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Original article

# Characterization of cultivated pumpkin (*Cucurbita moschata* Duchesne) landraces for genotypic variance, heritability and agro-morphological traits

Vincent Ezin *, Ulrich Herbert Gbemenou, Adam Ahanchede

*Department of Crop Production, Faculty of Agricultural Science, University of Abomey-Calavi, Abomey Calavi, Benin*

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ABSTRACT

*Cucurbita moschata* (Pumpkin) is a multipurpose species whose fruits and sometimes oil seeds are used for various purposes. It is low in calories and is a significant source of income. Despite the great potential of the pumpkin production and usage, there is no attention to establish the varieties grown in Benin for proper documentation. Therefore, the present study was carried out to (i) evaluate the agro-morphological variability of pumpkin accessions collected in Benin and (ii) investigate heritability, genetic gain, phenotypic and genotypic variances of the agronomic traits. Six landraces from one hundred and twenty accessions collected in Benin were sown and characterized. The agronomic experiment was laid out in a complete randomized block design with three replicates. Out of the twenty-seven quantitative descriptors measured, fifteen were found to be significant. The 50% emergence time ($p = 0.03$), the number of female flowers ($p = 0.02$), the seed width ($p = 0.05$) and the ratio seed width and length ($p = 0.01$) were significant. A highly significant difference was observed with the days to 50% flowering and the length of male flower stalks ($p = 0.002$), the average weight of one hundred seeds ($p = 0.009$). Fruit set at 50%, length of female flower peduncle, number of male flowers, mean fruit weight, fruit length, fruit diameter, average number of seeds per fruit and seed length were very highly significant ($p < 0.001$). Fruit color and shape, seed color and leaf color showed phenotypic variability. A positive correlation ($r = 0.76$; $p < 0.05$) was observed between average fruit weight and average number of seeds per fruit. Principal component analysis and Hierarchical Ascending Classification revealed three classes. Estimates of the phenotypic coefficient of variation were higher than estimates of the genotypic coefficient of variation for most characters. High heritability was observed for fruit diameter (96.73%), average fruit weight (96.46%) and fruit length (94.64%). High heritability associated with high genetic advance was observed for these traits. In sum, the genetic diversity observed within the landraces of pumpkin shows that there is possibility for further selection.



## 1. Introduction

*Cucurbita moschata* (Pumpkin) belongs to the genus *Cucurbita*, order Cucurbitales, family Cucurbitaceae, subfamily Cucurbitoideae and tribe Cucurbiteae (Jeffrey, 1990). The genus *Cucurbita* consists of 20–27 species Esquinas-Alcazar and Gulick (1983) of which five are cultivated (OECD, 2016). These are: *Cucurbita argyrosperma*, *Cucurbita moschata*, *Cucurbita maxima*, *Cucurbita pepo* and *Cucurbita ficifolia*. *Cucurbita moschata, Cucurbita maxima*, and *Cucurbita pepo* are more grown globally (OECD, 2016). According to Jeffrey (1990), the genus *Cucurbita* is indigenous to the Americas. The pumpkin is most likely cultivated in all tropical African countries (PROTA, 2018). It is an annual herbaceous plant, highly branched with creeping or climbing branches (OECD, 2016). It has an angular stem and a leaf blade with a broadly oval outline. These leaves are simple, alternate, without stipules and the petiole length vary between 9 and 24 cm, respectively. Flowering is asynchronous with male flowers that are solitary, it has 3 stamens with free threads, and the anther is usually supported by a long twisted organ with a very long peduncle (Agbagwa et al., 2007). According

* Corresponding author.
*E-mail addresses:* ishola.vincent@yahoo.com, awevincent@gmail.com (V. Ezin).

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to the same source, the female flower is always solitary and has 3 stigmas supported by a thick style, an inferior ovary and a short stalk. Yield and yield components such as average fruit weight, fruit diameter and fruit length are traits of interest for improving pumpkin productivity (Abdein et al., 2017). The positive correlation between the average fruit weight and the average number of seeds per fruit as reported by Mbogne et al., (2015) are key variables in crop improvement. The pumpkin seeds are highly valued and have a multitude usages in different parts of the world (OECD, 2016). The fruit is a large berry. The shape of the fruit of the pumpkin varies from globular to ovoid. The fruit stalk is hard, angular with five ribs clearly widened at the apex. It has obovoid and flattened seed shapes (PROTA, 2018).

Pumpkin is generally grown for its leaves, flowers, fruits, and sometimes for its oil seeds (Fu et al., 2006). It is a low-calorie vegetable suitable for any diet. In Latin America, the flowers are eaten as vegetables (Merrick, 1992) and (Nee, 1990). The vines and fruits are used as fodder for domestic animals (Noguera, 2002). According to González et al. (2001), the pumpkin is an important source of vitamin A (4 ± 20 mg / g). Thus, it plays a vital role in the fight against vitamin A deficiency, which affects more than 250 million children under five years of age worldwide (Mbogne et al., 2015). According to the work of Roura et al. (2007), it is has a large quantity of ascorbic acid (22.9 mg/100 g) and inhibits the development of degenerative diseases such as cancer, diabetes, cardiovascular and neurological diseases. But despite these strengths, it has been well established that pumpkin has been neglected by institutional research and improvement programs in Africa and more specially in Benin (PROTA, 2018).

Several studies have been conducted on *C. moschata* around the world. According to the work of Mbogne et al. (2015), fruit weight of *C. moschata* and *C. maxima* can be used as a good criterion for selecting individuals with many seeds. The seeds number per fruit is a discriminating trait in three Cucurbitaceae species (*Lagenaria siceraria*, *Citrullus lanatus* and *Cucurbita moschata*) grown in Congo-Brazzaville (Bembe et al., 2010). Qualitative traits such as fruit color and flesh color are discriminative traits of *C. moschata* genotypes in northern Bangladesh (Ahamed et al., 2011). The work of Agbagwa et al. (2007) showed that flowering is asynchronous in *C. moschata* and the ratio of male to female flowers is 9:1. Fruit shape is a complex and multigenic trait that distinguishes different accessions of *C. maxima* and *C. pepo* (Liu et al., 2013). The genes responsible for fruit shape are ''flatteners'' and ''elongators'' (Brown, 2002). *The study of* Abdein et al., (2017) in Egypt on combining ability and heritability of yield and yield component traits of pumpkin (*Cucurbita moschata* Poir.) under different conditions, showed that broad heritability ranged from 99.224 to 99.762% for fruit length and diameter. The number of fruits per plant, fruit weight, flesh thickness, polar circumferences of fruit and equatorial circumferences of fruit can be used as useful selection criteria to increase fruit yield per plant in pumpkin (Chaudhari et al. (2017). To the best of our knowledge, there are no scientific studies on pumpkin in Benin.

In Benin, many varieties of pumpkin are grown in different regions of the country. These varieties available on the market are not uniform. However, there is no information on agro-morphological traits, agronomic practices that can be used for standardizing the different varieties. The varieties present on the market may be landraces or introduced varieties. According to Djè et al. (2007), landraces of cultivated species are disappearing in the tropics. The disappearance of these varieties denotes genetic erosion and the narrowing of genetic diversity and loss of traits of interest that are essential in breeding and genetic improvement programmes. These landraces constitute an essential factor in the conservation of genetic resources, as they are adapted to local conditions and may have interesting characteristics (Grace et al., 2009). The real cause of this restriction in the area of cultivation of landraces is indeed the use of improved varieties with higher yield potential, which contributes to the reduction of the genetic diversity and erosion of traditional varieties. This reduction in the biodiversity of a species can reduce its flexibility and resistance to biotic and abiotic stresses (PNUD, 2015). Studies of crop diversity have clearly shown that traditional varieties, although less productive, are genetically more diverse than improved varieties (Brown and Munday, 1982). In response to the erosion of biological diversity, accessions of several species are collected and maintained in gene banks (Abdou, 2014). These species exhibit genetic variation that will determine the success or failure of any breeding program (Ibrahim, 2012). According to the same source, measuring genetic variation is an important step in any crop improvement program. Thus, the collection, characterization, evaluation and conservation of Beninese germplasm of pumpkin becomes a necessity. In view of the fundamental role of varietal diversity in the production and improvement of pumpkin, this study was established to fill the existing gaps on the phenotypic and genetic diversity of these landraces in Benin. More specially, this study aims at (i) evaluating the agro-morphological variability of pumpkin accessions collected in Benin and (ii) determining heritability, genetic gain, phenotypic and genotypic variances of the agronomic traits.

## 2. Materials and methods

### 2.1. Experimental site

The experiment was conducted at the International Institute of Tropical Agriculture (IITA) (6°25′3′′N, 2°19′46′′E) situated in the township of Abomey-Calavi, in the southern part of Benin Republic from August 25th to December 25th 2020. The climate is characterized by two dry seasons and two rainy seasons. Climatic data during the experiments were obtained at the IITA meteorological station (Table 1). The rainfall during the trial was about 392 mm with 35 days of rain over 4 months, with a relative humidity that varied on average from 70 to 99.87%. The soil is ferralitic in nature (Houdegbe et al., 2018).

### 2.2. Plant material

The plant material consists of 120 accessions of *Cucurbita moschata*. These seeds were collected from June to July 2020 and from farmers' granary and farms and market place during our surveys conducted on pumpkin in 6 southern and northern townships of Benin (Ezin et al., 2021) (Fig. 1). The collected seeds were stored in the envelopes and transported to the laboratory before using them in the experiment, which was carried out from August to December 2020. According to the producers the collected seeds were stored from their previous harvest of the year 2019. The 120 accessions collected in the study areas were grouped into 6 homogeneous lots (landraces) (Table 10). This grouping was performed based on two criteria: in the South, based on the local names that producers gave to the fruits and in the North based on the description of the varieties (shape and color of the fruits at physiological maturity). The choice of variety description in the North as a criterion for discrimination was due to the homogenization of local names different varieties. It should also be noted that the same variety was found both in the North and the South. But this variety is split into two different varieties since it was found in two different agro-ecological zones. The varieties were named respectively by: Vx.y with V (variety), x the zone number and y the variety number (Table 2).

**Table 1**
Climate data at IITA from August to December 2020.

| Month | Total rainfall (mm) | Average min temperature (°C) | Average max temperature (°C) | Average min relative humidity (%) | Average max relative humidity (%) |
|---|---|---|---|---|---|
| August | 0 | 24.1 | 28.44 | 70 | 89.01 |
| September | 203.6 | 23.66 | 28.52 | 73.32 | 90.76 |
| October | 146.3 | 24.11 | 29.85 | 70.76 | 91.56 |
| November | 12.2 | 24.94 | 31.79 | 88.05 | 95.21 |
| December | 29.9 | 24.88 | 31.82 | 99.68 | 99.87 |

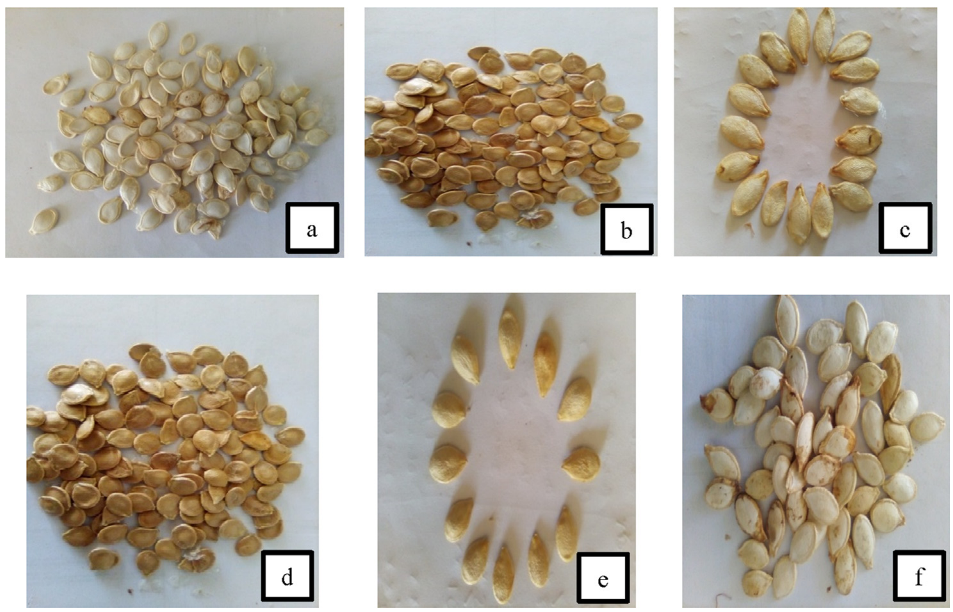


**Fig. 1.** The six local varieties of pumpkin used in the experiment. **(a)**: V0.1; **(b)**: V0.2; **(c)**:V1.3; **(d)**: V1.4; **(e)**: V1.5; **(f)**: V1.6.

**Table 2**
Collection area numbers.

| Zone | Number | Townships | Number |
|---|---|---|---|
| South | 0 | Dogbo | 1 |
| | | Djakotomey | 2 |
| | | Klouékanme | 3 |
| | | Aplahoué | 4 |
| North | 1 | Parakou | 5 |
| | | N'dali | 6 |

### 2.3. Experimental design

The agronomic experiment was arranged in a completely randomized single factor block design with three replications oriented North-East. Each block consisted of six elementary plots. Each elementary plot corresponds to a variety, i.e. the treatment. The spacing of 0.5 m between elementary plots 1 m between blocks was adopted. In each elementary plot, a spacing of 2 m × 2 m between rows and between blocks, resulting in four (4) rows with three (3) plants each were adopted. A basal dressing organic fertilizer (poultry droppings) at a dose of 100 Kg per block was applied. Two seeds per hill were sown in the soil at a depth of 2 cm on August 25th, 2020. Another sowing was done on September 2nd 2020 to fill the vacant stands due to non-germination of seeds. Then thinning of seedlings was done and only the most vigorous seedling was left at each stand. The Data were only collected from the six plants in the middle rows for each experimental unit so as to minimize the border effects.

### 2.4. Data collection

A total of 34 descriptors were measured and observed separately on leaves, vines, flowers, fruits and seeds. UPOV (2007) and IBPGR (1983) cucurbit descriptors were used to characterize the diversity of different varieties of pumpkin. Among these descriptors, there were 27 quantitative and 07 qualitative parameters. These descriptors include: days to 50% seedling emergence, length and width of cotyledons (cm), duration of vegetative phase; days to 50% flowering, number of male and female flowers, color of the flowers, length of the male and female flower stalks peduncle (cm), vines length (cm) and the number of leaves per plant, length and diameter of petioles (cm), leaf length and width (cm), leaf color, days to 50% fruiting, number of fruits, yield of fruits per plant (Kg), fruit yield per elementary plot was determined in t/ha, average fruit weight (Kg), diameter, length (cm) and shape of the fruits (at physiological maturity) from 10 random fruits from each experimental unit, fruit color and main flesh color were observed on physiologically mature fruits, average number of seeds per fruit on 10 fruits randomly selected at physiological maturity from each experimental unit, weight of one hundred seeds (g), seed width/length ratio, color of the seeds and shape of the seeds. The collection of the different qualitative descriptors (colour of flower, leaf, flesh, seed and fruit) was done on a standard scale based on a model for qualitative colour description and omparison by Zoe et al., (2011). Zoro Bi et al. (2003) determined three seed shapes based on the ratio of width and length. These are: elongated seeds (l/L < 1), round seeds (l/L = 1), and wide seeds (l/L greater than 1).

### 2.5. Data analysis

Analysis of variance (ANOVA) was performed to assess whether the variations observed at the variety level for all quantitative traits were significant. Ranking of means was performed in case of significant difference, according to the Student-Newman-Keuls (SNK) test at the 5% confidence level. The agricolae package was used to perform this test. The relationships between the different variables were determined using the correlation coefficients. Their significance was tested with the Pearson test using the package Hmisc. Principal component analysis (PCA) was used to determine relationships between individuals on a limited number of orthogonal axes. The Hierarchical Ascending Classification (HAC) grouped individuals into more or less homogeneous classes. The dendrogram was plotted using the Euclidean distance and Ward's aggregation method (minimization of the intra-class variance). These multivariate analyses were performed using the Factoshiny package. Genetic parameters were estimated from the components of the analysis of variance. Genotypic and phenotypic variances, genotypic and phenotypic coefficients of variation, broad heritability, and expected genetic gain were calculated according to the formulas used by Assefa et al. (2001), Hosseini et al. (2012), Johnson et al. (1955). All analyses were performed with R 3.6.2 software.

## 3. Results

### 3.1. Phenological parameters

The results of the analysis of variance of days to emergence of seedlings, show that there was a significant difference between varieties (F = 3.48; p = 0.03). The average days to emergence of seedling was 5.18 days for the variety V0.2 and 5.86 to 6.48 days respectively for varieties V0.1, V1.3, V1.4 and V1.6 (Table 3). It was 6.97 days for the variety V1.5. The analysis of variance of the vegetative phase period that reveals a non-significant difference between the different treatments (F = 2.72; p = 0.07). This vegetative phase varied from 34.76 to 48.97 days. There was a highly significant difference (F = 7.51; p = 0.002) between treatments for days to 50% flowering. 68.72 days after sowing were the longest days to flowering with V1.6. The variety V0.1 recorded the shortest days to flowering (41.21 days after sowing). There was total abortion of fruits from plants of variety V1.6, thus no fruit was harvested (Fig. 2). Days to 50% fruiting showed a highly significant difference (F = 176.8; p < 0.001) among varieties used. Fruit maturation ranged from 61.80 to 115.38 days after sowing.

### 3.2. Growth parameters

The length and width of the cotyledons ranged from 2.77 to 3.26 cm and 2.03 to 2.37 cm respectively (Table 4). The analysis of variance of these two traits shows a non-significant difference, with p = 0.35 (cotyledon length) and p = 0.26 (cotyledon width). The results show highly significant differences between varieties for male flower peduncle length (F = 8.05; p = 0.002). A highly significant difference (F = 18.77; p < 0.001) was also recorded for female flower peduncle length. For flower peduncle length, the highest value (9.93 cm) was obtained with the male flower of variety V0.2 while with the female flower; the variety V1.6 recorded the highest value (3.40 cm). The lowest values of the stalk lengths of the male and female flower were recorded in the varieties V1.3 (3.56 cm) and V1.4 (1.69 cm), respectively.

The results of the analysis of variance indicate that the treatments are not statistically different 10 weeks after sowing for vines length, petiole length and diameter, leaf length and width and number of leaves. Vines length and number of leaves ranged from 102.17 to 307.44 cm and 24.56 to 95.50 cm, respectively. The highest values for leaf length (21.67 cm), petiole length (18.84 cm) and petiole diameter (0.76 cm) were observed with variety V0.2. The variety V1.5 recorded the lowest values for leaf length (16.74 cm) and petiole diameter (0.42 cm). The lowest value for petiole length was observed in the variety V0.1 (12.42 cm). The width of the leaves varied from 16.59 to 20.44 cm (Table 4).

### 3.3. Characterization of leaves, flowers and fruits

In *Cucurbita moschata*, two colors of leaves were observed during the experiment: dark green leaves (60%) and green leaves mottled with white (40%) (Fig. 3). Flowering was asynchronous and all flowers were clear yellow (Table 5). The variety V0.1 recorded the highest ratio of male to female flowers (24.33: 4.44) while the lowest value of this ratio was recorded in the variety V1.4 (6.28: 1.39) (Table 6). The results of the statistical analysis for the number of male flowers show a highly significant difference between varieties (F = 11.28; p < 0.001) and the number of female flowers shows a significant difference between treatments (F = 4.36; p = 0.02).

Fruit yield per plant is dependent on the number of fruits and the average fruit weight at the individual plant level. The average fruit weight (1.87 kg) and numbers of fruits per plant (2.10) were recorded in the variety V0.2 with the highest fruit yield per plant (1.20 kg/m$^2$). The lowest values of number of fruits per plant, fruit yield per plant and average fruit weight were recorded in varieties V1.3 (1.06 fruits); V1.5 (0.30 kg/m2) and V0.1 (0.40 kg), respectively. The results of the analysis of variance of the number of fruits per plant (p = 0.44) and the fruit yield per plant (p = 0.20) indicate a non-significant difference between varieties. Average fruit weight showed a highly significant difference between treatments (F = 28.40; p < 0.001). Fruit yield per unit plot ranged from 2.33 to 11.33 t/ha. There was no significant difference between varieties in fruit yield per unit plot (p = 0.13). Fruit length ranged from 6.65 to 47.24 cm and fruit diameter from 10.12 to 66.20 cm (Table 6). The variety V1.3 recorded the highest values for both fruit length and diameter, while the variety V0.1 recorded the lowest values for these same parameters. Analysis of the results for fruit length and diameter indicates a highly significant difference between varieties (p < 0.001).

**Table 3**
Means of phenological parameters of pumpkin varieties.

| Varieties | DE 50% (JAS) | DVP (JAS) | DF 50% (JAS) | DFS 50% (JAS) |
|---|---|---|---|---|
| V0.1 | 5.86 ± 0.42 ab | 34.76 ± 0.30 a | 41.21 ± 0.60b | 61.80 ± 2.90c |
| V0.2 | 5.18 ± 0.30b | 42.59 ± 9.25 a | 64.27 ± 9.78 a | 114.11 ± 4.27 a |
| V1.3 | 6.33 ± 0.49 ab | 37.75 ± 1.68 a | 49.52 ± 2.49b | 86.06 ± 1.68b |
| V1.4 | 6.15 ± 1.05 ab | 47.52 ± 10.68 a | 66.27 ± 11.91 a | 115.38 ± 1.45 a |
| V1.5 | 6.97 ± 0.52 a | 39.77 ± 2.79 a | 53.93 ± 2.63 ab | 113.93 ± 4.08 a |
| V1.6 | 6.48 ± 0.20 ab | 48.97 ± 4.97 a | 68.72 ± 7.35 a | – |

**Note:** In the same column, values followed by the same letter are not significantly different at the 0.05 threshold according to the SNK test. No fruit was harvested from V1.6 due to fruits abortions DE50%: Days to 50% emergence, DVP50%: duration of vegetative phase at 50%, DF50%: days to 50% flowering, DFS50%: days to 50% fruit set.


**Fig. 2.** Fruit set of the variety V1.6. **(a):** beginning of fruit set; **(b):** fruit abortion.

**Table 4**
Means of growth parameters of pumpkin varieties.

| Varieties | LC (cm) | WC (cm) | LMFP (cm) | LFFP (cm) | VL (cm) | PL (cm) | PD (cm) | LL (cm) | LW (cm) | NL |
|---|---|---|---|---|---|---|---|---|---|---|
| V0.1 | 2.86 ± 0.55 a | 2.03 ± 0.36 a | 5.76 ± 0.37 bc | 2.03 ± 0.29 c | 269.56 ± 77.24 a | 12.42 ± 2.44 a | 0.61 ± 0.21 a | 18.37 ± 3.81 a | 17.80 ± 3.33 a | 66.44 ± 20.61 a |
| V0.2 | 2.98 ± 0.26 a | 2.37 ± 0.28 a | 9.93 ± 2.46 a | 2.62 ± 0.22 b | 307.44 ± 185.00 a | 18.84 ± 4.96 a | 0.76 ± 0.10 a | 21.67 ± 2.73 a | 20.44 ± 2.54 a | 95.50 ± 74.35 a |
| V1.3 | 3.04 ± 0.22 a | 2.03 ± 0.14 a | 3.56 ± 0.04 c | 2.04 ± 0.16 c | 187.89 ± 36.54 a | 14.98 ± 1.06 a | 0.59 ± 0.01 a | 20.19 ± 1.26 a | 18.36 ± 0.74 a | 72.00 ± 8.89 a |
| V1.4 | 2.77 ± 0.53 a | 2.13 ± 0.36 a | 6.28 ± 1.07 bc | 1.69 ± 0.33 c | 144.33 ± 85.35 a | 12.71 ± 2.77 a | 0.52 ± 0.19 a | 18.76 ± 3.89 a | 17.58 ± 3.86 a | 31.44 ± 11.66 a |
| V1.5 | 3.26 ± 0.13 a | 2.22 ± 0.10 a | 6.97 ± 1.52 b | 2.51 ± 0.29 b | 162.17 ± 63.16 a | 12.58 ± 2.68 a | 0.42 ± 0.18 a | 16.74 ± 5.02 a | 16.59 ± 5.28 a | 45.78 ± 12.29 a |
| V1.6 | 2.53 ± 0.43 a | 1.82 ± 0.24 a | 5.48 ± 0.47 bc | 3.40 ± 0.05 a | 102.17 ± 83.18 a | 12.84 ± 2.85 a | 0.70 ± 0.18 a | 19.67 ± 1.54 a | 19.57 ± 2.68 a | 24.56 ± 10.49 a |

**Note:** Means followed by the same alphabetic letters are not significantly different at the 0.05 level according to the SNK test. LC: Length of cotyledons, WC: width of cotyledons, LMFP: length of male floral peduncles, LFFP: length of female floral peduncles, VL: vines length, PL: petiole length,PD: petiole diameter, LL: leaf length, LW: leaf width, NL: number of leaves.

**Fig. 3.** Phenotypic variability in leaf color of *C. moschata.*

**Table 5**
Inter-varietal variation in qualitative traits of pumpkin.

| Varieties | FC | LC | FrS | FrC | MFC | SS | SC |
|---|---|---|---|---|---|---|---|
| V0.1 | Clear yellow | Dark green | Flattened | Yellowish orange | Clear yellow | Elongated | White |
| V0.2 | Clear yellow | Green leaves with white mottling | Globular | Pale green | Clear yellow | Elongated | Dark brune |
| V1.3 | Clear yellow | Dark green | Elongated | Clear yellow | Clear yellow | Elongated | Clear yellow |
| V1.4 | Clear yellow | Green leaves with white mottling | Globular | Pale green | Clear yellow | Elongated | Dark brune |
| V1.5 | Clear yellow | Dark green | Pyriform | Clear yellow | Clear yellow | Elongated | Clear yellow |

**Note:** FC: flower color, LC: leaf color, FrS: fruit shape, FrC: fruit color, MFC: main flesh color, SS: seed shape, SC: seed color.

In addition to the quantitative characters, the qualitative traits were observed. Four fruit shapes were recorded. They were globular or spherical shape (40%), elongated, flattened and pear-shaped represented 20% each. Fruits with a globular shape were observed with varieties V0.2 and V1.4, while fruits with an elongated, flattened and pear-shaped shape were found in varieties V1.3, V0.1 and V1.5 respectively (Table 5). Fruit color ranged from clear yellow (40%), pale green (40%) and yellowish orange (20%) (Fig. 4). The main flesh color of all varieties was clear yellow.

### 3.4. Seed characterization

The average number of seeds per fruit varied from 324.23 to 129.10 among all varieties (Table 7). Variety V1.3 had the highest value (324.23) followed by variety V0.2 (308.00). The lowest value of the average number of seeds per fruit was recorded in the variety V0.1 (129.10). The analysis of variance of number of seeds per fruit shows a highly significant difference between varieties ($F = 17.76$; $p < 0.001$). The average weight of one hundred seeds

**Table 6**
Means flower and fruit of pumpkin varieties.

| Varieties | NMF | NFF | NFP | FYP (Kg/m2) | FYE (t/ha) | AFW (kg) | FrL (cm) | FrD (cm) |
|---|---|---|---|---|---|---|---|---|
| V0.1 | 24.33 ± 2.75 a | 4.44 ± 1.0 a | 3.81 ± 4.12 a | 0.54 ± 0.81b | 4.33 ± 5.77 a | 0.40 ± 0.11c | 6.65 ± 1.39c | 10.12 ± 2.29 d |
| V0.2 | 10.06 ± 4.30b | 2.94 ± 1.87 ab | 2.10 ± 0.35 a | 1.20 ± 0.64 a | 11.33 ± 6.51 a | 1.87 ± 0.32b | 28.75 ± 4.01b | 55.84 ± 1.02 ab |
| V1.3 | 14.83 ± 1.89b | 2.11 ± 0.35b | 1.06 ± 0.10 a | 0.89 ± 0.04 a | 5.00 ± 1.00 a | 3.38 ± 0.09 a | 47.24 ± 12.99 a | 66.20 ± 2.02 a |
| V1.4 | 6.28 ± 1.07b | 1.39 ± 0.19b | 1.53 ± 0.10 a | 0.44 ± 0.17 a | 3.67 ± 1.15 a | 1.64 ± 0.67b | 23.52 ± 7.55b | 44.12 ± 9.91 bc |
| V1.5 | 13.17 ± 3.51b | 1.83 ± 0.44b | 1.47 ± 0.08 a | 0.30 ± 0.04 a | 2.33 ± 0.58 a | 1.12 ± 0.28b | 21.26 ± 4.27b | 36.45 ± 10.74c |
| V1.6 | 7.22 ± 5.16b | 1.78 ± 0.54b | No fruit | No fruit | No fruit | No fruit | No fruit | No fruit |

**Note:** Means followed by the same alphabetical letters are not significantly different at the 0.05 threshold according to the SNK test. NMF: number of male flowers, NFF: number of female flowers, NFP: number of fruits per plant, FYP: fruit yield per plant, FYE: fruit yield per elementary plot, AFW: average fruit weight, FrL: fruit length, FrD: fruit diameter.

ranged from 6.67 to 7.07 g in varieties V0.1 and V1.4, while this weight varied between 8.73 and 8.87 g in varieties V0.2 and V1.3. The highest value was recorded in the variety V1.5 (10.27 g). There was therefore a very significant difference between treatments ($F = 6.24$; $p = 0.009$). Seeds of high length were observed in the varieties V1.3 and V1.5 (1.51 cm) and those of high width were recorded only in the variety V1.5 (0.95 cm). While the seeds of small length were observed in the variety V1.4 (0.98 cm) and those of small width in the varieties V0.1 and V1.4 (0.71 cm). Seed length showed a highly significant difference between the different varieties ($F = 34.01$; $p < 0.001$). Seed width, shows a significant difference between treatments ($F = 3.30$; $p = 0.05$). Seed width and length ratio was significant ($F = 5.77$; $p = 0.01$) and allowed for determination of seed shape. This ratio ranged from 0.50 to 0.72 and was<1. Therefore, all seeds were elongated in shape (Table 5). In this study, three different seed colors were observed: white (20%), light yellow (40%) and dark brown (40%) (Fig. 1).

### 3.5. Analysis of relationships between descriptors

Thirty-four variables were measured and observed on the leaves, flowers, fruits and seeds of pumpkin. Given the high number of parameters, emphasis was put on the fifteen descriptors that showed a significant difference in the analysis of variance in order to highlight the discriminating agro-morphological parameters of the different varieties of *C. moschata* in Benin. Variety V1.6 was not included in the Principal component analysis (PCA) and the Hierarchical Clustering because we could not collect all data due to fruit abortion.

The analysis of the correlation matrix shows strong and significant correlations among phenotypic traits (Table 8). On the one hand, positive correlations were observed between the length of female flower peduncles and the length of male flower peduncles ($r = 0.60^{*}$); between 50% fruiting and days to 50% flowering ($r = 0.80^{***}$); between the number of female flowers and the number of male flowers ($r = 0.74^{**}$); between fruit length and fruit weight ($r = 0.88^{***}$); between fruit diameter and average fruit weight ($r = 0.85^{***}$) and fruit length ($r = 0.91^{***}$); between number of seeds per fruit and fruit weight ($r = 0.76^{**}$), fruit length ($r = 0.72^{**}$) and the fruit diameter ($r = 0.78^{***}$); between the seed length and days to 50% emergence ($r = 0.60^{*}$) and the average weight of one hundred seeds ($r = 0.68^{**}$); between seed width and days to 50% emergence ($r = 0.62^{*}$), 100-seed weight ($r = 0.66^{**}$) and seed length ($r = 0.68^{**}$), and between seed width and length and days to 50% flowering($r = 0.54^{*}$).

**Fig. 4.** Phenotypic variability of pumpkin fruits.

**Table 7**
Means seed parameters of pumpkin.

| Varieties | ANSF | AWOS (g) | SL (cm) | SW (cm) | SWLR |
|---|---|---|---|---|---|
| V0.1 | 129.10 ± 23.20 b | 6.67 ± 0.23 b | 1.09 ± 0.08 b | 0.71 ± 0.06 b | 0.65 ± 0.01 a |
| V0.2 | 308.00 ± 73.67 a | 8.73 ± 1.03 ab | 1.07 ± 0.13 b | 0.75 ± 0.18 b | 0.68 ± 0.12 a |
| V1.3 | 324.23 ± 19.08 a | 8.87 ± 0.12 ab | 1.51 ± 0.02 a | 0.76 ± 0.02 b | 0.50 ± 0.01 b |
| V1.4 | 264.63 ± 33.61 a | 7.07 ± 2.00 b | 0.98 ± 0.06 b | 0.71 ± 0.09 b | 0.72 ± 0.06 a |
| V1.5 | 133.07 ± 9.17 b | 10.27 ± 0.12 a | 1.51 ± 0.04 a | 0.95 ± 0.02 a | 0.63 ± 0.02 a |

**Note:** In the same column, the values followed by the same letter are not significantly different at the 0.05 at the 0.05 threshold according to the SNK test. ANSF: average number of seeds per fruit, AWOS: average weight of one hundred seeds, SL: seed length, SW: seed width, SWLR: seed width and length ratio.

**Table 8**
Correlation matrix of significant descriptors.

| | DE50% | DF50% | LMFP | LFFP | DFS50% | NMF | NFF | AFW | FrL | FrD | ANSF | ANOS | SL | SW | SWLR |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| DE50% | 1.00 | | | | | | | | | | | | | | |
| DF50% | 0.05 | 1.00 | | | | | | | | | | | | | |
| LMFP | −0.32 | 0.26 | 1.00 | | | | | | | | | | | | |
| LFFP | 0.01 | −0.10 | 0.60* | 1.00 | | | | | | | | | | | |
| DFS50% | 0.14 | 0.80*** | 0.44 | 0.22 | 1.00 | | | | | | | | | | |
| NMF | −0.07 | −0.87*** | −0.18 | 0.05 | −0.86*** | 1.00 | | | | | | | | | |
| NFF | −0.35 | −0.62* | 0.27 | 0.29 | −0.65** | 0.74** | 1.00 | | | | | | | | |
| AFW | 0.07 | 0.28 | −0.33 | −0.10 | 0.18 | −0.34 | −0.37 | 1.00 | | | | | | | |
| FrL | 0.04 | 0.15 | −0.18 | 0.09 | 0.25 | −0.35 | −0.38 | 0.88*** | 1.00 | | | | | | |
| FrD | −0.09 | 0.38 | 0.06 | 0.15 | 0.48 | −0.53* | −0.44 | 0.85*** | 0.91*** | 1.00 | | | | | |
| ANSF | −0.35 | 0.33 | 0.11 | 0.04 | 0.26 | −0.43 | −0.12 | 0.76** | 0.72** | 0.78*** | 1.00 | | | | |
| ANOS | 0.49 | 0.25 | 0.10 | 0.36 | 0.44 | −0.20 | −0.33 | 0.37 | 0.37 | 0.40 | −0.04 | 1.00 | | | |
| SL | 0.60* | −0.28 | −0.41 | 0.30 | −0.04 | 0.12 | −0.18 | 0.42 | 0.43 | 0.27 | −0.06 | 0.68** | 1.00 | | |
| SW | 0.62* | 0.14 | −0.05 | 0.42 | 0.31 | −0.12 | −0.19 | 0.02 | −0.03 | −0.04 | −0.29 | 0.66** | 0.68** | 1.00 | |
| SWLR | −0.07 | 0.54* | 0.37 | −0.04 | 0.36 | −0.30 | 0.01 | −0.44 | −0.56* | −0.38 | −0.18 | −0.20 | −0.54* | 0.23 | 1.00 |

**Note:** *: significant, **: very significant, ***: highly significant at the 5% level. DE50%: Day to 50 %emergence, DF50%: days to 50% flowering, LMFP: length of male floral peduncles, LFFP: length of female floral peduncles, DFS50%: Days to 50% fruit set, NMF: number of male flowers, NFF: number of female flowers, AFW: average fruit weight, FrL: fruit length, FrD: fruit diameter, ANSF: average number of seeds per fruit, AWOS: average weight of one hundred seeds, SL: seed length, SW: seed width, SWLR: seed width and length ratio.

On the other hand, negative and significant correlations were recorded between the number of male flowers and days to 50% of flowering (r = -0.87***) and days to 50% fruiting (r = -0.86***); between the number of female flowers and days to flowering (r = -0.62*) and days to 50% fruit set(r = -0.65**); between fruit diameter and number of male flowers (r = -0.53*) and between seed width and length ratio and fruit length (r = -0.56*) and seed length (r = -0.54*),.

The Principal Component Analysis (PCA) identified three main dimensions that explain 87.86% of the total variability. The PCA graph shows the projection of the variables in the factorial plane 1–2 (Fig. 5). Dimension 1 alone explains 38.39% of the total variability and was correlated in its positive end with days to 50% fruit set, average fruit weight, fruit length, fruit diameter, average number of seeds per fruit and average hundred-seed weight. It was correlated in its negative end to number of male and female flowers. It is the dimension of days to 50% fruit set and yield (size and quantity) of fruits and seeds. Dimension 2, which explains 27.82% of the variability, associates the days to 50% emergence and seed length at the positive pole. At the negative pole were the days to 50% flowering, the length of male flower peduncle and the ratio of width and length of seeds. This dimension could be interpreted as the dimension of seed quantity (size, shape) and male flower peduncle length. Dimension 3 is not shown in the figure but accounts for 21.66% of the variability and correlates with seed width and female flower peduncle length at the positive pole. This is the dimension of seed size/female flower peduncle length.

The Hierarchical Ascending Classification reveals the existence of three classes (Fig. 6). The clustering was done by taking into account the fifteen variables that were significant in the analysis of variance and the five varieties on which all descriptors were collected. The variety V0.1 was found in class I. This class is characterized by dark green leaves, yellowish orange fruits with a flattened shape and white seeds. In this class, the plants produce an average of 24.33 male and 4.44 female flowers respectively. Class II includes the varieties V0.2 and V1.4 characterized by green leaves mottled with white, globular fruits of light green color and dark brown seeds. This class had high mean values for the following parameters: days to 50% flowering, length of male flower peduncles, days to 50% fruit set, and number of seeds per fruit and seed length ratio. The other varieties, namely V1.3 and V1.5 are found in class III. This class remains more or less heterogeneous concerning some descriptors. It is characterized by dark green leaves, light yellow fruits with elongated and pear-shaped forms, light yellow seeds. Days to 50% emergence, the length of the female flower peduncles, the average weight of the fruit, the length and the diameter of the fruits, the average weight of one hundred seeds, the length and width of the seeds are in this class.

### *3.6. Genetic variability*

#### *3.6.1. Genotypic and phenotypic variance*

The mean squares of the genotypes are as high (except for the leaf length and width) as the mean squares of the error for the majority of the traits studied (Table 9). Genotypic variance ranged from 0 to 8428 while phenotypic variance varied between 0.01 and 8931. The highest genotypic and phenotypic variances were obtained with number of seeds per fruit. Days to 50% emergence (0.37), male flower peduncle length (4.41), female flower peduncle length (0.37), number of female flowers (1.26), fruit weight (1.22), hundred seed weight (2.14), seed length (0.07), seed width (0.01), seed width and length ratio (0.01), cotyledon width (0.04), cotyledon length (0.06), petiole length (7.52), petiole diameter (0.02), leaf length (3.49), leaf width (2.04), number of fruits per plant (1.17), fruit yield per plant (0.13), and fruit yield per unit plot (12.22) showed moderate phenotypic variances (<20). Seed width and seed

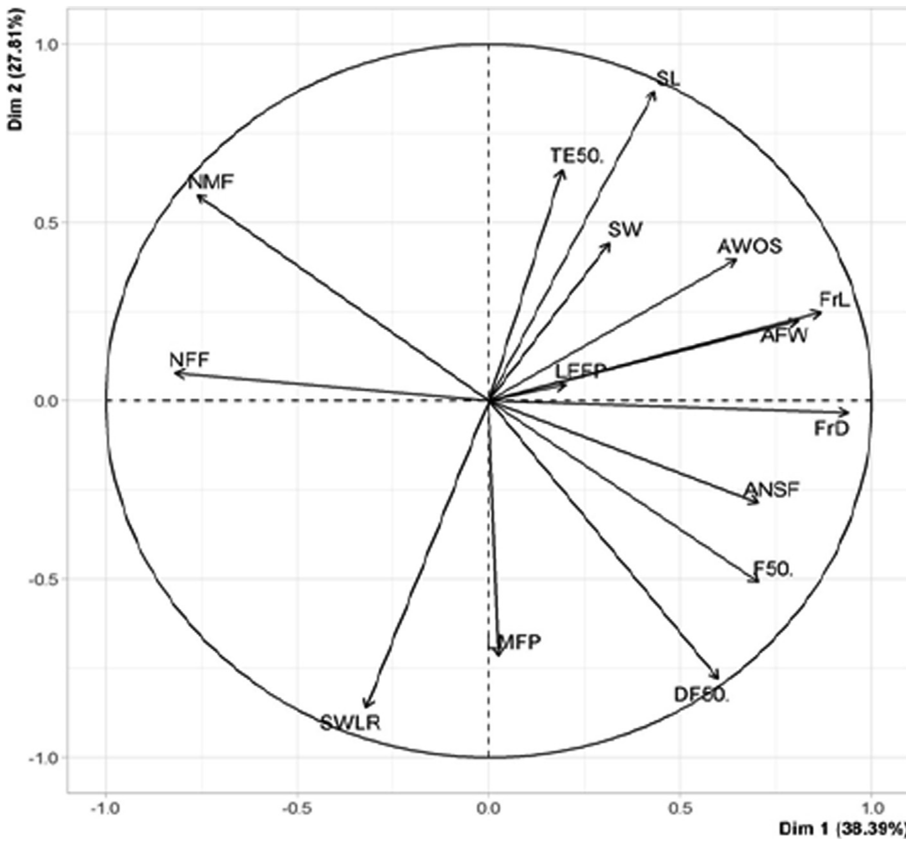


**Fig. 5.** Principal Component Analysis (PCA) graph.

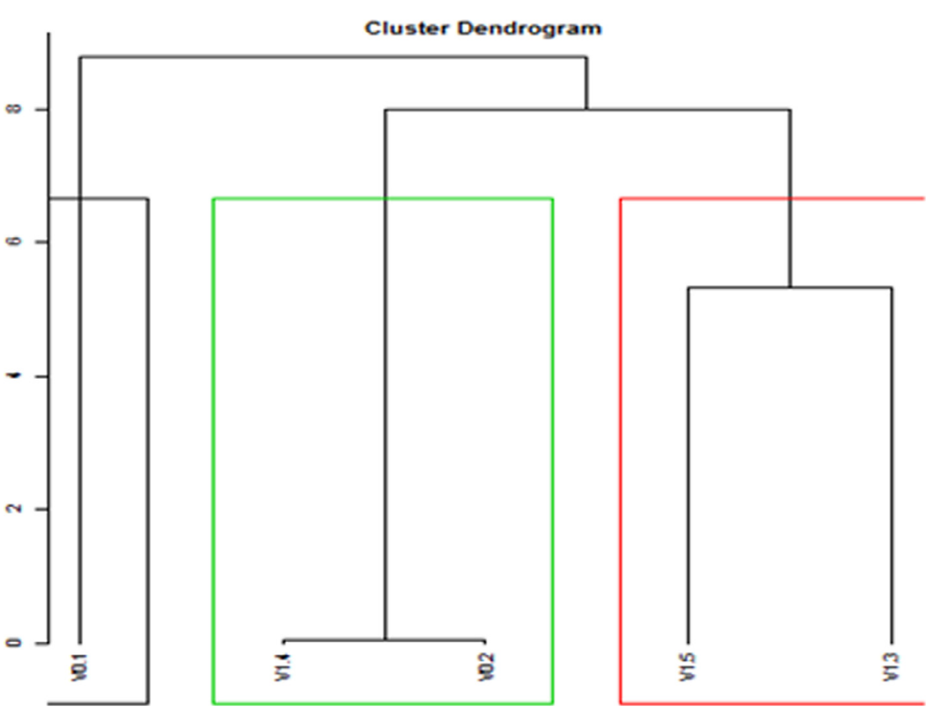


**Fig. 6.** Dendrogram of the 05 pumpkin varieties.

width and length ratio showed the lowest genotypic variances (0.01) while leaf length and width had the lowest phenotypic variance (0).

### 3.6.2. Coefficients of genotypic and phenotypic variation

High coefficients of genotypic and phenotypic variation were obtained for several traits (Table 9). In general, estimates of the

**Table 9**
Estimation of genetic parameters of pumpkin.

| | GV | PV | $H^2$ (%) | $\sqrt{GV}$ | $\sqrt{PV}$ | X | GCV (%) | PCV (%) | GA | GA (%) mean |
|---|---|---|---|---|---|---|---|---|---|---|
| DE50% | 0.27 | 0.37 | 71.43 | 0.62 | 0.72 | 6.26 | 9.90 | 11.50 | 1.06 | 16.92 |
| DF50% | 110.37 | 127.33 | 86.68 | 10.77 | 11.50 | 54.26 | 19.85 | 21.19 | 20.53 | 37.84 |
| LMFP | 3.86 | 4.41 | 87.60 | 1.96 | 2.10 | 6.50 | 30.17 | 32.32 | 3.79 | 58.33 |
| LFFP | 0.35 | 0.37 | 94.55 | 0.59 | 0.61 | 2.18 | 27.09 | 28.00 | 1.19 | 54.54 |
| DFS50% | 563.73 | 566.93 | 99.44 | 23.66 | 23.72 | 93.43 | 25.32 | 25.39 | 48.59 | 52.00 |
| NMF | 39.79 | 43.67 | 91.13 | 6.31 | 6.61 | 13.73 | 45.96 | 48.14 | 12.41 | 90.38 |
| NFF | 0.97 | 1.26 | 76.98 | 0.98 | 1.12 | 2.54 | 38.52 | 44.02 | 1.78 | 69.81 |
| AFW | 1.18 | 1.22 | 96.46 | 1.09 | 1.10 | 1.68 | 64.81 | 65.41 | 2.19 | 129.96 |
| FrL | 203.63 | 215.17 | 94.64 | 14.27 | 14.67 | 25.48 | 56.00 | 57.57 | 28.60 | 112.23 |
| FrD | 441.83 | 456.77 | 96.73 | 21.02 | 21.37 | 42.55 | 49.40 | 50.23 | 42.58 | 100.09 |
| ANSF | 8428.00 | 8931.00 | 94.37 | 91.80 | 94.50 | 231.81 | 39.60 | 40.77 | 183.71 | 79.25 |
| AWOS | 1.80 | 2.14 | 83.96 | 1.34 | 1.46 | 8.32 | 16.11 | 17.55 | 2.53 | 30.35 |
| SL | 0.06 | 0.07 | 95.00 | 0.24 | 0.26 | 1.23 | 19.46 | 21.08 | 0.51 | 41.26 |
| SW | 0.01 | 0.01 | 66.67 | 0.10 | 0.10 | 0.78 | 12.87 | 12.87 | 0.14 | 17.67 |
| SWLR | 0.003 | 0.01 | 50.00 | 0.05 | 0.10 | 0.64 | 7.84 | 15.69 | 0.10 | 16.16 |
| DVP | 22.44 | 35.51 | 63.21 | 4.65 | 6.00 | 41.21 | 11.28 | 14.56 | 7.81 | 18.96 |
| WC | 0.01 | 0.04 | 36.36 | 0.10 | 0.20 | 2.16 | 4.64 | 9.27 | 0.15 | 6.95 |
| LC | 0.01 | 0.06 | 21.05 | 0.10 | 0.24 | 2.98 | 3.35 | 8.04 | 0.10 | 3.49 |
| VL | 2785.00 | 6102.67 | 45.64 | 52.77 | 78.12 | 214.28 | 24.63 | 36.46 | 73.44 | 34.27 |
| PL | 4.42 | 7.52 | 58.73 | 2.10 | 2.74 | 14.31 | 14.68 | 19.15 | 3.32 | 23.17 |
| PD | 0.01 | 0.02 | 60.00 | 0.10 | 0.14 | 0.58 | 17.19 | 24.06 | 0.17 | 29.74 |
| LL | 0.00 | 3.49 | 0.00 | 0.00 | 0.00 | 19.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| LW | 0.00 | 2.04 | 0.00 | 0.00 | 0.00 | 18.15 | 0.00 | 0.00 | 0.00 | 0.00 |
| NL | 367.00 | 724.33 | 50.67 | 19.16 | 26.91 | 62.23 | 30.79 | 43.24 | 28.09 | 45.13 |
| NFP | 0.03 | 1.17 | 2.56 | 0.17 | 1.08 | 1.99 | 8.53 | 54.20 | 0.06 | 2.86 |
| FYP | 0.06 | 0.13 | 45.00 | 0.24 | 0.36 | 0.67 | 35.61 | 53.41 | 0.33 | 49.51 |
| FYE | 7.00 | 12.22 | 57.27 | 2.65 | 3.50 | 5.33 | 49.69 | 65.63 | 4.13 | 77.42 |

**Note:** GV: Genotypic variance, PV: Phenotypic variance, $H^{2:}$ Heritability, $\sqrt{}$GV: Standard deviation of genotypic variance, $\sqrt{}$PV: Standard deviation of phenotypic, X: Mean of the characters, GCV: Genotypic Coefficient of Variation, PCV: Phenotypic Coefficient of Variation, GA (%): Genetic gain.

phenotypic coefficient of variation were higher than estimates of the genotypic coefficient of variation for the majority of traits. But the seed width had a unique value for both coefficients (12.87%). The highest genotypic coefficient of variation (64.81%) was obtained with fruit weight while the trait fruit yield per unit plot recorded the highest value of phenotypic coefficient of variation (65.63%). The moderate genotypic and phenotypic coefficients of variation were recorded in days to 50% flowering, weight of hundred seeds, seed length, seed width, vegetative phase period, petiole length, petiole diameter, days to 50% emergence and seed width length ratio (11% to 20%). Low genotypic and phenotypic coefficients of variation were observed for the following traits: days to 50% emergence, seed width and length ratio, cotyledon length and width, leaf length and width, and number of fruits per plant (<11%).

### 3.6.3. Heritability in the broad sense

Days to50% fruit set (99.44%), fruit diameter (96.73%), fruit weight (96.46%), seed length (95%), fruit length (94.64%), female flower peduncle length (94.55%), number of seeds per fruit (94.37%), number of male flowers (91.13%), and length of male flower peduncle (87.60%) demonstrated high heritability (Table 9). Medium heritability was recorded in seed width and length ratio (50%), cotyledon width (36.36%), cotyledon length (21.05%), vines length (45.64%) and fruit yield per plant (45%). Leaf length and width (0%), number of fruits per plant (2.56%) show very low heritability.

### 3.6.4. Expected genetic gain

The expected genetic gain related to the mean of the GA trait (% mean trait) varied widely among the traits ranging from 0 to 129.96% (Table 9). The highest expected genetic gain was observed in fruit weight (129.96%), fruit length (112.23%) and fruit diameter (100.09%). Leaf length and width (0%), number of fruits per plant (2.86%), cotyledon length (3.49%) and cotyledon width (6.95%) had very low expected genetic gains.

## 4. Discussion

### 4.1. Phenological parameters

Some studies on *Cucurbita moschata* including agro-morphological evaluation of varieties (Ahamed et al., 2011; Aruah et al., 2010; Kiramana and Isutsa, 2017; Mbogne et al., 2015), floral biology (Agbagwa et al., 2007), genetic diversity analysis (Barboza et al., 2012) were carried out. *Cucurbita* species differ from one another by genetic constitutions and are therefore assessed using phenotypic parameters (Whitaker and Davis, 1962). However, no scientific study on Benin pumpkin landraces has been performed and documented but is accomplished through this study.

In this study, the days to emergence were between 5.18 and 6.97 days. These results are similar to those of (PROTA, 2018) who reported that the emergence of *C. moschata* seedlings is observed between 5 and 7 days after sowing. The days to emergence are also in agreement with the work of (OECD, 2016). This work revealed that the time of emergence is synchronous and the seeds germinated between 2 and 6 days after water imbibition and enzyme activities. Aruah et al. (2010) conducted a study on the variation of local accessions of the genus *Cucurbita* in Nigeria and reported that the days to emergence varied between 3 and 8 days. The significant differences in days to emergence among the different varieties could be attributed to the thickness of the seed coat and the different tissue layers of the seed or seed viability (Aruah et al., 2010). Seed size compensates for variation in environmental conditions and enhances the productive capacity of a plant (OECD, 2016). It is proportional to the food resources present in the cotyledon to support seedling emergence. The food reserves of smaller seeds are quickly depleted, affecting seed viability and vigor

(Haferkamp, 1988). In this study, large seeds (V1.5, V1.6, and V1.3) emerged late while small seeds (V0.1, V0.2 and V1.4) emerged earlier. We could also hypothesize that the late germination of small pumpkin seeds could be due to the decrease of their viability in farmer's storage facility as it can be poor at time. Dissimilar results were obtained in Kenya by Kiramana and Isutsa (2017) who reported early emergence in accessions with large seed sizes and late emergence in accessions with small seed sizes. According to the work of Djè et al. (2007), the days to emergence of seedlings above ground could vary with seed age or seeds store in poor storage facility/conditions. They showed that 18-month-old *Cucumis melo* seeds had emergence days of 6 and 8 days, while 3 months old seeds emerged between 5 and 14 days. We could hypothesize that the small seeds collected during the surveys we conducted might be less old than the large ones. As for the vegetative phase, it appears that the time of appearance of floral buds of the different varieties of *C. moschata* varies from 34.76 to 48.97 days after sowing. These results corroborate that of Bembe et al. (2010) who reported that the vegetative phase in *C. moschata* was 37 days after sowing. The non-significant difference in this trait could be explained by more or less similar vegetative traits in the different species of the Cucurbitaceae family (Bembe et al., 2010). Other work conducted on *Lagenaria siceraria* and *Cucumeropsis mannii*, revealed the vegetative phases that varied from 30 to 60 days, respectively (Soko et al., 2018; Zoro Bi et al., 2003).

The different variations observed in days to 50% flowering could be mainly due to intra-species variability. In this study, days to 50% flowering varied from 41.21 to 68.72. These results corroborate with Mendlinger et al. (1992) who stated that the collected accessions of pumpkin reached 50% flowering in the range of 57 to 88 days. According to the work of PROTA (2018), flowering begins 35–60 days after emergence and is more or less continuous. In the same vein, Agbagwa et al. (2007) reported that flowering of *C. moschata* occurred 56 days after sowing. In pumpkin, flowering is asynchronous with the opening of male flowers followed by the opening of female flower 2–3 weeks later (Agbagwa et al., 2007). In this study, the number of male and female flowers per plant ranged from 6.28 to 24.33 and 1.39 to 4.44, respectively. All varieties exhibited light yellow flowers. According to the work of Ahamed et al. (2011), the flower color of all accessions of pumpkin in northern Bangladesh was yellow. According to the work of Islam et al. (2016), the highest and lowest male to female flower ratio in Bangladesh were 12.11: 4.08 and 10.24: 3.27, respectively. In Nigeria, Aruah et al. (2010) showed that the highest male to female flower ratio within *Cucurbita* accessions was 66: 8.67. Agbagwa et al. (2007) had a male to female ratio of 9: 1 with yellow flowers. Our results, like theirs, showed that the number of male flowers was greater than the number of female flowers on a plant. But Kiramana and Isutsa (2017) presented contrary results. According to these authors, early female flowering was observed in 9 accessions of pumpkin in Kenya. McCormack (2005) indicated that it was not uncommon for *C. moschata* to bear female flowers first. The flower color was orange in most accessions of *C. moschata* in Kenya.

According to Janick and Paull (2008), the genus *Cucurbita* can exhibit a wide variation in the proportion of male and female flowers on a plant. Zomlefer (1994) reported that female flower production is often lower than male flower production. The sequence of floral development in pumpkin allows for cross-pollination of flowers (McCormack, 2005). Flower development is regulated by genetic makeup and environmental mechanisms (OECD, 2016). On the one hand, sexual expression in Cucurbitaceae is influenced by hormones produced in the plant (Kiramana and Isutsa, 2017). According to these authors, gibberellins promote the development of staminate flowers. Ethylene and auxins stimulate the development of pistillate flowers (Maynard, 2007). On the other hand, high temperature, high light intensity, and long days promote the production of male flowers while low temperature, low light intensity, and short days induce the development of female flowers (OECD, 2016).

This study found that days to 50% fruiting ranged from 61.80 to 120 days after sowing. The shortest days to 50% fruiting (61.80 days) was recorded in variety V0.1. It could be an early variety introduced through seed exchanges between producers from one country to another. The longest days to 50% fruit set (120 days) was observed in V0.2, V1.4 and V1.5. Ahamed et al. (2011) reported that fruit ripening occurred in the range of 103 to 123 days. In the present study, fruit ripening did not take place in variety V1.6. In fact, during fruit set, there was fruit abortion in plants of this variety. This could be due to abscission of fruits, which is a non-parasitic disease related to poor fruit fertilization with sometimes short flower life (Blancard, 2013). It is characterized by an extremely early cessation of development of young fruits. Fruit abscission is caused by the absence or insufficiency of pollen due to a limited number of male flowers, by climatic conditions not conducive to the activity of pollinating insects. In this study, the ratio of male to female flowers of the V1.6 was 7.22:1.78. Hand pollination of flowers was not carried out, as it could influence fruit yield. In this context, Djè et al. (2007) argued that very low fruit production could result from a low number of pollen grains deposited on the stigmas of female flowers. In this study, a second cycle of fruiting was observed for all varieties whose fruits reached physiological maturity and this could be due to the indeterminate growth related to the species. The work of OECD (2016) corroborates with this result. These authors state that the regeneration of the second fruiting cycle was attributed to genetic influence and adaptability to environmental conditions.

### 4.2. Growth parameters

Male flower peduncle was longer than female flower peduncle for all varieties. The difference in length of reproductive organs is to facilitate cross-pollination in *Cucurbita moschata* (OECD, 2016). It also serves as an indicator in the differentiation of male versus female flowers (PROTA, 2018).

All varieties exhibited multilateral vines and tendrils at internodes. According to OECD (2016), pumpkin has hard and slightly fluted vines. In this study, vines length ranged from 102.17 to 307.44 cm. The number of leaves varied from 24.56 to 95.50 leaves per plant in the tenth week after sowing. Aruah et al. (2010) obtained very large branche lengths and leaf numbers that ranged from 383 to 707 cm and 97.70 to 210, respectively. The results Ahamed et al. (2011) show that the vines length at harvest varied from 169.6 to 400.1 cm. This difference in results could be due to the mineral fertilizer application made in their trials. High soil fertility stimulates good vines growth and high leaf production. PROTA (2018) showed that plant growth of pumpkin is indeterminate under favorable conditions. They further stated that, under these conditions, vines can exceed 20 m in length. Maynard (2007) reported that a vine at nodes follows the same general growth pattern as the main stem. Variation observed for vines' length has been attributed to genetic constitution and gene expression (Kiramana and Isutsa, 2017).

Leaf length varied from 16.74 to 21.67 cm while leaf width varied from 16.59 to 20.44 cm in this study. Kiramana and Isutsa (2017) obtained leaf length and widths that ranged from 30.7 to 47.2 cm and 15 to 23 cm, respectively. Grubben and Ngwerume (2004) showed that the leaf length of *Cucurbita moschata* was between 9 and 24 cm. These authors indicated that the diameter of the leaves of *C. moschata* varied from 20 to 35 cm. All these results are more or less similar to the results of the agro-morphological characterization of landraces of pumpkin in Benin; however, the slight differences may be related to the environmental conditions and soil fertility.

### 4.3. Characterization of leaves and fruits

The present study revealed qualititative diversity in leaf color. The qualitative color description and comparison model of Zoe et al. (2011) was used for qualitative variables' measurement. All varieties had dark green leaf color except V0.2 and V1.4 which showed green leaves mottled with white. Agbagwa et al. (2007) observed leaves with white spots. According to Kiramana and Isutsa (2017), the leaves of most accessions were green, dark green and diverse in color. Aruah et al. (2010) state that leaves of pumpkin had a high variability. They can be light green, dark green, and variegated in color. They are tender and moderately lobed (OECD (2016). Morphological characteristics of the leaves such as shape, size, margin, and color are diagnostic and essential tools in the identification of pumpkin at the genus level (Kiramana and Isutsa, 2017). These characteristics help distinguish *C. moschata* from other cultivated *Cucurbita* species (Agbagwa and Ndukwu, 2004).

The varieties showed wide variation in fruit shape. The different fruit shapes recorded in this study were globular (40%) and flattened (20%), elongated (20%), pyriform (20%). Fruit shape was determined from the descriptors of *Cucurbita* (Esquinas-Alcazar and Gulick, 1983). Ahamed et al. (2011) observed elliptical, round and pyriform fruit shapes. Kiramana and Isutsa (2017) observed fruits with predominantly globular shapes while Aruah et al. (2010) recorded fruits with cylindrical, oblong and round shapes. This phenotypic variability in fruit shape is due to varietal diversity related to their genotype. The genes responsible for fruit shape are "flatteners" and "elongators" (Brown, 2002). In this study, fruit color ranged from light yellow (40%) to pale green (40%) to yellowish orange (20%). The main flesh color of all fruits was light yellow. Kiramana and Isutsa (2017) obtained fruits with green and gray colors, yet Aruah et al. (2010) observed fruits with bright orange, yellow, green, gray, and green spotted with white. Genotypes of pumpkin in northern Bangladesh have green, yellow, and brown fruit colors (Ahamed et al., 2011). Kiramana and Isutsa (2017) stated that fruit color is controlled by 3 loci (Gr, Mldg and B). According to Ahamed et al. (2011), the main color of the flesh can be yellow, orange, white and yellowish orange while Kiramana and Isutsa (2017) obtained fruits with only orange flesh. Brown (2002) showed that intense yellow and orange flesh colors are associated with high levels of carotenoids. Our results, like theirs, show high variability with respect to fruit-related quality traits.

In the present study, the number of fruits per plant ranged from 1 to 4. According to Aruah et al. (2010) the number of fruits per plant of Nigerian accessions varies between 4.67 to 8. Ahamed et al. (2011) recorded 2 to 16 fruits per plant in northern Bangladesh. Labrada et al. (1997) also reported that among 34 landraces, 25 were capable of producing more than 4–5 fruits per plant. The high number of fruits per plant at the genotype level of pumpkin in northern Bangladesh could be explained by three factors: improved genotypes and soil fertility and suitable environmental conditions. In this study, the highest average fruit weight was obtained in variety V1.3 (3.38 kg) while the range was from 0.40 to 3.38 kg. PROTA (2018) demonstrated that the average fruit weight depends on the type of cultivars and ranged from 1 to 10 kg. Bembe et al. (2010) recorded an average weight of 1.80 kg and this weight varied from 1 to 2.5 kg. All these results are more or less similar. The length and diameter of fruits varied from 6.65 to 47.24 cm and 10.12 to 55.84 cm, respectively. Ahamed et al. (2011) showed that fruit diameter varies from 46.3 to 77.1 cm among the genotypes of pumpkin in northern Bangladesh. It could be inferred that the average fruit weight is proportional to the fruit length and diameter. The estimated fruit yield per unit plot per hectare ranged from 2.33 to 11.33 t/ha. In Africa, cultivation practices related to pumpkin cultivation are still extensive and yields are low (PROTA, 2018). They further demonstrated that, with more cultural care a fruit yield of 15 t/ha is reasonable yet under low inputs the yield is about 5 t/ha. Improved cultivars are likely to have a fruit yield of 30 t/ha (PROTA, 2018). These authors believe that there has been no varietal selection for yield in Africa. Growers' assertion gathered during our survey showed the fruit harvest period varied between 2 and 6 months after sowing (Ezin et al., 2021) corroborates with our experimental results and those of OECD (2016) and PROTA (2018).

### 4.4. Seed characterization

In the present study, seed shape was determined based on the ratio of seed width to seed length. Zoro Bi et al. (2003) determined three seed shapes based on the ratio of width and length. These are: elongated seeds ($l/L < 1$), round seeds ($l/L = 1$), and wide seeds (l/L greater than 1). All seeds in this study are elongated. This result corroborates with that of Bembe et al. (2010). In the morphological characterization of pumpkin in Kenya, Kiramana and Isutsa (2017) obtained mainly elliptical shaped seeds. According to OECD (2016), pumpkin seeds can be oval or oval-elliptic. There is phenotypic variability in seed color. The different seed colors are dark brown (40%), light yellow (40%), and white (20%). Aruah et al. (2010) observed light brown and brown seed colors. Kiramana and Isutsa (2017) on the other hand obtained creamy yellow, yellow-white, brown, white and light brown seed colors.

The average number of seeds per fruit ranged from 129.10 to 324.23. Bembe et al. (2010) found that, the number of seeds per fruit varied from 250 to 300 with an average number of 272.40. dissimilar results by Islam et al., (2016) showed that the number of seeds per fruit varied from 61.67 to 162.77. As for Aruah et al. (2010), they obtained 214.70 to 523.70 seeds per fruit. The number of seeds produced per fruit varies by species and variety (OECD, 2016). High seed length and width put together lead to a high average hundred seed weight. In this study, both small and large seed sizes were observed. The average hundred-seed weight ranged from 6.67 to 10.27 g. This result is more or less similar to that of Bembe et al. (2010). Islam et al. (2016) obtained average hundred seed weights that range from 8.38 to 14.34 g while Aruah et al. (2010) recorded hundred seed weights that range from 10.10 to 53.00 g. From the analyses of the different descriptors, it is clear that there is an agro-morphological variability between the different varieties of pumpkin collected in Benin. The existence of this variability between varieties could be used in breeding programs for yield and resistance to diseases and insects and abiotic stress.

### 4.5. Analysis of relationships between descriptors

The days to 50% emergence depends on size of seeds and seed viability. It was observed that the days to 50% emergence were proportionally with seed length and width most importantly the availability of the nutrients, viability and vigor of the seeds. Contrary results were obtained by Aruah et al. (2010). Our findings demonstrated that days to emergence were negatively correlated with seed length. This could be due to the quality and age of seeds collected during our investigations. Increasing fruit length and diameter contributes to increasing fruit weight and number of seeds per fruit. There is a positive correlation between fruit weight and number of seeds per fruit. Variety V1.3 had the highest fruit weight (3.38 kg) and number of seeds per fruit (324.23) among all varieties. The lowest values were recorded in variety V0.1, with an average fruit weight of 0.40 kg and an average number of seeds per fruit of 129.10. Therefore, the fruit weight of *Cucurbita moschata* could be used as good criteria for selecting individuals with many seeds. These results confirm those of Mbogne et al. (2015) in Cameroon. Aruah et al. (2010) highlighted that it is obvious that fruits with more seeds will grow to a larger size. A high weight is one of the factors that contribute to increased fruit yield. In line with these observations, Priori et al. (2018) indicated that the marketing of fruit for consumption is related to fresh matter (fruit weight) which is a key characteristic for the consumer.

Principal Component Analysis was used to generate a correlation among quantitative variables. Combining all the quantitative descriptors that showed a significant difference in the analysis of variance with the qualitative variables (mainly fruit color and shape, seed color, leaf color) allowed discriminating the landraces of pumpkin in Benin. Moreover, the factorial design (1–2-3) of the Principal Component Analysis explains 87.86% of the total variability. This percentage of explained variation is higher than 50%. Therefore, the descriptors that showed a significant difference in the analysis of variance can be used to discriminate the different landraces of pumpkin in Benin.

### 4.6. Genetic variability

Measuring genetic variation and understanding how quantitative traits are transmitted are key steps in any crop improvement program (Ibrahim, 2012). Analysis of genetic parameters for all traits studied showed that phenotypic variance is greater than genotypic variance. These results are consistent with Abdein et al. (2017) on pumpkin, Dar and Sharma (2011) on tomato and Ibrahim (2012) on melon. The difference between phenotypic and genotypic variance for all traits was due to the environmental factors on the gene expression controlling these traits (Danbe et al., 2018). The coefficient of variation simply indicates the extent of total variability present in a trait (Béninga, 2007). Coefficients of genotypic and phenotypic variation were low below 11%, moderate between 11 and 20% and high above 20% (Sumathi et al., 2010). High phenotypic and genotypic coefficients of variation were observed for many traits except for the leaf length and width traits. For these two traits, the mean square of error is greater than the mean square of genotype. It could be deduced that these two characters do not explain the existing genetic variability within the varieties. Traits (fruit weight, fruit length, and fruit yield per unit plot) with high phenotypic coefficient of variation indicate a greater influence of environmental factors. In general, estimates of the phenotypic coefficient of variation were higher than estimates of the genotypic coefficient of variation for many traits. These results suggest that apparent variation is not only due to genotypes, but also to environmental influence (Ibrahim, 2012). But the little difference between these two coefficients shows that these traits were very little influenced by the environment (Lakshmana et al., 2009). Similar results were reported by Abdel-Rahman et al. (2011) on pumpkin, Ibrahim (2012); Tomar et al. (2008) and Torkadi et al. (2007) on melon, Dar and Sharma (2011) and Singh and Singh (2018) on tomato.

The estimation of heritability is of paramount importance and accurately indicates the expected heritable gain (Béninga, 2007). According to Johnson et al. (1955), heritability is high above 50%, low below 20%, and medium between 20% and 50%. In this study, broad heritability ranged from 0 to 99.44%. High heritability of traits reflects the poor influence of environmental factors on their expression (Danbe et al., 2018). In this case, the phenotype truly expresses the varieties genotype (Visscher et al., 2008). Therefore, these traits can be improved by selection (Ibrahim, 2012). These results more or less agree with those of Abdein et al. (2017) on pumpkin and Ibrahim (2012) on melon. These authors claim to have obtained very high broad-sense heritability. According to Ibrahim (2012), high heritability does not mean high genetic advance for a particular quantitative trait. Johnson et al. (1955) reported that the effectiveness of selection depends not only on broad-sense heritability, but also on genetic advance. Therefore, genetic advance was also estimated as a percentage. In the present study, high heritability combined with high genetic advance was found in traits such as average fruit weight, fruit diameter and fruit length. This indicates that these three traits were primarily governed by additive gene action (Johnson et al., 1955). Therefore, these traits can be selected and improved to increase the fruit and seed yield of pumpkin varieties in Benin. According to Béninga (2007), high heritability accompanied by low genetic advance for days to 50% emergence indicates the predominance of non-additive genetic actions that could be exploited through heterosis selection. These results are in harmony with those obtained by Abdel-Rahman et al. (2011), Abdein et al. (2017) and Chaudhari et al. (2017) on pumpkin, Ibrahim (2012) on melon.

## 5. Conclusion

Phenological and growth parameters through characterization of morphological traits, flowers, fruits and seeds of pumpkin landraces has revealed agro-morphological and genetic variability. Descriptors related to fruits and seeds could not be collected on variety V1.6 due to fruit abortion. The three PCA dimensions explain 87.86 % of the variability. The descriptors fruit color and shape, leaf color and seed color showed phenotypic variability. The varieties were grouped into three distinct classes. The data from this study showed the possibility of improving pumpkin by selection for the traits such as average fruit weight, fruit length and diameter. The high heritability and genetic advance of these traits reflect the additive action of genes in trait expression. Therefore, these data could be used in breeding programmes in Benin for pumpkin productivity and, thus contributing to improved food security and nutrition. This study deserves to be deepened by a molecular characterization taking into account a very high number of accessions.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix

(See Table 10).

**Table 10**
Distribution of accessions into varieties.

| Variétés | Nombres accessions | Numéro accessions |
|---|---|---|
| $V_{0.1}$ | 46 | $A_{1.1}$, $A_{1.2}$, $A_{1.3}$, $A_{1.4}$, $A_{1.5}$, $A_{1.6}$, $A_{1.7}$, $A_{1.8}$, $A_{1.9}$, $A_{1.10}$, $A_{1.11}$, $A_{1.12}$, $A_{2.13}$, $A_{2.16}$, $A_{2.17}$, $A_{2.18}$, $A_{2.20}$, $A_{2.21}$, $A_{2.22}$, $A_{2.23}$, $A_{2.24}$, $A_{2.25}$, $A_{3.27}$, $A_{3.28}$, $A_{3.29}$, $A_{3.30}$, $A_{3.32}$, $A_{3.33}$, $A_{3.34}$, $A_{3.36}$, $A_{3.37}$, $A_{3.38}$, $A_{3.39}$, $A_{3.40}$, $A_{3.41}$, $A_{3.42}$, $A_{3.43}$, $A_{3.44}$, $A_{4.47}$, $A_{4.48}$, $A_{4.53}$, $A_{4.54}$, $A_{4.55}$, $A_{4.56}$, $A_{4.57}$, $A_{4.58}$ |
| $V_{0.2}$ | 14 | $A_{2.14}$, $A_{2.15}$, $A_{2.19}$, $A_{2.26}$, $A_{3.31}$, $A_{3.35}$, $A_{4.45}$, $A_{4.46}$, $A_{4.49}$, $A_{4.50}$, $A_{4.51}$, $A_{4.52}$, $A_{4.59}$, $A_{4.60}$ |
| $V_{1.3}$ | 1 | $A_{6.120}$ |
| $V_{1.4}$ | 2 | $A_{6.89}$, $A_{6.105}$ |
| $V_{1.5}$ | 21 | $A_{5.63}$, $A_{5.64}$, $A_{5.68}$, $A_{5.69}$, $A_{5.80}$, $A_{6.90}$, $A_{6.91}$, $A_{6.92}$, $A_{6.93}$, $A_{6.94}$, $A_{6.95}$, $A_{6.96}$, $A_{6.97}$, $A_{6.98}$, $A_{6.99}$, $A_{6.100}$, $A_{6.101}$, $A_{6.102}$, $A_{6.103}$, $A_{6.104}$, $A_{6.106}$ |
| $V_{1.6}$ | 36 | $A_{5.61}$, $A_{5.62}$, $A_{5.65}$, $A_{5.66}$, $A_{5.67}$, $A_{5.70}$, $A_{5.71}$, $A_{5.72}$, $A_{5.73}$, $A_{5.74}$, $A_{5.75}$, $A_{5.76}$,$A_{5.77}$, $A_{5.78}$, $A_{5.79}$, $A_{6.81}$, $A_{6.82}$, $A_{6.83}$, $A_{6.84}$, $A_{6.85}$, $A_{6.86}$, $A_{6.87}$, $A_{6.88}$, $A_{6.107}$, $A_{6.108}$, $A_{6.109}$, $A_{6.110}$, $A_{6.111}$, $A_{6.112}$, $A_{6.113}$, $A_{6.114}$, $A_{6.115}$, $A_{6.116}$, $A_{6.117}$,$A_{6.118}$,$A_{6.119}$ |

This grouping was performed based on two criteria: in the South, based on the local names that producers gave to the fruits and in the North based on the description of the varieties (shape and color of the fruits at physiological maturity).

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