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**GENETIC RELATIONSHIPS IN CUCURBITA MAXIMA DUCHESNE GERMPLASM COLLECTED IN TUNISIA AS REVEALED BY MORPHOLOGICAL MARKERS**

**HAMDI Khawla, MOKRANI Khaoula, BEN-AMOR Jihen and TARCHOUN Neji**

Laboratory of Vegetable crops/ High Agronomic Institute of Chott Mariem, Sousse, Tunisia 4042

**ABSTRACT**

Squash is one of the most important crops in Tunisia. It showed great diversity in morphological characteristics, particularly leaf length and width, peduncle length, fruit weight, fruit shape, floral scar ratio, skin thickness, flesh thickness, fruit colour and seed shape. In the present research, 31 accessions of squash were collected from different provinces of Tunisia and phenotypic diversity in their fruit characters was assessed. The collection showed appreciable phenotypic variation in all the traits. Principal component analysis (PCA) and cluster were performed to determine relationships among populations and to obtain information about those fruit characters for the definition

of groups. The first two principal component axes accounted for 62.20% of the total multivariate variation among the accessions. The greater part of variance was accounted for by floral scar ratio, leaf length, leaf width and flesh thickness. Cluster analysis identified four different groups. The morphological characterization will be helpful to curators in the management and improvement of squash germplasm in Tunisia

**Keywords:** Squash, morphological characterization, accessions, PCA, cluster

**1. INTRODUCTION**

Cucurbitaceae is a large family including many species with an economic interest such as melon, watermelon, various squash and pumpkins. Many species are consumed in various forms, such as seeds, leaves, fruits and sometimes flowers in villages all over Africa. The genus *Cucurbita* L. (pumpkins and squash) is native to America where people cultivate it for more than 10000 years (Smith, 1989).

Species of this genus have become, in the 16th century, familiar and important in many European countries (Janick and Paris, 2006). Cucurbits have dispersed rapidly from Europe to other continents (Decker-Walters and Walters 2000, Paris, 2016) and there are three economically important species of *Cucurbita*, namely *Cucurbita pepo* L., *Cucurbita maxima* Duch. and *Cucurbita moschata* Duch., which have different climatic adaptations and are widely distributed around the world (Robinson and Decker-Walters, 1997, Wu et al., 2011). According to FAOSTAT (2017), the world's most cucurbit-producing countries are China, India, Russia, Ukraine, the United States of America and Iran with production of 7 297 540 respectively; 4 987

123; 1 232 162; 1 104 550; 863 460 and 603 629 tonnes in 2014. Much of the production of Cucurbitaceae was based on traditional local cultivars that have been maintained by farmers for centuries, such as Spain, Latin America and the Caribbean Africa (Gwanama et al., 2000, Lira-Saade, 2002, Ferriol et al., 2003).

For a long time, pumpkins have been used for traditional medicine in some countries such as China, Argentina, India, Mexico, Brazil and Korea, since the pulp of pumpkins and their seeds are rich not only in protein but vitamins, antioxidants, carotenoids and tocopherols (Stephenson et al., 2001).

In Egypt, summer squash is considered one of the most popular vegetable crops (Abd El-Hadi et al., 2012). Pumpkin seeds are also used in culinary practices mainly in the southern regions of Austria, Slovenia and Hungary (Murkovi'c et al., 1996). Turkey is also one of the main centers of diversity for cucurbits grown because of their adaptation to various ecological conditions resulting from natural selection and selection of farmers (Sari et al., 2008).

The genetic diversity of plant species must be evaluated primarily by the use of morphological markers (through the descriptors). According to Smith and Smith (1989), the essential elements in the classifications of cultivated species are morphological characters. These characters are considered as a main descriptive tool in the characterization of morphotype collections. Indeed, studies on phenotypic diversity within Cucurbita populations have been evaluated by Whitaker and Robinson (1986) and Hernandez et al. (2005).

In this study we present an analysis of the genetic variability in *C. maxima* collected from the major growing regions of Tunisia using the morphological descriptors.

## 2. MATERIALS AND METHODS

### Plant material

Mature fruits and seeds of squash accessions that were usually propagated and maintained by farmers themselves were collected. Moreover, thirty-one squash accessions were included in our investigation, including 6 Kerkoubi type, 14 Batati type, 9 Bejaoui type and 2 Kalaoui type derived from many prospection's missions during the harvest period from September 2014 to Mai 2015 from seven localities belonging to the main cultivation regions in Tunisia. Three fruits collected from the fields represent each accession. The collection and geographic position are shown in figure 1, table 1 and 2.

**Table 1: Season, type, number and origin of the local squash accessions collected**

Collecte season	Type	Accessions number	Délégation	Governorate	Latitude (N)	Longitude (E)	Altitude (m)
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<b>2014-2015</b>	Batati Sahel	5	Chott-Meriem	Sousse	35°54'22.21"N	10°32'47.81"E	6
		5	Sehline	Monastir	35°45'05"N	10°42'39"E	10
	Kerkoubi	2	Sidi-Bou-Ali	Sousse	35°57'24"N	10°28'23"E	8
		2	Sehline	Monastir	35°45'05"N	10°42'39"E	10
		2	Chott-Meriem	Sousse	35°54'22.21"N	10°32'47.81"E	6
	Bejaoui Sahel	3	Chott-Meriem	Sousse	35°54'22.21"N	10°32'47.81"E	6
		3	Sehline	Monastir	35°45'05"N	10°42'39"E	10
	<b>2015-2016</b>	Kalaaoui	1	Kalaat-Andalous	Ariana	37°033"N	10°11'7"E
Batati Nord		2	Kalaat-Andalous	Ariana	37°033"N	10°11'7"E	2
		3	Sidi-Hmeda	Siliana	35°57'28"N	9°32'57"E	800
Bejaoui Nord		1	Kalaat-Andalous	Ariana	37°033"N	10°11'7"E	2
		2	Sidi-Hmeda	Siliana	35°57'28"N	9°32'57"E	800
<b>Total</b>	<b>31</b>						

**Table 2: Local names and origins of the collected accessions**

<b>Accession</b>	<b>Local Name</b>	<b>Origine</b>
<b>Ker1</b>	Kerkoubi rouge	Chott-Meriem
<b>Ker2</b>	Kerkoubi rose foncé	Chott-Meriem
<b>Ker3</b>	Kerkoubi blanc	Sidi-Bou-Ali
<b>Ker4</b>	Kerkoubi jaune	Sidi-Bou-Ali
<b>Ker5</b>	Kerkoubi rouge	Sehline
<b>Ker6</b>	Kerkoubi rose foncé	Sehline
<b>Bat1</b>	Batati vert tacheté rose	Chott-Meriem
<b>Bat2</b>	Batati jaune	Chott-Meriem
<b>Bat3</b>	Batati blanc	Chott-Meriem
<b>Bat4</b>	Batati orange clair	Chott-Meriem
<b>Bat5</b>	Batati vert grisâtre	Gazze (Sehline)
<b>Bat6</b>	Batati vert	Gazze (Sehline)
<b>Bat7</b>	Batati blanc	Bhira (Sehline)
<b>Bat8</b>	Batati orange clair	Masjed-Aissa (Sehline)
<b>Bat9</b>	Batati jaune	Masjed-Aissa (Sehline)
<b>Bat10</b>	Batati tacheté blanc	Sidi-Hmeda (Siliana)
<b>Bat11</b>	Batati blanc	Sidi-Hmeda (Siliana)
<b>Bat12</b>	Batati vert	Kalaat andalous
<b>Bat13</b>	Batati vert grisâtre	Kalaat andalous
<b>Bat14</b>	Batati orange clair	Sidi-Hmeda (Siliana)
<b>Bej1</b>	Bejaoui vert	Masjed-Aissa (Sehline)
<b>Bej2</b>	Bejaoui vert grisâtre	Bhira (Sehline)
<b>Bej3</b>	Bejaoui blanc	Bhira (Sehline)
<b>Bej4</b>	Bejaoui vert	Chott-Meriem
<b>Bej5</b>	Bejaoui vert grisâtre	Chott-Meriem

<b>Bej6</b>	Bejaoui blanc	Chott-Meriem
<b>Bej7</b>	Bejaoui vert	Sidi-Hmeda (Siliana)
<b>Bej8</b>	Bejaoui vert claire	Sidi-Hmeda (Siliana)
<b>Bej9</b>	Bejaoui kalâa	Kalaat-Andalous
<b>Kal 1</b>	Kalaoui	Kalaat-Andalous
<b>Kal 2</b>	Kalaoui	Kalaat-Andalous

### Morphological characterization

The collected fruits were brought back to the laboratory of vegetable crops of the Higher Institute of Agronomic Sciences of Chott-Meriem, characterized based on IPGRI (International Plant Genetic Resources Institute, 1998) and UPOV (International Union for the Protection of New Varieties of Plants, 2007). The seeds were extracted, washed, dried, characterized and kept cold. For the accessions represented by seeds, the latter have been characterized and preserved. All seeds were treated with a fungicide and stored in cardboard packaging.

Twenty-two quantitative traits (cotyledon length and width, cotyledon ratio (L/W), stem diameter, branches number, nodes number, leaf length and width, leaf ratio, male and female flowers number, flower ratio, fruits number, fruit weight, peduncle length, floral scar length and width, floral scar ratio (L/W), bark thickness, flesh thickness, seeds number, seeds length and width) were measured with a ruler or caliper, fruit weight with a balance. Qualitative traits (length of main stem, leaf blade size, intensity of green colour of skin, fruit shape in longitudinal section, presence of neck, curving (longitudinal axis), profile at stem end, profile at blossom end, grooves, distance between grooves, depth of grooves, marbling, main color of skin, intensity of main color of skin, waxiness of skin, warts, Fruit: main color of flesh, seeds: color of coat) were evaluated based on scoring and coding.

### Statistical analysis

Data analyses were performed using the statistical procedures in SAS 9.1 software (SAS 1992). Simple statistics such as means and coefficient of variation were used on quantitative parameters in order to compare the variation between the studied accessions. A variance analysis (ANOVA) was performed and then the averages were compared by Duncan's multiple range test at 5% level. A Pearson correlation analysis was carried out to estimate the relationship between the studied variables. Different multivariate analyses were performed to evaluate the contribution of each quantitative and qualitative character to the total variation: Principal component analysis (PCA), factorial correspondence analysis (FCA) and hierarchical cluster analysis (HCA) were conducted on quantitative, qualitative and mixed data respectively.

### 3. RESULTS

#### 3.1. Quantitative characterization

##### Variance of the morphological characters

Statistical analysis showed significant differences between accessions collected for all parameters except skin thickness. Table 3 showed that leaf length and width vary respectively from 37.50 to 55.33 mm and from 21.86 to 31.46 mm. Kerkoubi type accessions from Chott-Meriem had the smallest leaves and the largest ones are found with the Bejaoui-Sahel type accessions. The leaf shape has been described using the Length / Width ratio. It is found that the Batati type of Sahel (Bat3, Bat4, Bat5, Bat6, Bat7, Bat8, Bat9 and Bat10) possess the higher ratios which reaches 2.01. Their leaves are long rather than broad. The average value of the fruit weight through the accessions is 7.60 kg and the average fruit weight of the individual accessions varies from 4.55 to 10.90 kg. The Bejaoui type accessions (Bej2, Bej3, Bej4 and Bej5) have the heaviest fruits. Concerning peduncle length, we noted that the Kerkoubi-Sahel type accessions (Ker4, Ker5 and Ker6) had the shortest peduncles and the Bejaoui Sahel type accessions (Bej2, Bej3, Bej4 and Bej5) had the longest peduncles. Moreover, the floral scar ratio varies between 0.43 and 0.80 with an average of 0.59. The lowest value was noted in the Kerkoubi type fruits, so, we noted that it had a rounded shape. In addition, the highest value is found in the fruits of Bejaoui, Batati and Kalaoui types accessions whose fruits are long.

Concerning the skin and flesh thickness, we obtained high values (0.32 and 6.49 mm respectively) in Kalaoui-type originated from Kalaat-Andalous while Batati-Sahel type fruits are characterized by lower values (0.11 and 3.92 mm respectively). In contrast, Batati type accessions have the biggest seed producer (337 seed per fruit) and the smallest were Kalaoui type accessions (119 seed per fruit). According to the UPOV (2007) standards, seed ratios are all lower or close to 0.5 which allows us to divide seeds between medium (Batati-Sahel and Bejaoui-Sahel types accessions) and small (remaining accessions). Seeds length and width varies from 1.26 to 1.97 cm and from 0.12 to 0.39 cm respectively.

**Table 3. Means comparison for quantitative traits in 31 Tunisian squash accessions**

Acc	PL	FW	FLSL	FLSW	FLSR	FTH	STH	SEN	SEL	SEW	SER
<b>Ker1</b>	7.30bcd	1.13f	8.26c	34.07cde	70.40def	0.12c	0.49a	5.23b	188.67hij	18.20def	3.32cdefg
<b>Ker2</b>	6.86bcd	0.91f	3.62c	34.00cde	77.95def	0.26c	0.43a	5.81b	176.00hij	19.64bcd	3.36cdefg
<b>Ker3</b>	9.50abc	0.86f	7.29c	38.45cde	75.05def	0.13c	0.49a	4.45b	337.33a	12.72klm	3.87cde
<b>Ker4</b>	6.34bcd	1.66ef	9.67c	41.70cde	72.40def	0.31c	0.57a	3.92b	201.00ghi	12.62klm	3.88cde

<b>Ker5</b>	10.90a	1.53f	11.33c	43.73cde	58.14def	0.22c	0.75a	4.81b	311.00ab	13.87klm	1.66efg
<b>Ker6</b>	7.13bcd	2.66def	6.07c	35.33cde	52.07ef	0.11c	0.69a	6.49b	119.00klm	14.26jkl	3.20efg
<b>Bat1</b>	5.13bcd	1.25f	8.17c	41.98cde	67.51def	0.15c	0.61a	5.43b	283.67abc	13.02klm	1.24fg
<b>Bat2</b>	6.27bcd	0.91f	9.00c	33.50cde	70.40def	0.33c	0.48a	7.79b	238.00efg	14.87ijk	2.69efg
<b>Bat3</b>	8.18bcd	1.20f	7.67c	33.54cde	63.03def	0.13c	0.53a	4.29b	276.67bcd	15.01hij	2.06efg
<b>Bat4</b>	9.20abc	1.40f	7.17c	49.14cde	82.51def	0.24c	0.59a	6.40b	262.33cde	18.41cde	3.76cde
<b>Bat5</b>	7.32bcd	1.08f	13.50c	43.15cde	57.39def	0.19c	0.75a	5.82b	73.33klm	20.22bcd	4.69b
<b>Bat6</b>	11.06a	0.33f	8.33c	37.10cde	70.47def	0.13c	0.52a	5.43b	102.00klm	16.81efg	2.31efg
<b>Bat7</b>	6.91bcd	1.66ef	6.83c	36.25cde	44.71f	0.13c	0.84a	5.71b	167.00ijk	16.97efg	3.23efg
<b>Bat8</b>	4.01cd	0.53f	7.00c	90.10abc	48.42f	0.43c	1.86a	6.71b	204.67fgh	20.35bcd	2.64efg
<b>Bat9</b>	7.41bcd	6.34ab	5.00c	44.86bcde	37.67f	0.13c	1.18a	5.38b	178.67hij	20.10bcd	3.75cde
<b>Bat10</b>	6.63bcd	0.46f	8.44c	64.33cde	46.57f	0.11c	1.38a	4.87b	174.00hij	20.00bcd	3.31efg
<b>Bat11</b>	8.15bcd	1.86ef	9.11c	89.98abc	52.41ef	0.34c	1.71a	2.83b	205.00fgh	20.59bc	3.46def
<b>Bat12</b>	6.83bcd	1.53f	5.84c	32.19de	46.69f	0.19c	0.69a	5.10b	117.00 klm	21.38b	4.17cd
<b>Bat13</b>	11.14a	2.80def	8.95c	50.06cde	44.04f	0.13c	1.13a	6.04b	177.00hij	21.51b	3.75cde
<b>Bat14</b>	8.59bcd	1.40f	7.66c	54.62cde	44.20f	0.21c	1.23a	6.58b	175.33hij	24.88a	11.60a
<b>Bej1</b>	10.66ab	1.60f	5.78c	13.68e	22.64f	0.11c	0.60a	2.86b	154.67jkl	15.64hij	3.50def
<b>Bej2</b>	6.80bcd	1.00f	10.22c	44.09cde	39.17f	0.29c	1.12a	5.87b	259.33def	11.81mn	2.47efg
<b>Bej3</b>	6.39bcd	5.93abc	75.52a	104.84a	151.21bcd	2.71ab	0.70a	43.39a	77.00klm	10.10n	2.49efg
<b>Bej4</b>	4.14bcd	4.54cd	58.95ab	96.11ab	148.79cde	4.02a	0.74a	28.93ab	67.00lm	16.30fgh	7.86b
<b>Bej5</b>	3.54cd	4.00cde	54.68ab	64.30cde	105.27def	2.83ab	0.44a	27.98ab	75.25klm	15.38ghi	7.22b

<b>Bej6</b>	4.19bcd	6.18abc	31.84bc	64.01cde	105.27def	2.21abc	1.96a	35.73a	54.88m	13.87klm	1.59efg
<b>Bej7</b>	3.73cd	7.06ab	32.37bc	61.54cde	98.40def	1.59bc	1.37a	27.55ab	105.75klm	12.37lm	1.63efg
<b>Bej8</b>	6.97bcd	7.50a	53.16ab	86.60bcd	191.02ab	2.90a	0.51a	40.71a	123.63klm	25.44a	12.48a
<b>Bej9</b>	2.56d	5.92abc	69.57a	83.33bcd	191.03ab	1.89bc	0.53a	22.39ab	141.50klm	16.00ghi	11.20a
<b>Kal 1</b>	9.74abc	5.14bc	66.84a	104.14a	212.07a	2.85ab	0.54a	37.97a	129.00klm	16.73efg	3.30efg
<b>Kal 2</b>	8.59bcd	2.27def	59.73ab	75.26bcd	159.52abc	2.54ab	0.48a	42.52a	176.70hij	24.08a	11.98a

Note: ACC: accession, PL: peduncle length, FW: fruits weight, FLSSL: floral scare length, FLSW: floral scare width, FLRS: floral scare ratio, FTH: flesh thickness, STH: skin thickness, SEN: seeds number, SEL: seeds length, SEW: seeds width, SER: seeds ratio

**Principal components of morphological characters**

Following principal component analysis, leaf length and width, peduncle length, and floral scar ratio are the parameters with the highest contributions in the construction of the first axis with values of 0.91, 0.89, 0.88 and 0.88 respectively. While the second axis is mainly composed of the flesh thickness with 0.90% of contribution (Table 4).

**Table 4. Principal component analysis (PCA) of characters associated with the squash (*C. maxima* Duch.) accessions**

	<b>F1</b>	<b>F2</b>
<b>Eigen value</b>	8,771	5,534
<b>Variabilité (%)</b>	38,135	24,063
<b>% cumulé</b>	38,135	62,197
<b>Traits</b>	<b>Eigen vectors</b>	
LFL	0,917	0,007
LFW	0,892	0,168
LFR	0,441	-0,272
<b>FW</b>	0,471	-0,210
<b>PL</b>	0,883	-0,369



<b>FLSL</b>	0,781	-0,370
<b>FLSW</b>	-0,647	-0,663
<b>FLSR</b>	0,883	0,366
<b>FTH</b>	0,008	-0,499
<b>STH</b>	-0,263	0,904
<b>SEN</b>	0,377	-0,597
<b>SEL</b>	-0,631	0,261
<b>SEW</b>	-0,641	-0,349
<b>SER</b>	-0,323	-0,491

According to Figure 3, the first two axis have the highest eigenvalues (8.77 and 5.53 respectively for F1 and F2). They reserve 62.20% of the total variability. The first axis occupies 38.13% of the observed variability. It was positively correlated with leaf and width length and floral scar ratio (l/L) and negative with seed length and width. It separates the accessions according to their leaf and floral scars forms.

The second axis explains 24.06% of the total variability. It is negatively correlated with the skin thickness, seeds number, and leaf ratio and positively correlated with flesh thickness and seeds length. It permit to differentiate the accessions according to their flesh thickness.

Considering axis 1, two groups of accessions can be distinguished (Fig. 4). The first group consists of accessions that had the longest broadest leaves and smallest seeds. Some accessions in this group had a high floral scar ratio (Bej4, Bej5, Bej6, Bej8 and Bej9) and others have high-weight fruits with long peduncles such as Bej1, Bej2, Bej3 and Bej7. The second group contains accessions with small leaves and long seeds (Ker3, Ker4, Ker5, Ker6 and Bat1) or wide seeds (Ker1, Ker2 and Bat2).

Following the second axis, the accessions were divided into two groups according to their flesh thickness. A thick flesh, a thin skin and a few seeds production characterize the first group. It contained six Batati type accessions originated from Siliana and Kalaat-Andalous (Bat11, Bat12, Bat13, Bat14, Kal1 and Kal2). While the second group contained the accessions having thick skin, accessions in this group were divided into two sub-groups; The accession Bat10 had a high grain yield and the rest of accessions (Bat3, Bat4, Bat5, Bat6, Bat7, Bat8 and Bat9) had a flat floral scar and broad leaves.

**3.2. Qualitative characterization**

A Factorial Correspondence Analysis (FAC) was performed to detect associations and oppositions between squash accessions and qualitative traits, by measuring their contribution to total inertia for each factor. Table 5 shows the eigenvalue and the cumulative percentage of the qualitative traits on the first three factors. Factor 1 accounted 25.80% of the total variance and a positive correlation with fruit shape, presence of neck, neck shape and neck length, groove depth, and waxiness of skin. Factor 2 accounted for 24.69% of the total variance and was positively correlated with profile at stem end, profile at blossom end, main skin color, flesh color, and warts.

**Table 5. Definition of the first three factors of FCA on the base of morphological qualitative characters of Tunisian squash landraces**

	<b>F1</b>	<b>F2</b>	<b>F3</b>
<b>Eigenvalue</b>	0,078	0,074	0,054
<b>Percentage (%)</b>	25,802	24,691	17,846
<b>Cumulative percent</b>	25,802	50,493	68,340
<b>Character</b>	Eigenvalue		
<b>SH</b>	0,447	-0,027	0,038
<b>PNC</b>	0,976	0,203	0,178
<b>SNC</b>	0,296	0,147	0,009
<b>LNC</b>	0,545	0,194	0,071
<b>CV</b>	-0,077	0,116	-0,083
<b>PSE</b>	-0,282	0,031	-0,122
<b>PBE</b>	-0,073	0,174	-0,215
<b>GV</b>	-0,052	0,117	-0,056
<b>DGV</b>	-0,040	0,059	-0,046
<b>DEGV</b>	0,033	-0,036	-0,037
<b>MR</b>	-0,284	-0,557	-0,087

<b>MCS</b>	-0,301	0,774	-0,394
<b>IMCS</b>	-0,020	-0,097	-0,009
<b>SCS</b>	-0,151	-0,771	-0,027
<b>MCF</b>	-0,054	0,040	-0,130
<b>WXS</b>	0,232	-0,164	0,164
<b>WR</b>	-0,576	0,336	1,082
<b>CCS</b>	-0,209	0,126	-0,139

Note: SH: shape, PNC: presence of neck, SNC: shape of neck, LNC: length of neck, CV: curving, PSE: profile at stem end, PBE: profile at blossom end, GV: fruit grooves, DGV: distance between grooves, DEGV: depth of grooves, MR: marbling, MCS: main color of skin, IMCS: intensity of main color of skin, SCS: second color of skin, MCF: main color of flesh, WXS: waxiness of skin, WR: warts, CCS: color of coat seeds

The scatter plot of the correspondence analysis in the plane defined by the first two factors (Fig.5) divided the squash accessions on the basis of the qualitative characteristics into three distinct groups. The first group (G1) included the accessions of site 2 (Sidi-Bou-Ali) and 3 (Chott-Meriem) characterized by fruits without necks, transverse broad elliptic shaped, slightly depressed profile at stem end, grooves usually present (84.61%), marbling absent to very weak, absence of waxiness and warts. In Sidi-Bou-Ali, 57.14% of skin fruits were light orange, 28.57% cream and 14.28% yellow. While in Chott-Meriem, accessions had skin fruits colored with light orange (33.33%), green (16.66%), yellow (16.66%), cream (16.66%) and orange brown (16.66%). Seeds in this group are cream, yellow and brown with the percentages 38.46, 30.76 and 30.76 % respectively.

The second group contains accessions from site 4 (Sidi-Hmeda) and 1 (Sehline, Masjed-Aissa and Behira) presented by fruits with large grooves. Accessions coming from Sidi-Hmeda had skin fruit colored with 85.71% green, 9.52% orange brown and 4.76% brown. Seeds are 50% cream colored and 50% brown colored. While, skin fruit in the accessions coming from Sehline had primary color cream (50%), green (40%) and yellow (10%). Seeds are cream, green and yellow (50, 40 and 10% respectively).

The third group includes accessions from site 3 (Kalaat-Andalus). Fruits in this group are pear-shaped and had strong neck. Grooves were medium and skin fruit was green with cream seeds (83.33%) or cream with brown seeds (16.66%).

### **Clustering of the Tunisian accessions**

The coefficients of genetic similarity between the 31 accessions collected vary between 0.93 and 1. According to Figure 6, there are four groups of accessions with a similarity coefficient 0.93.

Four accessions (Bat12, Bat13, Kal1 and Kal2) in the fourth cluster were well separated from the rest of accessions. The first cluster C-I is subdivided into three sub-clusters. C-I-1 consists of four Kerkoubi type accessions (Ker3, Ker4, Ker5 and Ker6) originating from Sidi-Bou-Ali and Sehline. Long seeds and small leaves characterize these accessions. C-I-2 contained two Kerkoubi-type accessions (Ker1 and Ker2) from Chott-Meriem and three Batati-type accessions (Bat8, Bat9 and Bat10) from Sehline. A broad floral scar and a thin flesh characterize this sub-cluster. C-I-3 consisted only of one accession (Bat7) from the Batati-type of Sehline, which had a thick skin. The second cluster C-II is subdivided into three sub-groups. C-II-1 is composed of four accessions (Bat2, Bat3, Bat4 and Bat5) of the Batati type originating from Chott-Meriem. It had broad seeds, a thick skin and a thin flesh. The accessions (Bat6, Bej8 and Bej9) belonged to the subgroup C-II-2. A thin flesh and a thick skin characterized this sub-group. C-II-3 contains only the Batati-type accession Bat1 from Chott-Meriem. It has long seeds and small leaves.

The third cluster C-III is subdivided into four sub-groups. C-III-1 consists of four Bejaoui-type accessions (Bej3, Bej4, Bej5, and Bej6) from Sehline with long leaves and broad floral scars. Two Bejaoui-type accessions (Bej1 and Bej2) from Sehline belonged to the sub-group C-III-2. High fruit weight and high grain yield characterize all of these accessions. Sub-cluster C-III-3 contains two accessions (Bat11 and Bat14) from the Batati type originating from Siliana. It had thick flesh and low grain yield. C-III-4 contained only one accession (Bej7) from the Bejaoui type originated of Siliana with long leaves, high weight and high grains yield.

The last cluster C-IV is subdivided into two sub-groups. It contained accessions with the thickest flesh, the lowest fruit weight and grain yield. C-IV-1 contained three accessions (Bat12, Kal1 and Kal2) originated from Kalaat-Andalous. In addition, C-IV-2 is formed only of one Batati-type accession (Bat13) from Siliana.

#### **4. DISCUSSION**

The morphological variability observed in this study is similar to several studies showing that, in domesticated plant species, morphological differences are often based on agronomic traits (Morimoto et al., 2005). Similarly, researchers have found that genetic diversity in local squash populations is high, including the shape, size and color of fruit, the number and size of seeds and flesh quality, color and thickness (Nerson et al., 2000, Ferriol et al., 2003, Paksoy and Aydin 2004). In another study by Liu et al. (2013), the morphological characterization of two collections (Chinese and Russian) of squash revealed a great genetic diversity. In the Cucurbitaceae family, the significant contribution of fruit and seed characteristics to morphological variability has been reported for watermelon (Maggs-Kolling et al., 2000, Gusmini, 2003), gourd (Morimoto et al., 2005), zucchini (Dey et al., 2006) and squash (Paris, 2001). Squash types with big seeds produce small fruits and fewest seeds number per fruit. Such a negative correlation between fruit and seed size and seed number per fruit could be attributed to regulatory behavior of plants, mainly because of limited external resources. The same observations were reported for cucurbits by Nerson (2005) and Ban et al. (2006). For example,

seeds length and width ranged between 1.26-1.97 and 0.12-0.39 cm. These results were near to the dimensions found by Iré et al. (2003) who studied three species of cucurbitaceae (*Citrullus* sp., *Cucumeropsis mannii* Naudin and *Lagenaria siceraria*). Seeds size reach 1.7 cm in length and 1 cm in width.

Principal Component Analysis (PCA) provided additional informations on the usefulness of defined group traits. Knowledge of the variation found in a crop species and how it is distributed is important for the development of breeding programs (Gil and Ron 1992, Balkaya and Ergün 2007).

With the greatest genetic diversity, squash have generally different characteristics, such as fruit shape, fruit weight and leaf size (Mohanty and Mishra, 1999). Leaf shape and fruit weight are used as an important characters on this work. In a similar work, Wehner (2008) used the leaf shape as a discriminating criterion in watermelon. Nerson (2002) has shown that fruit weight is a selection criterion between varieties.

In a similar study, PCA summarized the diversity of 20 morphological squash traits into three major factors: leaf factor (represented by leaf size), fruit factor (represented by fruit width), and quality factor (flesh thickness). These three main factors could be the priority indices in genetic material screening and genetic selection (Du et al., 2011).

The genetic diversity of local varieties is part of the economic value of global biodiversity and it has a primary importance for the future global productions (Stoilova et al., 2005). In addition, the conservation and maintenance of these genetic resources constitute a source of biodiversity for the breeding programs (Balkaya et al., 2005).

It was indicated that the useful strategies for ensuring efficient collection and management of genetic material are estimation of the genetic diversity and the determination of the relationships between collections (Bozokalfa et al., 2009).

The qualitative characterization proved that accessions have many significant differences. In a similar study, of genetic diversity, Du et al. (2011) showed that *C. moschata* in China had a wide variety of shapes, such as, round, oval and pear-shaped. It was probably due to the genetic variability of the species to adapt to various agro-ecological conditions.

## **5. CONCLUSION**

*Cucurbita maxima* Duchesne has a high importance in vegetable cultivation of Tunisia and it was so far under-concerned in terms of collection and characterization. In this study, the collection contributed to maximize the number of collected accessions from all productive zones in Tunisia.

During our research work, morphological characterization based on the quantitative and phenotypic traits of the different collected accessions indicates relationships between accession, geographical origin and morphological characteristics (proximity of ecotypes with the same

geographical origin or morphological characteristics). This characterization also facilitate the establishment of descriptive sheet of the characters of each accession.

Regarding the importance of some quantitative traits, we were able to distinguish accessions representing interesting agronomic characters. Batati-yellow originating from Sehline is the most seeds productive. The Bejaoui-type accessions from Sahel (Sehline and Chott-Meriem) had high-weight fruits. In addition, Kalaoui-type originating from Kalaat-Andalous and Batati-type originating from Siliana are characterized by thick flesh and thin skin.

Qualitative characterization revealed that accessions coming from Sahel of Tunisia have generally orange or crème skin fruits and yellow seeds. While accessions coming from Siliana and Kalaat-Andalous had green skin fruits and cream seeds.

This large genetic diversity must be exploited in improvement programs affecting both yield and quality. The generated information will be helpful to curators in the management and improvement of Squash germplasm in Tunisia.

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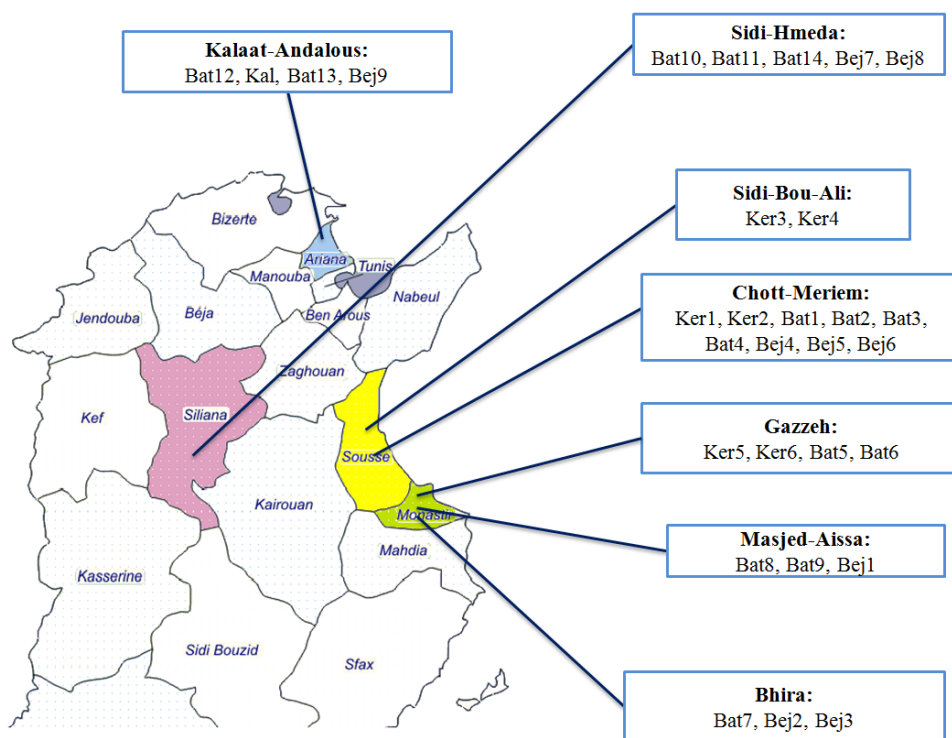
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**Figure 1. Geographic distribution of *Cucurbita maxima* collection used in this study. The names of provinces and locations are in bold.**

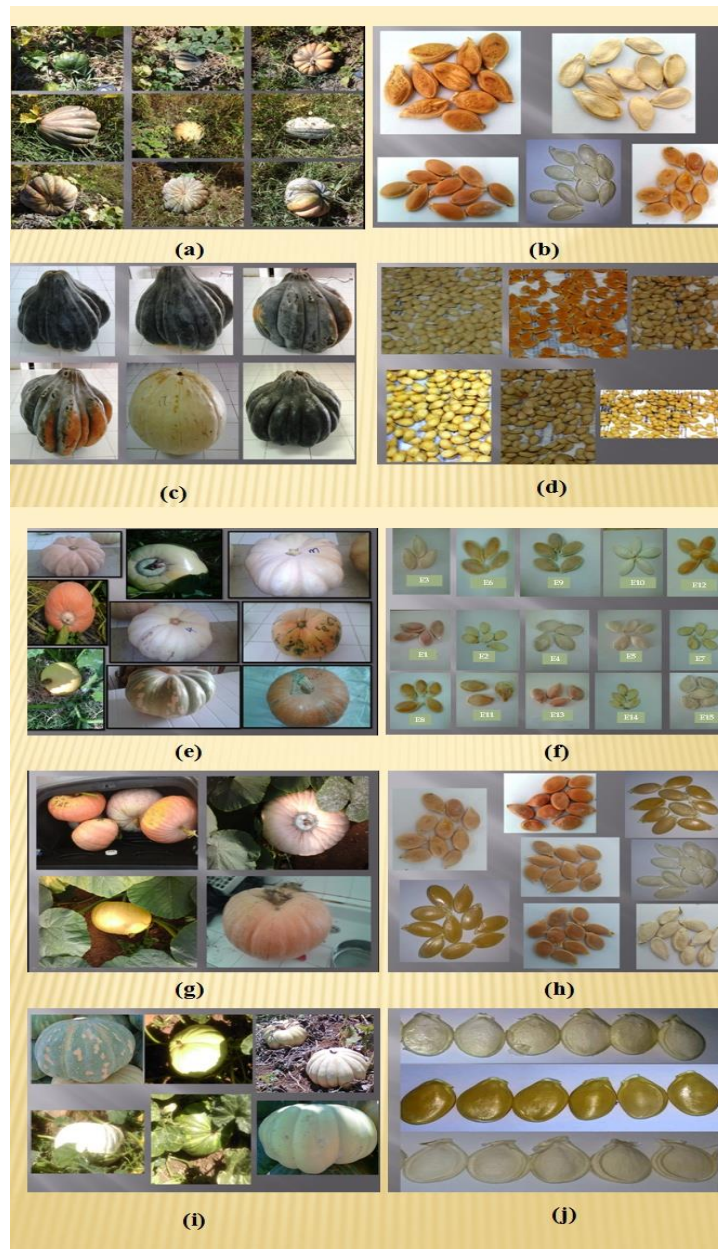


Figure 2. Fruits and seeds of different accessions collected from the different prospected zones

Note: (a) and (b) from Sidi-Hmeda Siliana, (c) and (d) from Kalaat-Andalous, (e) and (f) from Chott-Meriem, (g) and (h) from Sidi-Bou-Ali, (i) and (j) from Sehline

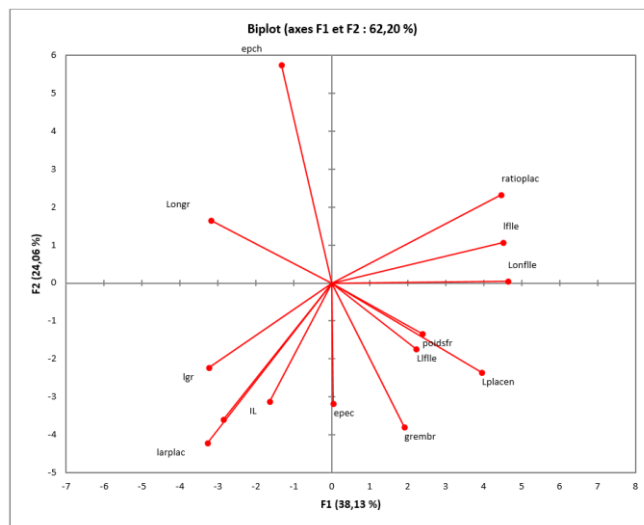


Figure 3. Projection of the quantitative characters on the plane generated by the first two axis

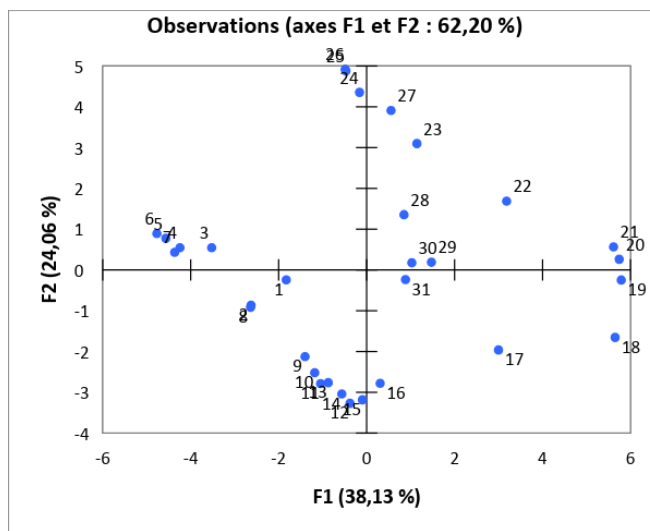


Figure 4. Diagram showing the relationships among the Tunisian accessions of *C. maxima* at the basis the first two principal component axes using morphological characterization

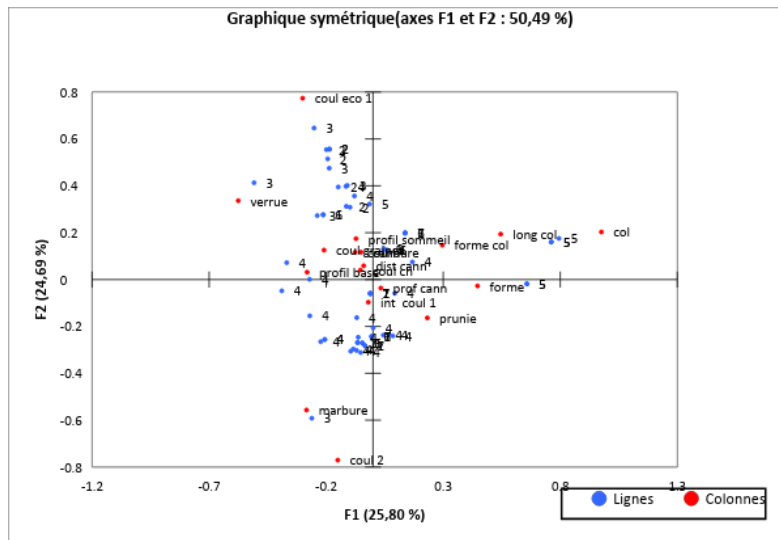


Figure 5. Scatter plot grouping of 31 Tunisian squash landraces based on the first two principal factors of FCA

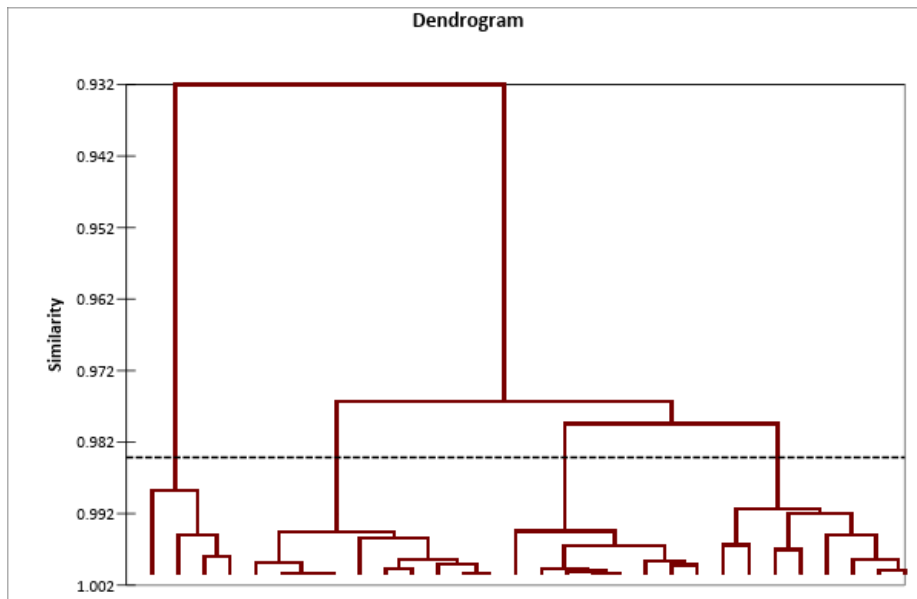


Figure 6. Dendrogram obtained from cluster analysis of thirty-one Tunisian squash landraces using the UPGMA