,064	0,023
EMPA026	-0,131	0,030
EMPaS14	-0,009	0,043
BPPCT034	0,557	0,066
EMPaSO2	0,067	0,025
CPSCT038	0,201	0,016
BPPCT037	0,299	0,029
EPPB4230	0,436	0,102
Mean	0,101	0,040
SE	0,058	0,006

The UPGMA dendrogram confirmed the presence of differentiation between breeding zones, although only in rare cases it was possible to observe a structuring linked to their geographical location. The 25 accessions were grouped into four clusters (G_{I-IV}) (Figure 1).

The STRUCTURE v 2.3.4 [9] was performed on the whole data set, the ΔK value corresponding to each K was calculated. The result showed that the maximum ΔK value appeared at K =3 (Figure S1), using Evano's method [12]. The four Tunisian wild sweet cherries were an admixture of the three studied groups with different contribution of each population. Similarly, the landrace "Bouargoub" was an admixture of three populations with a contribution that exceeds 50% from the wild group. The unknown UMM2 belongs to the European group (C2) and closely related to the Hungarian cultivar "Vera" with a contribution which exceeds 90% of the genetic background of the second group (C2). The two unknowns "UMM1" and "UMM3" belong to third group (C3) and were an admixture of three populations with different contribution of each population (Figure 2).

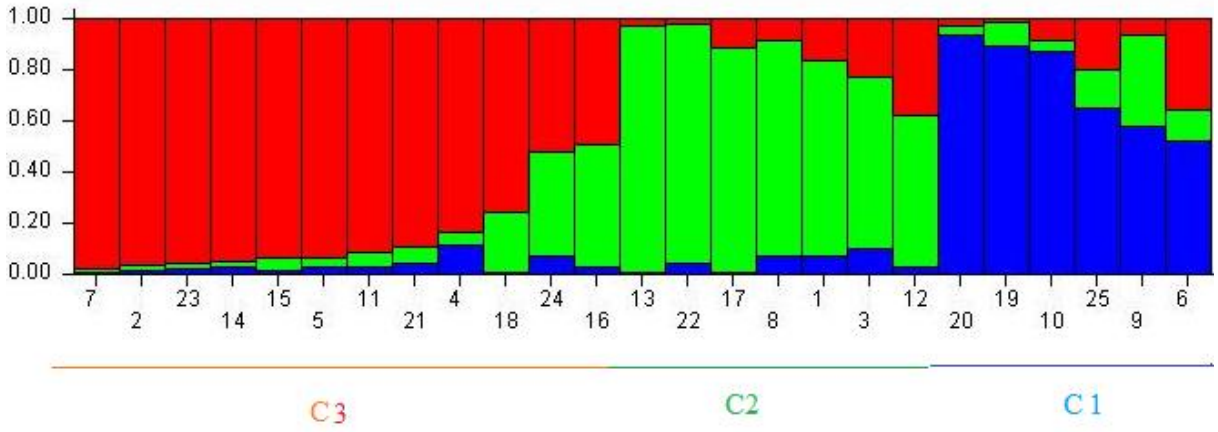

Figure 2. Structure bar plot results obtained on the whole set of data at K=3. Each vertical bar corresponds with a distinct genotype and different colors indicate the part of its genome assigned to each cluster.
1. Napoleon; 2.Van; 3.Moreau; 4.Sunburst; 5.Stella; 7.Burlat; 6.Bouargoub; 8.Adriana; 9.Carmen; 10.Ferrovia; 11.Newstar; 12.Earlybigi; 13.Vera; 14.Sweet early; 15.Early star; 16.Samba; 17.Back star; 18.Grace star; 19.Wild local Beja1; 20.Wild local Beja2; 21.Unknown Makthar 1; 22.Unknown Makthar 2; 23.Unknown Makthar 3; 24.Wild local Ain-Draham1; 25.Wild local Ain-Draham2.

DISCUSSION

The number of observed allele per locus (8.18) in 21cultivated sweet cherry are less than 37 alleles found by by Kacar et al. [13] when they genotyped 10 sweet cherry with 9 SSR primers. However, our result was almost higher than the result found by Clarke JB et al. when they used [14] sweet cherry cultivars for SSR analysis and determined 2 to 7 alleles per SSR primer. In the same way, the number of allele per locus for the local wild cherries (3.68) are almost the same that found by Vaughan SP et al. [15] when they used 10 SSR primers in 16 wild cherry accessions and they obtained 2 to 6 alleles. The average number of alleles per locus identified in this study was bigger than the identified number (2.8 alleles per locus) in the study of [16] when they genotyped 21 sweet cherry cultivars. The wild sweet cherries grown in Tunisia present for each microsatellite a number of alleles lower than the cultivated one. Thus, most likely reflects the fact that cultivated sweet cherry possess a wider basis of genetic diversity compared to wild Tunisian one, since they are the result either of different geographical origins and/or the result of different breeding program from different genotypes (Table 1). The effective number of alleles for the two groups, wild (Ne=3.03) and cultivated (Ne=3.8) is almost the same.

The primers BPPCT034 and BPPCT040 produced 5 and 6 alleles/locus respectively in the study of Turet-Sayar et al. [17], when they worked with 15 genotypes. In the present study these two primers produce 9 and 11 alleles respectively in 21 cultivated sweet cherries, which show variability in the number of alleles per locus for the same marker. This is affirmed by several research works Kaçar YA reported that, in sweet cherry, the number of alleles per locus ranges according to the number of genotypes which is confirmed by the present study [18]. The EMPAS10 and EMPAS02 primers recorded a very small number of Na alleles which was of the order of 1 and 5 alleles respectively in the studied wild cherries, whereas these markers showed a high number of 25 and 16 alleles per locus in the Vaughan SP et al. research when they genotyped wild cherry trees [15]. The primer EPDU3392 was most informative according to highest number of alleles in wild and cultivated sweet cherries with 6 and 11 alleles respectively (Table 2).

To distinguish Tunisian wild sweet cherries, 11 specific alleles were detected with 16 primer pair's cherries. The selected marker set was able to distinguish cultivated from wild sweet cherries, due to a number of unique alleles detected in this latter. Wild cherries could be distinguished from cultivated sweet cherries using specific allele in 10 loci among 16 used loci (Table 3). The specific alleles of local wild and cultivated sweet cherries can be selected in breeding program with the objective of adapting to climate change and ecological conditions. Studies carried out by Azizi-Gannouni et al. [19] have shown that the landrace sweet cherry "Bouargoub" has a low chill requirement and adapts well to mild winter. According to this finding, we can select the specific alleles of chill requirement. In the same way, the specific alleles of local wild sweet cherries can be selected for other needs.

Unlike the number of alleles, the observed level of heterozygosity was somewhat affected by domestication and breeding. The mean heterozygosity of the studied cultivated cherries (He=0.71) is higher than that reported for other Prunus species including peach [20] and apricot [21] using SSR markers.

We found differences between He and Ho in cultivated and wild sweet cherry populations which is not in accordance with previous research found by Dirlewanger et al. [16], Wunch A et al [16] and Ganopoulos et al. [22].

The use of the Shannon's diversity Index as an indicator of genetic diversity alongside other indicators analyzed by this study, confirm the most informative loci such as EPDU3392 and BPPCT040 in cultivated sweet cherries (I=1.58) and local wild cherries (I=1.12) respectively. The Shannon diversity index (I=1.37) for all studied population reveal a relatively low level of among-population genetic differentiation along with the absence of most genetic variation within presented populations (Table 2).

Despite the low sampling of wild cherries we noticed a slight increase in expected and observed heterozygosity for the EPDU3392 (He=0.81) and CPSCTO34 (Ho=1) primers (Table 2) which can be explained by domestication in order to improve fruit production and to promote self-

compatible varieties. Heterozygosity of genome depends on gametophytic self-incompatibility, which is controlled by multiallele S-locus [23,24]. Our study is based on a low number of self-compatible accession such as “Early star”, “Grace star”, “Newstar”, “Stella”, “Sunburst” [25] and Bouargoub [26].

This result agrees with the hypothesis suggesting a relatively moderate degree of genetic diversity in the collection of studied sweet cherry (*Prunus avium* L.), as a result of intra-collection variability. Furthermore, the EPPCU3090 locus seems to contribute the most to the diversity of genetic resources as soon as it presents the greatest number of observed heterozygosity (Ho=0.95) in the cultivated cherries. However, in the Tunisian wild cherries the locus CPSCT034 isolated from plum is the most indicative of this diversity. The molecular characterization of cultivated and wild sweet cherries can be used to enlarge the genetic diversity and to select the most efficient genotype with adaptive parameters for suitable growing zone or adaptations to climate change.

The value of Fis index for the primers EMPA002, CPSCT034, EPPCU3090, EMPA026 and EMPaS14 were negative (Table 4) and showed an excess of herozygosity, given that our studied accessions are in large part garnished by gametophytic self-incompatibility system. This characteristic prevents self-pollination and reduces inbreeding level.

Additional information was confirmed by the calculation of Pairwise Fst values to clarify the relationships among the 25 accessions (Table 4). The genetic differentiation of populations Fst is a measure of population differentiation due to genetic structure. According to Wright S [27] the average Fst value over all loci revealed a low genetic differentiation between Tunisian wild and cultivated sweet cherry groups (Fst=0.04). It was lower than the differentiation observed among the wild cherry populations (Fst =0.097) in the studied of Ganopoulos et al. [28].

The results of this study are expected results based on the open pollination of Prunus avium which explain the high degree of heterozygosity (0.59). In the same way, for the 25 accessions, the average inbreeding coefficient Fst value indicated a low to moderate level of differentiation among populations and a comparatively high level of genetic diversity. This value is in accordance with this found by De Rogatis et al. and Santi F et al. [29,30] when they genotyped Italian and Georgian wild cherries respectively.

Comparing landrace and Tunisian wild cherries in the one hand and landrace and the foreigner cultivated cherries on the other hand, the result showed two highest Fst value (0.207) and (0.18) respectively (Table S2). The high differentiation between the landrace and the local wild cherries can be explained by the specificity of genetic material for each genotype and lack of human modification of these latter. However, pairwaise Fst value between cultivated foreigner and local wild cherries are low (0.04) suggesting less differentiation between them. This low level of differentiation was an unexpected finding, since they belong to different geographical areas and do not have parental ties in common. Taking into account that prevalence of wild cherry in North Africa and possibly their presence in southern Europe and other geographic region in the world, it appeared sensible to expect the high degree of relatedness and, consequently the less differentiation between them. We cannot confirm the Tunisian originality of this species since it’s reported to have originated in an area between the Black Sea and the Caspian Sea [31] and is an early derivative of an ancestral *Prunus* native to central Asia [32]. The spread of sweet cherry (*Prunus avium*) in several geographical areas combined with different cultivation methods have contributed to the development of specific ecotypes. Each ecotype developed some features like cold hardiness, tree habit, fruit and leaf characteristics that allow its adaptation to each area [32].

Gene flow is very important for the dispersal and evolution of plant populations. In this study, gene flow was very high (Nm = 6) suggesting that a high genetic exchange or high gene flow may occur and led to a low genetic differentiation between the different accession of studied sweet cherries which is the same case of the study of Eltaher et al. [33].

In this study, the high value of this parameter can be explained by several phenomena. Indeed, the majority of the foreigner modern studied cultivars were the result of breeding programs using as parents cultivars from the same range of this study (Table 1). As well as, the synchronization of the blooming period showed variability in the dates and period of blooming for the landrace

“Bouargoub” and other foreigner cultivated sweet cherries [19]. Their blooming periods were superimposed on each other, which created the condition for their possible pollination according to pollen compatibility. Changes in ecological conditions may result in blooming overlap and increased gene flow among cultivated and wild populations [34,35] showed that differences in flowering time between wild and cultivated populations may be an efficient barrier to extensive genetic introgression.

The high level of gene flow can lead to genetic homogenization and alter fitness. Ganopoulos et al. mentioned that the absence of genetic pollution such as gene flow from sweet cherry orchards to wild cherry populations is very promising to guide future management and genetic conservation efforts in wild cherry [35].

The UPGMA cluster analysis based on genotypic data clearly showed the complexity of the relationship among Tunisian wild, landrace and cultivated foreigner sweet cherries, revealing similarities but also several apparent discrepancies. The dendrogram generated from UPGMA cluster analysis classified all studied accessions into four main groups which are depicted in Figure1.

The first main cluster (GI) contained all cultivated sweet cherry genotypes with high distribution, which was divided into two sub clusters. The first one (GI-1) included one unknown “UMM3” genotypes and 8 cultivated foreigner sweet cherries. Indeed, these latter were the result of a breeding program, one of whose parents was (were) “Burlat” and/or “Stella” (Table1). Also, the accession “UMM3” seems to be genetically close to the Italian early star cultivar or to be the result of breeding progmam from “Burlat” and/or “Stella” genotype (s) as one or two parents. The second sub cluster (GII-2) is composed by “Stella” and “Samba” which sharing a large part of the genomic sequence knowing that “Stella” was parent of this latter. The second group (GII) is made up of three Canadian cultivated sweet cherries, “New star”, “Van” and “Sunburst” which are the result of breeding program. “Van” and “Stella” are parents of “Newstar” and “Sunburt” with a mixed genetic background [25].The third group (GIII) is composed by the landrace “Bouargoub” and the three local wild cherries “WLAD1”, “WLB1”, “WLB 2” and two Italian cultivars, one of them is local cultivar (“Ferrovia”). Also, we found the unknown “UMM1”, “UMM2” and Hungarian cultivar “Carmen” (Figure 1). Consequently, molecular analyses demonstrated that Tunisian wild cherries and landrace are probably divergent on the genetic point of view. Forever, Tunisian landrace genotypes “Bouargoub” shared genetic backgound with Italian genotypes (“Adriana” and “Ferrovia”) given the geographic location on the Mediterranean shore. The unknown cultivated cultivar “UMM1” shows a kinship with “Adriana” which may suggest their common origins. However, the cultivated unknown cultivar “UMM2” is closely related to the three local wild cherries and to the landrace “Bouargoub”. These finding is in disagreement with result found by Gonopoulos et al. [35] who confirmed the distinction between wild and cultivated sweet cherries and point towards the absence of past hybridization.

In the forth group (GIV), we found the local wild cherries “WLAD 2” and three cultivated foreigner accessions which are French cultivar (“Moreau”), Germany cultivar (“Napoleon”) and Hungarian one (“Vera”). The reason why the Tunisian wild cherry “WLAD2” shared the maximum of genomic sequence with these three latter was not clear. It might be due to different methods of cultivation such as plant gathering and distribution. As a result, foreigner accessions cultivated in private farms are expected to be mixed and have lost their identifications. The presence of “Vera” in this group, one of whose parents is “Trusenszkaya 6” having Hungary as region of origin, is expected to improve genetic diversity.

Tunisian wild cherries showed high genetic variability since they belong to different clusters and consequently could be considered as a potential source of germoplasm to be exploited in sweet cherry improvement. Genetic polymorphism may be indicative of evolutionary adaptation which plays a key role for survival of population in the changing environment [36].

Based on the genetic population structure (Figure 2) the 25 accessions are divided into three subpopulations. Indeed, the Tunisian wild and the cultivated cherries were not distinguished according to their ecological and geographical origin. This finding is in accordance with research found by Ganopoulos et al. [27]. Only a part of studied accessions in group C1 was clearly clustered according to geographic origin, such as, Tunisian wild cherries (“WLB1”and “WLB2”) and landrace

"Bouargoub". With SSR markers, the degree of relation-ship between Tunisian wild and landrace germoplasm is evident.

In the present study, the SSR markers were able to detect differences in genetic background between wild and cultivated sweet cherries which are in accordance with other results found by [1,37]. These differences were possibly due to seed propagation, cross-incompatibility, natural hybridization and human selection [38] and repeated domestication. The second group C2 is made up only of European cultivated sweet cherries with only one of unknown origin "UMM2". This finding can be explained by their common genetic background.

The third group C3 was formed by 12 accessions and showed the closest genetic relationships. While, a portion of the genetic background of the Tunisian wild sweet cherry "WLAD1" was similar to the Canadian "Samba" hybrid cultivar with "Stella" as parents. This result is probably due to human moving cultivars to different sites during the past several thousand years of sweet cherry cultivation [27].

Intra specific variation observed within the four Tunisian wild sweet cherries which can be explained by the complexity in the development of the species. The belonging of the wild Tunisian cherry "WLAD1" to the red group lets us hypothesize that Tunisian wild sweet cherry "WLAD1" has a genetic background origin similar to the 10 other cultivated foreigner accessions and all accessions of the red group could have been originated from the same ancestral provenance (Figure 2).

CONCLUSION

A low value of genetic diversity parameters of the 25 accessions was maintained. These findings enhance future planning in robust breeding programs and contribute to enrich national and international cherry gene pool. A core collection can be constructed using the studied samples in order to conserve and to broaden the genetic base of sweet cherry breeding germoplasms.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

1. Mariette S, Tavaud M, Arunyawat U, Capdeville G et al. (2010). Population structure and genetic bottleneck in sweet cherry estimated with SSRs and the gametophytic selfincompatibility locus. *BMC Genetics*
2. Doebley JF, Gaut BS and Smith BD (2006). The molecular genetics of crop domestication. *Cell* 1309–21.
3. Zhuravlev YN, Reunova GD, Kats IL Muzarok TI et al. (2010). Genetic variability and population structure of endangered Panax ginseng in the Russian Primorye. *Chin Med*
4. Reunova G, Koren O, Muzarok TI, Zhuravlev Y et al. (2014). Microsatellite Analysis of Panax ginseng Natural Populations in Russia. *Chin Med*
5. Campoy JA, LerigoleurBalsemin E, Christmann H, Beauvieux R, et al. (2016). Genetic diversity, linkage disequilibrium, population structure and construction of a core collection of Prunus avium L. landraces and bred cultivars. *BMC Plant Biology*
6. Hamrick JL and Godt M (1996). Effets des traits d'histoire de vie sur la diversité génétique des espèces végétales. *Phil Trans R Soc Lond B*
7. Dirlewanger E, J QueroGarcı´a, Le Dantec L, Lambert P Mariette et al. 2010et al. (2012). Comparison of the genetic determinism of two key phenological traits, flowering and maturity dates, in three *Prunus* species: peach, apricot and sweet cherry. *Heredity*
8. Doyle JJ and Doyle JL (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochemical Bulletin 19:1115

9. Pritchard JK, Stephens M and Donnelly P (2000). Inference of population structure using multilocus genotype data. *Genetics.*
10. Weir BS and Cockerham CC (1984). Estimating F-statistics for the analysis of population structure. *Evolution*
11. Barton N H and Slatkin M (1986). A quasi-equilibrium theory of the distribution of rare alleles in a subdivided population. *Heredity*
12. Evanno G, Regnaut S and Goudet J (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study.
13. Kaçar Y A, Iezzoni AF and Çetiner MS (2005). Sweet cherry cultivar identification by using SSR markers. *J Biol Sci* 5: 616–19.
14. Clarke JB and Tobutt KR (2003). Development and characterization of polymorphic microsatellites from Prunus avium 'Napoleon'. *Mol Ecol*.
15. Vaughan SP and Russell K (2004). Characterization of novel microsatellites and development of multiplex PCR for large scale population studies in wild cherry; *Prunus avium*.
16. Dirlewanger E, Cosson P, Tavaud M, Aranzana J, et al. (2002). Development of microsatellite markers in peach *Prunus per sica* (L.) and their use in genetic diversity analysis in peach and sweet cherry (*Prunus avium* L.), *Theor Appl Genet*
17. TuretSayar M, Turkec A and Demir T (2012). Identification of Sweet Cherry Cultivars (*Prunus avium* L.) and Analysis of Their Genetic Relationship Using Microsatellite DNA Fingerprinting. *J Agric Sci*
18. Kaçar YA (2001). Classification of important sweet cherry (*Prunus avium* L.) and sour cherry (*Prunus cerasus* L.) cultivars and types growing in turkey by DNA fingerprinting methods, Çukurova Univ., thesis, Adana, Turke
19. AziziGannouni T, Campoy JO, QueroGarcía J, Barreneche T et al. (2017). Dormancy related traits and adaptation of sweet cherry in Northern Africa: A case of study in two Tunisian areas. *Sci Hortic*
20. Testolin R, Marrazzo T, Cipriani G, Quarta R et al. (2000). Microsatellite DNA in peach [*Prunus persica* (L.) Batsch] and its use in fingerprinting and testing the genetic origin of cultivars. *Genome*
21. Hormaza J I (2002). Molecular characterization and similarity relationships among apricot (*Prunus armeniaca* L.) genotypes using simple sequence repeats. *Theor App Genet*
22. Wunch A and Hormaza J I (2002). Cultivar identification and genetic fingerprinting of fruit tree species using DNA markers. *Euphytica*
23. Ganopoulos IV, Argiriou A and Tsaftaris AS (2010). Determination of selfincompatible genotypes in 21 cultivated sweet cherry cultivars in Greece and implications for orchard cultivation. *J Horticultural Sci & Biotechnol*
24. Farsad A and Esna Ashari M (2016). Genetic Diversity of Some Iranian Sweet Cherry (*Prunus avium*) Cultivars Using Microsatellite Markers and Morphological Traits. Cytol *Genet* diversity of sweet
25. Azizi-Gannouni T, Campoy JA, QueroGarcía J, Barreneche T, et al. (2018). Slocus cherry cultivars in Tunisia. *J New Sci Agr Tech*
26. Wright S (1946). Isolation by distance under diverse systems of mating. *Genetics*
27. Ganopoulos IV, Konstantinos K, IChatzicharisis O et al. (2011). Genetic diversity, structure and fruit trait associations in Greek sweet cherry cultivars using microsatellite based (SSR/ISSR) and morphophysiological markers. *Euphytica*
28. De Rogatis D, Ferrazzini F, Ducci1 S, et al. (2013). Genetic variation in Italian wild cherry (Prunus avium L.) as characterized by nSSR markers. *Forestry*
29. Santi F and Dufour J (2010). Differences between Georgian and French Wild Cherry Populations and Consequence for wild cherry breeding programmes. Silvae .*Genet*
30. Webster AD (1996). The taxonomic classification of Sweet and Sour Cherries and a brief history of their cultivation.
31. Iezzoni AF (2008). Cherries. The Encyclopedia of Fruits and Nuts, Wallingford, Oxfordshire
32. Eltaher S, Sallam A, Belamkar V, Emara, H A et al. (2018). Genetic diversity and population structure of F3:6 nebraska winter wheat genotypes using genotyping by sequencing. *Front Gen*
33. Ellstrand NC and Schierenbeck Kristina A (2006). Hybridization as a stimulus for the evolution of invasiveness in plants. *Euphytica*
34. Chatzicharisis I, MainouPlerou A, Chatzitheodorou I and Kazantzis K (2000). Naousa: Institute of Pomology. Monograph of sweetur cherry cultivars evaluated and cultivated in Greece.
35. Ganopoulos IV, Aravanopoulos FA and Tsaftaris A (2013). Genetic differentiation and gene flow between wild and cultivated *Prunus avium*: An analysis of moleculargenetic evidence at a regional scale. *Plant Biosys*
36. Stevens L, Salomon B and Sun GL (2007). Variabilité des microsatelliteset excès d'hétérozygotes chez Elymus trachycaulus populations de la ColombieBritannique au Canada. Système biochimique. *Ecol*
37. KhadiviKhub A, Zamani Z, Fattahi R and Wu¨nsch A (2014). Genetic variation in wild Prunus L. subgen. Cerasus germplasm from Iran characterized by nuclear and chloroplast SSR markers .*Trees*
38. Sefc KM, Steinkellner H, Wagner HW and Gloessl J et al. (1997). Application of microsatellite markers to parentage studies in grapevine. *Vitis*