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Agro-morphological characterization and assessment of variability in local germoplasm of *Cucumis melo* L. in Tunisia

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Abstract

The agro-morphological characterization is fundamental in order to provide information for genetic resources conservation and breeding programs. The present study was carried out to characterize six local varieties of melon (*Cucumis melo* L.) selected by the National Institute of Agronomic Research of Tunisia. The studied varieties were grown in the field in randomized block design with three replicates. A descriptor list with 21 (9 quantitative and 12 qualitative) characters related to stem, leaf, fruit and seed was adopted. Quantitative data underwent analysis of variance (ANOVA) and principal component analysis (PCA); qualitative data was subjected to factorial correspondence analysis (FCA). With the traits retained in the PCA and the dimensions obtained in the FCA, a cluster analysis was performed using the unweighted pair-group method of averages. Significant differences were noted for the totality of the quantitative traits and high degree of polymorphism was observed for almost all of qualitative characters. Cluster analysis and distribution of populations in the 1-2 plan of PCA and FCA separated the varieties in different group with a divergence of 'Fakous' (*Cucumis melo* var. flexuosus) variety from the other varieties.

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Introduction

Cucumis melo L. (2n=24) is the one of polymorphic species among the cucurbitaceae family (Decker-Walters *et al.,* 2002; Szamozi *et al.,* 2010). The species is generally known as melon, it is also called sweet melon, muskmelon, casaba, and cantaloupe (Nayar and Singh, 1998). Munger and Robinson (1991) defined six groups of melon: Cantaloup, Inodorus, Flexuosus, Conomon, Chito, Dudaim, and Momordica. A recent taxonomy of melon identified 16 groups (Pitrat *et al.,* 2000 a), five in subspecies agrestis (acidulous, chinensis, conomon, makuwa and momordica) and 11 in subspecies melo (adana, ameri, cantaloupensis, chandalak, chate, chito, dudaim, inodorus, flexuosus, reticulates and tibish). The genus melon considered originated from Africa (Kerge and Grum, 2000). Recently phylogenetic data demonstrated that *Cucumis* originated from Asia (Sebastian *et al.,* 2010; Telford *et al.,* 2011).It is located in tropical and subtropical regions and is grown in temperate climate (Pech *et al.,* 2007; Staub *et al.,* 2004).

Cucumis melo is thought to contain the most diverse varieties in the genus *Cucumis*. Genetic diversity in this species has been analysed using several morphological characters in (Laghetti *et al.,* 2008; Staub *et al.,* 2004; Escribano and Lazaro, 2009; Nasrabadi *et al.,* 2012; Trimech *et al.,* 2013) and molecular markers such as isozymes (Akashi *et al.,* 2002; McCreight *et al.,* 2004), amplified fragment length polymorphism (AFLP, Yashiro *et al.,* 2005), random amplified polymorphic DNA (RAPD, Lopez-Sesé *et al.,* 2003; Staub *et al.,* 2004; Sensoy *et al.,* 2007; Tanaka *et al.,* 2007; Yi *et al.,* 2009; Soltani *et al.,* 2010; Nhi *et al.,* 2010) and simple sequence repeat (SSR, Monforte *et al.,* 2003; Ya *et al.,* 2012).

In Tunisia, melon is among the main vegetable crops grown and consumed, therefore of its economic importance. It ranks second after watermelon (Jebari *et al.,* 2004). In 2011, 10447 ha were dedicated to this crop and its production amounted to 104482 tones (FAO, 2013). However local melon genetic resources are currently being lost due to severe genetic erosion caused by the replacement of local varieties by modern varieties and improper management and inadequate regeneration procedures of germoplasm collections. Therefore, a survey of the genetic diversity is necessary to encourage rational management and selection programs involving the local *Cucumis melo* germoplasm.

The aim of the present study was to determine the agro-morphological variation in six local varieties of melon selected by the National Institute of Agronomic Research of Tunisia and to provide useful information to facilitate the choice of genitors for melon breeding program.

Materials and Methods

Plant materiel and experimental design

Six local varieties of melon *(Cucumis melo L.)* selected by National Institute of Agronomic Research of Tunisia were used in this study. They consisted on 'Maazoun' (MAZ), 'Galaoui' (GAL),'Stambouli'(STM), 'Trabelsi' (TRB), 'Asli' (ASL) and 'Fakous' (FAK; *Cucumis melo* var. *flexuosus*).

The essay was carried out from March to August 2012 at the Manouba Support Station located in the North East of Tunisia (36°45'0"N, longitude 10°0'00"E).Seeds were germinated in polystyrene trays with a peat substrate. Twenty days after emergence, the most vigorous seedlings of each variety were transplanted to the field in three rows (replication) with an in-row spacing of 100 cm and a between-row spacing of 150 cm. The experimental area was fertilized before planting by 85 Kg Ammonitrate ha^{-1}, 70 Kg phosphoric acid ha^{-1}, 130 Kg potassium nitrate ha^{-1}, 80 Kg magnesium sulfate ha^{-1}. Other agronomic practices including irrigation, weeding and chemical insecticide treatments were conducted uniformly and as required in all plots.

Data collection and statistical analysis

Data was collected on 21 agro-morphological (9 quantitative and 12 qualitative) parameters related to stem, leaf, fruit and seed according to the combined standards of descriptor lists of IPGRI

(International Plant Genetic Resources Institute, 2003) and UPOV (International Union For the Protection of New Varieties of Plants, 2006) for melon (table 1). Observations were recorded on three randomly selected plants of each variety per replication. A numerical micrometer was used to measure length, diameter and thickness. Fruit and seed weight measures were made by using a balance. Seed weight was determined for 100 seeds in three replicates per variety. Leaf size was determined by the use of OPTIMAS 6.1 software (OPTIMAS, 1996).

Table 1. Agro-morphological traits used for local melon varieties characterization.

Descriptor / Trait	Acronym	Type	Source	State/Unit
Stem				
Internode thickness	IT	QN	IPGRI	cm
Internode length	IL	QN	IPGRI	cm
Leaf				
Leaf color	LC	QL	IPGRI	1 Light green, 2 Green, 3 Dark green
Leaf size	LS	QN	IPGRI	cm^2
Terminal lobe length	TLL	QN	UPOV	cm
Fruit				
Fruit shape	FS	QL	IPGRI	1 Globular, 2 Flattened, 3 Oblate, 4 Elliptical, 8 Elongate
Predominant Fruit skin color	PFC	QL	IPGRI	2 Light-yellow, 3 Cream, 4 Pale green, 5 Green, 6 Dark green, 8 Orange
Secondary fruit skin color	SFC	QL	IPGRI	2 Light-yellow, 4 Pale green, 5 green, 6 Dark green , 7 Orange, 8 Brown
Peduncle length	PL	QN	IPGRI	cm
Cork formation	CF	QL	UPOV	1 Absent, 9 Present
Flesh flavor	FF	QL	IPGRI	3 Insipid, 5 Intermediate, 7 Sweet
Flesh acidity	FA	QL	IPGRI	3 Low, 5 Intermediate, 7 High
Fruit splitting	FSp	QL	IPGRI	3 Low, 5 intermediate, 7 High
Fruit storage ability	FSA	QL	IPGRI	1 Low, 2 Intermediate, 3 High
Fruit diameter	FD	QN	UPOV	cm
Fruit weight	FW	QN	IPGRI	g
Seed				
Seed length	SS	QN	UPOV	cm
Seed weight	SW	QN	IPGRI	g
Seed color	SC	QL	UPOV	1 whitish, 2 cream yellow

QN: quantitative; QL: qualitative.

Data analyses were performed using the statistical procedures in SAS 6.1 software (SAS, 1990). For quantitative parameters, analysis of variance (on-way ANOVA) was used to determine differences between varieties. Comparison of the mean values was made using the Duncan's multiple range test ($P<0.05$).

Correlation between pairs of morphological characters was evaluated using Pearson's correlation coefficient (Snedecor and Cohran, 1968; Turna, 2003). Multivariate relationships among varieties were revealed through a Principal Component Analysis (PCA) for quantitative characters and Factorial Correspondence Analysis (FCA) for qualitative characters. With the traits retained in the PCA and the dimensions obtained in the FCA, a cluster analysis was performed using the unweighted pair-group method of averages (UPGMA; Sokal and Michener, 1958). This analysis was used to study patterns of variance and relationships among accessions, where accessions with close genetic distances were placed in close proximity in the dendrogram.

Results and discussion

The data obtained is extracted on the basis of 21 descriptors starting from six melon varieties at a rate of three replicates per variety. Based on measurements and morphological observations, the examined melon varieties showed a wide range of variability for almost all of the traits studied.

Quantitative traits variation

Analysis of variance applied on quantitative characters (table 2) showed that differences among cultivars for the totality of studied characters were significant ($P<0.05$) to highly significant ($P<0.001$). The Duncan's test at 5% revealed 3 to 5 groups of means depending on the descriptors. Fruit weight (FW), terminal lobe length (TLL), peduncle length (PL) and seed size (SS) were the most discriminating character. The genotype 'Fakous' consistently recorded significant ($P<0.05$) differences from the others varieties in many quantitative characters. It showed the highest value for leaf size (198.92 cm) and peduncle length (16.17 cm), but the lowest values for fruit diameter (3.43 cm), fruit weight (0.22 kg), seed size (1.04 cm) and seed weight (3.33 g). 'Galaoui' presented, however, the highest average values for internode thickness (0.97 cm), and the lowest average values for leaf size (112.75 cm^2), internode length (4.55 cm) and peduncle length (9.63 cm). Whereas 'Stambouli' had the lowest value for internode thickness (0.67 cm) and the highest value for internode length (7.76 cm) and seed size (1.35 cm). 'Trabelsi' presented the heaviest fruits (3.53 kg), the biggest (1.37 cm) and the heaviest seeds (5 g); whereas 'Asli' had the longest terminal lobes (6.79 cm). Significant differences between varieties were also reported in previous data (Henan et al., 2013) for phenolic and carotenoid contents. Generally, the highest rates were obtained for 'Galaoui' genotype. Relations between quantitative traits were expressed in correlation matrix in table 3. According to this table, 5 morphological features were significantly at correlated 0.05 or 0.01 significant level.

Table 2. Means comparison for quantitative traits in six local melon varieties.

Variety	IT (cm)	IL (cm)	TLL (cm)	LS (cm^2)	PL (cm)	FD (cm)	FW (kg)	SS (cm)	SW (g)
MAZ	0,90±0.02ab	7,64±0.91ab	3,34±0.17c	133,35±4.24c	12,69±1.16c	17,16±0.29ab	2,50±0.12d	1,93±0,89cd	4±0cb
GAL	0,97±0.08a	4,55±0.54d	6,31±0.43ab	112,75±2.17d	9,63±0.69d	16,50±1.32b	3,36±0.13b	1,21±0,36b	4,3±0,5ab
STM	0,68±0.06d	7,76±0.27a	3,33±0.15c	124,90±19.5cd	11,03±0.8d	15,00±0.0c	2,72±0.02c	1,35±0,92a	0,46±0,06ab
TRB	0,79±0.05c	6,19±0.1c	2,81±0.16c	117,40±9.08cd	14,33±0.79b	17,66±0.29ab	3,53±0.06a	1,37±0,35a	5±0a
ASL	0,85±0.02bc	6,62±0.66bc	6,79±0.26a	152,65±9.06b	13,08±0.71bc	17,17±0.29ab	2,65±0.09cd	1,11±0,41cb	4,3±0,5ab
FAK	0,85±0.02bc	6,63±0.59bc	6,12±0.43b	198,93±2.82a	16,17±0.88a	3,43±0.06d	0,22±0.0e	1,04±0,82d	3,3±0,6c
F-value	13.53*	12.38*	112.81**	32.26**	22.09**	271.29**	630.61**	14.20*	4.45*

Mean in the same column followed by the same letters are not significant different at $P<0.05$ according to Duncan's multiple range test.*significant at $P<0.05$, **highly significant at $P<0.001$; LS: Leaf size; TLL: Terminal lobe length; IL: Internode length; IT: Internode thickness; PL: Peduncle length; FD: Fruit diameter; FW: Fruit weight, SS: Seed size; SW: Seed weight (100 seeds).

The main positive correlation appeared as follows: Fruit weight with fruit diameter (r=0.94), seed weight (r=0.89) and seed size (r=0.64); leaf size with peduncle length (r=0.75); seed weight with fruit diameter and seed size (r=0, 79). On the other hand, strong negative correlation was detected between leaf size with fruit weight (r=-0.95), fruit diameter (r=-0.87), seed weight (r=-0.83) and seed size (r=-0.7); peduncle length with fruit weight (r=-0.66) and fruit diameter (r= -0.61). Negative correlation between leaves development and fruits development was also detected in tomato (Gautier et al., 2013) suggesting a competition for assimilates between vegetative parts and reproductive ones.

Table 3. Pearson's correlation coefficients between pairs of quantitative characters.

	LS	TLL	IL	IT	PL	FD	FW	SS	SW
LS[(a)]	1	0.47	0.23	0.02	0.75	-0.87*	-0.95**	-0.7	-0.83*
TLL	0.47	1	-0.51	0.53	-0.002	-0.34	-0.35	-0.44	-0.52
IL			1	-0.63	0.28	-0.07	-0.29	-0.25	-0.09
IT				1	-0.15	0.03	0.006	-0.45	-0.41
PL					1	-0.61	-0.66	-0.45	-0.41
FD						1	0.94**	0.38	0.79
FW							1	0.64	0.89
SS								1	0.79
SW									1

(a) See table 1 for character abbreviation.* significant at 5%, ** significant at 1%.

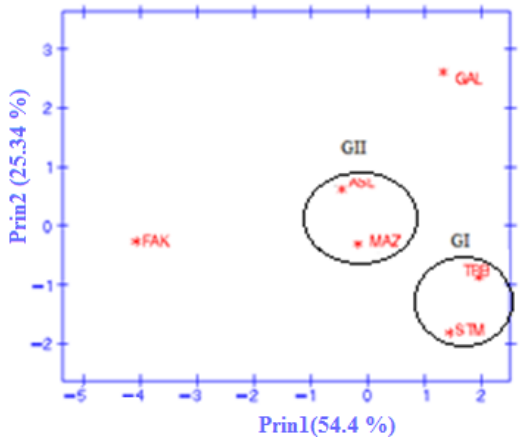

Fig. 1. Distribution of melon varieties in the 1-2 plan of Principal component analysis based on quantitative traits.

Information about the correlation and linkage among different horticultural characteristics is of primary importance in the field of crop improvement. Linkage relationships can be used to increase breeding efficiency by allowing earlier selection and reduction plant population size during selection (Nasrabadi et al., 2012).

Fig. 2. Variation in fruit shape and fruit skin color in the studied melon varieties.

Results of the principal component analysis for the 9 quantitative characters indicated that the first three axes explained 89.86% of the observed phenotypic diversity (Table 4). The first principal component (Prin1) explained 54.5% of the total variance and was positively correlated with fruit weight (FW), fruit diameter (FD), seed size (SS) and seed weight (SW)

but negatively correlated with leaf size (LS) and peduncle length (PL). Prin2 explained 25.34% of total variance and was positively correlated with terminal lobe length (TLL). Prin3 explained 9.85% of total variance and was positively associated with internode thickness (IT), internode length (IL) and fruit diameter (FD).

Table 4. Definition of the first three components of PCA on the base of morphological quantitative characters of local melon varieties.

Principal component	Prin 1	Prin 2	Prin 3
Eigenvalue	4.90	2.28	0.88
Proportion	54.50	25.34	9.85
Cumulative %	54.50	79.83	89.86
Character[(a)]		Eigenvalue	
LS	-0.44	-0.08	-0.10
TLL	-0.22	0.47	-0.16
IL	-0.07	-0.05	0.48
IT	-0.08	0.05	+0.30
PL	-0.30	-0.02	-0.11
FD	0.39	0.08	0.44
FW	0.43	0.12	0.11
SS	0.35	-0.1	-0.62
SW	0.42	-0.12	-0.13

(a) See table 1 for character abbreviations

The projection of varieties in the plan defined by the two first principal components is presented in Fig. 1. 'Fakous' separated clearly from the other genotypes and was located on the left side of the PCA graph while the other varieties were in the right side. 'Galaoui' also seemed to diverge significantly from the rest of melon varieties and was located on the upper part of the PCA graph. These results are expected since the two varieties consistently exhibited significant differences as compared to the other varieties for the majority of quantitative characters (Table 2). 'Trabelsi' and 'Stambouli' on one hand, 'Asli' and 'Maazoun' on the other hand were very close and grouped together in the PCA graph (Group I and Group II, respectively). The first group varieties showed significant similarities in many characters i.g. leaf size, terminal length size, seed size and seed weight; whereas the second group varieties showed significant similarities in all quantitative traits except in leaf size and terminal lobe length.

Qualitative traits variation

Variation in qualitative characters in the six melon varieties is summarized in table 5. The variability in qualitative characteristics was very important and allowed the distinction between varieties for the totality of parameters expect for fruit splitting (FSp) which was a non-polymorphic character. Fruit shape (FS), fruit skin color (PFSC and SFSC, Fig. 2), flesh color (FC) and flesh texture (FT) were the most discriminating characters.

Table 5. Variation in morphological qualitative characters of melon varieties.

Variety	LC[(a)]	FS	PFSC	SFSC	FLC	CF	FT	FF	FA	FSA	FSp	SC
MAZ	2	3	4	6	3	1	1	7	3	1	3	2
GAL	2	1	3	8	6	9	2	5	3	1	3	2
STM	1	4	2	5	6	9	3	7	3	2	3	2
TRB	2	2	2	5	4	9	1	5	3	2	3	2
ASL	2	4	2	2	6	1	5	7	3	2	3	2
FAK	3	8	6	4	5	9	1	5	5	2	3	1

(a) See table 1 for character abbreviations and states

Interaction between varieties and qualitative characters was analyzed by Factorial Correspondence Analysis (FCA). The FCA scatterplot according to the two first factors (54.66% of the total variation) was reported in Fig. 3.

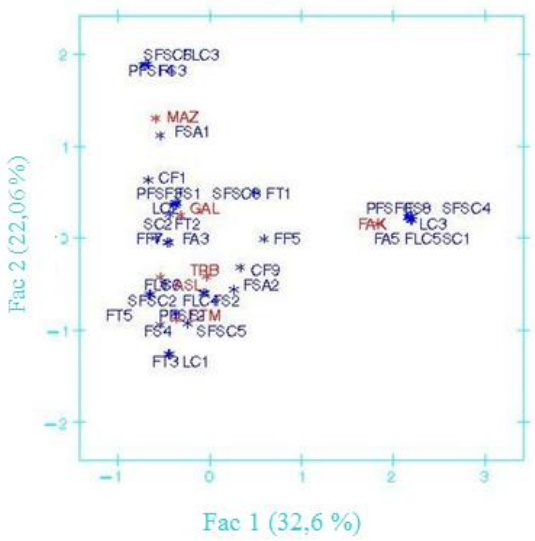

Fig. 3. Distribution of melon varieties in the 1-2 plan of factorial correspondence analysis based on qualitative traits.

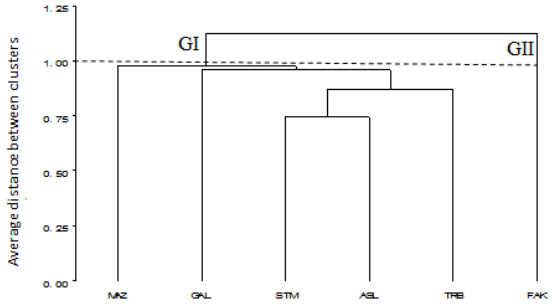

Fig. 4. Dendrogram obtained from cluster analysis of local melon varieties using the UPGMA.

In agreement with the PCA results, 'Fakous' clearly stood apart from the rest of melon varieties. 'Maazoun' and 'Galaoui' seemed to diverge from the others varieties whereas 'Trabelsi', 'Stambouli' and 'Asli' were grouped together. 'Fakous' is marked by leaf with dark green color, elongate fruits with dark green skins, green flesh with an intermediate acidity and whitish seeds. 'Maazoun' was distinguishable by fruits with oblate shape, pale green predominant skin, dark green secondary skin and cream flesh; whereas 'Galaoui' was characterized by fruits with globular shape, cream predominant skin, brown secondary skin and grainy-firm texture. Grouping of 'Trabelsi', 'Stambouli' and 'Asli 'is an indicative of morphological similarities in many qualitative characters such as a light-yellow predominant fruit skin color, a low fruit acidity and an intermediate fruit storage ability.

Based o the PCA and FCA analyses, it should be emphasized that the cumulative proportion of the variation revealed was relatively high, suggesting that all the traits studied were efficient for the melon varieties characterization. This was confirmed by variance analysis conducted on quantitative traits.

Differentiation among varieties based on all examined traits

A cluster dendogram (Fig. 4) combining quantitative and qualitative characters was carried out in order to study the general pattern of variance and to establish relationship among the six melon varieties. At an average distance of 1.0, hierarchical clustering process leads to two major groups. The first group (GI) included 'Maazoun', 'Galaoui', 'Stambouli', 'Trabelsi' and 'Asli' whereas the second one (GII) contained the single variety Fakous. The nearest varieties in the dendogram are Stambouli and 'Asli' with 'Trabelsi' being close to them, followed by 'Gaaloui' and 'Maazoun' in a larger distance.

According to Pitrat et al. (2000 b), *Cucumis melo* var. *flexuosus* or 'Snake melon' has a low similarity with each other varieties of melon and represented other group of melon.

Conclusion

Morpho-agronomical traits considered in this study showed a large variability in six local melon varieties. Results obtained could be used to establish a catalogue of local melon varieties. Further studies involving molecular markers could be very promising.

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