

Phenotypic Variability of Local Squash (*Cucurbita maxima* Duch.) Grown in the Passoré Province of Burkina Faso

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To cite this article:

Soumbougma Benoît, Kiébré Mariam, Tiama Djakaridia, Kabré V. Nikodème, Kaboré Boukaré, Kiébré Zakaria, Bationo-Kando Pauline. Phenotypic Variability of Local Squash (*Cucurbita maxima* Duch.) Grown in the Passoré Province of Burkina Faso. *American Journal of Agriculture and Forestry*. Vol. 11, No. 1, 2023, pp. 29-36. doi: 10.11648/j.ajaf.20231101.15

Received: February 6, 2023; Accepted: March 4, 2023; Published: March 16, 2023

Abstract: Squash (*Cucurbita maxima* Duch) is one of the vegetables-fruits grown and used in human nutrition and in the treatment of many diseases in Burkina Faso. The main objective of this study is to contribute to a better knowledge of the phenotypic variability of the local squash grown in the Passoré province. To this end, an agro-morphological characterization was carried out in a randomised complete block design with three replications under rain-fed conditions in a Sudanese-Saharan climate. A total of 32 variables, including 21 quantitative variables and 11 qualitative variables relating to the vegetative development of the plant and fruit yield, were measured. This study has shown the existence of a phenotypic diversity on the color and form of fruits and agro-morphological diversity based on the female characters flowering time (FLF), male flowering time (FLM), fruit length (LFT) and fruit diameter (DMF) organized in three (3) morphological groups. The Group 1 is composed of early flowering plants with small leaves and fruits and late maturity of fruits. Group 2 is the opposite of group 1 and is made up of accessions with large fruits and big seeds. As for Group 3 it is composed of high performance accessions with large leaves and fruits of high length and diameter.

Keywords: Local Squash, Variability, Phenotype, Passoré, Burkina Faso

1. Introduction

The term squash refers to several species of the Cucurbitaceae family, usually grown for their edible fruits or sometimes for their oilseeds. Squash has an essential role in the diet of rural communities as well as in traditional medicine [1]. It is used either fresh as a dessert [2] or cooked as a main dish or ingredient in pies, soups, stews and in bakeries [3]. It is also used in the preparation of marinades and juices [2]. In addition to its nutritional role, squash is a vegetable-fruit rich in nutrients essential to the proper functioning of the body. Biochemical analyses have shown that squash is rich in water, bioactive components (with 80% of β -carotenes), as well as polysaccharides, dietary fibres,

proteins and minerals. It is also a source of vitamins B6, K, E, A and C [4]. Seeds are rich in fatty acids whose linoleic acid is the main component [5, 6].

Squash (*Cucurbita maxima* Duch) is a more widely grown species [7]. Its growing system requires at least a temperature of 25°C during the growing period and low humidity [8]. In Burkina Faso, its growing conditions are very poorly documented and the existing diversity within the plant is still unknown. The main objective of this study entitled phenotypic variability of the local squash (*Cucurbita maxima* Duch.) grown in the Passoré province of Burkina Faso is to know the genetic diversity of squash grown in the Passoré province. It is specially meant for (i) identifying the discriminating characters, (ii) establishing the level of

variability of the species in the Passoré province, and finally (iii) establishing the organization of this variability.

2. Material and Methods

2.1. Plant Material

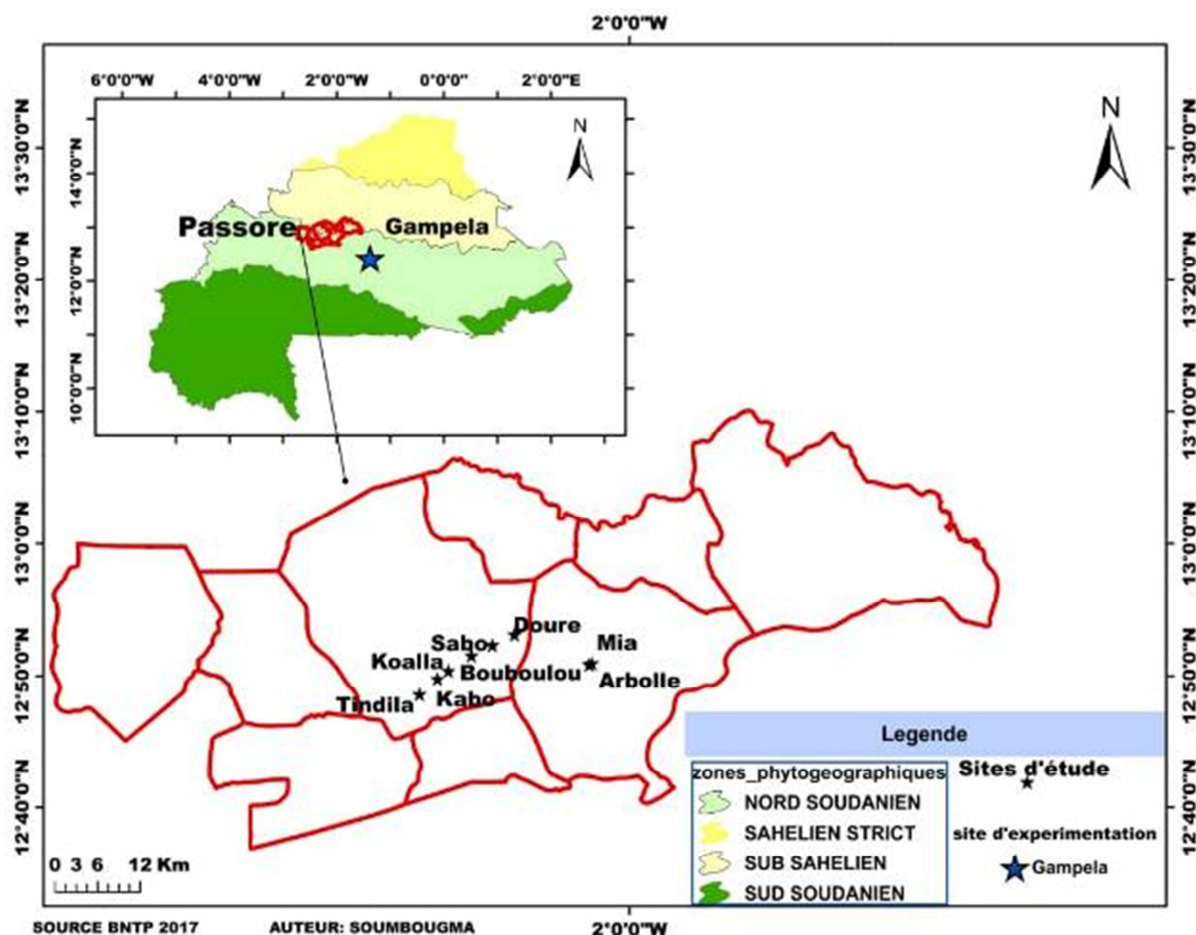


Figure 1. Map of the collection area.

The plant material is composed of twenty (20) accessions collected in two districts (Yako and Arbolle) in the Passoré province. Among these 20 accessions, 10 are from the districts of Yako and 10 from the districts of Arbolle.

2.2. Experimental Site, Experimental Setup and Growing Practices

A trial was conducted during the 2017-2018 crop year at the Institut du Développement Rural (IDR) experimental station located in Gampela following a randomised complete block design with three replications. Gampela is located about twenty kilometres from Ouagadougou on the Ouagadougou-Niamey stretch. In each repeat, each accession has been sowed in a row of two seeds/hole. Row-space and hole-space are of 2 metres. The spaces between repeats are of 2 metres too. Before sowing on July 12th 2018, the plot was ploughed with a tractor, fertilized with organic manure at a rate of 50 t/ha and then levelled with a hoe. The first weeding took place on 01 August, 21 days after sowing. During this hoeing, a

weeding was carried out with one plant per hole. At the same time, a second fertilization with manure was made with 30 g of organic manure from small ruminants. Two other weeding operations were carried out upon request.

2.3. Data Collection

Thirty-two (32) variables, eleven (11) of which are qualitative and twenty-one (21) quantitative, selected mostly from the descriptor of *Cucurbita mochatata* Duch proposed in 2007 by UPOV, were used to characterize the accessions.

The observations on the eleven (11) qualitative variables were spread throughout the plant development cycle. These variables were about leaf blade margin, fruit color (CFT), mesocarp color (CDM), seed color (CDG) and peduncle color (CDP), fruit longitudinal section shape (FFT), position of fruit maximum diameter (PDM), fruit longitudinal axis curvature (CLF), fruit base profile (PSB), fruit apex profile (PSF) and fruit stria (CAF). For the quantitative variables, the measurement techniques are reported in Table 1.

Table 1. Quantitative characteristics of *C. maxima* and measurement techniques.

Parameters	Measurement techniques
Male flowering date (FLM) [day].	Determined by counting the number of days between sowing and male flowering of at least 50% of the plants on the whole line containing the accession
Female flowering date (FLF) [day].	Déterminée par comptage du nombre de jours qui sépare le semis et la floraison femelle d'au moins 50% des pieds sur toute la ligne comportant l'accension
The number of days to maturity (MAT) [days].	Determined by counting the number of days between sowing and fruiting of at least 50% of the plants on the entire line containing the accession
the number of branches (NDR)	Assessed by counting the total number of fruits on the three selected stands
the length of the internodes (END) [cm].	Assessed by measuring three internodes of the three selected feet with a double decimeter from the third leaf
the diameter of the stem (DTG) [cm].	Evaluated by measuring three selected feet on the line with a double decimeter from the third leaf
the length of the leaf (LOF) [cm].	Assessed by measuring three (3) mature leaves per plant, measured from the extension of the leaf at the base to the tip of the leaf and along the main vein using a double decimeter
the width of the leaf (LAF) [cm].	Measured on three (3) mature leaves per plant, it is measured in the middle of the leaf and perpendicular to the main vein using a double decimeter
the length of the petiole (LOP) [cm].	Measured on three (3) mature leaves per plant from the stem insertion to the base of the leaf blade using a double decimeter
Petiole diameter (DPE) [cm].	Measured on three (3) mature leaves per plant using a caliper
the weight of the fruit (PFT) [kg].	Assessed by weighing three (3) ripe fruits per plant
Fruit diameter (DFT) [cm].	Measured on three (3) fruits, it is measured on the widest part of the fruit using a caliper
length of fruit (LFT) [cm].	Measured on three (3) fruits, it is the distance between the apex and the base where the peduncle is contacted using a double decimeter
Mesocarp thickness (EDM) [cm].	Measured on three (3) fruits, it is measured on the widest part of the flesh, from the bark to the carpel case, using a double decimeter
the length of the peduncle (LDP) [cm].	Measured on three (3) ripe fruits with a caliper
diameter of the peduncle (DPL) [cm].	Measured on three (3) ripe fruits with a caliper

2.4. Data Analysis

The qualitative data underwent a descriptive analysis through the calculation of frequencies. For the quantitative data, an analysis of variance (ANOVA) was carried out to identify the discriminating characteristics in order to assess the level of variability. Principal component analysis (PCA) was performed to determine the main associations between the variables and to extract the most representative ones for the grouping of accessions by hierarchical ascending classification (HAC) using Ward's aggregation method. The groups resulting from this organization were characterized through discriminating factorial analysis (DFA). The Newman-Keuls test of separation of means at the 5% threshold was performed in order to identify the characteristics that discriminate the groups resulting from the HAC.

3. Results and Discussion

3.1. Results

3.1.1. Description of Variability Using Qualitative Characteristics

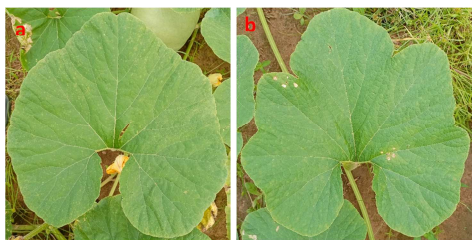


Figure 2. Aspects of the leaf blade margin: whole blade (a); weakly incised blade (b).

On the basis of the leaf blade margin, two morphotypes were observed (Figure 2). These are the morphotype with a full blade margin (Figure 2a) which represents 64% of the collection and the morphotype with a weakly incised blade margin, representing 36% of the collection (Figure 2b).

Observations made on the fruits show a variability in color and form. Thus, on the basis of fruit color, 4 modalities were observed (Figure 3). The fruits were either orange in color with a frequency of 48% of the accessions analyzed, either yellow (28%), or light yellow (18%) or green, which represents 6% of the collection.

Observations made on the fruits show a variability in color and form. Thus, on the basis of fruit color, 4 modalities were observed (Figure 3). The fruits were either orange in color with a frequency of 48% of the accessions analyzed, either yellow (28%), or light yellow (18%) or green, which represents 6% of the collection.



Figure 3. Variability of squash fruit color: orange (a); yellow (b); light yellow (c); green (d).

As for the shape of the longitudinal section of the fruit (Figure 4), seven (07) modalities were observed. These are the average transverse elliptical form (Figure 4a) observed in 4% of the collection, the rounded form (Figure 4b) corresponding to 24% of the accessions, the ovoid form (Figure 4c) with a frequency of 20% of the material analyzed, the quadrangular form (Figure 4d) (22%), the

trapezoid form (Figure 4e) (4%), the pyriform form (Figure 4f) (18%) and the fruits with a cylindrical longitudinal section (Figure 4g) represented by 8% of the collection.



Figure 4. Different form of squash fruit: cross-elliptical medium form (a); rounded form (b); ovoid (c); quadrangular (d); trapezoid (e); pear-formed (f); cylindrical (g).

The cylindrical to spongy stalks were either yellow in color corresponding to 70% of the collection (Figure 5a) or green representing 30% of the collection (Figure 5b).



Figure 5. Color of peduncle: yellow (a), green peduncle (b).

At the seed level (Figure 6), three modalities were observed, namely white (Figure 6a), grey (Figure 6b) and brown (Figure 6c) representing respectively 46%, 28% and 26% of the collection analyzed.



Figure 6. Variability in seed color of squash: white (a); grey (b); brown (c).

3.1.2. Description of Variability Using Quantitative Characteristics

Table 2. Average performance of the analyzed accessions.

Variable	Minimum	Maximum	Average	CV%	F	Pr > F
FLM (day)	40	79	63	11.89±7.46	1.13 ^{ns}	0.369
FLF (day)	52.000	85.000	67	11.96±7.95	0.56 ^{ns}	0.908
LAF (cm)	14.167	33.333	23.541	18.41±4.33	2.98**	0.003
LOF (cm)	14.333	32.733	23.705	18.95±4.49	2.27**	0.020
LOP (cm)	10.500	23.167	15.577	16.10±2.51	3.49**	0.001
LPL (cm)	2.000	6.000	3.356	23.96±0.80	0.87 ^{ns}	0.613
DPL (cm)	0.500	3.000	1.357	36.17±0.49	1.85 ^{ns}	0.061
END (cm)	8.500	42.333	13.667	36.06±4.93	1.19 ^{ns}	0.322
DTG (cm)	0.400	0.967	0.635	22.50±0.14	2.03*	0.037
LFT (cm)	12.000	49.500	25.100	28.96±7.27	3.02**	0.003
DMF (cm)	9.500	26.500	19.202	21.92±4.21	1.53 ^{ns}	0.142
MAT (day)	82	109	98	7.019±6.87	0.97 ^{ns}	0.499
PFT (kg)	1.200	21.500	6.287	66.48±4.18	0.90 ^{ns}	0.588
EDM (cm)	1.000	4.500	2.679	26.28±0.70	1.42 ^{ns}	0.183
NDR	0	26	5	93.02±5.03	0.85 ^{ns}	0.634
IDG (mm)	8.173	11.710	9.730	7.87±0.77	3.19**	0.002
LDG (mm)	14.010	18.397	16.344	6.94±1.14	2.61**	0.008
EDG (mm)	1.730	4.333	2.703	25.77±0.70	2.81**	0.005
DPE (cm)	0.467	1.333	0.864	21.95±0.19	3.35**	0.001
PDG (g)	1.930	24.500	13.464	38.64±5.20	0.70 ^{ns}	0.793

FLM: 50% male flowering; FLF: 50% female flowering; LAF: leaf width; LOF: leaf length; LOP: petiole length; LPL: peduncle length; DPL: peduncle diameter; END: internode length; DTG: stem diameter; LFT: fruit length; DMF: maximum fruit diameter; MAT: cycle at maturity; NFP: number of fruits per plant; PFT: fruit weight; EDM: mesocarp thickness; NDR: number of branches; IDG: seed width; LDG: seed length; EDG: seed thickness; DPE: petiole diameter; PDG: weight of 100 seeds; *: significant difference at 5%; **: highly significant difference at 5% level; CV: Coefficient of Variation; F: Fisher's F; F pro: probability of F; ±: standard error.

The results of the analysis of variance (Table 2) show that nine characters out of the twenty-two discriminate significantly between accessions at the 5% threshold, thus indicating the existence of agro-morphological variability within the collection. This variability was characterized by high amplitudes. Thus, the number of days to 50% male flowering with an average of 63 days varied from 40 to 79

days after sowing (DAS) and that of female flowering varied from 52 to 85 DAS with an average of 67 days. At flowering time, the width of the leaf blade varied from 14.17 to 33.33 cm with an average value of 23.54 cm. The length of the petiole varied from 10.50 to 23.17 cm with an average value of 15.58 cm. As for the diameter of the stem, it varied from 0.4 cm to 0.97 cm with an average

value of 0.64 cm. At maturity time, the fruits have a length between 12 cm and 49.5 cm. The average value of the fruit length is 25.10 cm. The length of the seeds varied from 14 to 18.40 mm with an average of 16.34 mm. The average value for seed width is 9.73 mm compared to 2.70 mm for seed thickness. These high amplitudes resulted in high coefficients of variation, i.e. over 30% for some characteristics. Thus, high values of the coefficient of variation were observed for petiole diameter (CV = 36.17%), internodes (CV = 33.44%), number of fruits (CV = 56.20%), fruit weight (CV = 66.48%), number of branches (CV = 93.02%) and seed weight (CV = 38.64%). On the other hand, they are low (CV < 30%) for the other sixteen (16) traits.

3.2. Organization Diversity

3.2.1. Association Between Quantitative Characteristics

Table 3. Correlations between variables and factors.

Main component	F1	F2	F3	F4
Eigenvalue	5.52	4.45	2.17	1.98
Total Variance (%)	24	19.34	9.42	8.63
Cumulative total variance (%)	24	43.34	52.77	61.39
NPL	-0.589	0.538	0.031	-0.029
FLM	0.616	-0.125	0.031	-0.302
FLF	-0.322	0.060	0.154	0.586
LAF	0.941	0.158	-0.166	0.053
LOF	0.901	0.331	-0.100	0.091
LOP	0.678	-0.082	-0.213	0.094
LPL	0.162	0.144	-0.280	0.299
DPL	0.541	0.259	-0.114	-0.133
END	-0.397	0.587	0.397	0.221
DTG	0.778	0.138	0.215	0.220
LFT	0.161	0.352	-0.289	-0.493
DMF	0.446	0.059	0.560	-0.309
MAT	0.107	0.123	0.453	0.659
PFT	-0.175	0.917	0.078	-0.002
EDM	0.121	0.724	-0.036	-0.240
NDR	-0.020	0.871	-0.053	-0.084
IDG	0.059	0.405	-0.544	0.558
LDG	-0.250	0.599	-0.329	0.137
EDG	0.198	-0.456	-0.308	0.303
DPE	0.767	0.304	0.379	0.111
PDG	0.464	0.006	-0.503	0.113

NPL: number of lifted clusters; FLM: 50% male flowering; FLF: 50% female flowering; LAF: leaf width; LOF: leaf length; LOP: petiole length; LPL: peduncle length; DPL: peduncle diameter; END: internode length; DTG: stem diameter; LFT: fruit length; DMF: maximum fruit diameter; MAT: cycle to maturity; PFT: fruit weight; EDM: mesocarp thickness; NDR: number of branches; IDG: seed width; LDG: seed length; EDG: seed thickness; DPE: petiole diameter; PDG: weight of 100 seeds

The results of the principal component analysis (PCA) carried out on the basis of the 21 variables show that the first four (04) lines with eigenvalues of 5.52; 4.45; 2.17 and 1.98 respectively explain 61.39% of the total variability (Table 3). Thus, axis 1 with 24% of the total inertia positively associates the characters leaf width ($r = 0.941$), leaf length ($r = 0.901$), petiole length ($r = 0.678$), petiole diameter ($r =$

0.767) and stem diameter ($r = 0.778$). It is negatively correlated with the number of the holes grown ($r = -0.589$). Axis 1 can therefore be defined as the axis of vegetative development. Axis 2, which accounts for 19.34% of the total variance, positively associates the characters internode length ($r = 0.587$), fruit weight ($r = 0.917$), mesocarp thickness ($r = 0.724$) and number of primary branches ($r = 0.871$). It was negatively correlated with the seed thickness parameter ($r = -0.456$). Axis 3, with 9.42% of the total inertia, is positively correlated with maximum fruit diameter ($r = 0.560$) and negatively correlated with seed weight ($r = -0.503$). Axes 2 and 3 can be considered as production axes. Axis 4, which accounts for 8.63% of the variability, positively associates the characters date at maturity ($r = 0.659$), date 50% female flowering ($r = 0.586$) and seed width ($r = 0.558$) and negatively the parameter fruit length ($r = -0.493$). Axis 4 can be considered as the cycle axis.

3.2.2. Organization of Squash Diversity in the Passoré Province

The dendrogram resulting from the hierarchical ascending classification (HAC) carried out on the weighted averages of the Euclidean distances gives a structuring of the variability of the twenty (20) accessions in three (3) morphological groups G1, G2 and G3 (Figure 7). These three groups are made up of 5, 5 and 10 accessions respectively.

3.2.3. Characterization of Morphological Groups from CAH

Figure 8 shows that axis 1 with 73.92% inertia and axis 2 with 26.08% inertia explain 100% of the total variability. The relationship of the groups with the axes shows that group 1 is negatively correlated with axis 1, which opposes it to group 2. Group 3 is positively correlated with axis 2. Furthermore, the Mahalanobis Mc Lachlan, (1992) distance squares (Table 4) from this analysis show that the groups are significantly different from one another at the rate of 5%. They also show that groups 2 and 3 are the most distant (27.03%). The results of the analysis of variance combined with the Newman-Keuls test (Table 5) indicate that the characters 50% male flowering, fruit length, maximum fruit diameter and mesocarp thickness significantly discriminate the three groups. Thus, Group 1 is characterized by plants with small leaves, small cylindrical fruits, early flowering and late maturity. This group is made up of low-performance accessions. Group 2 is characterized by plants with medium performance. It includes accessions with medium diameter, round fruits containing large seeds. Group 3 includes the high performance accessions, characterized by large leaves with long fruits of high maximum diameter. Thus, these accessions have large cylindrical fruits with a high mesocarp (flesh) diameter.

Table 4. Results of the Mahalanobis distance discrimination test.

	1	2	3
GI	0		
GII	13.86**	0	
GIII	24.26**	27.03**	0

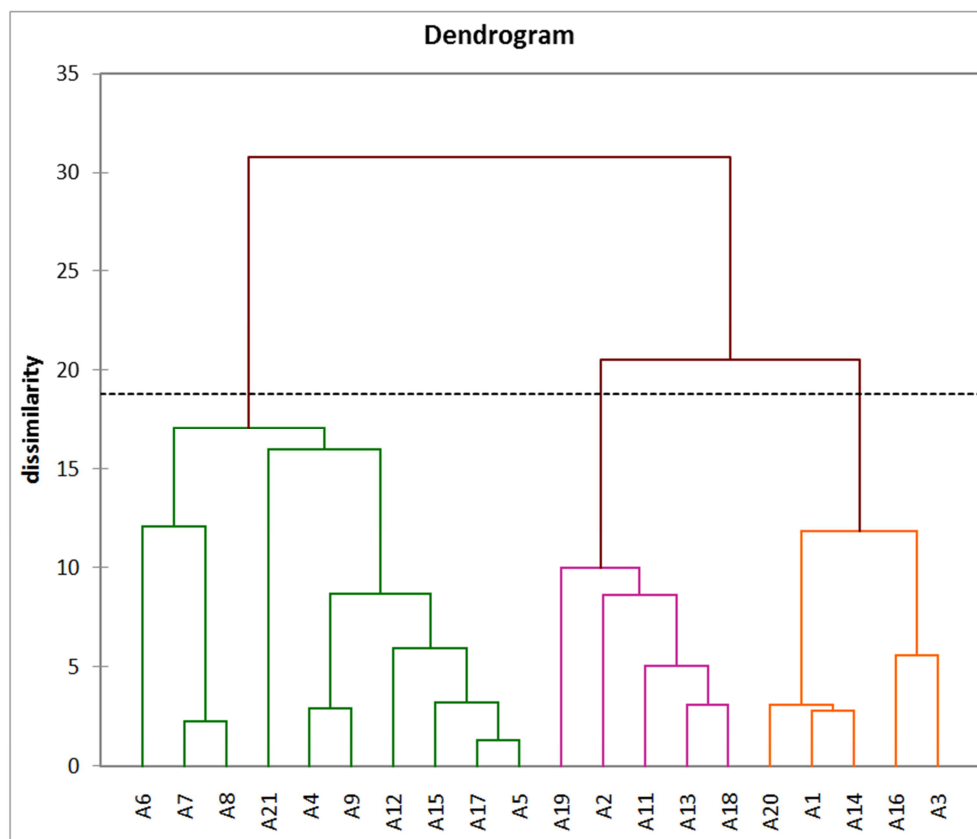


Figure 7. Dendrogram from the hierarchical ascending classification of the twenty (20) accessions.

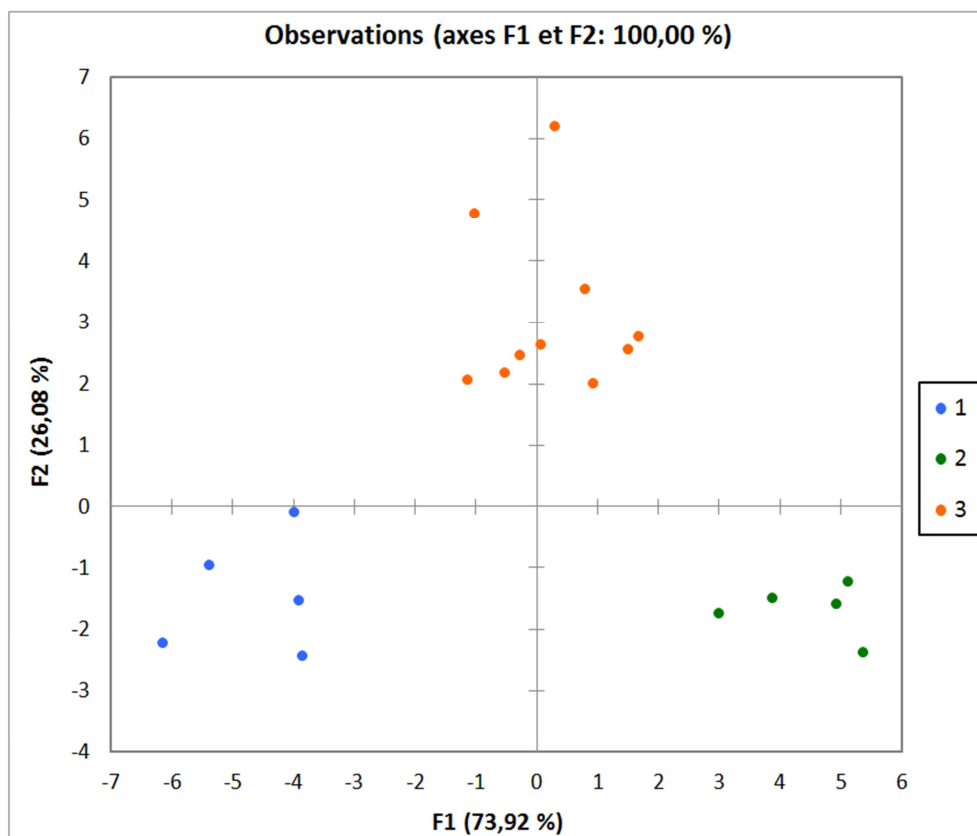


Figure 8. Projection of the three (03) agro-morphological groups in the map formed by the first two axes of the Discriminating Factor Analysis (DFA).

Table 5. Average performance of the 3 groups from the Hierarchical Ascending Classification (HAC).

Characters	Group 1	Group 2	Group 3	R ²	F
	5	5	10		genotype
NPL	2.200 ^a	1.800 ^a	2.050 ^a	0.045	0.678 ^{ns}
FLM	56.900 ^b	62.500 ^{ab}	66.550 ^a	0.537	0.001 ^{**}
FLF	63.400 ^a	70.100 ^a	66.950 ^a	0.230	0.108 ^{ns}
LAF	22.227 ^a	20.083 ^{ab}	25.487 ^a	0.318	0.039 [*]
LOP	14.900 ^a	15.350 ^a	16.442 ^a	0.116	0.350 ^{ns}
LPL	3.300 ^a	3.355 ^a	3.457 ^a	0.017	0.862 ^{ns}
LFT	24.600 ^a	19.550 ^a	28.125 ^a	0.273	0.067 ^{ns}
DMF	16.200 ^a	18.450 ^{ab}	20.325 ^a	0.298	0.049 [*]
MAT	95.900 ^a	99.000 ^a	95.550 ^a	0.097	0.419
EDM	2.590 ^b	2.130 ^{ab}	2.905 ^a	0.482	0.004 ^{**}
IDG	9.806 ^a	9.433 ^a	9.763 ^a	0.052	0.638 ^{ns}
EDG	2.551 ^a	3.236 ^a	2.557 ^a	0.232	0.106 ^{ns}

NPL: number of emerged clusters; FLM: 50% male flowering; FLF: 50% female flowering; LAF: leaf width; LOP: petiole length; LPL: peduncle length; LFT: fruit length; DMF: maximum fruit diameter; MAT: cycle to maturity; EDM: mesocarp thickness; IDG: seed width; EDG: seed thickness; *: Significant difference at 5%; **: Highly significant difference; ns: not significant

3.3. Discussion

The round form of the peduncle observed for all accessions analyzed suggests that the local squash grown in the Passoré province belongs to the same species. According to Gagnon *et al.*, the form of the peduncle is a distinguishing parameter between the different species of the *Cucurbita* genus most commonly grown [9]. According to them, the species *C. maxima* is characterized by a rounded peduncle, with a spongy appearance without marked ribs. Therefore, the local squash collection of this study would belong to the species *Cucurbita maxima*. Moreover, the characteristics of the leaves, namely the entire or slightly incised margin of the blades of the whole collection, confirm the belonging of the accessions to the species *Cucurbita maxima* Duch. Indeed, the leaf margin is one of the important criteria in the classification of species of the genus *Cucurbita*. The leaves of *Cucurbita maxima* are generally whole or slightly incised. However hand, those of *Cucurbita pepo* are deeply incised and those of *Cucurbita moschata* are weakly incised and spotted with white [10].

The existence of several discriminating characters and the structuring of the accessions into three phenotypic groups indicate the existence of morphological variability in the accessions. Thus, the variation in fruit form, color, length, diameter and size could be attributed to genetic factors within the accessions. According to Nee *et al.*, and Abdullah *et al.*, *Cucurbita* produce fruits of different sizes depending on the genetic makeup [11, 12]. According to Okombe *et al.*, reported that quality traits are under the control of additive effect gene with about 30 genes coding for quality traits in cultivated *Cucurbita* species [13]. Earlier studies by Chung *et al.*, Du *et al.*, and Tarchoun *et al.*, also found variability in fruit form and color [14-16]. The greater variability observed in the characteristics of the fruit could be justified by the fact that the fruit is the organ of interest, and therefore morphological variability is maintained by the local population, which is managed according to preferences.

Taking into consideration the plant's reproductive system, the existence of intermediate forms would result from natural crosses between different morphotypes of the same species.

Thus, the variability observed within the accessions analyzed could be partly due to the plant's mode of reproduction. Indeed, squash has a mixed mode of reproduction dominated by allogamy [17]. This mode of reproduction favours trait recombination and intra-population heterogeneity. According to Sagnard *et al.*, reproductive biology, pollen dispersal capacity, breeding practices and seed exchange also play a role in structuring diversity [18].

Furthermore, the structuring of the morphological diversity observed offers possibilities for genetic improvement of the species, either by direct selection or by hybridization between groups. Thus, the organization of diversity indicates that accessions in morphological group 3 perform well compared to groups 1 and 2 and would be potential progenitors of many leaf biomass and fruit yield traits in a selection process. The characters that most discriminate the morphological groups formed are male flowering date, fruit length, maximum fruit diameter and mesocarp thickness. Thus, hybridizations between group 2 plants that produce large fruits and group 3 plants that produce long fruits would result in long-fruited varieties with large sizes. Furthermore, the very strong and positive association between the number of primary branches, fruit weight, mesocarp thickness and axis 2 is an indication of a linear relationship between these parameters. This means that the number of branches would increase with the number of fruits. This relationship is very important because the same plant provides leaves and fruits, all of which are used in the diet. Another important relationship is that between fruit weight and mesocarp diameter. This indicates that both parameters grow in the same direction. Thus the heavier the fruit, the more mesocarp (flesh) it contains, which is used for food preparation. These positive correlations observed between these traits can facilitate genetic improvement because improving one trait also improves the other.

4. Conclusion and Prospects

This study highlighted the existence of significant agro-morphological diversity within the collection of squash

accessions grown in the Passoré province. This observed variability is more noticeable with the fruit quality traits. Interesting correlations between the traits of interest were also noted. The study also showed a structuring of squash accessions into three (3) agro-morphological groups whose characters that most discriminate the morphological groups formed are flowering date, fruit diameter, fruit length.

The study also showed great morphological heterogeneity among the squash accessions of Passoré. The information from this study can be used as a springboard to extend the study on samples of the whole country and to further studies.

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