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Genetic diversity of Tunisian melon (*Cucumis melo*. L) landraces and their relationships with introduced varieties as assessed by simple-sequence repeat (SSR) markers

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The genetic diversity and the relationships among Tunisian melon landraces and introduced varieties belonging to different varietal groups were estimated using six simple-sequence repeat (SSR) markers. All loci were polymorphic and provided a total of 56 alleles, with an average of 9.33 alleles per locus. The allelic frequencies differed according to accessions, and particular alleles were found within several accessions. The polymorphism information content (PIC) values ranged from 0.568 to 0.866, with an average of 0.754, and the level of the genetic diversity differed according to sites. The genetic differentiation among landraces fit a model of isolation by distance; that among introduced varieties and landraces was high suggesting a low level of gene flow between the two sets of melons. The dendrogram based on Nei's genetic distances produced two major groups of accessions. The *inodorus* introduced varieties were distinctly different from all other accessions. The *dudaim* accessions were clearly separated from the *reticulatus* ones which were dispersed among local landraces grouped according to their geographical origin. The close molecular relatedness between local landraces and *reticulatus* accessions indicates that local melons have been presumably developed from the group *reticulatus* introgressed with the *inodorus* one. Based on these findings, conservation strategies of landraces are discussed.

Key words: *Cucumis melo*, genetic diversity, introduced varieties, SSR markers, Tunisian melon.

INTRODUCTION

Melon (*Cucumis melo* L., Cucurbitaceae) exhibits a wide range of variation in floral (that is, sex type), vegetative and fruit (that is, size, flesh color, rind color and form) traits (Stepansky et al., 1999). Jeffrey (1980) subdivided the species into two subspecies according to the hypanthian hairiness: *C. melo* ssp. *agrestis* (Naud.)

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Pangalo with sericeous ovaries, and *C. melo* ssp. *melo* with pilose ones. At present, the most adopted classification of melon is that of Munger and Robinson (1991) who divided the species into a single wild variety, *C. melo* var. *agrestis* Naud., and six cultivated ones: var. *cantalupensis* Naud. also including former var. *reticulatus*, *inodorus* Jacq., *conomon* (Thunb.) Makino, *chito* and *dudaim* (L.) Naud., *flexuosus* (L.) Naud., and *momordica* (Roxb.) Duthie et Fuller.

The genetic diversity of melon has been assessed using phenotypic (Escribano and Lazaro, 2009; Szamosi et al., 2010), isozymic (Staub et al., 1997; Akashi et al., 2002; McCreight et al., 2004) and molecular markers, including mainly RAPDs (Lopez-Sesé et al., 2003; Yi et al., 2009), AFLPs (Garcia-Mas et al., 2000; Frary et al., 2013; Shamasbi et al., 2014) and simple-sequence repeat markers (SSRs) (Garcia-Mas et al., 2004; Tzitzikas et al., 2009; Kim et al., 2010). The latter are frequently used because of their reproducibility, multiallelic nature, codominant inheritance and good genome coverage. They have provided species-specific allele patterns in melon (Morales et al., 2004) and considered as useful markers for assessing the genetic diversity and the relationships among melon genotypes (Yildiz et al.,2011; Escribano et al., 2011; Roy et al., 2012; Raghami et al., 2014, for some recent ones).

In Tunisia, melon is mainly cultivated in open fields with 104482 tones of production and10447 ha harvested area in 2011 (Henane et al., 2013). The main cultivated areas are in regions of Beja, Jendouba, Sfax, Gafsa, Tozeur's oasis, Gabes, Kairouan, Sidi Bouzid and the Sahel. Most commercial melons, sold in the local markets, are the introduced Charentais, Galia, Yellow Canary and Pineapple varieties. They replace the most known landraces such as Abdelaoui, Beji, Bouricha, Bouzemzouma, Chefli, Kasbar, Souri, Stambouli and ancient introduced varieties (Maazoun and Galaoui) which risk genetic erosion (Elbekkay et al., 2008). At present, landraces are cultivated for self supply in scattered family fields (Novikoff, 1952; Jebbari et al., 2004). Numerous studies linked mainly to viral and fungal diseases, salt stress, *in vitro* tissue culture and production of various Tunisian melons had been reported (Hamza et al., 2007; Jabnoun-Khiareddine et al., 2007; Mnari-Hattab et al., 2009; Rhimi and Boussaid, 2012). Little attention has been paid to the conservation of this germplasm, the knowledge of its genetic diversity and its relationship with the other Mediterranean melon landraces (Mliki et al., 2001; Trimech et al., 2013; Henane et al., 2013). In the present study, we used SSRs to determine levels of polymorphism and patterns of genetic structure among 26 Tunisian melon landraces collected from different geographical areas. This information, jointly to that previously performed on the same landraces using morphological traits, is crucial to understand better their genetic structure and conceive conservation and improvement programs.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 26 local accessions were assessed. Fruits were collected in open fields, based on mature fruit characteristics, in three geographical regions belonging to the upper-arid and lower-arid bioclimatic zones (Figure 1): Monastir (Mz1–Mz6, Mn1–Mn4, Mk1–Mk8, Chmz and Chmk), Mahdia (Chb) and Tozeur's oasis (Tz1–Tz5). Seeds were sown on the same field (Manouba; 36° 48' 49''N; 10° 3' 25''E; rainfall 450 mm/year; altitude 42 m). Two ancient introduced varieties Galaoui (Gal, presumably from Turkey) and Maazoun (Maa, unknown origin) cultivated in Bizerte region were also included. Yellow Canary (Casaba market class type) was added as a reference variety. Several accessions, based on morphological traits (that is, size and shape of fruits, color of skin and flesh, sex expression type), have been tentatively assigned to three Munger and Robinson's (1991) varietal groups: *inodorus* (Mz1-Mz6), *reticulatus* (Chb) and *dudaim* (Chmz and Chmk). The three introduced varieties belong to *reticulatus* (Gal) and *inodorus* (Maa and YC) groups. Local name, sites of collection, putative classification and main morphological characteristics of accessions are given in Table 1.

DNA extraction and SSR analysis

For each accession, three fruits were considered based mainly on shape and rind aspect (color and degree of corking). Their seeds were germinated on a filter paper at 22°C at 16 h photoperiod (cool white fluorescent lamps, 5 $Mm^{-2}s^{-1}$). Total genomic DNA was isolated from each of six plantlets per accession using the CTAB method according to Garcia-Mas et al. (2000). The DNA concentration was spectrophotometrically estimated and its quality was checked by analytic agarose minigel electrophoresis.

A set of six SSR markers developed by Katzir et al. (1996) and Danin-Poleg et al. (2001) were used to assess the genetic diversity within and among accessions (Table 2). All microsatellites were amplified using a gradient thermal cycling (Palm Cycler) in a final volume of 15 µL containing 1X Taq buffer, 2.5 mM $MgCl_2$, 200 µM dNTPs, 0.2 µM of each forward and reverse primers (Metabion International), 1U *Taq* DNA polymerase (Promega) and 30 ng/µL genomic DNA. The amplification program consists of a preliminary denaturing step at 94°C for 30s, followed by 30 cycles, each of which has a denaturing step at 94°C for 30 s, an annealing step for 60 s at a temperature adjusted according to the requirement of each primer, and an extension step for 1 min. The PCR product analysis was performed using the Experion DNA 1K Analysis kit and the Experion automated electrophoresis station according to manufacturer's instructions. The chips were prepared with the gel stain mix and then pressurized. 1 µL of PCR product and 5 µL of buffer were loaded into sample wells. The DNA 1 K ladder, included in the kit, was used for accurate quantitation and alignment of samples. At the end of the run, SSR band profiles appeared in a simulated gel and the amplification product size estimates were given by the Experion software.

Data analysis

The genetic variation for each locus including observed (Ho) and expected (He) heterozygosities, and the observed (na) and effective (ne) number of alleles per locus were estimated using PopGene 1.31 software (Yeh et al., 1999). The polymorphism information content polymorphism information content (PIC) ($\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2 - 2\sum_{i=1}^{n-1}\sum_{j=i+1}^{n} p_i^2\, p_j^2$, where, p_i and p_j are the frequencies of the i^{th} and j^{th} allele, respectively and n represents the

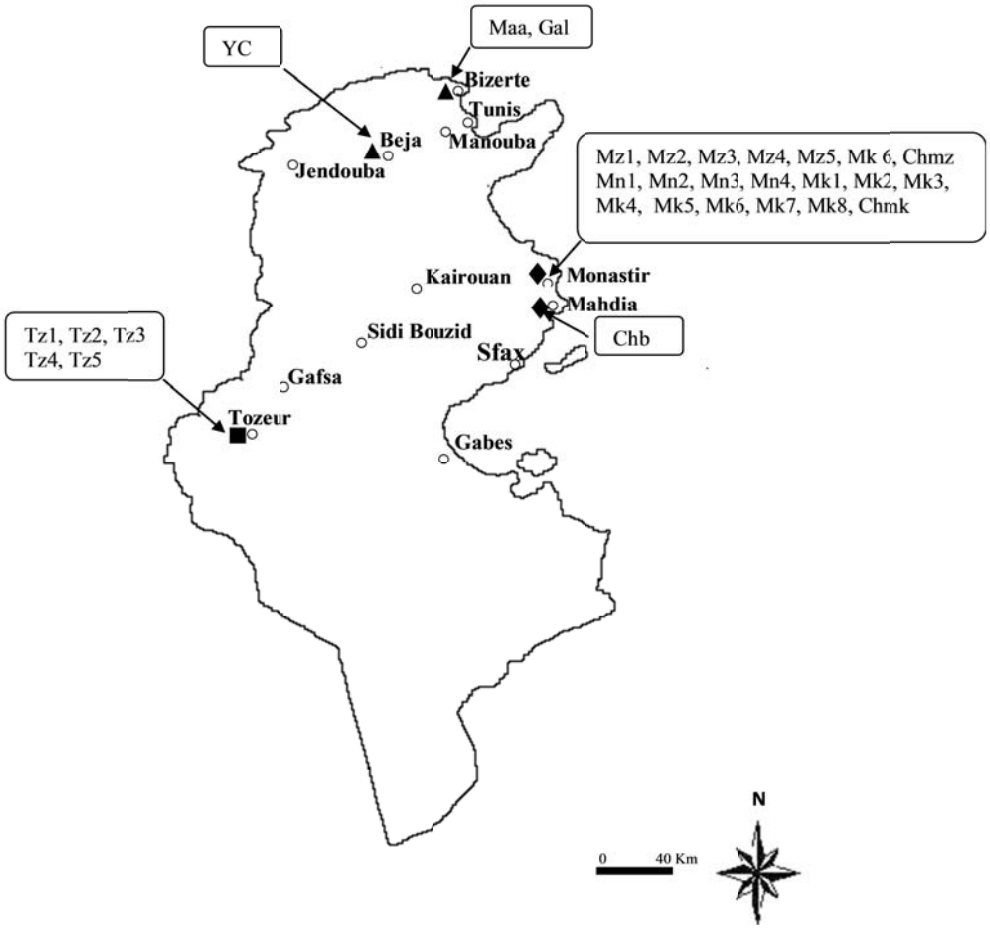

Figure 1. Map of Tunisia: geographical distribution of the 29 samples analyzed. Symbols indicate the bioclimatic zone: ▲ Sub-humid, ♦Upper arid, ■ Lower arid, ○ Department.

number of alleles) was calculated according to Botstein et al. (1980) using the Power Marker software (Liu and Muse, 2005).

The genetic variation within collection site was estimated by allelic frequencies, mean number of alleles per polymorphic locus (Ap), percentage of polymorphic loci (P%) and averages of observed (Ho) and expected heterozygosities (He) using Biosys-1 software package (Swofford and Selander, 1981). Deflection from Hardy-Weinberg (HW) expectation was assessed by the F_{IS} inbreeding coefficient. The significance of deficit or excess of heterozygotes was tested by randomizing alleles among individuals. Wright's (1951; 1965) *F*-statistics (F_{IS}, F_{ST} and F_{IT}) were estimated for each locus according to the method of Weir and Cockerham (1984) implemented in the computer program FSTAT 2.9.3.2 (Goudet, 2001). Standard errors were obtained by jackknifing over populations and loci, and the significance of indices was tested after randomizations.

The genetic structure among accessions was estimated by F_{ST} calculated between accession-pairs within sites or between accessions grouped according to their geographical region. The correlation between matrices of F_{ST} and geographic distances (Km) among sites of collection was estimated by the Mantel test (Mantel, 1967), and the gene flow between accessions was evaluated by Nm $[Nm = (1 - F_{ST})/4F_{ST}]$ (Wright, 1951; Slatkin and Barton, 1989).

Estimates of genetic relationships between all accessions were obtained by Nei's (1972) genetic distances. A dendrogram, based on these distances, was generated using the unweighted pair group method with arithmetic averages (UPGMA) (Swofford and Selander, 1981).

RESULTS

Genetic diversity

The total number of observed alleles (na) was 56 across the six loci. It ranged from 5 (CMTC123) to 12 (CMGA172), with an average of 9.33 alleles per locus (Table 3). The effective number of alleles (ne) varied from 2.72 (CMTC123) to 8.22 (CMTAA166), with an average of 5.50. The PIC value, estimating the discriminatory power of loci, ranged between 0.568 (CMTC123) and 0.866 (CMTAA166) with a mean of 0.754. The observed (Ho) and expected (He) heterozygosities among loci were 0.388 ($0 < Ho < 0.885$) and 0.786 ($0.633 < He < 0.878$), respectively. A significant deficiency of heterozygotes was shown for three loci.

The distribution of allele frequencies (Data not shown) indicated that particular alleles (numbered alphabetically

Table 1. Accessions of *C. melo*: Collection sites, names, their assignment to Munger and Robinson's (1991) varietal groups and their main morphological traits.

Geographical region	Collection site	Accession name	Code	Varietal group	Main morphological traits (Trimech et al., 2013)				
					Sex expression	Fruit			Seed size
						Form	Surface	Peduncle	
Monastir	Mazdour (Mz)	Yellow Hab Rched [a]	Mz1	i	a	el	n w, net, spo	n d	s
		Green Hab Rched [a]	Mz2	i	a	el	n w, net, spo	n d	md
		Mazdour 1[b]	Mz3	i	a	ov	n w, net, spo	n d	s
		Mazdour 2[b]	Mz4	i	a	el	n w, net, n spo	n d	s
		Mazdour 3[b]	Mz5	i	a	rn	n w, net, spo	n d	s
		Mazdour 4[b]	Mz6	i	a	el	n w, net, spo	n d	md
		Chemoum [a]	Chmz	du	a	ov	n w, n net, spo and ste	d	v s
	Menzel Nour (Mn)	Menzel Nour 1[b]	Mn1	n at	a/m	el	n w, net, spo	n d	md
		Menzel Nour 2[b]	Mn2	n at	a	ov	n w, net, n spo	n d	md
		Menzel Nour 3[b]	Mn3	n at	a/m	ov	n w, net, n spo	n d	md
		Menzel Nour 4[b]	Mn4	n at	a	rn	n w, net, spo	d/ n d	l
	Moknine (Mk)	Moknine 1[b]	Mk1	n at	a	ov	n w, net, spo	n d	md
		Moknine 2[b]	Mk2	n at	a	ov	n w, net, spo	n d	md
		Moknine 3[b]	Mk3	n at	a	ov	n w, net, spo	n d	l
		Moknine 4[b]	Mk4	n at	a	ov	n w, net, spo	n d	md
		Moknine 5[b]	Mk5	n at	a	ov	n w, net, spo	d/ n d	l
		Moknine 6[b]	Mk6	n at	a	ov	n w, net, spo	d/ n d	l
		Moknine 7[b]	Mk7	n at	a	ov	n w, net, spo	d/ n d	md
		Moknine 8[b]	Mk8	n at	a	rn	n w, net, spo	d/ n d	l
		Chemoum [a]	Chmk	du	a	ov	n w, n net, spo and ste	d	v s
Mahdia	Chiba (Chb)	Chiba[b]	Chb	r	a	ov	n w, net, n spo	d/ n d	md
Tozeur	Tozeur (Tz)	Tozeur 1[a]	Tz1	n at	m	el	n w, net	d	md
		Tozeur 2[a]	Tz2	n at	m	el	n w, net	d	md
		Tozeur 3[a]	Tz3	n at	m	el	n w, net	d	md
		Tozeur 4[a]	Tz4	n at	m	el	n w, net	d	md
		Tozeur 5[a]	Tz5	n at	m	el	n w, net	d	md
Bizerte	Bizerte	Galaoui[a]	Gal	r	a	ov	n w, net, n spo	n d	v l
		Maazoun[a]	Maa	i	a	rn	w, n net, spo	n d	v l
Beja	Beja	Yellow Canary[mk]	YC	i	a	ov	w, n net, n spo	n d	v l

[a]Local name given by the farmer [b]name given according to the location: market class, **i**: *inodorus*, **du**: *dudaim*, **r**: *reticulatus*, **n at**: not attributed, **m**: monoecious, **a**: andromonoecious, **el**: elliptical, **ov**: ovoid, **rn**: round, **w**: wrinkled, **n w**: not wrinkled, **net**: netted, **n net**: not netted, **spo**: spotted, **n spo**: not spotted**, ste**: stepped, **d**: dehiscent, **n d**: not dehiscent, **v s**: very small (< 7 mm), **s**: small (7≤ s<9 mm), **md**: medium (9≤md<11mm), **l**: large (11≤ l<13mm), **v l**: very large (≥13 mm).

Table 2. Characteristics of SSR markers used for the assessment of the genetic diversity of *Cucumis melo* accessions (Danim-Poleg et al., 2001).

SSR designation	Core repeat motif and number of repeats	Expected size (bp)	Number of detected alleles
CMACC146	$(ACC)_9$	[128-152]	3 - 11
CMTAA166	(TAA)9N8(GA)9(AT)3	[145-193]	2 - 16
CMTC123	(TC)9(TTTC)2	[96-108]	2 - 5
CMTC168	(TC)14	[178-204]	4 - 9
CMGA172	(GA)9	[106-136]	3 - 10
CMGA104	(GA)14AA(GA)3	125	6

Table 3. Polymorphism detected with the six SSR markers in all *Cucumis melo* accessions

Locus	na	ne	Ho	He	PIC	F_{IS}	F_{ST}	F_{IT}
CMACC146	7.000	3.176	0.075	0.685	0.630	0.842**(0.053)	0.324**(0.074)	0.892**(0.034)
CMTAA166	11.000	8.222	0.660	0.878	0.866	-0.146^{ns}(0.090)	0.340**(0.051)	0.244**(0.083)
CMTC123	5.000	2.725	0.000	0.633	0.568	1.000**(0.000)	0.695**(0.069)	1.000**(0.000)
CMTC168	11.000	5.409	0.092	0.815	0.792	0.846**(0.067)	0.279**(0.046)	0.888**(0.048)
CMGA172	12.000	7.045	0.885	0.858	0.843	-0.442^{ns}(0.059)	0.294**(0.045)	-0.019^{ns} (0.059)
CMGA104	10.000	6.447	0.615	0.845	0.827	-0.058^{ns}(0.115)	0.316**(0.037)	0.276** (0.085)
Mean	9.333 (2.733)	5.504 (2.182)	0.388 (0.377)	0.786 (0.102)	0.754	0.219**(0.257)	0.361**(0.054)	0.503**(0.174)

na and ne: Observed and effective number of alleles, respectively. Ho and He: observed and expected heterozygosities, respectively. F_{IS}, F_{ST}, F_{IT}: Wright's F-statistics. ^{ns}: not significant, ** highly significant (P<0.001) based on 1000 randomizations. Standard deviations are between parentheses.

a, b, c… according to their size) occurred exclusively in some accessions: CMACC146-g (Mz2: 33.3%), CMTC168-d (Tz3: 25%, Tz4: 16.7%), CMTC168-c (Tz3: 16.7%), CMTC168-e (Tz4: 25%, Gal: 8.3%), CMGA172-a (Mn2: 50%, Mn3: 25%), CMGA104-a (Tz1: 41.7%, Tz2: 16.7%, Tz5: 16.7%), CMGA104-b (Mn3: 50%, Mn4: 33%), CMGA104-c (Mz5: 16.7%, Mz6: 60%). Alleles CMTC168-a, CMTC168-b, CMGA172-l were detected only in Chmz and Chmk accessions. The recent introduced variety YC showed two specific alleles CMTAA166-e (25%) and CMTC123-a (66.7%). The ancient introduction Gal shared the allele CMTC168-e with Tz4.

The level of the within-site genetic diversity differed according to accessions (Table 4). The highest variation was observed for Mn1 (Ap=3.2, P%=100, Ho= 0.417), Mk6 (Ap=3.2, P%=100, Ho= 0.500), Mk3 (Ap=3.2, P%=100, Ho= 0.333), Mz5 (Ap=3.2, P%=100, Ho= 0.333) and Tz2 (Ap=3.2, P%=100, Ho=0.389). The majority of accessions exhibited an excess of homozygotes with the highest level noted in Moknine (F_{IS}= 0.381) and the lowest in Tozeur (F_{IS}=0.035). Chemoum (Chmz and Chmk) and Chiba (Chb) landraces exhibited less variation than Menzel Nour (Mn), Moknine (Mk) and Tozeur (Tz) ones. Compared to landraces, the varieties YC (Ap=2.3, P%=66.7, Ho=0.500), Maa (Ap=2, P%=66.7, Ho=0.500) and Gal (Ap=1.8, P%=33.3, Ho=0.083) were less polymorphic.

A high inter-site genetic variation was observed (Table 4). The highest level of genetic diversity was recorded in Mn (Ap=2.95, P%=91.6, Ho= 0.269) and Mk (Ap=2.90, P%=91.6, Ho= 0.364)

Table 4. Genetic diversity parameters for accessions within and between sites of collection.

Accession	Ap	P%	Ho	He	F_{IS}
Mz1	2.0 (0.4)	66.7	0.500 (0.224)	0.414 (0.134)	-0.208^{ns}
Mz2	2.5 (0.4)	83.3	0.500 (0.224)	0.538 (0.117)	0.077^{ns}
Mz3	1.8 (0.3)	66.7	0.111 (0.111)	0.364 (0.121)	0.714**
Mz4	1.8 (0.2)	83.3	0.333 (0.172)	0.384 (0.084)	0.143^{ns}
Mz5	3.2 (0.5)	100	0.333 (0.172)	0.606 (0.083)	0.474**
Mz6	2.7 (0.5)	100	0.400 (0.200)	0.589 (0.056)	0.369*
Mean	2.3 (0,38)	83,3	0.212 (0,183)	0.482 (0.010)	0.262
Mn1	3.2 (0.6)	100	0.417 (0.171)	0.644 (0.066)	0.375*
Mn2	2.8 (0.3)	100	0.417 (0.171)	0.629 (0.038)	0.359*
Mn3	2.8 (0.6)	83.3	0.444 (0.205)	0.530 (0.120)	0.175^{ns}
Mn4	3.0 (0.6)	83.3	0.333 (0.172)	0.520 (0.118)	0.355*
Mean	2.95 (0.52)	91.6	0.269 (0.180)	0.580 (0.086)	0.316
Mk1	3.0 (0.4)	100	0.444 (0.205)	0.621 (0.056)	0.304*
Mk2	2.8 (0.5)	83.3	0.333 (0.211)	0.551 (0.122)	0.417*
Mk3	3.2 (0.3)	100	0.333 (0.172)	0.677 (0.045)	0.457**
Mk4	2.5 (0.4)	83.3	0.278 (0.181)	0.505 (0.109)	0.474*
Mk5	3.0 (0.5)	100	0.389 (0.200)	0.616 (0.061)	0.391*
Mk6	3.2 (0.7)	100	0.500 (0.224)	0.667 (0.068)	0.268*
Mk7	2.7 (0.6)	83.3	0.361 (0.163)	0.482 (0.137)	0.270*
Mk8	2.7 (0.4)	83.3	0.278 (0.165)	0.500 (0.102)	0.468**
Mean	2.9 (0.5)	91.6	0.364 (0.190)	0.577 (0.087)	0.381
Tz1	2.5 (0.3)	83.3	0.361 (0.163)	0.505 (0.105)	0.305*
Tz2	3.2 (0.5)	100	0.389 (0.176)	0.596 (0.070)	0.324*
Tz3	2.8 (0.5)	100	0.583 (0.176)	0.566 (0.068)	-0.034^{ns}
Tz4	2.2 (0.3)	83.3	0.556 (0.159)	0.424 (0.101)	-0.351^{ns}
Tz5	2.8 (0.6)	83.3	0.611 (0.200)	0.515 (0.132)	-0.209^{ns}
Mean	2.7 (0.4)	90.0	0.500 (0.174)	0.521 (0.095)	0.035
Chb	2.0 (0.5)	50	0.250 (0.171)	0.313 (0.148)	0.217^{ns}
Chmz	1.5 (0.2)	50	0.333 (0.211)	0.232 (0.110)	-0.500^{ns}
Chmk	2.3 (0.3)	83.3	0.389 (0.196)	0.432 (0.113)	0.091^{ns}
Mean	1.9 (0.25)	66.65	0.361 (0.203)	0.332 (0.111)	-0.409
Gal	1.8 (0.5)	33.3	0.083 (0.057)	0.210 (0.133)	0.625*
Maa	2.0 (0.4)	66.7	0.500 (0.224)	0.364 (0.131)	-0.429^{ns}
YC	2.3 (0.6)	66.7	0.500 (0.224)	0.439 (0.149)	-0.154^{ns}
Total mean	2.56 (0.277)	82.75	0.388 (0.182)	0.497 (0.099)	0.219**

and the lowest in Mz (Ap=2.3, P%=83.3, Ho= 0.212). Although their geographical proximity, Moknine (Mk), Menzel Nour (Mn) and Mazdour (Mz) accessions showed significant levels of genetic variation.

Genetic structure among accessions

F_{ST} values at loci levels ranged from 0.279 (CMTC168) to 0.695 (CMTC123), with a mean of 0.361 (Table 3), and a significant differentiation was scored between pairs of most accessions from the same site (data not shown). A high differentiation between sites was recorded (Table 5). The accession Chb displayed high F_{ST} values with those from Tz and Mn (F_{ST}= 0.259). Pairwise samples from Mk, Mn and Mz exhibited low but significant differentiation ($0.042<F_{ST}<0.058$). The gene flow scored among these accessions ranged between 4.060 (Mz-Mn) and 5.702 (Mz-Mk) (data not shown). The most level of differentiation (FsT ranged from 0.293 to 0.639) and the lowest gene flow ($0.141<Nm<0.624$) were observed among Chemoum (Chmz and Chmk) and all other

Table 5. Nei's genetic distance (above diagonal) and F_{ST} (below diagonal) values between melon accessions analysed.

Accession	Mz	Mn	Mk	Chb	Tz	Chm	Gal	Maa	YC
Mz		0.065	0.047	0.173	0.091	0.285	0.335	0.245	0.256
Mn	0.058*		0.053	0.300	0.081	0.293	0.355	0.308	0.292
Mk	0.042*	0.043*		0.248	0.113	0.317	0.305	0.275	0.284
Chb	0.157*	0.259*	0.215*		0.260	0.382	0.492	0.266	0.565
Tz	0.098*	0.080*	0.118*	0.259*		0.255	0.348	0.365	0.349
Chm	0.294*	0.293*	0.300*	0.513*	0.286*		0.577	0.527	0.586
Gal	0.304*	0.306*	0.261*	0.630*	0.334*	0.639*		0.531	0.564
Maa	0.226*	0.266*	0.238*	0.419*	0.338*	0.589*	0.640*		0.438
YC	0.232*	0.249*	0.241*	0.586*	0.324*	0.598*	0.619*	0.514*	

accessions. The highest F_{ST} values between local and introduced melons were recorded between the pairs Chb-Gal (F_{ST}=0.630) and Chb-YC (F_{ST}=0.586); the lowest ones were scored between Mz-YC (0.232) and Mz-Maa (0.226) (Table 5). The level of gene flow among the two sets of melons was low (0.147<Nm<0.856). The Mantel test revealed a significant correlation (r= 0.811; p= 0.0033) between matrices of F_{ST} and geographical distances among sites (Mz, Mn, Mk, Chb and Tz), indicating an isolation per distance.

Nei's genetic distances differed between accessions within sites. They ranged from 0.133 to 0.569 in Mz, from 0.193 to 0.465 in Mn, from 0.106 to 0.395 in Mk and from 0.125 to 0.323 in Tz. The two Chm accessions (*dudaim* group) exhibited the most distance with all other accessions (D ranged from 0.255 to 0.586) for the remnant accessions. The highest inter-site genetic distance occurred between Chb and Mn (D=0.300) and between Chb and Tz (D=0.260) (Table 5). The lowest divergences were noted between pairwise accessions Mk-Mz (D=0.047) and Mk-Mn (D=0.053). Nei's genetic distances between local accessions (grouped according to their collection site) and introduced varieties were relatively high (0.245<D<0.565) (Table 5); the highest value was recorded among Chb (*reticulatus* group) and YC (*inodorus* group) accessions. A relative genetic affinity between Mz, Maa and YC accessions, belonging to the *inodorus* group was observed (0.245<D<0.256).

The dendrogram, based on Nei's (1972) genetic distances separated accessions into two main clusters (I and II) globally similar to those previously found based on morphological data (Trimech et al., 2013). The group I (Figure 2) was formed by the introduced *inodorus* varieties Maa and YC. The group II could be subdivided into two sub-clusters. The first one (II.1) included the two *dudaim* (Chmz and Chmk), Tz, and mixed accessions from Mk, Mz, Mn and Chb. In the second sub-cluster (II.2) emerged the variety Gal well separated from the remnant accessions.

DISCUSSION

Previous study on Tunisian melons showed high morphological variation (for example, color of skin and flesh, shape and size of fruits, sex expression) within and among landraces. The segregation of landraces was globally concordant with their geographical origin and botanical group (Trimech et al., 2013; Henane et al., 2013). In the current study, we reported the use of six melon specific SSR markers (Danin-Poleg et al., 2001; Szabò et al., 2005; Kaçar et al., 2012) for assessing the genetic diversity and relationships among 26 landraces, two ancient (Maa and Gal) and one recent (YC) introduced varieties.

All SSR markers used were polymorphic, and detected different levels of polymorphism. The highest number of alleles and PIC values over all accessions were detected by CMGA172 (12 alleles, PIC= 0.843). The average of observed alleles over all loci (na = 9.33) was high compared to that identified in melons using SSRs. However it was within the range of values (from 3.5 to 17) found in earlier *Cucumis melo* genetic diversity studies using SSR markers (Monforte et al., 2003; Dhillon et al., 2007; Fergany et al., 2011). Large discrepancies between observed (0.000<Ho<0.885) and expected (0.633<He<0.878) heterozygosities due to genetic drift and a low level of gene flow among landraces were recorded. Loci CMACC146, CMTC123 and CMTC168 did not conform to Hardy-Weinberg expectations (0.842<F_{IS}<1.000) and exhibited increased differentiation among accessions relative to the other loci. SSRs allowed for melons the detection of specific genotype alleles (Morales et al., 2004; Parvathaneni et al., 2011). In our study, several identified alleles could be useful for the characterization and management of Tunisian melon. CMTAA166-e and CMTC123-a were unique to the *inodorus* YC variety, CMTC168-a, b and CMGA172-l were specific to the *dudaim* Chemoum (Chm) landrace. Other alleles (that is, CMGA104-a, b, c; CMACC146-a, g; CMTC168-c, e; CMGA172-a) detected at relatively high frequencies, could help to differentiate accessions within and among sites.

Mk, Mn, Mz and Tz landraces were more polymorphic (P% ranged from 83.3 to 91.6) than the local melons Chb and Chm or the introduced varieties YC, Maa and Gal (P% ranged from 50.0 to 66.5). The high genetic diversity

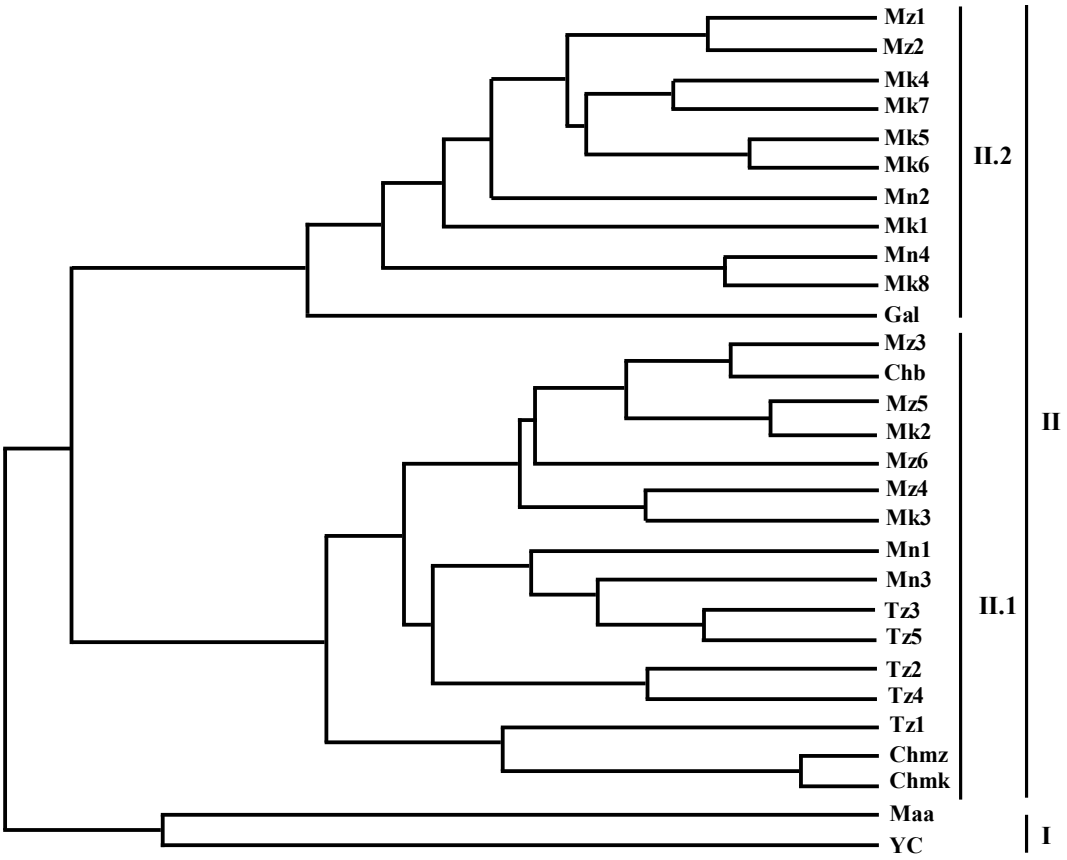

Figure 2. Dendrogram based on Nei's (1972) genetic distances between accessions-pairs.

of landraces within sites should result from the initial wealth of genotypes rather than from crossing among individuals since a significant deficiency of heterozygotes ($0.035<F_{IS}<0.381$) was revealed within most sites. This suggests that farmers have presumably selected genotypes from related growers.

A geographic pattern of genetic differentiation among Tunisian melons, similar to that previously reported for other origins (Roy et al., 2012; Raghami et al., 2014), was revealed. The differentiation among the geographically close Mk, Mn and Mz accessions (7-20 Km distant from each other) was less important than that noted among these accessions and Tz ones separated with a minimum distance of 400 km Local and introduced varieties exhibited high levels of differentiation suggesting low levels of gene flow among them. The *reticulatus* Chb and Gal, the *inodorus* YC and Maa and the *dudaim* Chm accessions were highly differentiated and more isolated from the remnant landraces. The distinction between the two ancient introduced varieties Gal, and revealed also by morphological data (Henane et al., 2013, Trimech et al., 2013), indicates the maintenance of reproductive barriers among them despite their ancient cultivation in the same or overlapping geographical area.

Nei's genetic distances among Mk, Mn, Mz and Tz accessions were low (from 0.047 to 0.113) (Table 5). The high genetic similarity between these accessions and their significant genetic structure suggest that genetic drift has historically been a major influence in shaping their differentiation. The dendrogram based on these distances showed a clear divergence: i) among local *dudaim*, *reticulatus* and introduced *inodorus* accessions, and ii) between the northern (Mk, Mn and Mz) and the southern (Tz) landraces suggesting limited level of seed exchange mainly due to geographic isolation. The northern Mk, Mn and Mz (*inodorus* group) accessions were dispersed within two sub-clusters but near the *reticulatus* Chb and Gal accessions. The high variation among Mn, Mk and Mz and their close affinity with *reticulatus* accessions indicate that landraces were likely developed from a broader germplasm base. They might be reviewed as composed of a number of ancient and genetically diverse origins including mainly *reticulatus* genotypes presumably introgressed with *inodorus* ones. The topology of the dendrogram based on SSR markers was globally concordant with that produced from morphological traits (that is, leaf shape and size, sex expression, aroma and morphology of fruits) which showed a clear distinction among local and introduced accessions and among Chm and all other landraces

(Trimech et al., 2013). The similar differentiation of landraces obtained from morphological traits (mainly fruit traits) and that identified by SSRs, with the latter being more effective in detecting polymorphism has been previously reported in several papers (Parvathaneni et al., 2011; Sestili et al., 2011; Yildiz et al., 2014).

Conclusions

Tunisian local melons have been drastically affected by genetic erosion caused mainly by the disappearance or the substitution of agrarian ecotypes or old cultivars by elite ones. Our results, based on the assessment of the genetic diversity and genetic structure among landraces and introduced varieties give information to guide conservation strategy. A significant differentiation among accessions within and among sites even geographically near, and among landraces and recent introduced varieties was observed. Thus, conservation strategies are urgently required. Samples with high genetic diversity and private alleles should firstly be conserved. The preservation of accessions from Mz, Mn and mainly those from Mk showing the highest level of genetic variation is necessary. Collection of seeds from these localities should be made rather within than among sites since a high within-site diversity and a significant differentiation among sites were observed. Accessions from Tz with less genetic variation are thought to be more vulnerable to environmental changes. Their narrow geographic range and their isolation from northern accessions increase genetic drift. Thus conservation management is urgently required to preserve their genetic diversity.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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