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Assessment of the genetic diversity of some local squash (*Cucurbita maxima* Duchesne) populations revealed by agro-morphological and chemical traits

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Abstract – Tunisia is one of the most important diversity centers for cultivated cucurbits characterized by their adaptation to climatic changes. Many local varieties have resulted from natural or farmers' selection. The aim of this study was to characterize fifteen local accessions of squash collected from different regions of the country. '*In situ*' and '*ex situ*' characterization were undertaken based on 30 parameters related to seeds, growth, flowers and fruit characteristic. This agro-morphological characterization was carried out based on UPOV and IPGRI descriptors. Total sugar, β-carotene pigment, and chemical parameters were also analyzed. Results revealed a considerable genetic variability for most of the agro-morphological parameters. Morphological variation was most apparent in fruit characteristics. In fact, most populations in this study had transverse broad elliptical fruit (75.6%), a medium skin thickness (89.12%), a medium flesh thickness (57.89%) and a big cavity diameter (94.7%). PCA helped to produce a dendrogram which classifies these local varieties into three homogenous groups. Analysis of the biochemical constituents revealed a significant difference between these groups for all the traits, except magnesium content. These results represent the first report on the characterization of squash in Tunisia, which will serve to the management, conservation and use of local genetic resources.

Keywords: squash, local varieties, genetic diversity, characterization.

1. Introduction

Cucurbita spp., commonly known as cucurbits, includes many economically and nutritionally important vegetable crops cultivated worldwide, such as cucumber, melon, watermelon, pumpkins, gourds, and squashes (Robinson and Decker-Walters, 1997). In this field, China and India are the world leading producers. Squash (*Cucurbita maxima* Duchesne) is one of the economically and nutritionally important vegetable crops in Tunisia and many other African countries. Local varieties of squash known as populations are largely produced in different regions of Tunisia. This species is often represented by local accessions (Bejaoui, Batati, Kerkoubi, Kalaaoui ...). In 2015, the area of 2523 ha was cultivated, producing 92450 tonnes of fresh fruits from different regions of Tunisia. Ariana, Nabeul, Kasserine, Gafsa and Sidi-Bouzid are the major producing regions, respectively covering production areas of 514, 507, 250, 245 and 250 ha (DGPA, 2015). The cucurbit family displays a rich diversity of many traits, and was among the first to be examined in sex expression analyses, vascular biology studies, and analysis of some fruit ripening mechanisms. Squashes have considerable impact on human nutrition, being appreciated for their medical properties (Shokrzadeh et al., 2010). In addition to the use of their flesh flowers and leaves, their seeds are also consumed as snacks, providing a source of edible oil and protein for human and animal consumption. They also have an array of applications in the pharmaceutical industry. The economic value of *Cucurbita* spp. as rootstocks for overcoming soil-generated diseases in cucurbits is significant. Squash is an important vegetable, mainly valued for its long storage life fruits, which can be consumed after six months of their harvest (Fita et al., 2007). *Cucurbita* genus (2n = 2 × = 40), which includes squashes, gourds and pumpkins, has been scarcely studied. It contains some of the earliest domesticated plant species (Smith, 2005). Despite the agricultural and biological importance of cucurbits, knowledge of their genetic diversity and genome has been very limited till now. Genomic characterization efforts have been largely focused on cucumber and melon, and only recently has focus started to be directed to the whole

genome range of the *C. sativus* L. (Gonzàlez et al., 2010). In crop improvement programme, determining the extent of genotypic variability among geographical areas is important (Muralidhara and Narasegowda, 2014). Quantitative and qualitative determination of the degree of variation of traits present in genetic resources is important for vegetable breeding programs (Escribano et al., 1998). Morphological characterization is the first step in the description and classification of genetic resources (Balkaya et al., 2009). Only a few studies have focused on morphological and/or molecular diversity analysis in connection with this species. In addition, very few molecular analyses have been conducted to estimate genetic relationships in squash (Ferriol et al., 2004).
Tunisia is a major cucurbits producer in the world. It is home of a diversity of cultivated cucurbits characterized by their adaptation to climatic changes. The local varieties have resulted from natural processes and farmers' practices. This large genetic variability of local squash populations can partly account for the great heterogeneity which is recorded in terms of agro-morphological characteristics. The objectives of this study are (i) the agro-morphological characterization of fifteen populations collected from different regions of Tunisia, and (ii) the analysis of genetic variation among these local varieties using the PCA.

2. Materials and methods

2.1. Plant material

Local squash populations (accessions) as seed and/or fruits were collected between July and September 2012. A total of 15 accessions (traditional populations) of squash (Fig.1) maintained by local farmers were collected from the main regions of squash production.

2.2. Field characterization (in situ)

Samples of about 300 seeds, 8 -12 fruits, and 7-10 leaves were collected from different sites and taken to the laboratory. Shape, colour and form of seeds and shape and area of leaves were recorded (Tab. 1). Regarding fruit traits, we identified width, diameter, skin thickness, peduncle length, flesh thickness, skin colour and flesh colour. General characteristics of the collected plant material are as follows (Fig.1).

Table 1: Squash types characteristics

Type	Accession number	Leaf description	Fruit description	Seed description
Batati	Seven	- big -heart shaped	-Smouth pericarp -diverse colours: bright orange, yellow, white and green -transverse elliptical or globular shaped -thick peduncle	-large -cream
Kerkoubi	Six	-big -rigid -heart shaped	-smooth pericarp -skin colour: orange -flesh colour orange or yellow -round shaped -thick peduncle	-flat -oval -white
Bejaoui	Two	-big -dark green -dotted with a few whitish spots	-main skin colour: green -flesh colour: dark orange -cylindrical or elliptical shaped -medium flesh thickness	-round -cream

Fig. 1. Diversity of fruit size, shape, and colour of the *Cucurbita maxima* accessions collected from different zones of Tunisia

2.3. Field evaluation (ex situ)

2.3.1. Agro-morphological characterization

The field evaluation was carried out in the experimental station of the Higher Institute of Agronomy Chatt-Mariem in 2012-2013. This experimental site was located at 35º55`N, 10º38`E. The soil of the experimental area was sandy loam with a pH of 7.35. The experiment was conducted through a Randomized Complete Block Design (RCBD), with three replications for phenotypic evaluation. To minimize the cross pollination, a minimum of 500 m were applied between the different accessions. Seeds were sown on both sides of the channel with a spacing of 2m between channel lines and 1.5m between plants. Irrigation, the recommended NPK fertilizer doses and cultural practices along with plant protection measures were applied according the plant needs to raise an ideal crop. The fruits were harvested at a marketable stage. For the morphological characterization, the standards descriptor sets for *Cucurbitacea* recommended by the IPGRI (2007) and the UPOV (2016) were used. Five plants per block were selected, after discarding the border plants at both ends, and were examined for morphological traits. The following characters were recorded or calculated for each accession: seed parameters (Length, Width, L/W, weight of 100 seeds), leaf parameters (Number, shape, form), flower parameters (female and male flowers number, FF/MF ratio, sepal length), and fruit parameters (weight, shape, peduncle length, skin thickness, flesh thickness, cavity diameter, seed number).

2.3.2. Biochemical characterization

The β-carotene pigment was extracted according to the procedure recommended by Minguez et al. (1993). Ten grams of each sample of the flesh fruits were mixed with 1 g of sodium bicarbonate and then homogenized and extracted with acetone. The extracts were mixed and the acetone was evaporated at 35 ° C until 50 ml of final volume was reached. The concentrate was transferred to an ampoule and mixed with 100 ml of ethyl ether. A NaCl solution (10%) was added to separate the phases and transfer the pigments to ether. This solution was treated several times with a solution of Na_2SO_4 anhydrous (2%) to remove all the water. The ether layer was evaporated to dryness at 30 ° C. The dry residue was then dissolved in a methanol (1: 1, v / v) ether mixture, filtered through a membrane (0.45 mm) and analyzed by HPLC. The preparation of the standard curves to evaluate the β-carotene pigment was carried out by pure β-carotene (Sigma Chemicol Co.) using the following concentrations: 3.12-6.25-12.5 and 25 Mg / g (Delia et al., 2004). The basic cations (K^+, Mg^{2+} and Ca^{2+}) were extracted after burning the sample in a muffle furnace. Dilution with a mixture of HCL/HNO3 was made, and analysis was carried out using an atomic absorption spectrophotometer according the method of Benton and Vernon (1990).

2.4. Statistical analyses

Analysis of variance was performed for all morphological, chemical and biochemical parameters using the SAS Software (version 9.2). Means comparison was conducted using Duncan's multiple range test at the level of 5%. Principal component analysis (PCA) and a hierarchical numeric classification were performed to determine the relationships between the agro-morphological traits and to group the fifteen accessions into homogenous classes.

3. Results and discussions

3.1. Agro-Morphological characterisation of the fifteen squash accessions

3.1.1. Seed characteristics

Table 2 and figure 2 show the qualitative and quantitative traits of the accessions' seeds**.** Statistical analyses revealed a highly significant difference for all traits (length, width, ratio L/W and the weight of 100 seeds). Concerning seed length, the accessions (E7), (E9) (E10), (E12) are characterized by the longest seeds ranging from 2.65 to 2.78cm, while the shorter lengths are found with the accessions (E3), (E5) and (E14), which range from 1.76 to 1.80cm. For seed width parameter the minimal width (0.9cm) was recorded in the accession (E5) while accession (E13) has the biggest width (1.44cm). A significant variability in the weight of 100 seeds was observed in the studied accessions. The weight varies from 14 to 46g for (E5) and (E13), respectively. This difference is attributed to seeds' dimensions and to embryo quality. To determinate seed shape, we calculated the ratio (length / width).

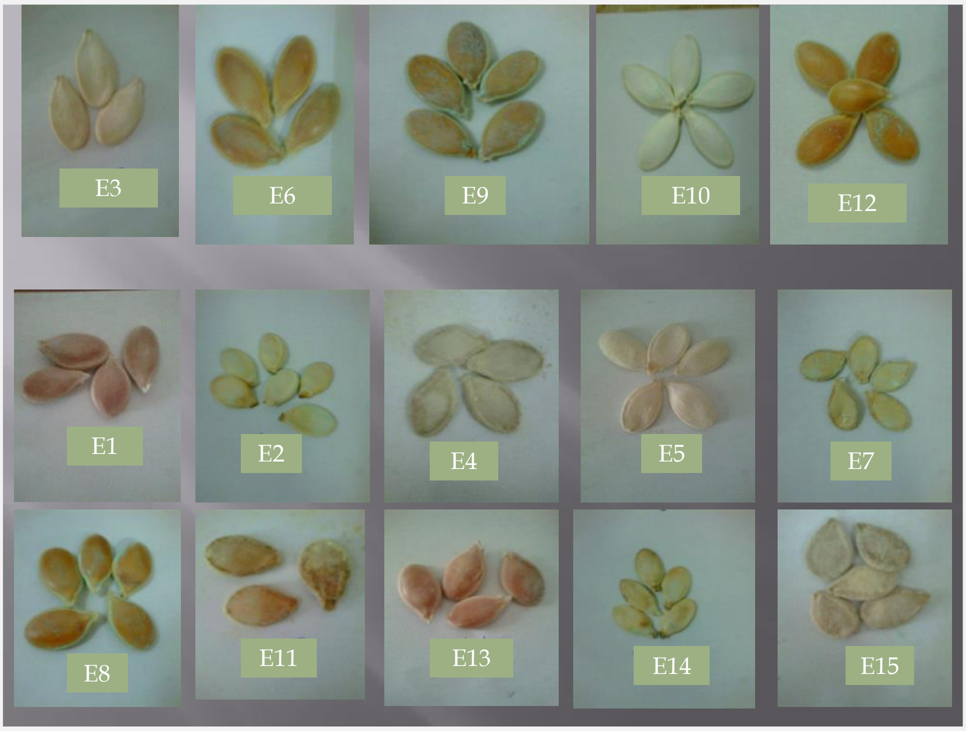

Fig. 2. Seeds characteristics of the fifteen squash populations

Using the IPGRI descriptor, the variability in seed shape between the studied accessions was noted (Fig. 2). Two groups of seeds were identified: seeds with elliptical form (E3, E6, E9, E10 and E12) and round seeds (E1, E2, E4, E5, E7, E8, E11, E13, E14 and E15). These results are not in accordance with those reported by Muralidhara and Narasegowda (2014) who found that *Cucurbita moschata* and *Cucumeropsis mannii* seeds had flat form with grooved edges. According to the UPOV standard, three colours have been identified: (1) white, (2) cream, and (3) light brown. In this respect, Ajuru and Okoli (2013) noted that the colour, size and shape of watermelon seeds are gene-controlled characters, a set which is already identified.

Table 2: Seed characteristics of the fifteen squash populations used in the study

Accession	Type	Length (cm)	Width (cm)	Colour	(L/W)	Weight of 100 seeds (g)
E1	Batati	2 .41f*	1.41abc	3	1.71 e	40 c
E2	Batati	2.57f	1.43ab	2	1.80de	46 a
E3	Batati	1.80h	1. 34de	2	2.10 b	43 b
E4	Kerkoubi	2.27g	1 .25gh	1	1.81cde	28 f
E5	Bejaoui	1.80h	0 .90j	1	1.80ed	14 h
E6	Kerkoubi	2 .31g	1 .23gh	3	2.08b	30 e
E7	Batati	2.78a	1 .38bcd	2	1.93c	42 b
E8	Batati	2.30g	1.21h	3	1.85cd	26 g
E9	Kerkoubi	2 .78a	1 .28fg	3	2.21 a	42 b
E10	Batati	2 .73ab	1 .37cde	1	2.08b	45 a
E11	Batati	2.45ef	1.34de	2	1.84cd	42 b
E12	Kerkoubi	2.68ab	1.32ef	3	2.05b	42 b
E13	Kerkoubi	2.66bc	1.44a	3	1.84cd	46 a
E14	Bejaoui	1 .76h	1.10i	2	1.59f	15 h
E15	Kerkoubi	2.45ed	1 .30cde	1	1.80ed	37 d

*Averages in the same colon, followed by the same letter are not statistically different at 5% (Duncan test).

3.1.2. Leaf characteristics

The statistical analysis shows that there is a significant difference related to leaf parameters. Table 3 demonstrates that the leaves number per plant varies from 35 to 68. A large leave number generates a good photosynthetic activity and, consequently, a better nutrition of future fruits. The average of the petiole length ranges from 29.03 to 48.65cm. Indeed, the Batati and Kerkoubi type accessions have the highest average, which exceeds 42 cm, while the lowest are found in the Bejaoui type accessions. Leaves with the longest petioles are most exposed to the light, and therefore have a good photosynthetic activity. The fastest growing accessions were then identified, namely the (E1), (E2), (E3) Batati type and the (E4) Kerkoubi type.
To describe leaf form, the ratio (leaf length/ leaf width) was used. The accession (E9) from the Kerkoubi type has the highest ratio in order of 1.27 while the others accessions have a ratio <1, explaining that their leaves are broad rather than long. Moreover, the correlation analysis revealed that the width of the leaves is negatively correlated with the length / width ratio (r = -0.50 **). These results show that most accessions have oval leaves. From the IPGRI and the UPOV descriptors, the leaves of all the accessions are large, green, and have a trilobate shape, except for the Kerkoubi type, which has heart-shaped leaves with a few whitish stains. Indeed, leaf shape was considered by Wehner (2008) as a criterion, which is genetically controlled in watermelon and which can serve as a discriminator between varieties.

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Table 3: Leaves number per plant (LN) and petiole length (PL)

Accession	Type	LN	PL
E1	Batati	59 abc*	42.06bcde
E2	Batati	68.7 a	40.16 cde
E3	Batati	57.7 abc	40.87 cde
E4	Kerkoubi	62.1 ab	41.80bcde
E5	Bejaoui	50.5 bcde	29.03 g
E6	Kerkoubi	56 bcd	35.84ef
E7	Batati	52.4 bcde	48.65a
E8	Batati	55.2 bcd	46.47abc
E9	Kerkoubi	41.6 ef	43.33abcd
E10	Batati	45.6def	48.27 ab
E11	Batati	49.7 cde	42.80abcd
E12	Kerkoubi	41.9 ef	43.98abc
E13	Kerkoubi	35.7 f	36.83def
E14	Bejaoui	42.8ef	31.46 gf
E15	Kerkoubi	50.5 bcde	43.38abcd

*Averages followed by the same letter are not statistically different at 5% (Duncan test)

3.1.3. Flower characteristics

Comparing the female flower numbers, we have seen that the first growing accessions includes (E1), (E2), (E3), (E7) (E8) from the Batati type and (E4) (E6) (E9) (E12) from the Kerkoubi types (Table 4). For the male flower sepals, we have two groups. The first cluster contains 12 accessions the sepal length of which exceeds 3 cm ((E1) (E3) (E4) (E5) (E7) (E8) (E9) (E10) (E11) (E12) (E13) (E14)). The second cluster includes the accessions (E2), (E6) (E15), which show shorter sepal length. The various types of squash have the same petal colour (yellow). This coloration has a pollination role. In fact, the female flower number was positively correlated with the ratio female flowers number divided by male flower number (r= +0.83**) but the male flower number was negatively correlated with this ratio (r= -0.43**). The male flower numbers outnumbered the female flower numbers during the experimental period for all the accessions. This finding is contrary to the results reported by Wehner (2008) on *C. pepo*, which indicated that both male and female flowers formed simultaneously right from the outset. This difference is attributed to species differences. In terms of sex related features, we noted that there was a difference in sex appearance, with the male flowers emerging before female flowers. Both the male and female flowers had a yellow colour, which backs up the findings of Agbagwa and Ndukwu (2004), who argued that this colour attracts insects for pollination in *Cucurbita moschata*.

Table 4: Flower characteristics: number of female flower (FF), male flower (MF), ration FF/MF, male and female flower sepal length (MFSL, FFSL)

Accession	FF	MF	FF/MF	MFSL (cm)	FFSL (cm)
E1	11.86 abc*	97.23 ab	0.13 abcd	3.30 abcd*	3.09 bc
E2	14 ab	100.97 ab	0.14 abcd	3.06 bcd	2.77 c
E3	12.63 ab	111.03 a	0.12 bcd	3.88 a	2.94 c
E7	13.70 ab	94.63 ab	0.15 abc	3.14 abcd	2.67 cd
E8	11.33 abc	82.17 abcd	0.16 abc	3.20 abcd	3.10 bc
E10	9.57 bcd	79.00 abcd	0.14 abcd	3.47 abc	3.32 bc
E11	6.33 de	77.33 abcd	0.08 de	3.24 abcd	3.68 ab
E4	14.43 a	90.63 abc	0.18 ab	3.13 abcd	3.18 bc
E6	12.90 ab	97.9 ab	0.132 abcd	2.97 cd	2.78 c
E9	12 .87 ab	98.27 ab	0.13 abcd	3.30 abcd	2.95 c
E12	13.33 ab	78.42 abcd	0.18 a	3.19 abcd	3.91 a
E13	6.33 de	68 bcde	0.094 cd	3.22 abcd	2.89 c
E15	7.74 cd	39.5 e	0.03 e	2.74 d	1.90 e
E5	5 .68 e	59.18 dce	0.03 e	3.09 abcd	2.94 c
E14	5 .82 e	72.93 bcde	0.03 e	3.69 ab	2.18 ed

*Averages followed by the same letter are statistically different at 5% (Duncan test).

3.1.4. Fruit characteristics

The skin colour of the collected accessions is mainly orange (45.71%), green (42.85%) and white (11.42%), the flesh colour is generally found to be orange (68.57%). Periphery and cavity diameter show also great variation, ranging from 70.67 to 120.5cm and from 15 to 24.7cm, respectively. In terms of skin thickness and flesh thickness, there are not significant differences among the populations. They show a range of 3 to 7.3mm for flesh thickness and 0.1 to 0.3mm for skin thickness (Table 5). The average value of fruit weight across accessions is 7.86kg and average fruit weight of individual accessions varies from 5.04 to 12.6kg. The highest variability in fruit weight was in the Bejaoui accessions (10.85kg). Similar results were obtained by Sharma and Rao (2013), who showed a large variability in fruit weight compared to earlier studies, with the weight mean for a collection of Indian squash accessions ranging between 1.5 kg and 2 kg. Therefore, weight could be used as a reliable criterion for the selection of individuals. Most of the populations in this study have broad-transverse-elliptical (75.61%), (16.20%) or globular fruit (8.19%). This result is consistent with the results obtained by Wehner (2008), which proved that the variability in fruit forms is a genetic variability criterion. Moreover, the average fruit height for the 15 accessions was 20.25 cm, whereas the Batati accessions height mean was 21.32cm. Concerning peduncle length length, the longest one was observed in morphotype E3 from the Batati type (11cm). In a study of pumpkin, Yadegari et al. (2012) noted that there was a positive correlation between yield and fruit weight. The Batati type has the highest seeds number while the Kerkoubi type has the lowest value. Concerning seed number, it is found to vary between 104 and 380 seeds. A positive correlation between weight of fruits and number of grains is noted (R=+0.63). A similar positive correlation between seed number and fruit weight has been reported for watermelon (Nerson, 2002).

Table 5: Fruit characteristics of the fifteen squash accessions: weight (kg), height (cm), Periphery (cm), peduncle length (cm), Skin thickness (cm), Flesh thickness (cm), Cavity diameter (cm) and Seeds number

Ecotype	**Type**	**Weight (kg)**	**Height (cm)**	**Periphery (cm)**	**Peduncle length (cm)**	**Skin thickness (cm)**	**Flesh thickness (cm)**	**Cavity diameter (cm)**	**Seeds number**
E1	Batati	10.5 a*	16.46 b*	105.6 abc	9.4*±3.61	0.30±0.07	5.3±0.70	22.0±1.70	380±75.00
E2	Batati	6.8 b	20.67 ab	93.67 abc	6.6±0.57	0.23±0.11	7.2±3.55	20.0±2.59	206±47.07
E3	Batati	9.8 a	22.88 ab	101.3 abc	11.0±2.65	0.10±0.06	4.5±2.70	17.0±1.30	145±86.70
E7	Batati	8.3 b	22.75ab	92.38 abc	6.0±0.75	0.10±0.23	5.8±1.08	24.7±1.25	104±56.61
E8	Batati	7.6 b	22.17 ab	90.92 abc	7.8±3.88	0.20±0.09	4.5±2.80	15.0±1.50	342±75.80
E10	Batati	11.9 a	20.50 ab	118.00 ab	8.5±0.7	0.13±0.05	4.3±0.57	24.5±3.90	214±48.28
E11	Batati	7.8 b	23.83 a	89.17 abc	6.7±2.45	0.20±0.03	5.0±1.32	23.7±1.61	202±85.71
E4	Kerkoubi	7.85 b	21.25 ab	80.75 bc	6.83±0.77	0.13±0.05	5.8±2.83	20.7±1.25	226±37.18
E6	Kerkoubi	9.50 a	20.5 ab	111.25 ab	9.0±1.73	0.17±0.05	3.9±0.75	21.2±0.28	170±58.96
E9	Kerkoubi	6.5 b	19.83 ab	90.83 abc	5.5±0.7	0.10±0.07	3.0±0.70	21.7±7.023	261±83.11
E12	Kerkoubi	5.04 b	18.00 ab	70.67 c	3.5±1.6	0.10±0.04	3.0±0.70	21.3±1.06	200±29.12
E13	Kerkoubi	6.9 b	18.67 ab	95.67 abc	5.0±2.83	0.10±0.08	3.5±2.40	19.5±6.30	364±78.80
E15	Kerkoubi	6.55 b	19.75 ab	120.50 a	7.0±0.6	0.15±0.07	4.2±1.62	20.2±3.89	205±120.20
E5	Bejaoui	9.15 ab	16.63 b	93.25 abc	8.3±3.21	0.20±0.06	5.3±2.23	23.0±1.30	290±89.30
E14	Bejaoui	12.6 a	19.88 ab	95.00 abc	2.0±0.7	0.10±0.06	7.3±0.76	21.3±3.21	251±95.69

n= 5 fruits per accession, * means± SD

3.2. Principal Component Analysis

A principal component analysis (PCA) was carried out in order to describe the relationships or variations that exist between the fifteen local populations of squash. The first axis explains 61.79 % of the total variation while the second axis explains 17.53%. The two axes can be retained to describe the variability among the squash accessions.

3.2.1. Correlation between variables and axes

The variables related to seeds, leaves, flowers and fruits are correlated to the two main axes differently (Tab.6). Seed traits (width, L/W ratio and weight of 100 seed) are positively and significantly correlated with axis 1 while seed length is correlated with axis 2. Leaf traits (leaf number and petiole length) are correlated with axis 1. For flower characteristics, except for the length of the male flower sepals, which is correlated with axis 2, all other traits are positively correlated with axis 1. Traits related to the fruit are in general correlated with axis 1 (except for skin and flesh thickness which are correlated with axis 2). Axis 1 gives information on the quantitative traits of seeds, leaves and fruits while Axis 2 comprises quantitative variables for seed length, female and male flower number, fruit weight, skin and flesh thickness and seed number.

Table 6. Correlation of variables within the first two axis

Variable	Axis 1	Axis 2
Seed length	-0.479	0.532*
Seed width	0.852*	-0.526
Seed ratio L/W	0.834*	-0.505
Weight of 100 seeds	0.745*	-0.405
Leaf number	0.83*	0.072
Petiole length	0.832*	-0.506
Female flowers number	0.629*	0.574*
Male flowers number	0.734*	0.439*
FF/MF	0.835*	0.118*
MFSL	-0.499	0.552*
FFSL	0.597*	-0.118
Fruit weight	0.915*	0.269*
Fruit shape	0.906*	-0.165
Peduncle length	0.623*	-0.365
Skin thickness	-0.469	0.352*
Flesh thickness	0.023	0.63*
Cavity diameter	0.904*	-0.034
Seeds number	0.470*	0.565*

3.2.2. Ascending Hierarchical Classification of accessions

To fully appreciate the agro-morphological diversity of the squash accessions, an ascending hierarchical classification was generated on the basis of the parameters evaluated with the SAS software. This classification helped to obtain a dendrogram which regroups the accessions into statistically homogenous classes. An analysis of the dendrogram reveals that there are three classes (Fig.6). The first class is composed of two accessions (E5 and E14). The second class consists of ten accessions (E2, E3, E1, E7, E11, E10, E4, E15, E8 and E6) while the third class contains three accessions (E9, E12 and E13). Figure 6 presents the numerical hierarchical classification of the different accessions. Group 1 (accessions from the Bejaoui type) is characterized by late flowering, shortest seed length, lowest fruit height, and lightest weight. This group opposes both Group 2 and 3. Group 2 (all accessions from the Batati type and 3 accessions from the Kerkoubi type) is characterized by high quantitative values, such as fruit diameter and weight. In group 3 (3 accessions from the Kerkoubi type), accessions have the lightest fruit weight. In fact, within the Cucurbitaceae family, an important morphological description of the fruits, leaves, and grains has been noted for water melon (Gusmini, 2003), as well as for gourd (Morimoto et al., 2005) and pumpkin (Mbogne et al., 2015).

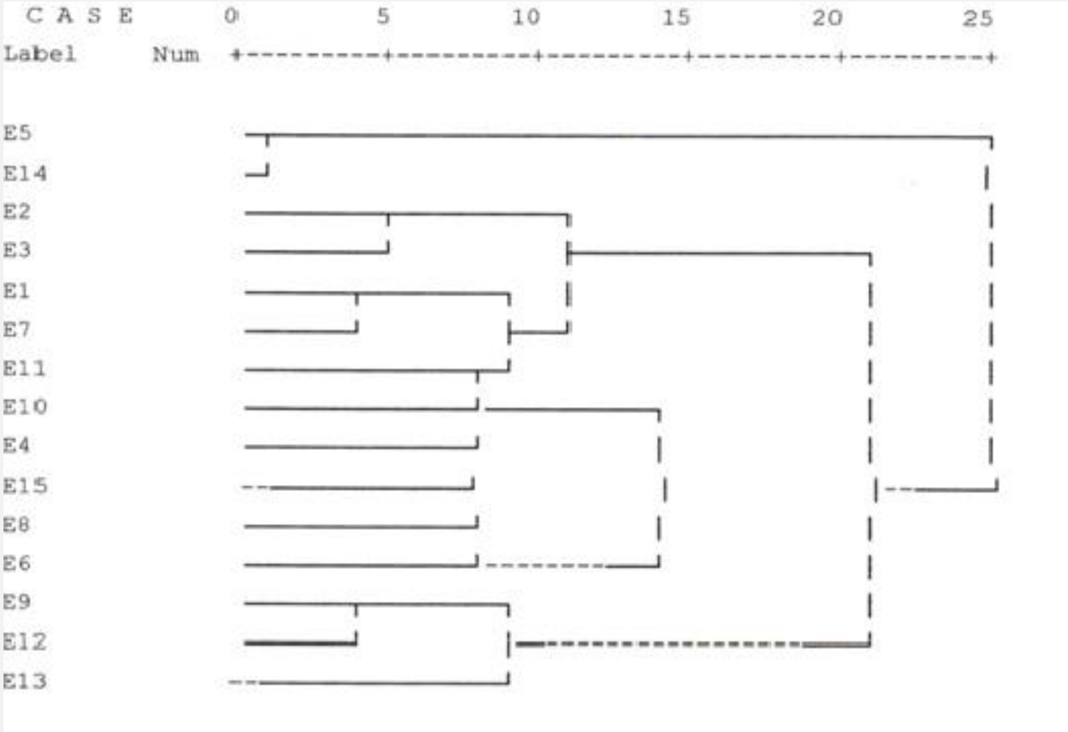

Fig. 6. Dendrogramme of quantitative and qualitative variables

3.3. Chemical and biochemical characterisation

The chemical and biochemical composition including those of antioxidant components such as carotenoids were analyzed for different accessions of *Cucurbita maxima* cultivated in Tunisia. Table 7 presents the analysis of variance followed by structured chemical and biochemical parameters. It can be noted that there is significant difference between morphotypes for all the parameters ($P < 0.05$), with the exception of magnesium. The highest values (272.82 mg/g) are found in group 3 for carotenoids. In the pumpkin fruit, carotenoids are responsible for the development of colour from yellow at young stage to orange at the ripening stage. Similar results for carotenogenesis during ripening were also reported in *C. Moschata* (Jacobo-Valenzuela et al., 2011), and their values showed great variability. This finding is consistent with the variation in total carotenoids reported in different studies, and might be due to agricultural factors (Setiawan et al., 2001). The total sugars in the fruit flesh ranges from 1.74% (group 1) to 5.15% (group 3) and the average value for the 15 accessions of pumpkin tested is 3.55% of the fresh weight of the fruit (Table 7). The fruits of E15 (Kerkoubi type) are characterized by the highest total level of sugars (5.15%), while E11 (Batati type) feature the lowest sugar level (1.74%). In connection with this point, Danilchenko et al. (2004) indicated a variability of sugar content for 8 cultivars ranging from 2.23% to 12.24%, while Biesiada et al. (2009) reported a range between 3.35% and 6%. The content of sugars in the fruits of squash depended on the genotype as well as on starch hydrolyzation during fruit storage. The potassium and calcium contents are found to vary significantly. Fruits in group 3 have the highest contents (2.13% and 0.98% respectively), while fruits in group 1 have the lowest values. Equal values of magnesium are noted for the three groups of accessions.

Table 7. Chemical parameters for the three squash groups revealed by CPA

Groups	β-carotene content (mg/g)	Total sugar (%)	K (%)	Mg (%)	Ca (%)
Group 1	91.67c	1.74ab	1.035b	1.25a	0.22b
Group 2	191.63b	3.79b	1.567ab	1.415a	0.95a
Group 3	272.82a	5.15a	2.13a	1.24a	0.98a
Mean	185.37	3.55	1.58	1.32	0.72
CV (%)	12.48	21.53	45.59	57.3	48.36

Averages followed by the same letter are not statistically different at 5% (Duncan test).

The results of the present study show a great variability of morphological and chemical characteristics across a range of local Tunisian pumpkin accessions. A similar finding is reported by Sensoy et al. (2007), who recorded a large genetic variation in Turkey's melon genotypes.

4. Conclusion

In this study, a morphological characterization comprising 30 parameters was conducted on the seeds, leaves, flowers, and fruits of fifteen local Tunisian accessions of pumpkin. As for the biochemical characterization, it was limited to total sugar content, carotenoids, and the mineral elements K, Mg, and Ca. These parameters have enabled us to draw the following conclusions: Seeds show a wide variability across the studied accessions with respect to their form, shape, and weight. The Kerkoubi and Batati types differ from the Bejaoui type in relation to seeds' form, weight, and vegetative mass. In addition, the Kerkoubi type differs from the Batati type by an early flowering and fruiting pattern. The Bejaoui type is the latest to flower and yield fruits. An ascending hierarchical classification shows that the morphotypes of pumpkin collected from different regions of Tunisia can be classified in three groups. The first group (Group 1) is characterized by late flowering, shortest seed length, lowest fruit height, and lightest weight. The second group (Group 2) is characterized by high quantitative values, such as diameter and fruit weight. The third group (Group 3) is intermediary between the two other groups. Chemical and biochemical characterization of the three groups of morphotypes reveals a significant difference only for carotenoids. Finally, the study shows a great morphological heterogeneity among the different morphotypes in the different regions. The information acquired through this study in Tunisia can be exploited by breeders to select and improve on pumpkin production.

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