Genetic variation of Saba senegalensis Pichon (Apocynaceae) and few nutritional values

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GENETIC VARIATION OF SABA SENEGALENSIS PICHON (APOCYNACEAE) AND FEW NUTRITIONAL VALUES

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ABSTRACT

Saba senegalensis is a wild liana naturally growing in the sudan savanna region of West Africa, which fruits are commonly eaten by local population and also used as food additive because of the ascorbic acid it contains. However, very little information is available on its genetic diversity and nutritional values. The main objective of this study was to value the level of morphological diversity and some nutritional components of the species. In order to better control the species characterization, survey was carried in two departments (Korhogo and Tengrela) of Côte d'Ivoire savannas region. Morphological traits were measured on 10 fruits collected on 30 tufts of Saba distributed on 3 sites. Some averages were calculated from 10 fruits collected on each of the sampled tufts. Parameters as the content in vitamin C, in phosphorus and in total sugars were measured out according to various suitable dosage methods. Descriptives statistics results showed that the length of the fruit varied from 6,7 to 12,3 cm, the weight of the whole fruit ranged from 173,8 to 491.14 g, the weight of the pulp varied from 63,47 to 216,53 g and the number of nuts by fruit lay from 10 to 45. The Principal Component Analysis (PCA) revealed 75.12% of the total variance expressed on the 2 factorial axes. The Hierarchical Ascending Classification (HAC) allowed structuring and

marking saba populations in 3 phenotypic groups. Different dosages showed that, vitamin C content in saba fruits varied from 34.8 mg to 67.5 mg /100. Phosphorus content ranged from 97.75 mg to 215.9 mg/100. Also, total sugars content varied from 26.71 g to 31.87g/100. However, genetic and nutrirional characterization of saba resources upon a big scale are necessary.

Keywords: Cote d'Ivoire, Phenotypic Diversity, Saba Senegalensis, Nutritive Value

INTRODUCTION

The Saba tree is an upwardly mobile plant of tropical West Africa and the western Sudan. Typically, this woody vine clambers up the fringes of the forest. *S. senegalensis* Pichon (*Apocynaceae*) wildely known under its synonyms Landolphia senegalensis is a wild liana up to 40 m long, often shrub like; trunk up to 20 cm in diameter. The species growing in various ecological conditions (from 100 mm to 1300 mm annual rainfall), but mainly distributed in riverine areas and open woodland of Africa [1]. Its well met in the sudanian savannas as in the Guinean savannas of Africa. Its native of Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Mali, Niger, Senegal, Tanzania [1, 2].

Regarding *S. senegalensis*, some preliminary studies on seed pregermination treatment were mentioned by [3]. The species is hermaphroditic liane flowers all year through. Inflorescence a 3-30 flowered lax cyme, peduncle 2.5-6 cm long, pedicels 2.5-8 mm long. Sepals shortly apiculate. 1-1.5 times as long as wide, corolla with a yellow throat; tube 5-9 times as long as the

calyx. Stamens inserted 3.5-6 mm above the corolla base; filaments 0.4-1 x 0.1 mm, anthers 1-2 x 0.2-0.5 mm, ovary often ribbed, glabrous with ca 30 ovules, style 1.5-3 mm long, pistil-head 1.7-2 mm long, basal part up to 1.8 mm long [2].

During the last 10 years, *Saba* fruit juice has remarkably gained popularity among urban consumers in many countries like Burkina Faso, Côte d'Ivoire, Mali, Senegal, Guinée etc. In Cote d'Ivoire the latex is used as an adhesive for poison preparations for arrows and the leaves are eaten to stop vomiting. In Senegal the leaves are prepared in sauces and condiments as an appetizer with a salty taste. Bark decoctions are taken for dysenteriform diarrhoea and food-poisoning. Crushed leaf infusion has haemostatic/antiseptic usage and the powdered root efficacious on children's burns. Fruits are eaten as a sterility treatment [1, 2, 4].

In parts of Africa, they are important to the rural economy, and many are trucked or carried in baskets to sell in the cities. These colorful treats are, for example, widely

consumed in Burkina Faso, Côte d'Ivoire, Mali, Senegal and the Gambia. These fruits are frequently seen for sale in markets across West Africa. In Mali, Burkina Faso, and Côte d'Ivoire it is also common to see young boys and girls selling clusters of them along the roadways.

Saba fruits are orange on the outside and pale yellow inside. They have a thick, rough rind. Most are about the size of a medium to small orange. In taste, they are acidic, almost like a strange form of citrus, which they nearly match in vitamin C content. It is used for various needs such us food additive because of the ascorbic acid it contains. The juice from these fruits is regarded as extremely healthful. In addition to a normal nutritional content for fruits, some have vitamin C levels approaching orange. At least one species, Landolphia hirsuta, provides good levels of provitamin C and A [5].

This has led to growing market for this wildly grown plant. In Côte d'Ivoire, the trade of saba fruits becomes more and more flourishing from rural zones toward the big urban centers where they are sold at 0.7 – 0.8 $ US the Kg. The quantities marketed to urban centers in 2008-2009 were estimated at 461,320 kg (unpublished source).

Despite the vastness of the resource, the species is rarely included in development activities. Indeed, intensive and uncontrolled exploitation of *S. senegalensis* fruits, combined with low rate of natural regeneration has led to the drastic depletion of this species and to our knowledge, very little study have undertaken on its genetic variability and its nutritional component content. The objective of this study was to evaluate morphological diversity and to measure some nutritional properties of *S. senegalensis* in Côte d'Ivoire across savanas region.

MATERIAL AND METHODS

Study Area

This study was conducted in the Sudanian zone of Côte d'Ivoire located between 09°31'04.8N and 10°30'04 N, and 005°32'58 and 006°24'02 E. It is subdivided in two main phyto geographical zones. The Sudanian zone which is located in the extreme North. The annual mean rainfall vary from 900 to 1200 mm of rain per year and the relative humidity varies from 18 % during the harmattan period (December-February) to 99 % in August. The temperature varies from 24°C to 31°C. The rainfall in the Sudano-Guinean zone is unimodal, from May to October, and last for about 113 days with an annual total varying from 1100 mm to 1500 mm. The annual temperature ranges from 25°C to 29°C, and the relative humidity from 31% to 98%. The vegetation of the Sudano-Guinean transition zone is characterized by a mosaic of

woodland, dry dense forests, tree and shrub savannas and gallery forests [6].

Morphological Data Collecting Methods

Plant material used in this study was tufts of liana Saba (**Figure 1**). Morphological diversity of the species has been studied in three stands sampled in three sites including one in the Sudanian zone and two in the Sudano Guinean zone. The choice of two sites in the Sudano Guinean explained by the size of the area. Indeed, the department of Korhogo is 2 times larger than Tengrela's. To avoid doing the study on the same population, a distance of 50 km has been observed between the two collection sites. The study sites selected around villages and their geographical coordinates are shown in **Table 1**. Morphological characteristics of each crumb vine were studied at the above-mentioned sites. In each population, 10 tufts were randomly sampled about an interval at least of 25 m to avoid possible effects of inbreeding. Ten tufts of saba were measured in the Sudanian zone, 20 in the Sudano Guinean's. In this study, 10 mature fruit of saba were collected on every clumb and quantitative describers were analyzed. The length of the fruit (LFRU), the width of the fruit (WFRU), the geometric diameter of the fruit (GDFRU) and the thickness of the cockle (THCOC) were measured on every fruit. All these data have been collected with the help of a foot

to slide with a precision of ± 0,1 cm. Thereafter, the weight of the fruit (WFRU), the nut (WNOI) and the weight of the cockle (WCOC) were measured. All this weighed were led with Sartorius balance of range 2200 g with the precision of ± 0, 01. From those measures, some specific reports susceptible to help towards saba populations characterization were calculated: [Width of the fruit / length of the fruit] and [Weight of the walnuts / Weight of the fruit]. And finally the number of walnut by fruit (NNOI) was counted.

Nutritional Data Collecting Methods

Nutritional components were measured out on a sample of 10 fruits of saba collected on the urban market of Abidjan. The ascorbic acid of formula ($C_6H_8O_6$) present in aqueous solution has reducers properties. In most fresh foods the amounts of dehydroascorbic acid are very low and for many purposes the measurement of ascorbic acid alone may be adequate. The method used was the colorimetric method [7] involving the reaction with 2, 4-dinitrophenyl hydrazine measured both ascorbic and dehydroascorbic acid, improved. Twenty grams of fruit pulp were ground in 40 ml of distilled water and then filtered. To 10 ml of crude extract were added 10 ml metaphosphoric acid / acetic acid. The mixture was centrifuged at 4200 rpm for 15 min. It was collected, 1 ml of the float

solution was determined by 2, 6-dichlorophenol indophenol to turn the color to the pink champagne persistent (Ve). A volume of 1 ml metaphosphoric acid / acetic acid was determined (V0). 1 ml of vitamin C (1mg/ml) is determined (Vs). Each test was performed three times: Vitamin C = 1 mg (Ve - Vo) x20 x 100/ 1 ml (Vs-V0) x 10

Ve: volume of the sample determined by the dichlorophenol-indophenol

Vs: volume meta phosphoric acid / acetic acid determined by the dichlorophenol-indophenol

V0: volume of vitamin C determined by the mother-dichlorophenol indophenol

According to the standard [8]. For the extraction solutions of hydrochloric acid is prepared, a 1: 3 and the other à1: 9. At the ash obtained after incineration 5 ml of hydrochloric acid (1: 3) were added to the heat and steam for 1 hour and cool to room temperature. Then 5 ml of hydrochloric acid (1: 9) were added and the mixture is added to 50 ml with distilled water. About itself determination, to 0.1 ml of the extract, 0.9 ml of distilled water and 3ml reagent vanadate molybdate were added. Everything was well mixed and was put in a spectrophotometer at 410 nm against a witness. The rate of phosphorus was determined from a calibration straight line in the same conditions.

The method for the extraction of soluble sugars ethano-soluble was used [9]. In a centrifuge tube, 0.5 g of sample were placed in 10 ml of ethyl alcohol 80° GL. The mixture was agitated for 2 min, and centrifuged at 3000 trs/min for 15 min. The float solution was recovered in another tube which 2 ml of lead acetate (10 %) was added and agitated to 3000 trs/min centrifuged for 15 min. The float solution was collected and 2 ml solution of oxalic acid (10 %) are added and centrifuged at 3000 trs/min during 15 min. The lastest float solution was collected and put to evaporation on a sand bath to remove as much alcohol. The residual volume was completed to 5 ml with distilled water. Determination of total sugars by the phenol-sulfuric method. In the presence of phenol and sulfuric acid concentrated sugars dehydrate forming furfural which reacts with phenol to give a yellow complex whose intensity is proportional to the concentration of total sugars present in the solution. For a volume of 0.1 ml of the extract, 0.9 ml of distilled water, 1 ml of phenol and sulfuric acid were added. The mixture was agitated and let cool slightly on the bench. The reading of the Optic density (OD) was made on a spectrophotometer at a wavelength of 490 nm. For the determination of sugars, the tubes were

prepared as in the case of the calibration curve.

Data Analyses Methods

Statistical analysis was performed using the descriptive statistics (maximum, average, minimum, coefficient of variation…) were calculated. The quantitative data were subjected to principal component analysis (PCA) in order to determine associated variables with each other. PCA is a multivariable descriptive analysis that allows for the construction of axes that maximize the variances of the individuals which participated in their construction in order to explain the observed variation [10, 11]. PCA is a statistical technique that reduces the number of variables into a small number of synthetic variables by a linear combination of the original variables that explained most of the variation. All variables measured in this work were normalized (The data were centred and reduced) before being subjected to the analysis.

Correlations studies were carried between pairs of variables to verify if they were interrelated. For each pair of variables, the correlation coefficient (r) of Pearson was calculated. The Student (T) significance test was used to define the link between the variables and verify the absence on meaningful correlation between the variables hypothesis.

A dendrogram was constructed based on dissimilarity relation between observations from the averages pondered of the euclidienne distances while applying the Ward algorithm [12]. A first structuring was led by a Hierarchized Ascending Classification (AHC). Data was also submitted to Discriminative Factorial Analysis (DFA). This last analysis permitted to verify the hypothesis of equality of inter group covariance. The results of Fisher test associated to the squares of Mahalanobis distances between groups permitted to test the funding of the regroupings achieved in the AHC [12, 13]. All these analyses were achieved using XLSTAT 2007.6 version software, Copyright 1995-2007, a mark deposited of AddinSoft.

RESULTS

Description of Saba Population Using Statistics Element

For each quantitative traits, descriptive statistics as minimum, average, maximum, coefficient of variation and average interval of confidence were calculated **(Table 2)**. The results showed that the length of the fruit varied from 6,7 to 12,3 cm ; the weight of the whole fruit ranged from 173,8 to 491.14 g ; the weight of the pulp varied from 63,47 to 216,53 g and the number of nuts by fruit swung from 10 to 45. Meanwhile ratio [width / length] of the fruits that reflects the shape of the fruit

ranged from 0.60 to 1.06. The report [PNOI / PFRU] varied from 0.33 to 0.60. Descriptive statistics carried on quantitative traits showed that, every average value was located inside its interval of confidence (**Figure 2**). With 30 %, of coefficient of variation the number of nuts by fruit, was the most discriminant variable. Data were dispersed around the average. On the other hand, the width of the fruit and the geometric diameter were little dispersed around their average. Indeed, each variable (NNOI, PNOI, ECOQ, PFRU, PCOQ), take individually can be usedfor an effective morphological desciption of saba population

Relationship Between Variables

The principal component analysis performed on the quantitatives traits showed that the first two axes explained 75.12 % of the variance expressed. The trait: LFRU, WFRU, GDFRU, WFRU, WCOC, WNOI were strongly correlated to the first axis and explained 51.90 % of the total variation (**Table 3**). The [Width of the fruit / length of the fruit], the [Weight of the walnuts / Weight of the fruit] and the thickness of the cockle are also correled with the second axis and explained 23.22 % of the total variation. However, the quality of the representation of each variable in the correlation circle (**Figure 3**) confirms the performance of the traits measured. The P-Value gotten following the Bartlett test is lower than the level of significance of 5 % showed that correlations between the variables are meaningful. These great interrelationships between the variables showed that quantitative traits analysed are redundant. A little number of variables can be used for big scale characterization.

Structuring and Consolidation of Saba Population

Dendrogram obtained from the truncation at 100 % of dissimilarity allowed to distinguish two major phenotypic groups within the sample investiguated trees. Also, at 30 % of dis similarity, three phenotypic classes were distinguished (**Figure 4**).

The class 1 consists of 16 individuals against 10 and 04 respectively for the second and the third classes. The Wilks test applied witth significance alpha = 0.05, and a P-value belowing 0.0001, permitted to disallow the null hypothesis of equality of 3 groups vectors. So, these 3 phenotypic groups represented biological entities.

Nutritional Properties of *Saba senegalensis* Fruits

The **Table 4** summarise some of saba fruits nutritionnal component content. The vitamin C (ascorbic acid) contained in saba fruits pulp varied from 34.8 mg/100 to 67.5 mg/100. Phosphorus content ranged from 97.75 mg / 100 to 215.9 mg/100. Total sugars varied from 26.71g/100 to 31.87g/100.

DISCUSSION

Based on the currently available information, we underline some aspects of *S. senegalensis*. The species contribute substantially to plant diversity in Côte d'Ivoire savannah's, but also in many other savannahs around West Africa. The present study is a previous necessary step to define a species genetics resource's characterization strategies. The originality of this work lies in the fact that, to our knowledge, litle genetic diversity survey has been carried on Saba in Côte d'Ivoire. The analysis in main component permitted to structure the diversity observed. Indeed, 75.12% of morphological variability have been revealed on the first plan. All the variables granted in this survey, contributed meaningfully to the revelation of morphological diversity explained. But the length and the width of the fruit, the number of nut by fruit, the weight of the fruit and nuts seems the most plausible. The choice of the length and the width of the fruit explain itself by their specific report importance in the determination of the fruit shape. As for the weight of the fruit and nut, the specific report permitted to estimate the indication of yield in seed of every fruit. Otherwise, the strong dependence of the quantitative characters to the environmental conditions posed the problem of their use through different climatic zone. Their use on a big

scale seems therefore inopportune. It is why in the continuation of this survey, it would be important to associate to the quantitative parameters measured here, the qualitative describers which have agronomic interest. Such results using morphological traits have been gotten on *V. paradoxa* [14, 15] and on *D. microcarpum* [16].

The hierarchical ascending classification was used to mark the sample into three groups that have been consolidated by the discriminating factor analysis (**Figure 5**). To some extent this could explain the existence within the species, several species or varieties. However, in the absence of standardized rules, three phenotypic groups were identified in relation with specific report [width length of the fruit on the fruit]. Three main shaped-fruits of Saba each corresponding to a phenotypic class defined. The class 1 was composed by elongated shape-fruit, the class 2 by ovoid-shaped fruit and the class 3 by round-shape fruit (**Figure 6**). The fruit is said rounded if the the specific report [width length of the fruit on the fruit] was placed side 1. It is said ovoid if the report was placed side 0.50 and lying when ratio was placed side 0. The three phenotypic classes defined hiding in fact a qualitative polymorphism which is not reflected in quantitative measures.

The data obtained in this study allows to consider several research axes. The duration

of the life cycle and the reproduction biology of the species is still poorly understood. The use of neutral markers such as microsatellites or RADP will be a significant contribution in the assessment of genetic diversity and in estimating the rate of outcrossing of the species especially the floral morphology suggests that saba is preferentially autogamous. It would also be possible to extend this survey to other parklands distributed in different climatic zones and to other countries. A survey on genetic diversity using molecular and biochemical describers seems an interesting perspective for this research as this will permit us to get complementary and useful information.

This result showed that, vitamin C contained in saba fruits pulp varied from 34.8 mg/100 to 67.5 mg/100. Phosphorus content ranged from 97.75 mg / 100 to 215.9 mg/100. Total sugars varied from 26.71g/100 to 31.87g/100. Other food plant species are consumed by the people, the animals and the birds in flora. The nutritional value of plant food is also an argument for recovery. In general, the use of these plants is related to their richness in nutrients (proteins, minerals, etc.). Nutritional value of wild crop plants was carried by various authors. These species overflow with nourishing elements and natural vitamins [17, 18]. The consumption

of saba fruits will contribute to maintain nutritional balance for rural population. The valuing of all other plant food can improve the nutritional quality of local populations rations. They should be adapted to the specific conditions of every middle according to population's cultural authenticity. A possible success of such action will require a rational management of these resources. Some studies deserve to be led in order to valorize these wild crop plants for human and animal food considering vitamin C content, the availability and the abundance. These wild crop plants can be real sources in ascorbic acid for rural populations. Genetic selection and domestication are important for turning the species into fruit crops. Finding quality types and developing horticultural methods that speed up production are two keys to success. But studies on the toxicity, the chemical composition and extraction methods should be undertaken before popularizing these plants.

S. senegalensis may be especially important for rural population where regular modern medicines and various other products are not easily available and accessible. Indeed, the reliance on the forest by indigenous peoples is often mentioned as a reason for rain forest conservation [19]. In some regions the knowledge of useful plants is disappearing more rapidly than the plants

themselves. If no efforts are made to conserve and study both the biological and cultural diversity, a potential resource of new medicines for human disease, food crops for international trade, and indigenous management systems will disappear together with the forests.

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Table 1: Geographical Characteristics and Number of Samples Collted by Site

Sites	Number of Samples	Altitude (m)	Latitude (N)	Longitude (W)
Tengrela/San	10	387	10°30'04	006°24'02
Korhogo/Latahaha	10	350	09°33'52	005°32'58
Korhogo/ Benguebougou/ Benguebougou	10	357	09°31'04.8	005°48'39.7

Table 3: Correlations Between Variables and Principal Components

	F1	F2	F3
LFRU	0.733	-0.493	-0.415
WiFFRU	0.834	0.378	0.313
WidFRU/LFRU	-0.153	0.746	0.625
GDFRU	0.800	0.292	0.325
NNU	0.875	0.215	-0.265
WeFRU	0.959	-0.169	0.046
WeNU	0.926	0.268	-0.163
WeNU/WFRU	0.210	0.780	-0.421
WeCOC	0.813	-0.483	0.212
THCOC	0.213	-0.552	0.624

Table 4: Content in Vitamin C, in Phosphorus and in Total Sugars of Saba Fruits

Samples of Saba	Ascorbic Acid (mg/100g)	Phosphorus Rate (mg/100g MS)	Total Sugar Content (mg/100g)
Minimum	34.80	97.75	26.71
Average	46.00	156.80	29.29
Maximum	67.80	215.9	31.87

Figure 1: Clumb of vine (*Saba senegalensis*)

Table 2. Descriptive Statistics for Quantitative Traits Studied

Statistics	LFRU	WiFRU	WiFRU/LFRU	GDFRU	NNU	WeFRUWei	WeiNUT	WeNU/WeF RUUruWei	WeCOC	THCOCTh
Number of observations	30	30	30	30	30	30	30	30	30	30
Minimum	6.70	5.90	0.63	6.00	10.00	173.80	63.47	0.33	83.43	0.40
Maximum	12.30	8.50	1.06	8.60	45.00	491.14	216.53	0.60	225.31	1.00
Median	8.68	7.50	0.87	7.48	27.00	296.57	146.32	0.53	135.98	0.68
average	8.86	7.51	0.86	7.44	27.53	298.17	150.50	0.50	142.67	0.67
CV (%)	12	08	11	08	30	24	27	14	23	26
Bound inf. of the average (95%)	8.44	7.27	0.82	7.21	24.45	270.54	135.19	0.48	130.00	0.60
Bound sup. of the average (95%)	9.27	7.74	0.89	7.67	30.62	325.81	165.82	0.53	155.34	0.73

NOTE: LFRU : Lengh of the Fruit; WiFRU : Widht of theFruit; GDFRU : Geometrical diameter of the fruit; NNU : Number of Nut; WeFRU : Weight of the Fruit; WeNU: Weight of the Nut; WeCOC : Weight of Cokle; THCOC : Tthickness of the Cockle
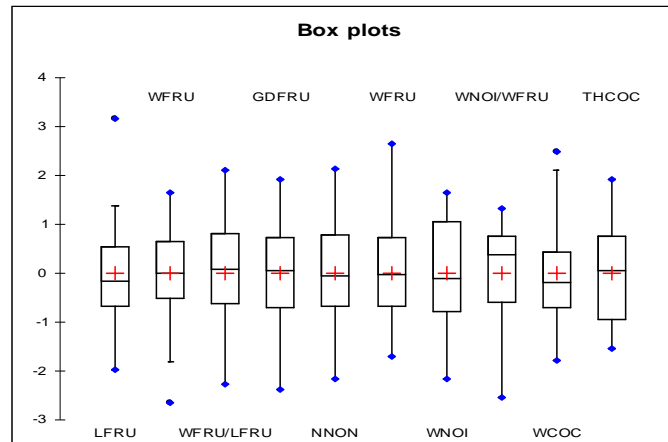

Figure 2: Box Plots Showing the Average, Median and the Bounds of Confidence Intervals of the Mean
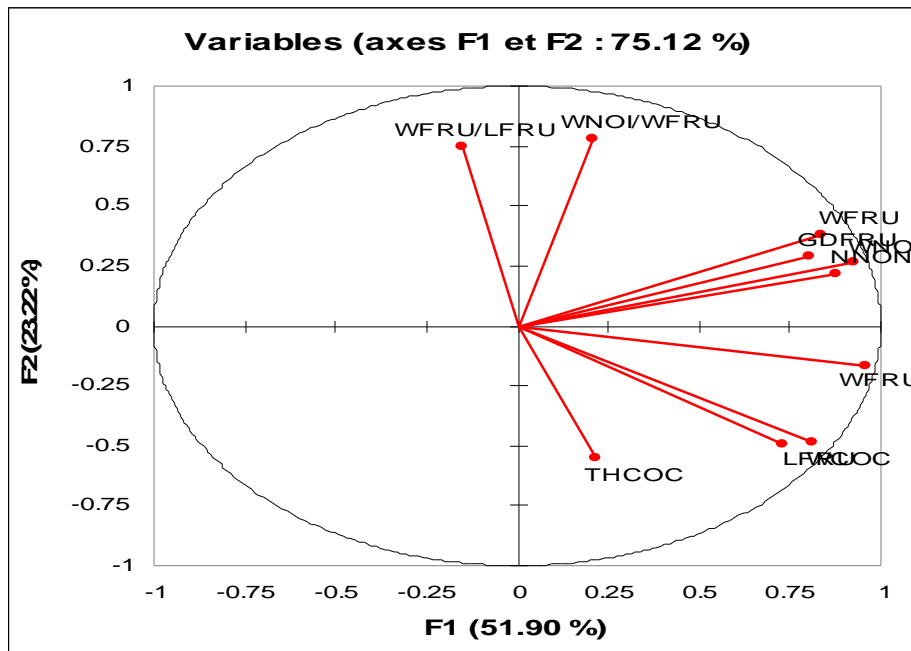
Figure 3: Quality of Quantitative Traits Studied Representation on the Correlation Circle in Plan 1 & 2 of the PCA
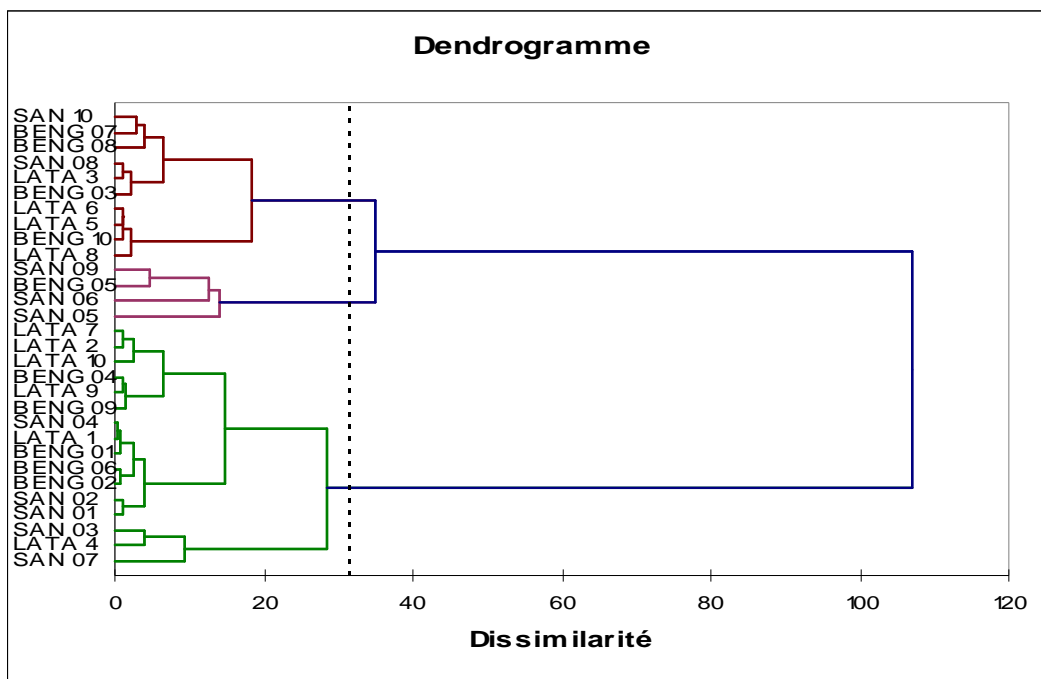

Figure 4: Clustering the Observations of the Sample from the Traits Analysed
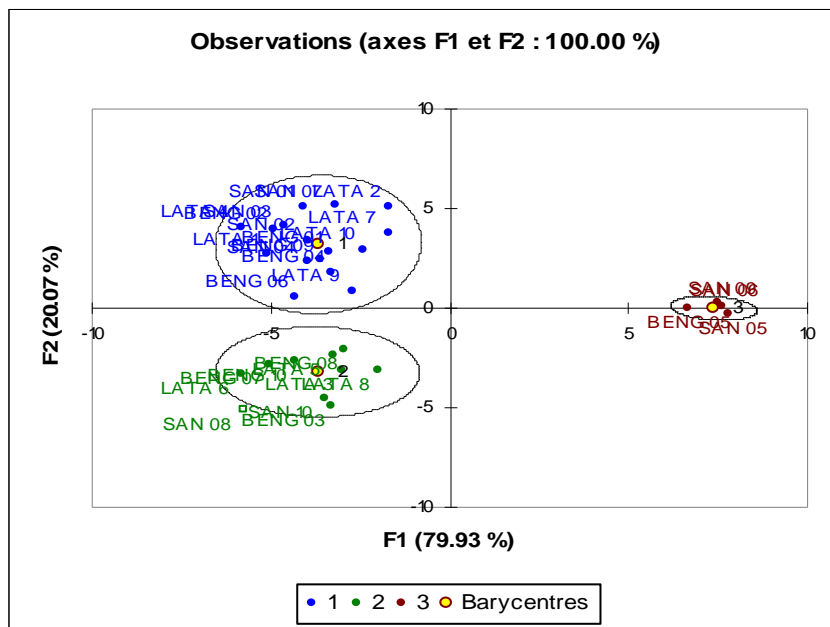
Figure 5: Representation of Individuals and Groups Obtained after Factor Analysis Discriminant
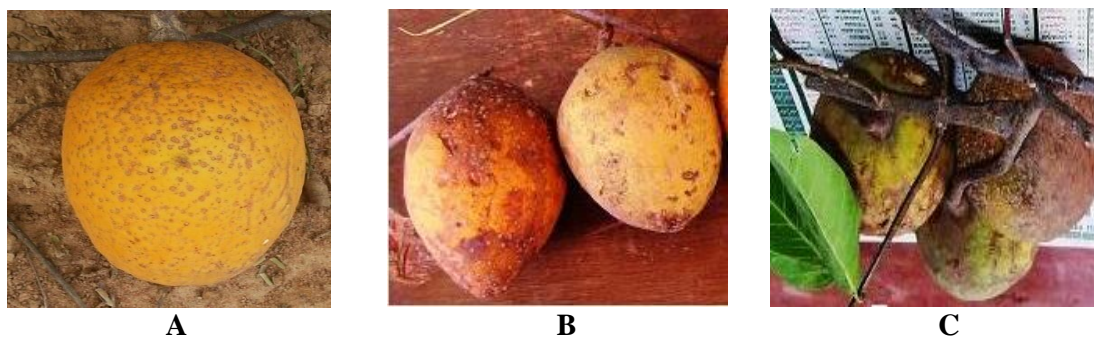

Figure 6: Variability observed in the Shape of the Fruit (A: Elongated-Shapes Fruits; B: Ovoid- Shaped Fruits; C: Rounded-Shaped Fruits)