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SHORT COMMUNICATION

Genetic variation in Tunisian melon (*Cucumis melo* L.) germplasm as assessed by morphological traits

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Abstract The morphological variation and the systematical status of 28 traditional melon including two ancient varieties collected from different geographical and bioclimatic areas of Tunisia were assessed based on 34 qualitative and quantitative traits. Yellow Canary was used as a reference variety. Significant differences among accessions within and among sites of collection were revealed for the majority of traits. The accessions from the Sahel were more polymorphic than those from Tozeur's oasis. However, the classification of the local accessions according to Munger and Robinson's varietal groups did not allow clear segregation of several of them because of their high heterogeneity. The principal component analysis (PCA) performed on all measured traits, allowed a distinction among the introduced varieties clustered according to their varietal group, and the local accessions grouped separately according to their geographical origin and bioclimatic zone. Mahalanobis distances among PCA's groups were significant. The correlation found between Mahalanobis distance matrix and geographical distance matrix among origins corroborated isolation per distance. Conservation strategies should be made appropriately according to origins. Accessions from the Sahel showing the highest polymorphism within and among sites of collection should be firstly preserved.

Keywords Classification · Conservation · *Cucumis melo* · Genetic variation · Morphological traits · Tunisian melon

Introduction

The genus *Cucumis* (subgenus *melo*, Cucurbitaceae) is an important vegetable crop cultivated in temperate and warm climates (Decker-Walters et al. 2002; Garcia-Mas et al. 2004). Its origin appears to be Africa (Pitrat et al. 2000; Janick et al. 2007). *Cucumis melo* L. is the most polymorphic species of the genus (Stepansky et al. 1999; Eduardo et al. 2007; Pech et al. 2007; Szamosi et al. 2010).

Jeffrey (1980) subdivided the species into two subspecies according to the hypanthian hairiness: *C.*

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melo ssp. *agrestis* (Naud.) Pangalo, with sericeous ovaries and *C. melo* ssp. *melo* with pilose ones. Munger and Robinson (1991) divided the species into a single wild variety, *C. melo* var. *agrestis* Naud., and six cultivated ones: var. *cantalupensis* Naud., *inodorus* Jacq., *conomon* (Thunb.) Makino, *dudaim* (L.) Naud., *flexuosus* (L.) Naud. and *momordica* (Roxb.) Duthie et Fuller. Recently, Pitrat et al. (2000) identified 16 melon groups assigned respectively to *C. melo* ssp. *agrestis*: var. *conomon* Thunb., *momordica* Roxb., *acidulus* Naud., *makuwa* Makino, *chinensis* Pangalo, and *C. melo* ssp. *melo*: var. *chate* (Hasselquist) Filov, *flexuosus* L., *tibish* Mohamed, *cantalupensis* Naud., *reticulatus* Ser., *inodorus* Jacq., *adana* Pangalo, *chandalak* (Pangalo) Filov, *ameri* Pangalo, *dudaim* L. and *chito* Morren.

In Tunisia, melon is mainly cultivated in open fields (8060 ha) in regions of Beja, Jendouba, Sfax, Gafsa, Tozeur's oasis, Gabes, Kairouan, Sidi Bouzid and the Sahel. At present, the most used cultivars (all introduced) are Charentais (Pancha F1), Galia (Gallicum F1, Alma F1), Yellow Canary (Jango F1, Gold mine F1) and Pineapple melon (Afamia F1) characterized by both high productivity and high sugar content. These improved cultivars replace, in commercial production, the most known traditional varieties Maazoun (ancient introduction from Turkey) and Galaoui (unknown origin) which are endangered and confined to small areas (e.g. Bizerte and Beja regions). Landraces such as Beji, Bouricha, Kasbar, Souri, Stambouli, Bouzemzouma, Chefli, Gaouane and Abdelaoui were abandoned, except in scattered family fields for self supply (Novikoff 1952; Jebbari et al. 2004). The present study reports the phenotypic variation of Tunisian melon accessions collected from different geographical and bioclimatic areas. The assessment of the morphological variation within and among landraces based on leaf, flower, seed and mature fruit traits helps to precise their classification within melon cultivar groups and conceive conservation and improvement strategies.

Materials and methods

Plant material and experimental methods

Twenty-eight Tunisian melon accessions, based on mature fruit characteristics. (e.g. shape, size, flesh color, rind texture, and aroma presence) were assessed. The accessions were harvested in July 2009 in open fields from five departments: Monastir (Mz1–Mz6, Mn1–Mn4, Mk1–Mk8, Chmz and Chmk), Mahdia (Chb), Bizerte (Gal and Maa) and Tozeur's oasis (Tz1–Tz5). Yellow Canary (Casaba market class type) was added as a reference variety (YC). The local name and the main ecological traits of the collection sites were reported in Table 1. The accessions were tentatively assigned to Munger and Robinson's (1991) varietal groups. Seeds from each fruit of each accession were isolated, dried and germinated initially in alveolar plates covered with peat moss. Seedlings were transferred to the field at the three leaf stage. The assay was carried out from March to August 2010 in the Experimental Station of the SAM (Support Station of Manouba; 36° 48′ 49″N; 10° 3′ 25″E; rainfall 450 mm/year; altitude 42 m). The experimental design was a randomized complete block with two replications. Each block consisted of 29 rows of 15 m long with a distance of 1 m between samples and 1.5 m between rows. Irrigation was applied every 5 days and hand weeding was applied when necessary.

Traits measured

Thirty-four characters related to flower (X1), leaf (X2–X4), fruit (X5–X33) and seed (X34) morphology were assessed (Table 2). Thirty of them were estimated according to the UPOV (The International Union of the Protection of New Varieties of Plants) descriptors. Additional traits such as aroma presence (X14), fruit weight (X28), total soluble solids content (X27) (measured as a Brix) and placentas number (X33) have been also scored. Three plants, representing the most frequent type per accession and per replicate were considered to assess variation of leaf, seed and fruit traits. The sex expression was estimated using 15 plants per accession and per replicate.

Data analysis

A variance analysis was performed to test differences of traits within and among origins. Duncan's test was used to provide significant differences between averages of traits. The general divergence among accessions was estimated using principal component analysis (PCA), based on all traits. The genetic distance among the different groups identified by the PCA was estimated by Mahalanobis distances D^2 [$(D^2(\mathrm{i/j}) = (\bar{\mathrm{x}}_\mathrm{i} - \bar{\mathrm{x}}_\mathrm{j})$ $\mathrm{COV}(\bar{\mathrm{x}}_\mathrm{i} - \bar{\mathrm{x}}_\mathrm{j})$, where $\bar{\mathrm{x}}_\mathrm{i}$ and $\bar{\mathrm{x}}_\mathrm{j}$ the mean vector of the observations in group i and j respectively] (Fisher

Table 1 Accessions of *C. melo*: collection site, bioclimatic zone, accessions names and their assignment to Munger and Robinson's (1991) varietal groups

Department	Collection site	Accession name	Code	Varietal groups	Bioclimatic zone[c]	Latitude	Longitude	Altitude (m)
Monastir	Mazdour	Yellow Hab Rched[a]	Mz1	i	Upper arid	35° 46′ 38″ N	10° 49′ 34″ E	16
		Green Hab Rched[a]	Mz2	i				
		Mazdour 1[b]	Mz3	i				
		Mazdour 2[b]	Mz4	i				
		Mazdour 3[b]	Mz5	i				
		Mazdour 4[b]	Mz6	i				
		Chemoum[a]	Chmz	d				
	Menzel Nour	Menzel Nour 1[b]	Mn1	na	Upper arid	35° 41′ 47″ N	10° 46′ 21″ E	10
		Menzel Nour 2[b]	Mn2	na				
		Menzel Nour 3[b]	Mn3	na				
		Menzel Nour 4[b]	Mn4	na				
	Moknine	Moknine1[b]	Mk1	na	Upper arid	35° 39′ 10″ N	10° 56′ 18″ E	19
		Moknine 2[b]	Mk2	na				
		Moknine 3[b]	Mk3	na				
		Moknine 4[b]	Mk4	na				
		Moknine 5[b]	Mk5	na				
		Moknine 6[b]	Mk6	na				
		Moknine 7[b]	Mk7	na				
		Moknine 8[b]	Mk8	na				
		Chemoum[a]	Chmk	d				
Mahdia	Chiba	Chiba[b]	Chb	r	Upper arid	35° 30′ 9″ N	11° 2′ 45″ E	2
Tozeur	Tozeur	Tozeur1[a]	Tz1	na	Lower arid	33° 55′ 27″ N	8° 7′ 52″ E	43
		Tozeur 2[a]	Tz2	na				
		Tozeur 3[a]	Tz3	na				
		Tozeur 4[a]	Tz4	na				
		Tozeur 5[a]	Tz5	na				
Bizerte	Bizerte	Maazoun[a]	Maa	r	Sub-humid	33° 55′ 27″ N	8° 7′ 52″ E	43
		Galaoui[a]	Gal	r				
Beja	Beja	Yellow Canary[mk]	YC	i	Sub-humid	36° 43′ 31″ N	9° 11′ 31″ E	211

mk market class, *i inodorus*, *d dudaim*, *r reticulatus*, *na* not attributed

[a] Local name given by the farmer

[b] Name given according to the location

[c] Defined according to Emberger's Q2 pluviothermic coefficient (1966)

1936; Hebrant 1974). The Statistical Analysis System program (SAS 2002) with GLM, PRINCOMP and CANDISC procedures was used to perform the statistical analyses. Mantel's test (Mantel 1967), using the zt program (Bonnet and Van de Peer 2002), was performed to estimate the correlation between matrices of Mahalanobis and geographic distances (Km) among origins of collection.

Results

Preliminary classification of accessions

The analyzed accessions have been tentatively assigned to Munger and Robinson's (1991) varietal groups based of flower and mature fruit traits. The main observed fruit types in each accession were

Table 2 Analyzed traits for all accessions

Trait class	Code	Designation	Unit, interval or class
Flower	X1	Sex expression	1 Monoecious, 2 andromonoecious
Leaf	X2	Development of lobes	3 Weak, 5 medium, strong 7
	X3	Length of terminal lobe	3 Short, 5 medium, long 7
	X4	Dentation of margin	3 Weak, 5 medium, strong 7
Fruit	X5	Position of maximum diameter	1 Toward stem end, 2 at middle, 3 toward blossom end
	X6	Shape in longitudinal section	1 Ovate, 2 medium elliptic, 3 broad elliptic, 4 circular 5 quadrangular, 6 oblate, 7 obovate, 8 elongated
	X7	Ground color of skin	1 White, 2 yellow, 3 green, 4 grey
	X8	Intensity of ground color of skin	3 Light, 5 medium, 7 dark
	X9	Density of patches	1 Absent or very sparse, 3 sparse, 5 medium, 7 dense, 9 very dense
	X10	Warts	1 Absent, 9 present
	X11	Strength of attachment of peduncle at maturity	1 Very weak, 3 weak, 5 medium, 7 strong, 9 very strong
	X12	Shape of base	1 Pointed, 2 rounded, 3 truncate
	X13	Shape of apex	1 Pointed, 2 rounded, 3 truncate
	X14	Aroma	1 Absent, 9 present
	X15	Grooves	1 Absent or very weakly expressed, 2 weakly expressed, 3 strongly expressed
	X16	Width of grooves	3 Narrow, 5 medium, 7 broad
	X17	Depth of grooves	1 Very shallow, 3 shallow, 5 medium, 7 deep, 9 very deep
	X18	Color of grooves	1 White, 2 yellow, 3 green
	X19	Creasing of surface	1 Absent or very weak, 3 weak, 5 medium, 7 strong, 9 very strong
	X20	Cork formation	1 Absent, 9 present
	X21	Thickness of cork layer	1 Very thin, 3 thin, 5 medium, 7 thick, 9 very thick
	X22	Pattern of cork formation	1 Dots only, 2 dots and linear, 3 linear only, 4 linear and netted, 5 netted only
	X23	Density of pattern of cork formation	1 Very sparse, 3 sparse, 5 medium, 7 dense, 9 very dense
	X24	Main color of flesh	1 White, 2 greenish white, 3 green, 4 yellowish white, 5 orange, 6 reddish orange
	X25	Firmness of flesh	3 Soft, 5 medium, 7 firm
	X26	Width of flesh in longitudinal section	Cm
	X27	Total soluble solids	°Bx
	X28	Weight	g
	X29	Length	Cm
	X30	Diameter	Cm
	X31	Ratio length/diameter	
	X32	Pistil scar diameter	Cm
	X33	Number of placentas	
Seed	X34	Seed size	1 Very small, 3 small, 5 medium, 7 large, 9 very large

reported in Figure 1 (added as Electronic Supplementary Material).

All accessions showed pubescent ovaries, and therefore they belong to the. *Cucumis melo* ssp. *melo*. Yellow Canary (YC) and Maazoun (Maa) varieties as previously reported, belong to the group *inodorus* (Jebbari et al. 2004; Pech et al. 2007). Accessions, collected in Mazdour (Mz1–Mz6) were also included in this group. They were andromonoecious and produced non aromatic fruits with long shelf life, pointed peduncle, large pistil scar (Mz1 and Mz5), greenish white flesh and green skin with green spots (Mz1, Mz2, Mz5 and Mz6) or yellow patches (Mz3). Galaoui (Gal) and Chiba (Chb) with netted rind and orange flesh fruits were included in the group *reticulatus*. Chemoum (Chmz and Chmk), characterized by small size, strong typical aroma and velvety skin (with brown stripes) fruits has been assigned to the group *dudaim.* Menzel Nour (Mn1–Mn4) and Moknine (Mk1–Mk8) accessions were high polymorphic and have not been classified. Their flower and fruit morphological traits did not fit the typical description one of Munger and Robinson groups (1991) (e.g. elevated weight >12 kg for Mn3, downy skin for Mk3 and Mk4, large pistil scar and netted skins with brown patches for Mk4 and Mk8). Tozeur accessions (Tz1–Tz4) were monoecious and characterized by long, orange, downy skins and white flesh fruits. Their assignment to one of Munger and Robinson's groups was problematic because of their ovary pubescence and monoecy.

Morphological traits variation among accessions within and among collection sites

The analysis of variance revealed significant differences for the examined traits among accessions within and between origins. The mean values of traits per origin were reported in Table 1 (added as Electronic Supplementary Material).

Morphological traits variation within collection sites

The majority of qualitative traits (X1–X25, and X34) differed significantly among accessions within origins. The highest variation was shown among Moknine accessions followed by those from Menzel Nour (24 and 18 out of the 26 traits were polymorphic). The level of variation was less important for Mazdour and Tozeur accessions (14 and 13 out of the 26 traits).

A significant variation within origins was also observed for the quantitative traits except the flesh width (X26), total soluble solids content (X27), fruit weight (X28) and length (X29), ratio fruit length/fruit diameter (X31), pistil scar diameter (X32) and placentas number (X33).

Morphological traits variation among collection sites

Significant differences among origins were observed for the majority of the qualitative traits except X5, X10, X13 and X20 (Table 1 added as Electronic Supplementary Material). The sex expression (X1) distinguished monoecious Tozeur accessions, from the remnant accessions all of which were andromonoecious (except Mn1 and Mn3 which were monoecious and andromonoecious). The development of leaf lobes was weak ($3.0 < X2 < 3.1$) for accessions Tz1–Tz5, Chb, Mk1, Mk2, Mk3 and Mk6, medium ($X2 = 5.3$) for Mk4, Mk5, Mk7, Mk8 and Menzel Nour (Mn1- Mn4), and deep ($X2 = 6.5$) for those from Mazdour. The position of the maximum diameter (X5) was at the middle for all accessions, except for Tz3 and Tz4 with a maximum diameter towards blossom (22.0 and 25.0 %, respectively) and Tz1 with a large diameter towards stem end (11.0 %). Warts were generally absent ($3.2 < X10 < 5.5$), except for Mz2 (77.8 %), Mn4 (85.7 %), Mk3 (62.5 %), Mk5 (90.0 %), Mk6 (88.9 %), Mk7 (64.3 %) and Tz5 (58.8 %). Accessions from Mazdour (Mz) and Menzel Nour (Mn) have no tendency to peduncle abscission ($6.4 < X11 < 8.5$). The external aroma characterized mainly Chb fruits ($X14 = 8.1$). The creasing of surface was rarely present ($1.0 < X19 < 2.2$). The skin corking (X20) was observed in all fruits. However the density of cork layer (X23) differed according to origins; the highest density was observed at Tozeur ($X23 = 8.0$) and Chiba ($X23 = 6.8$), while the lowest at Mazdour ($X23 = 4.1$), Moknine ($X23 = 4.0$) and Menzel Nour ($X23 = 4.4$). The firmness of the flesh was medium for most accessions ($4.3 < X25 < 6.2$).

The lowest flesh thickness was detected in accessions from Tozeur ($X26 = 3.1$ cm) while, the highest in those from Mazdour ($X26 = 4.6$ cm). The total soluble solids content for Mazdour and Chiba fruits (4.7°Bx and 4.5°Bx) was higher than those for Menzel Nour ($X27 = 3.9$°Bx), Moknine (3.9°Bx) and Tozeur (3.1°Bx). Fruits from Menzel Nour had the highest weight ($X28 = 3561$ g). The smallest pistil scar diameter was observed in accessions from Tozeur ($X32 = 0.7$ cm), while the largest in Chiba ($X32 = 2.5$ cm). The placentas number (X33) were 3 (the majority of accessions), 4 (Chiba) and 5 (Tozeur).

Seeds (X34) varied significantly in size, shape and color (Table 1; Figure 2 added as Electronic

Supplementary Material). They were white and medium ($4.0 < X34 < 4.7$) and small ($3.0 < X34 < 4.8$) for Tozeur and Mazdour accessions, respectively. Seeds from Moknine ($4.9 < X34 < 6.8$) and Menzel Nour ($5.4 < X34 < 7.6$) were medium to large, while those from Chiba were medium.

The two landraces Chemoum (Chmz and Chmk) were distinctive by sweet and smelling flavor ($X14 = 9$), low weight ($97.8\ g < X28 < 112.3\ g$) and small seeds ($X34 = 1$).

The introduced varieties were characterized by large seeds (X33) and low peduncle abscission at maturity (X11). Galaoui and Maazoun showed rounded and truncated apex (X13), respectively. The netted skin (X20) was perceivable only for Galaoui, while the wrinkled surface (X19) was present in both Maazoun and Yellow Canary. The latter showed the highest total soluble solids content (7.5°Bx) compared to all other varieties (Table 1 added as Electronic Supplementary Material).

Differentiation among accessions based on all measured traits

The PCA plot according to the two first components, accounting to 49.68 % of the total variation, was reported in Fig. 1. The first component (25.07 % of the inertia) was correlated to the traits development and length of terminal leaf lobe (X2 and X3), strength of attachment of peduncle (X11), firmness and width of flesh (X25 and X26), presence of aroma (X14) and traits of grooves (X15, X16 and X18). The second component (19.61 % of the total variation) was defined by cork layer pattern and density (X22 and X23), fruit length (X29), sex expression (X1) and patches density (X9). Six accession groupings that give support to the existence of a high heterogeneity among Tunisian melon landraces were observed. The first (I) and the second (II) groups include the Sahel (Mz, Mn, Mk and Chb) and Tozeur accessions, respectively. The third group (III) is formed by accessions Chmz and Chmk. The ancient introductions Galaoui (Gal) and Maazoun (Maa), clearly stand apart (Groups IV and V), and were well separated from the sixth group (VI) Yellow Canary (YC).

Mahalanobis distances (D^2) between group-pairs produced by the PCA plot were highly significant ($P < 0.0001$), and correlated with those of geographical distances between origins (Mantel's test; $r = 0.631$, $P < 0.001$) corroborating an isolation per distance. The highest D^2 value was scored between groups II and VI ($D^2 = 201.42$), the lowest between I and IV ($D^2 = 37.19$) and I and II ($D^2 = 53.27$) groups (Table 3). Mahalanobis distances among groups IV, V and VI corresponding to the three introductions ranged from 87.99 to 182.79. The distances between Chemoum (III) and the groups I and II were lower ($61.96 < D^2 < 94.14$) than those scored with groups IV, V and VI ($116.87 < D^2 < 182.79$).

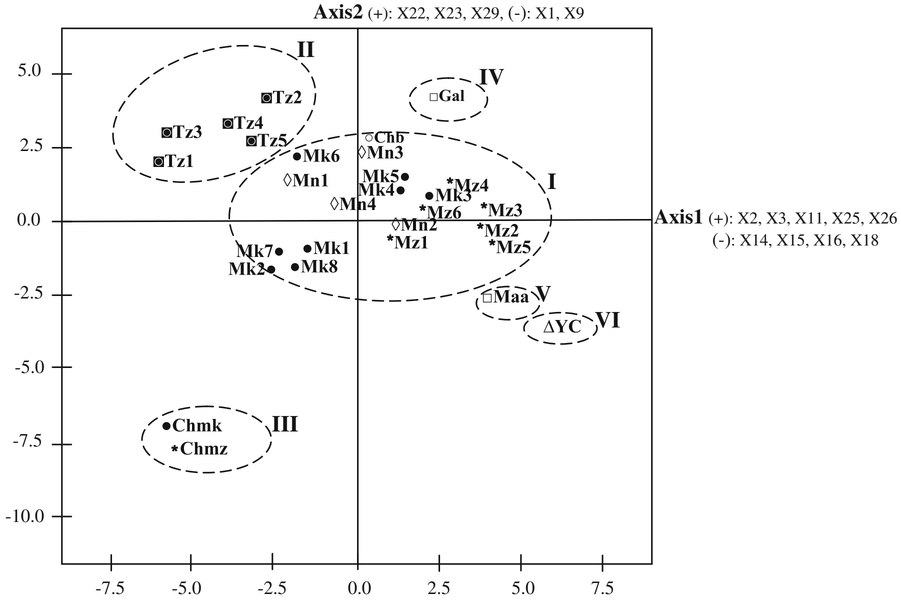

Fig. 1 PCA plot according to axes 1 and 2 based on all analyzed traits for all accessions. Symbols and letters indicate location and accession code, respectively (see Table 1) X1- X29: Variables contributing positively (+) or negatively (−) to the definition of PCA axes with Eigen-values equal to or higher than 0.2

Table 3 Squared Mahalanobis distances between group-pairs identified by the PCA plot

	Shl (I)	Tz (II)	Chm (III)	Gal (IV)	Maa (V)	YC (VI)
Shl (I)	0					
Tz (II)	53.27	0				
Chm (III)	61.96	94.14	0			
Gal (IV)	37.19	85.11	126.55	0		
Maa (V)	60.83	106.19	116.87	87.99	0	
YC (VI)	122.40	201.42	182.79	140.81	97.76	0

Shl sahel accessions (Mz, Mn, Mk and Chb), *Chm* chemoum accessions (Chmz and Chmk)

All distances among group-pairs are highly significant ($P < 0.0001$)

Discussion and conclusions

Morphological, biochemical and molecular markers have been used extensively to describe the variability of melon landraces. Most studies showed a high variation of the three sets of traits among accessions within and among geographical regions (Mliki et al. 2001; Lotti et al. 2008; Luan et al. 2008; Szamosi et al. 2010).

A total of thirty-four qualitative and quantitative traits related to leaf, flower, fruit and seed characteristics were used to assess the variation of Tunisian melon landraces from different geographical and bioclimatic regions. A high variation within and among origins was observed for the majority of the measured traits. The ancient introduced varieties Galaoui and Maazoun as previously reported (Jebbari et al. 2004) have been assigned to the group *reticulatus* and *inodorus,* respectively. Chemoum, characterized by a small size, strong typical aroma and velvety skin was included in the group *dudaim*. Accessions from Tozeur, Moknine and Menzel Nour, could not be clearly classified within one of Munger and Robinson's varietal groups. The trait pubescence of ovary, used as a key for the definition of melon subspecies, places them within *C. melo* ssp. *melo*. However, the varietal group should be defined based on combined morphological and molecular data.

The replacement of andromonoecy by monoecy in Tozeur accessions is an important breeding goal since monoecious accessions could be used as female parent for the exploitation of heterosis for earliness and yield (Kesavan and More 1991; Kim et al. 2010).

Accessions from Mazdour, assigned to group *inodorus,* are similar to Spanish cultivars Piel de Sapo and Tendral (Escribano and Lazaro 2009). This resemblance could result from an ancestral common origin.

All accessions showed low total sugar contents, thus they could be considered as primitive (Burger et al. 2010). However, accessions from Mazdour, with their thick flesh as well as the accessions Chiba and Chemoum, with their unique aroma, could be useful for breeding programs.

The PCA plot based on all measured traits allowed clear distinction between accessions from the Sahel (Upper arid bioclimate) and those from Tozeur (Lower arid bioclimate). These accessions clustered separately from the three introduced varieties, suggesting the singularity of the local landraces. The traits peduncle attachment strength, flesh width and firmness, fruit length, cork formation, sex expression, surface creasing, patches density, warts and grooves are the main traits contributing to the discrimination among accessions.

Our study on the genetic variation in Tunisian melons, gives information to guide conservation strategy. The used morphological traits allowed a high differentiation among local and introduced varieties indicating a low level of gene flow among them due probably to a low hybridization level as a result of differential floral biology and genetic drift. Artificial pollination should be made for further information. The high differentiation among the two sets of accessions allows also to think that they have undergone different life histories. A high differentiation was also shown among the Sahel and Tozeur landraces indicating a low level of gene flow between them due to both geographical isolation and limited seed exchange. Within geographical regions, the highest level of variation was observed within and among Sahel's origins and the lowest within Tozeur's ones. Thus, the conservation *in situ*, allowing the maintenance of interaction between landraces and local selective forces (abiotic and biotic forces) should be considered appropriately according to the geographical region. *Ex-situ* conservation should firstly include all accessions from the Sahel, even those geographically close. The high variation observed in Moknine and Menzel Nour implies that the within site seed collection should be favored to capture most of the genetic diversity. The conservation of Tozeur accessions is urgently required. Their specific distribution (oasis fields) and distinction from the other landraces may indicate several adaptations that could be used in selection programs. The analysis of molecular markers combined with adaptative traits should be conducted to deepen the assessment of the genetic variation and the classification of landraces within Munger and Robinson's groups and to suggest appropriate conservation strategy.

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