Title:

Morphological and allozyme variation in a collection of *Lagenaria siceraria* (Molina) Standl. from Côte d'Ivoire

Summary title:

Genetic diversity of *Lagenaria siceraria*

Abstract:

This study described the intraspecific variation of thirty edible-seed *Lagenaria siceraria* germplasm accessions from the University of Abobo-Adjamé. These accessions were collected from three (Centre, East and South) geographical zones of Côte d'Ivoire. Selection based on seed size by the farmers has resulted in subdividing the species into two cultivars: large-seeded and small-seeded. The morphological diversity study of the collection included 18 accessions and 24 traits. The multivariate analysis of variance (MANOVA) showed a significant difference between the two groups of cultivars. Principal component analysis on 13 traits pointed out variations among individuals, mainly on the basis of flower, fruit, and seed size. Dendrogram with UPGMA method allowed clustering of the cultivars. The genetic structure analysis among accessions using allozyme makers showed the following values: 18.95 % for the proportion of polymorphic loci (*P*), 1.21 for the number of alleles (*A*) and 0.053 for observed heterozygosity (*Ho*). The level of the within accessions genetic diversity (H_S = 0.188) was higher than the genetic variation among accessions (D_{ST} = 0.082). The estimates of *F*-statistics indicated a low level of genetic differentiation between accessions (F_{ST} = 0.298). Such a value suggested that *L. siceraria* maintains about 30 % of its genetic variation among accessions. Nei genetic distances between the two cultivars were also low (0.002), indicating that cultivars were genetically similar enough to belong to the same genetic group.

Keywords: *Lagenaria siceraria*; cucurbit; isozyme variation; morphological variation; cultivar.

Variation morphologique et enzymatique de la collection de *Lagenaria siceraria* (Molina) Standl de la Côte d'Ivoire

Résumé :

Cette étude décrit la variabilité intraspécifique de trente accessions de *Lagenaria siceraria* à graines consommées de la collection de l'Université d'Abobo-Adjamé. Ces accessions proviennent de trois zones géographiques de la Côte d'Ivoire (Centre, Est et Sud). La sélection opérée par les paysans sur la base de la taille des graines subdivise cette espèce en deux cultivars : le cultivar à petites graines et le cultivar à grosses graines. Les études morphologiques impliquent 18 accessions et 24 caractères morphologiques. L'analyse multivariée de variance (MANOVA) a montré une différence significative entre les deux cultivars. L'analyse en composantes principales portant sur 13 caractères morphologiques a révélé une variation entre les individus analysés, principalement sur base de la taille des fleurs, fruits et graines. Le dendrogramme construit avec la méthode UPGMA a permis un regroupement des cultivars. L'analyse de la structure génétique basée sur les marqueurs allozymiques a donné les valeurs suivantes : 18.95 % pour le pourcentage de loci polymorphes (*P*), 1.21 pour le nombre moyen d'allèles (*A*) et 0.053 pour l'hétérozygotie observée (*Ho*). La diversité génétique intra-accession (H_S = 0.188) est plus élevée que la diversité génétique inter-accessions (D_{ST} = 0.082). Les estimations des *F*-statistiques indiquent un faible niveau de différentiation génétique entre les accessions (F_{ST} = 0.298), suggérant seulement 30% de variation entre les accessions. La distance génétique de Nei entre les deux cultivars est également faible (0.002), indiquant que les deux cultivars sont génétiquement similaires et pourraient appartenir au même groupe génétique.

Mots clés : *Lagenaria siceraria*; cucurbites; variabilité enzymatique; variabilité morphologique; cultivar.

1. Introduction

Lagenaria siceraria (Molina) Standl is an important crop in tropical Africa. Some cultivars of this species with others of the Cucurbitaceae family are called Egusi, in Benin, Nigeria, and pistachio in Côte d'Ivoire. Although these crops are neglected and underutilized in Africa, their cultivation is widespread in most West African countries (Achu *et al.*, 2005; Enoch *et al.*, 2006; Zoro Bi *et al.*, 2006).

These cucurbits are prized for their oilseeds and are consumed as soup thickener. Their seeds are good sources of lipids and proteins (Achu *et al.*, 2005; Loukou *et al.*, 2007). Like other neglected and underutilized crops in Africa, cucurbits have numerous agronomic and economic potentials. They are well adapted to extremely divergent agroecosystems and to various cropping systems. They are also characterized by minimal inputs. Increased production and use of these cucurbits can result in food security and diversify small farmer's income (Chweya, Eyzaguirre, 1999; Williams, Hap, 2002).

In developing countries, the exploitation of local resources is certainly a way to achieve the objective of food security, particularly for a fast-growing population. However this requires the preservation and availability of a high level of genetic diversity of these resources (Given, 1987). This conservation constitutes a challenge for crops such as cucurbits that are endangered and have been neglected by national research programs. To this regard prerequisite information on genetic pattern is needed to make appropriate decision.

In spite of the economic importance of edible seeds of *L. siceraria* in Côte d'Ivoire, knowledge of its genetic diversity and differentiation is very poor. In order to fill up this gap, it is crucial to collect the genetic resource available at country level and to characterise the genetic diversity of the collected accessions on the basis of several markers.

The objective of this study is to characterise the collection of *L. siceraria* from different geographical zones in Côte d'Ivoire and available at Abobo-Adjamé University. The variability

will be assessed among accessions and cultivars, using in particular morphological and enzyme markers. In our investigations, we decided to use biochemical markers, such as allozymes, because they are relevant to identify the heterozygosity and to better know the genetic structure of the species. Results obtained from this characterisation would contribute to sample fruits and seeds in the most representative areas, to conduct further molecular analysis and to define appropriate sampling strategies for the conservation of *L. siceraria* genetic resources in Côte d'Ivoire.

2. Material and Methods

2.1 Plant material and collection sites

Thirty accessions (**Table 1**) of *L. siceraria* were selected from the germplasm collection maintained at the University of Abobo-Adjamé (Abidjan, Côte d'Ivoire). An accession is a sample collected in one field or obtained from one farmer's stock. The selected accessions were identified by alpha-numeric codes. A minimum distance of 25 km separated two consecutive collecting sites in each zone. The seed samples were collected mainly in three geographical zones (South, East, and Centre) of Côte d'Ivoire. The geographical coordinates and ecological traits of sites of the collecting missions are as follow (Zoro Bi *et al.*, 2005):

- The southern zone which is localized between latitudes 4°41 N-6°00 N and longitudes 4°00 W-7°30 W. In this zone, rainfalls are abundant (annual mean > 2000 mm) and mean annual temperature is 28°C, with annual amplitude of 5-10°C. Vegetation is mainly represented by the tropical rain forest, with mangrove on the coastal side.

- The eastern zone which is limited by latitudes 6°00 N-8°00 N and longitudes 3°00 W-5°00 W. This zone is characterized by the transitional woodland savannas, with several blocks of semi-deciduous forests. Rainfalls vary from 875 to 1910 mm, with an annual mean of 1250 mm; the annual mean temperature is 27°C.

- The central zone which is limited by latitudes 6°00 N-8°00 N and longitudes 5°00 W-7°00 W. Annual rainfalls vary from 800 to 1400 mm, with an annual mean of 1200 mm; the annual mean temperature is 27°C. The vegetations are made of various woodland savannas with extended ranges of herbaceous areas.

Accessions used in this study are from two landraces known as small-seed and large-seed. For the morphological study 18 accessions with 12 small-seeded and 6 large-seeded accessions were used. For allozyme analyses, thirteen small-seeded and six large-seeded accessions were used. The number of seeds used per accession for both morphological and allozyme analyses are indicated in **Table 1**.

2.2. Morphological characterisation

2.2.1. Study site and experimental design

The study was carried out in Abidjan district from May 2007 to November 2007. The experimental site was located in Akouedo village in Abidjan suburb, between latitudes 5^{o}17'N-5^{o}31'N and longitudes 3^{o}45'W-4^{o}22'W. In this zone, rainfalls are abundant (annual mean > 2000 mm) and the mean temperature is 28°C, with annual amplitude of 5-10°C. The field lay out was a completely randomised design, with three replications. Each replicate consisted of a 30 m x 27 m plot containing 90 plants (i.e., the eighteen accessions), each accession being represented by 5 plants. The planting distance was 3 m between and within rows with 1.5 m of edges. Two consecutive plots were spaced by 3 m. Manual weeding was carried out during plant development.

2.2.3. Morphological data and analyses

Morphological diversity was characterized using standard descriptors for cucurbits: twenty-four characters were chosen among those published for *Citrullus lanatus* (Thunb.) Mansf (Maggs-Kölling *et al.*, 2000) and *Lagenaria siceraria* (Morimoto *et al.*, 2005) (**Table 2**).

Multivariate analysis of variance (MANOVA) was performed with SAS software package (SAS, 1999) to investigate the difference between the two cultivars. Principal Components Analysis (PCA) with Statistica software package (Statistica, 1995) was applied to further describe morphological variation among accessions and between cultivars. PCA is particularly relevant to describe dataset by combining correlated variables into factors. Prior to PCA, the average values of the traits were standardised according to the formula:

$$standardised\ data = \frac{sample\ estimates - mean}{standard\ deviation}$$

This standardisation is required to reach the same scale for all the characters (Dagnelie, 1998). Data were averaged across individuals for each accession, and UPGMA (Unweighted Pair Group Method with Arithmetic) dendrograms were computed by Statistica software package (Statistica, 1995) to describe the relationships between accessions. To determine morphological differences among accessions from the three zones of the collecting missions, multivariate analysis of variance was carried out.

2.3. Allozyme analysis

2.3.1. Electrophoresis

Isozyme assays were conducted on cotyledonary tissue. The selected seeds were sown in field and cotyledons were taken from each 3-4 day old seedling. A quantity of 0.01 g of cotyledonary tissue from each seedling was ground in 0.045M TRIS-HCl, pH 7.1 (Knerr *et al.*, 1989; Staub *et al.*, 1997). Plant tissue was held at -20°C until the horizontal starch gel electrophoresis was performed according to Zoro Bi (1999).

Gels were prepared using 60 g of hydrolysed potato starch from Sigma **(**Sigma # S-5651**)** and 15 g of sucrose that were dissolved in 600 mL of buffer. The continuous morpholine-citrate, pH 6.1 was employed for electrophoresis.

Four enzyme systems were used to study electrophoretic variation: alcohol dehydrogenase (ADH, E.C. 1.1.1.1), malate dehydrogenase (MDH, E.C. 1.1.1.37), malic enzyme (ME, E.C. 1.11.1.7), shikimate dehydrogenase (SKDH, E.C. 1.1.1.25). The techniques for histochemical staining procedures were those reported by Zoro Bi (1999) with Lima bean (*Phaseolus lunatus* L.). For each enzymatic system, the presumed loci were numbered in ascending order from the anode. For each isozyme, the most common allele was referred to as 100 and the other alleles were named according to their migration distance in millimetres using the standard (Koenig, Gepts, 1989).

2.3.2. Data analysis

Allozymes data analysis was performed using the computer program GENSURVEY (Vekemans, Lefèbvre, 1997). Statistics of genetic diversity within and among accessions were calculated: percentage of polymorphic loci at 5% level (*P*), mean number of alleles per locus (*A*), observed heterozygosity (*Ho*), genetic diversity (*He*) corrected for small sample size (Nei, 1978) and Wright's *F* [$F = (1-Ho/He)$], the inbreeding coefficient which measures the deviation of the population genotypic composition from Hardy-Weinberg (H-W) expectations. Deviation from Hardy-Weinberg equilibrium at each locus in each accession and heterogeneity in alleles frequencies among accessions were tested by chi-square (χ^2) using the computer program GENEPOP (Raymond, Rousset, 1995). Genetic structure of the accessions was investigated using Nei's genetic diversity analysis on polymorphic loci (Nei, 1973). The genetic differentiation among accessions and cultivars was also estimated by partitioning the total genetic diversity (H_T) into gene diversity within accessions or cultivars (H_S) and among accessions or cultivars (D_{ST}) i.e. $H_T = H_S + D_{ST}$. The degree of genetic differentiation (G_{ST}) was calculated as D_{ST}/H_T. Wright's fixation index within population (F_{IS}), among populations (F_{ST}) and total genetic differentiation (F_{IT}) were calculated to demonstrate the relative distribution of genetic variation among and within accessions and cultivars (Wright, 1965). The number of

migrants into accessions per generation (*Nm*) was estimated (Wright, 1951). Cultivars divergence was estimated using Nei's genetic distance (Nei, 1978).

3. Results

3.1. Morphological variation

Comparison of morphological traits using a multivariate analysis of variance (MANOVA) showed a significant difference between the two cultivars of *Lagenaria siceraria* (F = 45.21; P < 0.001). As shown in **Table 3**, this difference is based on sixteen traits: male flower diameter (MFD), female flower diameter (FFD), female flower peduncle length (FFLP), limb length (LL), limb width (LWI), plant length (PL), number of branches (BN), days to fruit maturity (FM), fruit weight (FWE), seed cavity diameter (SCD), fruit width (FWI), fruit length (FL), number of seed/fruit (NS), seed length (SL), 100-seeds weight (100-SWE), tegument percent (TP). The large-seeded cultivar gave the highest values for all these traits with the exception of the number of branches from the central taproot (BN) and fruit maturity (FM).

A minimum list of descriptors was selected from the establishment of a correlation matrix (**Table 4**). When we observed a high positive correlation (> 0.70) between two variables, only one was retained to avoid redundancy (**Table 4**). Consequently 13 variables: seed emergence time (ET), days to the first tendril appearance (TT), days to the first female flower (FF), male flower diameter (MFD), female flower peduncle length (FFPL), days to the first fruit maturity (FM), fruit width (FWI), number of seeds per fruit (NS), seed length (SL), seed width (SWI) 100-seeds weight (100-SWE), harvest index (HI), and tegument percent (TP) were used for the morphological characterisation of the accessions.

The first two principal components accounted for 46.398% of the total variability (eigenvalues > 1). Male flower diameters (MFD), fruit width (FWI), number of seeds (SN), 100-seeds weight (100-SWE) are correlated with the first component (representing 32.762 % of the total variability). These variables are negatively correlated with PC1. The second component

(13.636% of the variability) is mainly linked to the seed emergence times (ET) with a positive correlation. **Figure 1** shows the correlation circle of the two first principal components analysis.

On the basis of these traits, two morphological groups were formed (**Figure 2**). The group 1 included individuals from the small-seeded cultivar, characterised by late maturity and small size of female flower peduncle. The group 2 consisted of individuals of large-seeded cultivar, characterised by large diameter of petals opening, and heavy fruits of large sections with many seeds per fruit.

UPGMA cluster analysis of morphological differentiation among accessions computed from a matrix of pairwise Euclidean distances showed two distinct groups (**Figure 3**). Group I included all accessions belonging to the large-seeded cultivar and two accessions (NI174, NI091) from the small-seeded cultivar. Group II included only accessions from the small-seeded cultivar. Consequently, dendrogram analysis showed that the two cultivars were morphologically dissimilar since only two accessions (11%) were inappropriately clustered.

The multivariate analysis of variance (MANOVA) applied to the small-seeded cultivar obtained from the South and East showed significant differences between the two zones (F = 23.25; P < .001). This result is mainly due to 10 characters out of 24 measured: emergence time (ET), tailspins time (TT), male flowering time (MF), female flowering time (FF), male flower diameter (MFD), limb width (LWI), plant length (PL), days to fruit maturity (FM), number of fruits/plant (FN), seed cavity diameter (SCD) (**Table 5)**. For the large-seeded cultivar obtained from the Centre and the East, the multivariate analysis also showed a significant difference between the two areas (F = 21.88; P < 0.001). This difference is due to seven characters out of the 24 measured: emergence time (ET), male flowering time (MF), limb length (LL), limb width (LWI), days to fruit maturity (FM), seed cavity diameter (SCD), fruit width (FWI) (**Table 5**). These data suggest that the morphological differences between accessions belonging to the same cultivar but collected in distinct geographical zones are not important.

3.2. Allozymes variation and genetic structure

Among the four enzyme systems investigated, ADH revealed three loci with a total of four alleles, ME showed only one locus with one allele, MDH showed two loci with three alleles and SKDH revealed one locus with two alleles. Two loci (Adh-2 and ME) produced unclear patterns and were discarded. Thus five loci were taken into account for further analysis. Two loci were polymorphic and diallelic: *Mdh-2* and *Skdh*, whereas *Mdh-1*, *Adh-1* and *Adh-3* were monomorphic.

The proportion of polymorphic loci per accession (*P*) varied from 0 (e.g.., NI060) to 40% (e.g.., NI388) with a mean of 18.95%. The mean number of alleles (*A*) per locus varied from 1.0 (e.g., NI210) to 1.4 (e.g., NI248) with an average of 1.2, indicating a low allelic richness. A low genetic diversity was also observed. Indeed, the average observed heterozygosity was 0.053 ranging from 0 (e.g., NI249) to 1.4 (e.g., NI241) and the average expected heterozygosity (*He*) was 0.073 ranging from 0 (e.g., NI329) to 0.202 (e.g., NI276). The observed mean heterozygosity (*Ho* = 0.053) was similar to the average expected heterozygosity. This suggested that the populations were at Hardy-Weinberg equilibrium. According to the Chi-square test, 66.67% of the accessions were not significantly different from zero ($\alpha = 0.05$) confirming the Hardy-Weinberg equilibrium hypothesis. This indicated that most accessions did not deviate from random union of gametes.

The total gene diversity (H_T) was 0.270 **(Table 6)**. For *Mdh-2* locus, the largest proportion of diversity was attributable to the within-accessions component (H_S = 0.243; D_{ST} =0.022). However, for *Skdh* locus, difference between the within-accessions and the among-accessions components of diversity (H_S = 0.133; D_{ST} = 0.141) was not significant. On the other hand, the coefficient of gene differentiation (G_{ST}) between accessions was estimated at 29.8%, indicating that most of the total genetic diversity in this species was within accessions rather than among accessions. From **table 6**, the mean inbreeding index (F_{IT}) for the 19 accessions was 0.505. This relatively high value showed important deficiency in heterozygosity. The average F_{IS} of 0.306 indicated a slight deficit of heterozygotes within accessions. On the other hand, the

average F_{ST} of 0.299 showed a low genetic differentiation among accessions. The mean number of migrants per generation (*Nm*) based on Wright's equation was 0.963. Such a value indicated that on average, one individual migrated in a given accession (seed stock or field) per generation.

At the cultivar level (**Table 7**), the average of total gene diversity (H_T) and intra-cultivar genetic diversity (H_S) were 0.300 and 0.298, respectively. The inter-cultivar genetic diversity (D_{ST}) and the coefficient of gene differentiation among cultivars (G_{ST}) were 0.002 and 0.007 respectively. According to these results, the degree of genetic differentiation between cultivars was very low (only 0.7%). *F*-statistics for the two cultivars indicated a relatively high mean inbreeding index (F_{IT} = 0.569), showing an important deficiency in heterozygosity. A high value was also obtained for F_{IS} (0.567). The proportion of the total genetic diversity among cultivars (F_{ST} = 0.006) was very low. A low value was also estimated for Nei genetic distances between the two cultivars, with D = 0.002, indicating that cultivars were genetically similar enough to belong to the same genetic group.

4. Discussion

4.1. Morphological variation

Our study showed morphological heterogeneity within the collection of *L. siceraria* of Abobo-Adjame University in Côte d'Ivoire. According to the multivariate analysis of variance, difference between the two groups of accessions with small and large seeds size was significant. Fruit and seed shape and size are reported to be highly variable in the genus *Lagenaria* (Bisognin, 2002). In most rural African communities, the landraces of *L. siceraria* are generally distinguished by their fruit size and shape, and designated by common name according to these morphological differences (Morimoto *et al.*, 2005). In Côte d'Ivoire, farmers refer to seed size when designating cultivars of edible-seeded *L. siceraria* (Zoro Bi *et al.*, 2006). Also, in the

cucurbit family, the significant contribution of fruit and seed traits to morphological variability has been reported for watermelon (Maggs-Kölling *et al.*, 2000; Gusmini, 2003), bottle gourd (Morimoto *et al.*, 2005) and squash (Paris, 2001).

However, in estimating the relative contribution of the different traits in the overall phenotypic variation among the 18 accessions, the first two principal components (PCs) explained 46.398% of the diversity obtained from the 13 selected traits. The variability explained by the first PC alone (32.762%) was mainly due to variations in fruit (FWI), seed (NS, 100-SWE) and flower sizes (MFD). In the cucurbit family, the significant contribution of fruit and seed traits to morphological variability has been reported for watermelon (Maggs-Kölling *et al.*, 2000; Gusmini, 2003). In spite of a variability explained by the first two principal components inferior to 50%, a relative separation was observed between the two cultivars (**Figure 2**). The presence of one individual (NI276-L5) from the large seeded accession (NI276) in the group 1 made up of small-seeded accessions could result from accidental mixture by farmers or collectors. However Morimoto *et al.* (2005) reported the difficulty to classify the landraces of *L. siceraria* into distinct groups, because of the high and continuous morphological variation in the species. The difference between the two studies results from the fact that we analyse only two edible-seed cultivars while Morimoto *et al.* (2005) analysed several forms of *L. siceraria* and its wild relatives.

A strong positive correlation between the weight of the fruits and the number of seeds was observed in our study. The same positive correlation was also noted in watermelon (Nerson, 2002). Therefore the fruit weight of *L. siceraria* could be used as a good criteria to select individuals with higher number of seeds. This result is congruent with findings of Enoch *et al.* (2006).

The phenotypical variation between the two cultivars of *L. siceraria* from Côte d'Ivoire seemed to be uncorrelated to the different regions where samples were collected. Two hypotheses could explain such a result: 1) the occurrence of an important seed flow; 2) the

similarity of the cultivation history among the collecting zones. According to Montes-Hernandez and Eguiarte (2002), human activities significantly buffer genetic variability between plants occurring in different geographical regions. This argument could be supported by the fact that most often farmers exchange seeds among themselves. Fruits and seeds are selected to constitute planting material for the next season. In addition, parts of the harvested products are marketed and contribute to the movement of seeds between regions.

4.2 Genetic diversity

In general, the intra-accession polymorphism indices estimated in this study were low (*P* = 18.95%; *A* = 1.21; *He* = 0.073). These indices are similar to those reported by Decker-Walters *et al*. (1990) on *Cucurbita maxima* Duch. (*P* = 11.5%; *A* = 1.43 ; *He* = 0.039) and on *Cucurbita pepo* L. (*P* = 19.3%; *A* = 2.24; *He* = 0.068). However values from *L. siceraria* were smaller than those calculated in four *Cucurbita* taxa by Montes-Hernandez and Eguiarte (2002): *P* = 100%; *A* = 2.08; *He* = 0.407 for *C. argyrosperma* ssp. *sororia* Huber., *P* = 93%; *A* = 2.5; *He* = 0.391 for *C. argyrosperma* ssp. *Argyrosperma* Huber., *P* = 97%; *A* = 2.06; *He* = 0.496 for *C. moschata* Duch. and *P* = 92%; *A* = 2.08; *He* = 0.366 for *C. pepo*. The lack of RAPD diversity (known to be highly polymorphic) was also found in southern African landraces of *L. siceraria* (Decker-Walters *et al.,* 2001) and in several accessions of two *Citrullus* species: *C. lanatus* and *C. colocynthis* (L.) Schrad. (Levi *et al.,* 2001). These results confirm our study and pointed out a narrow genetic basis in this species.

In spite of the very low number of loci and individuals analysed in this study, two hypotheses could explain the low allelic richness: the selection favouring homozygote individuals and the founder effect. However, the reproductive biology of indigeneous cucurbit avoiding selfing makes the first hypothesis (homozygotes selection) improbable. Indeed, *L. siceraria* is monoecious (staminate and pistilate flowers are separated) and flowers are pollinated by various insects. Thus this species is bound to experiment insect-mediated cross pollination

which promotes random-mating, buffering homozygotes selection (Wright, 1951). This argument was supported by the high percentage of accessions (66.67%) not significantly different from Hardy-Weinberg equilibrium. The last hypothesis: the founder effect, due to farmer's seeds selection approach, is most likely the major cause of the low allelic richness. In particular, in the collecting sites, generally a low number of seeds is usually taken from the farmer's stock, or are obtained from local markets, resulting to the genetic variability depletion (Nei *et al.*, 1975).

In our study, trends of variation were not similar with the application of the two markers: morphology and allozymes. Differentiation among cultivars was much higher for morphological traits than for allozymes. Djè *et al.* (1998) found similar results for sorghum landraces of northwestern Morocco. During the selection process, farmers and breeders favour phenotypic diversity, in order to meet varietal adaptation to diverse cropping systems and consumer's requirements. Considering the low genetic variability observed in our *L. siceraria* collection, one can assume that allozyme markers were not powerful enough to capture the genetic basis of the morphological variation, probably due to complex and multigenic inheritance of fruit and seed traits in cucurbits (Guner, Whener, 2004).

4.3 Genetic structure and gene flow

The mean within-accession gene diversity index (H_S = 0.188) was similar to those reported by Hamrick and Godt (1997) for cross-pollinated plants. Indeed, these authors showed that intra-population gene diversity index varied between 0.103 and 0.266. The degree of genetic differentiation between accessions (G_{ST} = 0.298) was also similar to those reported for many cross-pollinated plants, G_{ST} = 0.234 (Hamrick, Godt, 1997). The low values of G_{ST} in the present study could result from the high level of gene flow among accessions, in particular the frequent seed exchange among farmers. However, our G_{ST} value was lower than the value estimated in

Schizopepon bryoniaefolius Maxim (G_{ST} = 0.68), a wild Cucurbitaceae (Akimoto *et al.*, 1999). Differences between our study and Akimoto *et al.* (1999) study could be attributed to the different floral biology between the two species. *L. siceraria* is monoecious (males and females flowers are separated) and predisposed to predominantly outcrossing, while *Schizopepon bryoniaefolius* is androdioecious (with hermaphrodites flowers) which favours selfcrossing.

On average, F_{IS} showed a significant deficiency of heterozygosity ($F_{IS} > 0$) for all accessions. The proportion of the total genetic diversity found among accessions (F_{ST} = 0.299) was higher than that described by Hamrick and Godt (1989) in animal-pollinated species (F_{ST} = 0.187), and almost similar to the larger group including the cross-pollinated plants (F_{ST} = 0.234). The gene flow estimation (Nm = 0.963) and the low genetic differentiation between accessions (F_{ST} = 0.299) confirmed the high rate of gene exchange between accessions.

5. Conclusion

This study has allowed a better knowledge of the cultivated *L. siceraria* collection of the University of Abobo-Adjamé (Abidjan, Côte d'Ivoire). Morphological characterisation showed significant difference between two cultivars: small-seeded and large-seeded cultivars. Isozyme electrophoresis data indicated a lower genetic heterogeneity among accessions than within accessions. Therefore, during the collecting missions, it is recommended to collect a sufficient number of seeds or fruits within each accession, which is better than attempting to collect as many accessions as possible. However the low number of analysed loci and individuals suggest that analysis of additional accessions is required before a definitive conclusion can be performed. On the other hand, many questions still remain to be solved. A in-depth knowledge of reproductive biology of this species and the use of DNA markers are required. Indeed, molecular markers can be an effective means to determine genetic relatedness among cultivars and among accessions present in *L. siceraria* germplasm collection. These markers are generally more polymorphic than isozymes. Results obtained in studying floral biology and molecular variability will help us to define sampling strategies and optimum sample size for the management of *L. siceraria* genetic resources.

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(37 Réf)

Table 1.

Codes	Cultivars	Collection site	Collection zone	Geographic coordinates	Sample size for morphological analysis	Sample size for allozyme analysis
NI060[a]	small seeds	Assiè-Assasso	East	6°39'N-4°11'W	-	10
NI090[b]	small seeds	Assiè-Koumassi	East	6°42'N-4°10'W	5	-
NI091[b]	small seeds	Assiè-Assasso	East	6°39'N-4°11'W	5	-
NI106[b]	large seeds	Laviara	Centre	7°00'N-6°30'W	5	-
NI109[b]	small seeds	Assiè-Assasso	East	6°39'N-4°11'W	5	-
NI157[a]	small seeds	Assiè-Koumassi	East	6°42'N-4°10'W	-	10
NI174	small seeds	Assiè-Assasso	East	6°39'N-4°11'W	5	11
NI185	small seeds	Agoua	South	5°46'N-3°58'W	5	12
NI199[b]	small seeds	Ahouakoi	South	5°46'N-3°55'W	5	-
NI200[a]	small seeds	Ahouakoi	South	5°46'N-3°55'W	-	10
NI210[a]	small seeds	Akin	South	5°42'N-3°32'W	-	6
NI215[a]	small seeds	Danguira	South	5°39'N-3°45'W	-	8
NI219[b]	small seeds	Danguira	South	5°39'N-3°45'W	5	-
NI224[b]	small seeds	Abié	South	5°49'N-3°56'W	5	-
NI227	small seeds	Danguira	South	5°39'N-3°45'W	5	17
NI228[b]	small seeds	Danguira	South	5°39'N-3°45'W	5	-
NI239	small seeds	Danguira	South	5°39'N-3°45'W		10
NI241[a]	small seeds	Abié	South	5°49'N-3°56'W	-	10
NI248[a]	small seeds	Kodiossou	South	5°45'N-3°46'W	-	8
NI249	small seeds	Danguira	South	5°39'N-3°45'W	5	15
NI260[b]	small seeds	Kodioussou	South	5°45'N-3°46'W	5	-
NI276	large seeds	Bondoukou	East	7°05'N-5°03'W	5	10
NI283[b]	large seeds	Koumala	East	7°05'N-5°01'W	5	-
NI304	large seeds	Laoudiba	East	7°04'N-5°00'W	5	10
NI328[a]	large seeds	Kouassi N'Dawa	Centre	7°00'N-6°33'W	-	8
NI329[a]	large seeds	Kouassi N'Dawa	Centre	7°00'N-6°33'W	-	10
NI341[b]	large seeds	Flakiè	East	7°07'N-5°02'W	5	-
NI347[a]	large seeds	Flakiè	East	7°07'N-5°02'W	-	12
NI354	large seeds	Tefrôh	East	7°05'N-5°02'W	5	11
NI388[a]	small seeds	Grand Alépé	South	5°28'N-3°46'W	-	12

[a] Accessions used only for the analysis of allozymes
[b] Accessions used only for the analysis of morphological traits
Sample size is the number of seeds sown per accession

Table 2.

Characters	Codes	Type and period of observation	Sample size (n)	
			Large seed	Small seed
Emergence time (days) [a]	ET	Number of days from sowing to cotyledonary leaf opening	30	60
Tailspins time (days) [a]	TT	Number of days from sowing to first tailspin production	30	60
Male flowering time (days) [a]	MF	Number of days from sowing to first male flower opening per plant	30	60
Female flowering time (days) [a]	FF	Number of days from sowing to first female flower opening per plant	30	60
Male flower diameter (cm)	MFD	Diameter of petals, measured at flower opening	150	300
Female flower diameter (cm) [b]	FFD	Diameter of petals, measured at flower opening	150	300
Male flower peduncle length (cm) [b]	MFPL	Measured at flower opening	150	300
Female flower peduncle length (cm) [b]	FFPL	Measured at flower opening	150	300
Limb length (cm) [b]	LL	Measured after formation of the first fruit	150	300
Limb width (cm) [b]	LWI	Measured after formation of the first fruit	150	300
Plant length (m) [a]	PL	Measured 120 days after planting	30	60
Number of branches [a]	BN	Total number of branch per plant at 120 days after planting	30	60
Days to fruit maturity (days) [a]	FM	Number of days from sowing to first mature fruit per plant	30	60
Number of fruits/plant [a]	FN	Total number of fruits at plant maturity	30	60
Fruit weight (g) [b]	FWE	Weight of the mature fruits	150	300
Seed cavity diameter (cm) [b]	SCD	Measured on the mature fruits	150	300
Fruit width (cm) [b]	FWI	Measured on the mature fruits	150	300
Fruit length (cm) [b]	FL	Measured on the mature fruits	150	300
Number of seed/fruit [b]	NS	Total number of seeds per fruit	150	300
Seed length (cm) [c]	SL	Measured after drying seeds to 5% moisture content	600	1200
Seed width (cm) [c]	SWI	Measured after drying seeds to 5% moisture content	600	1200
100-seeds weight (g)	100-SWE	Weight of 100 seeds taken for a given individual after drying seeds to 5% moisture content	2 to 3	1 to 2
Harvest index [a,d]	HI	Measured after drying seeds to 5% moisture content per fruit.	150	300
Tegument percent (%) [c]	TP	Measured after drying seeds to 5% moisture content	600	1200

[a]: measurement on each plant per cultivar, [b]: measurement on five organs per plant, [c]: measurement on twenty seeds per fruit

[d]: harvest index calculated as the ratio of grain yield to aboveground dry matter and the weight of the fruits.

n is the number of measurements carried out for each trait

Table 3.

Characters	Cultivars		Parameters of statistical tests	
	Large seeds	**Small seeds**	***F***	***P***
ET (d)	6.00 ± 1.14	5.86 ± 1.31	0.16	0.688
TT (d)	30.52 ± 7.25	30.34 ± 5.56	0.01	0.912
MF (d)	47.45 ± 12.75	48.59 ± 11.23	0.12	0.726
FF (d)	73.85 ± 8.92	71.14 ± 16.44	0.48	0.489
MFD (cm)	7.31 ± 0.54	5.61 ± 0.68	93.80	<0.001
FFD (cm)	6.52 ± 0.48	5.02 ± 0.49	123.64	<0.001
MFPL (cm)	19.98 ± 3.62	18.67 ± 3.28	1.41	0.240
FFPL (cm)	6.14 ± 0.99	5.01 ± 0.99	16.97	<0.001
LL (cm)	19.18 ± 2.11	12.05 ± 4.42	47.98	<0.001
LWI (cm)	16.68 ± 3.25	13.19 ± 2.42	21.37	<0.001
PL (m)	11.26 ± 3.48	5.98 ± 2.27	48.11	<0.001
BN	1.33 ± 0.57	2.61 ± 1.27	18.84	<0.001
FM (d)	128.71 ± 4.34	140.01 ± 9.41	26.78	<0.001
FN	5.00 ± 3.62	3.94 ± 2.60	1.63	0.207
FWE (g)	1497.46 ± 379.98	668.61 ± 300.91	82.74	<0.001
SCD (cm)	9.57 ± 1.33	7.94 ± 1.27	21.20	<0.001
FWI (cm)	13.64 ± 1.67	11.30 ± 1.06	41.62	<0.001
FL (cm)	17.62 ± 3.64	11.97 ± 1.76	62.23	<0.001
NS	319.66 ± 58.68	203.80 ± 51.68	60.32	<0.001
SL (cm)	1.96 ± 0.16	1.78 ± 0.11	26.61	<0.001
SWI (cm)	1.26 ± 2.08	0.72 ± 0.04	2.43	0.125
100-SWE (g)	20.42 ± 3.38	12.75 ± 1.79	56.26	<0.001
HI	0.04 ± 0.01	0.042 ± 0.01	0.04	0.848
TP (%)	37.36 ± 4.52	32.08 ± 3.58	23.53	<0.001

d: number of days; cm: centimetre; g: gram, %: percentage

Table 4.

Variables	ET	TT	MF	FF	MFD	FFD	MFPL	FFPL	LL	LWI	PL	BN	FM	FN	FWE	SCD	FWI	FL	NS	SL	SWI	100-SWE	HI	TP
ET	1.000	0.320	0.463	0.295	-0.062	0.058	-0.206	0.258	-0.040	0.198	-0.080	-0.202	0.019	-0.158	0.011	-0.264	-0.124	0.105	0.090	0.126	0.017	0.087	0.246	-0.208
TT		1.000	0.459	0.329	-0.013	**0.701**	-0.219	0.216	-0.019	-0.021	-0.013	-0.271	-0.129	-0.280	0.099	-0.158	0.046	0.240	0.103	-0.103	-0.087	0.154	-0.072	0.005
MF			1.000	**0.721**	-0.121	0.114	-0.229	0.279	-0.058	0.255	-0.215	-0.311	0.245	-0.319	-0.057	-0.323	-0.284	0.008	-0.101	-0.099	-0.043	0.050	0.249	-0.115
FF				1.000	-0.145	0.157	-0.134	0.337	0.041	0.138	-0.044	-0.222	0.081	-0.148	0.039	-0.242	-0.155	0.218	-0.065	0.027	0.069	-0.013	0.084	-0.213
MFD					1.000	0.422	0.477	0.345	**0.763**	**0.704**	0.446	-0.257	-0.260	0.245	0.462	0.403	0.442	0.417	0.482	0.463	0.109	0.440	-0.029	0.643
FFD						1.000	0.379	0.437	0.484	0.468	0.494	-0.484	-0.360	0.112	0.451	0.423	0.476	0.401	0.417	0.420	0.128	0.437	0.149	0.408
MFPL							1.000	**0.754**	0.487	0.497	0.479	-0.050	0.105	0.303	0.403	0.348	0.326	0.306	0.206	0.361	-0.105	0.305	-0.276	0.342
FFPL								1.000	0.439	0.284	0.416	-0.430	-0.519	0.039	0.419	0.269	0.395	0.430	0.444	0.413	0.146	0.409	0.178	0.215
LL									1.000	0.462	0.428	-0.374	-0.314	0.308	0.491	0.497	0.452	0.491	0.458	0.449	0.174	0.451	0.053	0.411
LWI										1.000	0.298	-0.273	0.129	0.228	0.322	0.020	0.088	0.322	0.300	0.378	-0.107	0.292	0.090	0.334
PL											1.000	-0.397	**0.713**	0.441	0.478	0.494	0.420	0.447	0.434	0.408	-0.003	0.418	-0.058	0.546
BN												1.000	**0.756**	-0.113	-0.282	-0.289	-0.318	-0.291	-0.309	-0.207	-0.133	-0.318	-0.159	-0.213
FM													1.000	0.095	-0.520	-0.285	-0.480	-0.474	-0.531	-0.440	-0.143	-0.402	0.078	-0.156
FN														1.000	0.163	0.327	**0.795**	0.060	0.271	0.189	-0.092	0.052	0.007	0.349
FWE															1.000	0.490	0.496	0.401	**0.710**	0.423	0.081	**0.794**	-0.239	0.481
SCD																1.000	0.429	0.395	0.449	**0.721**	0.110	0.435	-0.007	0.380
FWI																	1.000	0.485	0.408	0.418	0.091	0.402	-0.091	0.377
FL																		1.000	0.481	0.430	**0.710**	0.497	-0.183	0.353
NS																			1.000	0.386	0.040	0.449	0.252	0.389
SL																				1.000	0.292	0.413	-0.103	0.251
SWI																					1.000	-0.007	-0.114	0.088
100-SWE																						1.000	0.069	0.394
HI																							1.000	-0.165
TP																								1.000

Values of correlations in bold are significant

Table 5.

Characters	Cultivar with small seeds		Parameters of statistical tests		Cultivar with large seeds		Parameters of statistical tests	
	Zone East	Zone South	*F*	*P*	Zone East	Zone Centre	*F*	*P*
ET (d)	6.60 ± 1.59	5.33 ± 0.73	10.32	0.003	5.666 ± 0.840	8.000 ± 0.000	22.170	<0.001
TT (d)	33.00 ± 7.05	28.43 ± 3.20	6.89	0.013	29.858 ± 7.650	34.533 ± 1.514	1.070	0.314
MF (d)	55.05 ± 14.83	43.97 ± 3.65	10.91	0.002	44.388 ± 10.691	65.800 ± 8.146	10.790	0.004
FF (d)	79.43 ± 21.10	65.21 ± 8.54	7.81	0.008	73.029 ± 7.257	78.80 ± 17.446	1.080	0.31
MFD (cm)	5.02 ± 0.57	6.03 ± 0.38	40.39	<0.001	7.254 ± 0.562	7.64 ± 0.295	1.310	0.267
FFD (cm)	4.89 ± 0.58	5.12 ± 0.40	2.17	0.150	6.483 ± 0.471	6.786 ± 0.589	1.010	0.328
MFPL (cm)	18.760 ± 3.15	18.95 ± 3.45	0.03	0.868	19.583 ± 3.213	22.366 ± 5.583	1.56	0.226
FFPL (cm)	6.18 ± 1.18	6.11 ± 0.87	0.04	0.848	5.094 ± 1.003	4.53 ± 1.006	0.80	0.381
LL (cm)	11.05 ± 6.68	12.76 ± 1.27	1.33	0.256	19.594 ± 1.989	16.706 ± 0.503	6.010	0.024
LWI (cm)	11.93 ± 2.96	14.08 ± 1.42	8.39	0.006	15.732 ± 2.375	22.366 ± 0.952	22.000	<0.001
PL (m)	4.59 ± 1.56	6.97 ± 2.20	12.77	0.001	11.672 ± 3.577	8.766 ± 1.101	1.870	0.186
BN	2.47 ± 1.12	2.71 ± 1.38	33.000	0.572	1.388 ± 0.607	1.000 ± 0.000	1.180	0.291
FM (d)	133.53 ±10.65	144.63 ± 4.66	18.11	<0.001	127.000 ± 0.000	138.955 ± 2.114	780.690	<0.001
FN	2.20 ± 0.56	5.19 ± 2.77	16.88	<0.001	5.222 ± 3.843	3.666 ± 1.527	0.46	0.504
FWE (g)	656.16 ± 425.34	677.50 ± 177.82	0.04	0.837	1488.760 ± 389.557	1549.666 ± 386.174	0.06	0.804
SCD (cm)	7.41 ± 0.88	8.30 ± 1.39	4.71	0.037	9.974 ± 0.955	7.166 ± 0.152	24.740	<0.001
FWI (cm)	11.07 ± 1.14	11.46 ± 0.99	1.23	0.274	13.997 ± 1.525	11.478 ± 0. 320	7.790	0.011
FL (cm)	12.36 ± 2.19	11.69 ± 1.36	1.27	0.268	17.646 ± 2.347	17.430 ± 9.252	0.01	0.927
NS	213.87 ±58.07	196.57 ± 46.73	0.98	0.329	321.518 ± 61.595	308.516 ± 44.418	0.12	0.732
SL (cm)	1.77 ± 0.09	1.78 ± 0.12	0.12	0.726	1.966 ± 0.171	1.942 ± 0.093	0.06	0.816
SWI (cm)	0.72 ± 0.04	0.72 ± 0.05	0.001	0.93	1.328 ± 2.24	0.859 ± 0.081	0.12	0.271
100-SWE (g)	12.62 ± 2.28	12.84 ± 1.39	0.13	0.720	21.443 ± 7.032	20.923 ± 2.154	0.02	0.902
HI	0.05 ± 0.02	0.03 ± 0.01	2.72	0.108	0.043 ± 0.010	0.039 ± 0.008	0.500	0.489
TP (%)	30.22 ± 3.57	33.45 ± 2.94	008.83	0.054	36.794 ± 4.684	41.106 ± 0.910	2.42	0.136

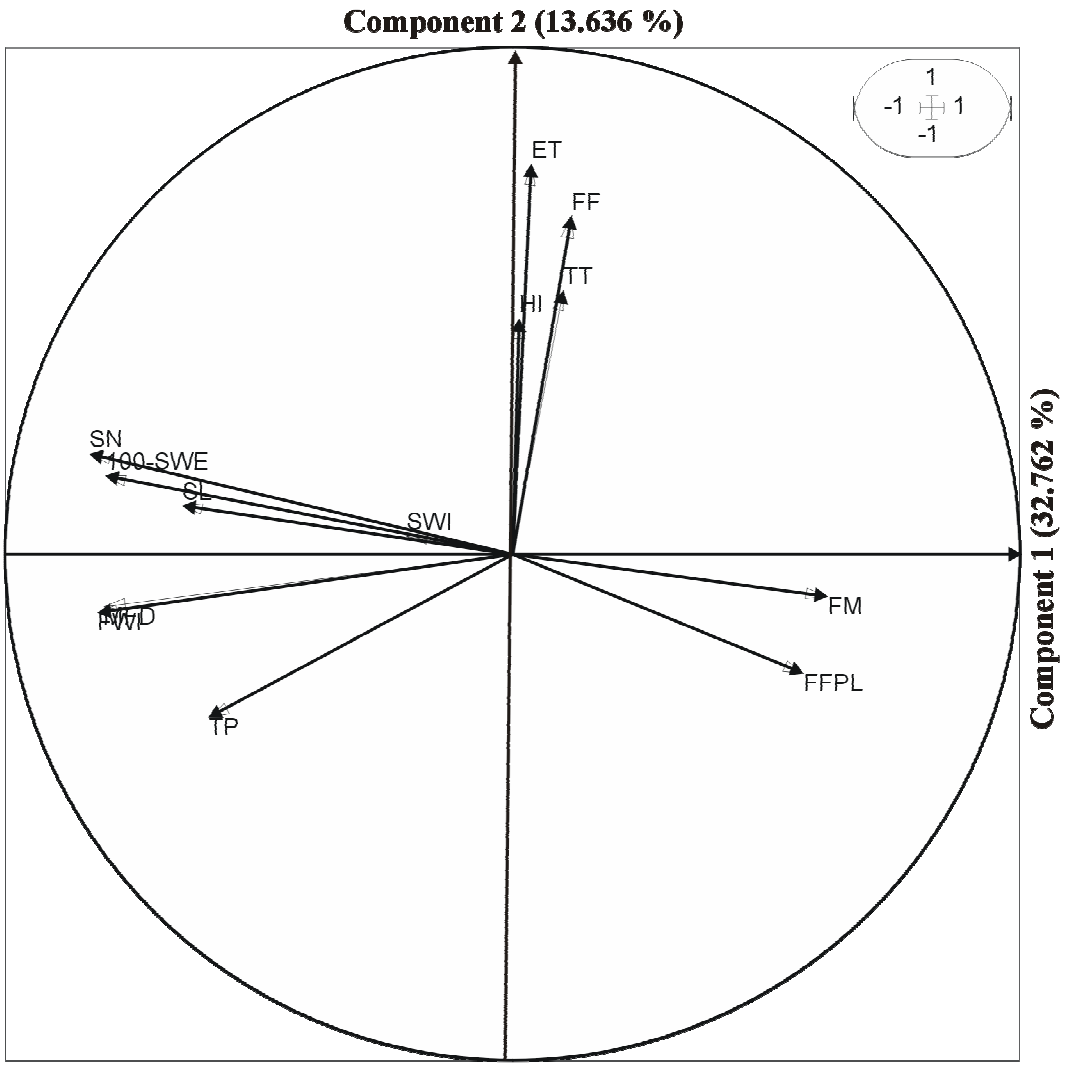
Table 6.

	Nei genetic diversity indices				***F* statistics**			**Gene flow**
Locus	H_T	H_S	D_{ST}	G_{ST}	F_{IT}	F_{IS}	F_{ST}	*Nm*
Mdh-2	0.265	0.243	0.022	0.082	0.331	0.272	0.082	0.997
Skdh	0.274	0.133	0.141	0.514	0.680	0.340	0.515	0.928
Mean	0.270	0.188	0.082	0.298	0.505	0.306	0.299	0.963
Std	0.006	0.078	0.084	0.305	0.247	0.048	0.306	0.049

Notes: H_T, the total genetic diversity; H_S, the genetic diversity within accessions; D_{ST}, the genetic diversity among accessions; G_{ST}, the among accessions gene differentiation coefficient; F_{IT}, the mean inbreeding coefficient of a set of accessions; F_{IS}, the fixation index related to non-random mating within populations; F_{ST}, the inter-accession genetic differentiation due to genetic drift; *Nm*, the gene flow estimate according to Wright's (1951) equation; and Std, the standard error.

Table 7.

	Nei genetic diversity indices				***F* statistics**		
Locus	H_T	H_S	D_{ST}	G_{ST}	F_{IT}	F_{IS}	F_{ST}
Mdh-2	0.291	0.290	0.001	0.003	0.477	0.479	0.003
Skdh	0.309	0.304	0.003	0.009	0.661	0.655	0.009
Mean	0.300	0.297	0.002	0.006	0.569	0.567	0.006
Std	0.013	0.010	0.001	0.004	0.130	0.124	0.004


Component 2 (13.636 %)
Component 1 (32.762 %)
1
-1
1
-1
ET
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100-SWE
SL
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FFPL
TP

Figure 1

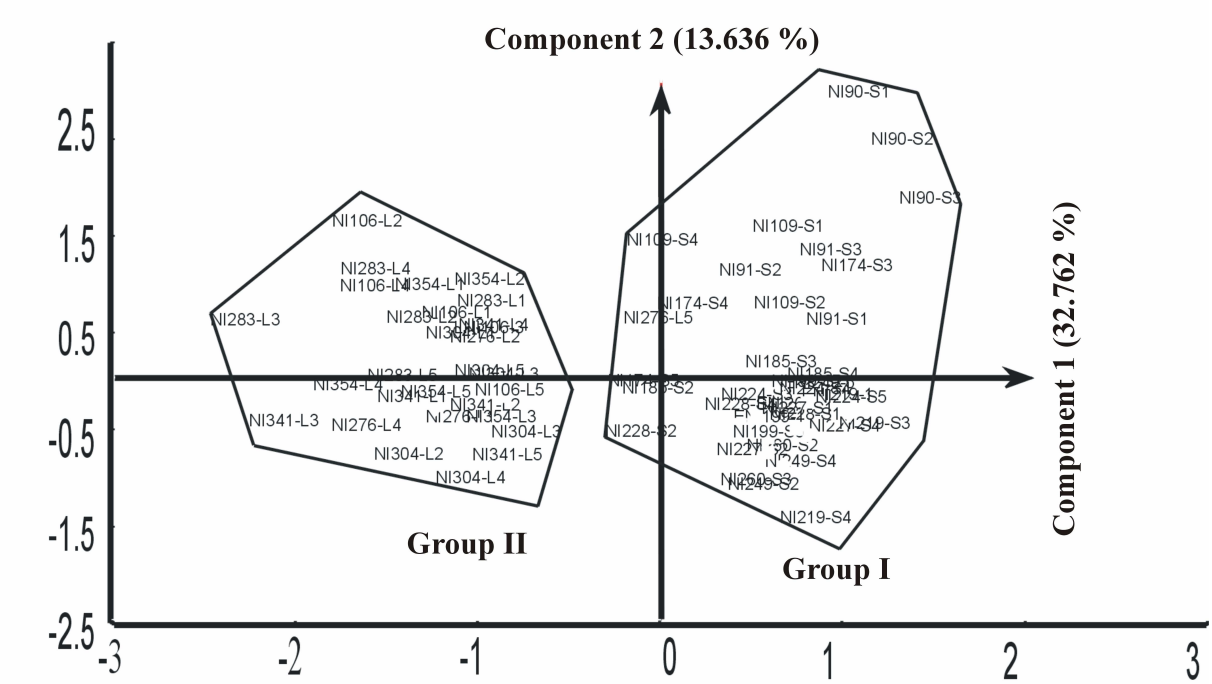
Component 2 (13.636 %)
Component 1 (32.762 %)
Group II
Group I
NI90-S1
NI90-S2
NI90-S3
NI109-S1
NI109-S4
NI91-S3
NI91-S2
NI174-S3
NI174-S4
NI109-S2
NI91-S1
NI185-S3
NI219-S4
NI106-L2
NI283-L4
NI354-L2
NI283-L1
NI283-L3
NI354-L4
NI106-L5
NI341-L3
NI276-L4
NI304-L3
NI304-L2
NI341-L5
NI304-L4
2.5
1.5
0.5
-0.5
-1.5
-2.5
-3
-2
-1
0
1
2
3

Figure 2

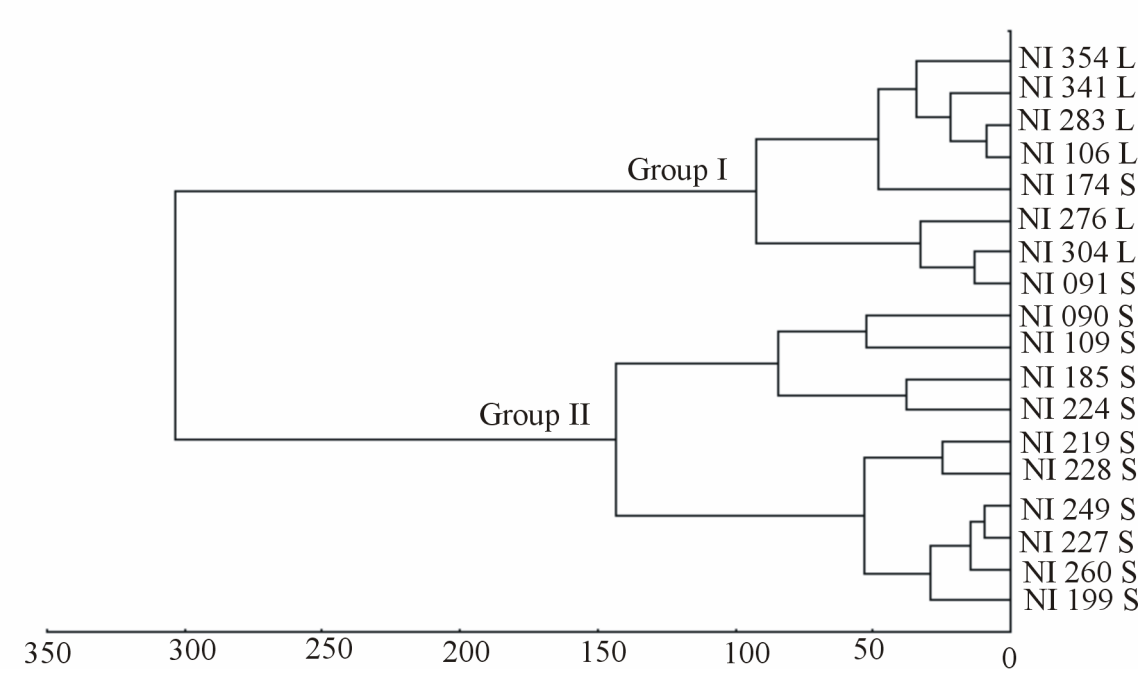
NI 354 L
NI 341 L
NI 283 L
NI 106 L
NI 174 S
NI 276 L
NI 304 L
NI 091 S
NI 090 S
NI 109 S
NI 185 S
NI 224 S
NI 219 S
NI 228 S
NI 249 S
NI 227 S
NI 260 S
NI 199 S
Group I
Group II
350
300
250
200
150
100
50
0

Figure 3

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Figure 1. Correlation circle of variables measured in the two first principal components of the PCA - Cercle de corrélation des variables mesurées chez les deux premières composantes de l'ACP

Figure 2. Accession distribution according to components 1 and 2 of the PCA based on morphological traits from *Lagenaria siceraria* collection (L: large seed; S: small seed) – Répartition des accessions suivant les axes 1 et 2 de l'ACP sur base des traits morphologiques de la collection de *Lagenaria siceraria* (L: grosse graine; S: petite graine)

Figure 3. Dendrogram illustrating morphological similarities among 18 accessions (L: large seed; S: small seed) from *Lagenaria siceraria* collection – Dendrogramme illustrant la similarité morphologique de 18 accessions (L: Grosse graine; S: Petite graine) de la collection de *Lagenaria siceraria*.