

Agro-morphological characterization of preselected cashew (*Anacardium occidentale* L.) mother trees in Benin farmer's plantations

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Abstract: One of the main causes of low cashew nut yields in African countries is the lack of improved planting materials used for plantation establishment. It is therefore necessary to efficiently select planting material to increase cashew nut yields. The objective of this study was to assess the agro-morphological diversity of preselected cashew mother trees for facilitating their further selection in cashew plantations in Benin. Thirty-one variables were measured. These variables, including 12 quantitative and 19 qualitative, were selected in cashew descriptors developed by Bioversity International. Statistical tools were performed for the assessment of the 394 cashew mother trees preselected in 23 administrative districts originating from the four major cashew nut production regions in Benin: Atacora-Donga, Borgou, Zou-Collines and Plateau. A Factorial Analysis on Mixed Data and a Hierarchical Cluster Analysis (HCA) were carried out to group the individuals. Finally, a morpho-metric characterization of the different groups based on quantitative discriminant descriptors from a multivariate analysis of variance (MANOVA) was carried out. The HCA result on mixed data showed three significant morpho-metric groups of cashew mother trees. Discriminant stepwise analysis carried out on

all 31 morpho-metric descriptors showed that 15 were found to be the most discriminating (5 quantitative and 10 qualitative descriptors) of the morphometric groups ($P < 0.001$). The leave vein crossing, hermaphrodite flower rate, apples unit weight, abnormal flower number and nut basis shape showed significant positive or negative correlations. These results suggest the existence of an important phenotypic variability among Beninese cashew accessions that could be used in cashew tree breeding program being implemented in Benin.

Keywords: cashew planting material, phenotypic variability, varietal breeding, Benin Republic

Introduction

Cashew (*Anacardium occidentale* L.) is nowadays an important export crop in Africa with 55% of world production. From 2000 to 2016, cashew nut production in Africa is booming, rising from 400,000 to 1,800,000 metric tons of cashew raw nuts (ACA, 2017). West Africa became the first cashew nut production area in the world with 1,350,000 metric tons of cashew raw nuts in 2015 (RONGEAD, 2015). Benin ranked seventh in the world in terms of cashew nut production with 90,000 metric tons in 2011 (ACA, 2012). From 2014 to 2015, this production increased from 81,004 metric tons to 82,354 metric tons. In 2015, cashew planted areas in Benin were estimated at 285,567.7 ha (Adégbola and Crinot, 2016). In Benin, cashew is the second most important agricultural export product after cotton. Cashew nut exports are booming, rising from 36,487 metric tons of raw nuts exported in 2001 (PAC/DCM/SESP, 2009) to 146,332 metric tons in 2011 (ACA, 2012). The cashew sector accounts for 8% of Benin's export earnings and 25% of Benin's agricultural export incomes. The cashew is produced in 8 of the 12 departments of Benin namely: Alibori, Atacora, Borgou, Collines, Couffo, Donga, Plateau and Zou (Adégbola and Crinot, 2016).

However, cashew production in Benin and West Africa is still facing several constraints. Among these, the lack of research on cashew planting material breeding and the unavailability of high-quality planting material for plantation establishment. Consequently, plantation yields are low, between 3 to 6 kg/tree compared to 10 to 15 kg/tree in major cashew producing countries such as India, Brazil, Viet-Nam and Tanzania (Masawe, 2010; Tandjiékpon, 2010).

Aiming to overcome these constraints, the Central Agricultural Research Center (CRA-Centre) of the National Agricultural Research Institute of Benin (INRAB)

initiated and started since 2011 a cashew breeding program development in Benin like in other African countries such as Tanzania and Ghana (Masawe, 1994; Masawe, 2006; Dadzie *et al.*, 2014; N'Djolossè *et al.*, 2015). An important way to increase the productivity of the crop and improve the quality of nuts is to select desirable genotypes from the existing variations and to use the superior materials identified in the breeding programs (Aliyu and Awopetu, 2007). The selection and characterization of high-performant mother trees in the farmer's cashew plantations are the first two important steps in breeding process. Identification and genetic relationships in cashew is difficult because of the lack of morphological and floral traits of the plant materials. There were a lot of variations among the variety due to the cross pollination (Samal *et al.*, 2004). Some studies were made on morphological, agro-morphological and genetic characterization in many countries such as India, Brazil, Venezuela, Malawi and Tanzania, both in unknown cashew populations and in selected cashew clones and hybrids (Samal *et al.*, 2003, 2004; Sindoni *et al.*, 2005; Chipojola *et al.*, 2009; Dasmohapatra *et al.*, 2014). In Benin, a general study was carried out on morphological characterization of accessions of cashew trees (Chabi Sika *et al.*, 2015); but it didn't focus on selected trees based on their agronomic performances.

The present study highlights the agro-morphological characterization of preselected cashew mother trees in Benin. It aims to assess the phenotypic and agronomic diversity of the preselected cashew mother trees in the farmer's plantations for further steps in ongoing cashew breeding in Benin.

Material and methods

Study area

The study was carried out in the four main cashew production regions in Benin: Atacora-Donga in the Northwest, Borgou in the Northeast, Zou-Collines and Plateau in the Central part. A total of 23 administrative districts were covered. This area is located within latitude ranges of 7°–11° N and longitude ranges of 1°23'–3°40' E (Figure 1) and includes 3 of the 8 agro-ecological zones of Benin.

Three types of climates are observed in this area. The agro-ecological zone III, covering the Borgou department and two districts of Atacora (Kouandé and Péhunco), has a Sudanese-type climate (monomodal rainfall) with an annual rainfall

ranging between 900 and 1,300 mm. The agro-ecological zone IV has a Sudano-Sahelian and Sudano-Guinean climate (monomodal rainfall) with an annual rainfall ranging between 1,000 and 1,300 mm. The agro-ecological zone IV includes Atacora department (apart from districts of Kouandé, Kérrou and Péhunco) and Donga department apart from Bassila district. The agro-ecological zone V includes the Collines department and the districts of Djidja, Kétou, Aplahoué, Bassila and South-Tchaourou. The climate is Sudano-Guinean type (bimodal rainfall) in the South and a monomodal rainfall in the North with an annual rainfall ranging between 1,000 and 1,200 mm (Boko, 1992).

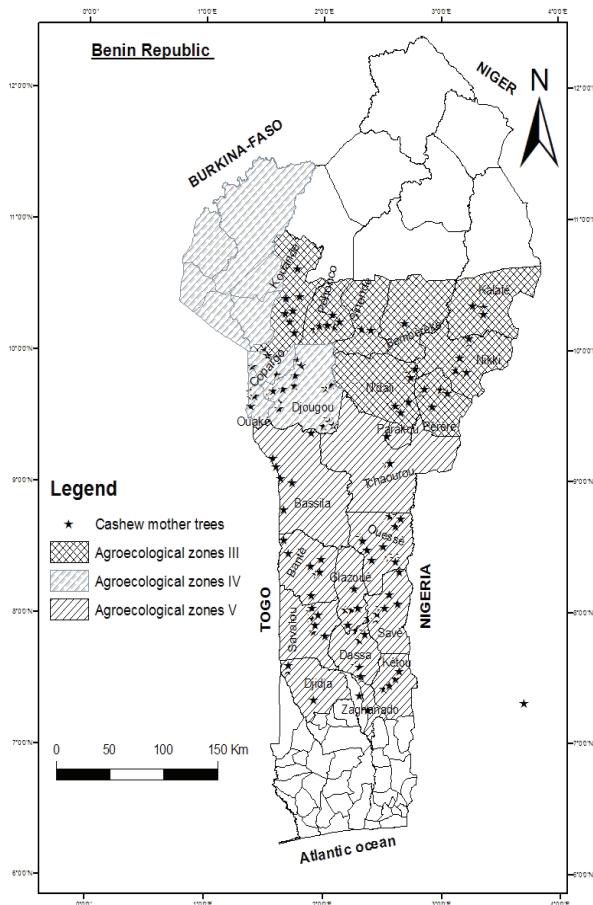


Figure 1- Study area showing the agro-ecological zones covered and the sites of preselected cashew trees

Material

Cashew mother trees used in this study were identified from January to march 2013 in farmer's plantations in the 23 covered districts of Benin. The current study focused on high-performant mother trees that were preselected in 2015 after two years assessment of their agronomic performances (N'Djolossè *et al.*, 2015).

The technical and agricultural equipments used during the data collection were: 30 m-ribbon, 25 kg balance, 5 kg electronic balance, caliper and pair of scissors. The table 1 provides moreinformation on materials used to measure each of 31 studied variables.

Methods

Data collection

Agronomic and morphological data were collected

Agronomic data were cashew nuts yield, nuts weight and kernels rate. The nut production data of the identified cashew mother trees were collected from 2013 to 2015. Each year, the produced nuts were harvested and weighed per tree from mid-January to mid-April at the frequency of 15 days. At the middle of the harvest period, a sample of 1.5 kg of nuts was taken from each of cashew mother tree's production for nuts unit weight and kernel rate determination. For measurement of these last two parameters, a sample of 1 kg of nuts was weighed using an electronic balance and the number of nuts contained in this sample was counted. Then, all the nuts of each sample were opened one by one with a pair of scissors specially designed for shelling raw cashew nuts and the kernels were removed and weighed using the electronic balance. The average nut weight was obtained by dividing the weight of the 1 kg of nuts by the number of nuts contained in the sample (1 kg). The kernel ratio was obtained by dividing the weight of the kernels by the weight of the nuts and multiplying the quotient by 100.

Morphological data were derived from cashew descriptors developed by Bioversity International (IBPGR, 1986). Twenty-eight variables, including 9 quantitative and 19 qualitative, were measured on diverse organs of each selected tree such as trunk and crown, leaves, flowers, nuts and apples. The table 1 presents the morphological variables measured and the tools used to measure them.

Table 1 - Descriptors used in assessing agro-morphological variability in cashew mother trees under selection in Benin

TRAIT TYPE	CHARACTER ASSESSED	ACRONYMS	UNIT	MEASUREMENT MATERIAL
Trunk and crown	Tree height	Hatot	Meter	30 m-ribbon
	Crown diameter	DiamCo	Meter	30 m-ribbon
	Tree habit	ArchitA	-	Direct observations and comparison with descriptor schemes (DOCDS)
	Crotch angle of main branches	InserB	-	DOCDS
Leaves	Angle of leaf petiole relative to stem	InserP	-	DOCDS
	Leaf Shape	Forfe	-	DOCDS
	Leaf apex shape	Bofe	-	DOCDS
	Leaf cross-section	CroNer	-	DOCDS
	Color of young leaves	CoJeFe	-	Direct observations
Flowers	Number of male flowers	Nbflm	-	Counting
	Number of hermaphrodite flowers	Nbflh	-	Counting
	Number of abnormal flowers	Nbfls	-	Counting
	Flower sex ratio	Txflh	Percent-age	calculation
	Appearance of panicles	Aspp	-	DOCDS
	Inflorescence shape	StrInf	-	DOCDS
	Elaboration of Inflorescence	ForInf	-	DOCDS
	Flower color	Cofl	-	Direct observations
	fructification form	Forfru	-	Direct observations
	Nut tint	TeiN	-	Direct observations
Nut	Nut shape	ForN	-	DOCDS
	Shape of nut base	ForbaN	-	DOCDS
	Attachment of peel to kernel	FacD	-	Hand appreciation
	Nut yield	RdYN		25 kg-balance
	Nut weight	PdsUN		5 kg-electronic balance
	Kernel rate	TxAm		Pair of scissors, calculation

Apples	Attachment of nut to apple	Sepnpo	-	Torsion with hand
	Apples length	Longpo	-	Caliper
	Apple diameter	Diampo	-	Caliper
	Apple weight	PdsUPo	Gram	Electronic balance
	Mature apple shape	Forpo.1	-	DOCDS
	Shape of apple base	Forbapo	-	DOCDS
	Mature apple color	Coulpo	-	Direct observations

Statistical analysis

Data processing was focused on a data matrix (row x column) consisting of 394 individual cashew mother trees and 31 variables of which, 12 were quantitative and 19 qualitative. A classification on mixed data (qualitative and quantitative) was carried out in two stages on the data matrix: (1) a Factorial Analysis on Mixed Data (FAMD) was carried out to produce an intermediate representation of the data; (2) then, a Hierarchical Cluster Analysis (HCA) was carried out based on the "representative" factors of the FAMD. The morpho-metric group number of cashew trees was determined using the criterion of the partition having the greatest inertia (Lamarange, 2016). A discriminating canonical analysis was performed on the line-column matrix constituted by the individual trees identified by their morpho-metric group and the selected descriptors after a stepwise discriminant analysis. A morpho-metric characterization of the different groups was performed on the basis of the quantitative discriminant descriptors from a multivariate analysis of variance (MANOVA). Correlation test of Pearson was performed to test the link between quantitative discriminant variables. Pearson's Chi-squared test was carried out to infer cashew mother trees' distribution in the three agroecological zones studied. The statistical analyses were carried out with the software R3.4.0 (R Core Team 2017). The best.cutree function of the JLutils package was used to determine the number of morpho-metric groups. The greedy.wilks function of the KlaR package was used for stepwise discriminant analysis while the lm and candisc functions of the candisc package were used for discriminant canonical analysis (model definition and analysis execution). The cor function was used for the correlation test.

Results

Variation of morphological descriptors of cashew mother trees

The coefficients of variation of morphological descriptors of cashew mother trees show that most of the descriptors are more dispersed around the averages ($CV > 25\%$) for all variables, especially for abnormal flowers number ($CV > 100\%$) (Table 2). The gap around the kernel rate is low ($CV < 10\%$), which indicates a low dispersion of fruit morphological descriptors (kernel) around the average for all individuals (Table 2).

Table 2 - Mean and coefficient of variation (CV) of morphological descriptors for all cashew mother trees.

VARIABLES	ACRONYMS	UNIT	MEAN	COEF.VAR (%)
Nut yield	RdYN	kg	40.06	38.20
Nut weight	PdsUN	g	7.24	35.77
Kernel rate	TxAm	%	28.78	7.78
Tree height	Hatot	m	7.86	26.90
Crown diameter	DiamCo	m	12.54	28.68
Apple length	Longpo	cm	7.16	17.89
Apple diameter	Diampo	cm	4.64	16.33
Apple weight	PdsUPo	g	74.12	34.72
Male flowers number	Nbflm	-	26.57	60.72
Hermaphrodite flowers number	Nbflh	-	6.53	67.28
Abnormal flowers number	Nbfls	-	2.00	105.63
Flower sex ratio	Txflh	%	20.42	64.33

Coef.var (%) : Coefficient of variation

Determination of morpho-metric groups and discriminant descriptors of cashew mother trees

Results of the Hierarchical cluster analysis on mixed data showed three significant morpho-metric groups of cashew mother trees (Figure 2). The first group was formed with 137 individuals, the second group comprised 176 individuals while the third contained 81 individuals (Figure 3).

Stepwise discriminant analysis carried out on all the morpho-metric descriptors of the cashew mother trees showed that 15 of the 31 studied descriptors, 5 quantitative and 10 qualitative descriptors, were found to be the most discriminating ($F > 0.02$, $P < 0.001$).

Results of the discriminatory canonical analysis carried out to identify the characteristics of cashew individuals mother trees allowing morpho-metric groups discrimination revealed that the first two canonical axes were globally significant ($P < 0.05$) with 65.89% for the first axis and 34.11% for the second. Correlations analysis between each of the two axes and the initial variables made it possible to deduce that leaf cross-section (CroNer), flower sex ratio (Txflh) and to a lesser extent, apples weight (PdsUPo), abnormal flowers number (Nbfls) and shape of nut base (ForbaN) were significantly correlated with the first axis (Table 3). Leaf cross-section and nuts weight showed significant negative correlations while other variables showed significant positive correlations. The axis 2 has no significant correlation with the variables. The joint representation of the cashew mother trees identified by their group in the first factorial plane formed by the canonical axes 1 and 2 (Figure 4) has shown that the first axis separates the group G1 from the other two G2 and G3. This position allows us to conclude that the cashew mother trees of the first group (G1) are characterized to a lesser extent by a high nuts unit weight and are distinguished from the two other groups (G2 and G3) individuals by the leaf's veins crossing. In contrast, cashew mother trees of G2 and G3 groups are characterized by a high flower sex ratio, and to a lesser extent by a high number of abnormal flowers and are distinguished from of the first group's trees (G1) by the shape of nuts base.

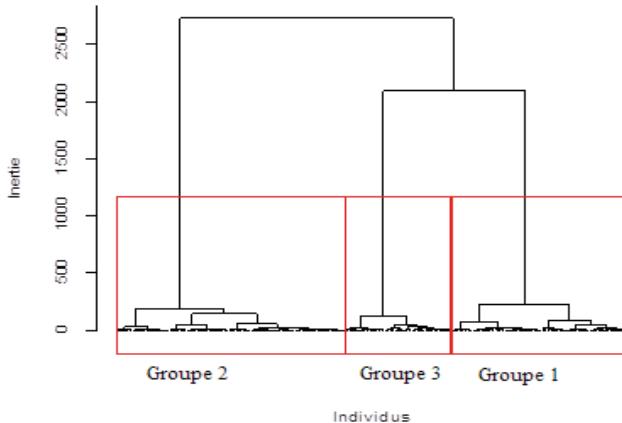


Figure 2- Dendrogram showing the morpho-metric groups of preselected cashew mother trees used during the study.

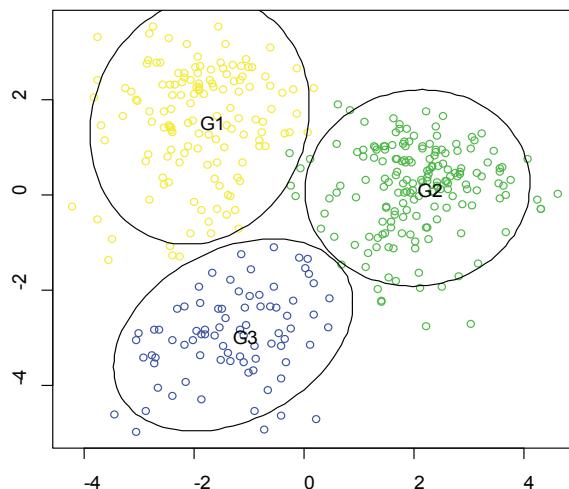


Figure 3- Morpho-metric groups of cashew mother trees projection on the plan defined by the two first axes.

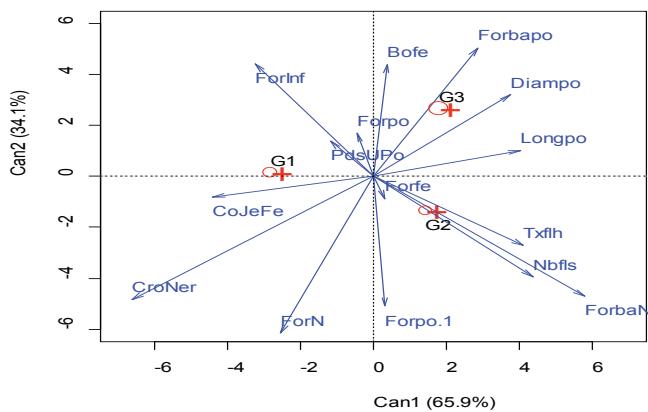


Figure 4 - Joint representation of cashew mother tree's groups and their characteristics

Table 3- Correlation between the initial variables and the two canonical axes

VARIABLE	CAN1	CAN2
Bofe	0.12	0.26
CoJeFe	-0.14	-0.11
CroNer	-0.65	-0.29
Diampo	0.35	0.15
ForbaN	0.48	-0.32
Forbapo	0.06	0.25
Forfe	0.06	-0.17
ForInf	-0.28	0.34
ForN	-0.14	-0.39
Forpo	0.01	0.24
Forpo.1	-0.01	-0.31
Longpo	0.39	0.02
Nbfls	0.42	-0.25
PdsUPo	-0.44	0.09
Txflh	0.53	-0.22

Characteristics of morphological groups of cashew mother trees

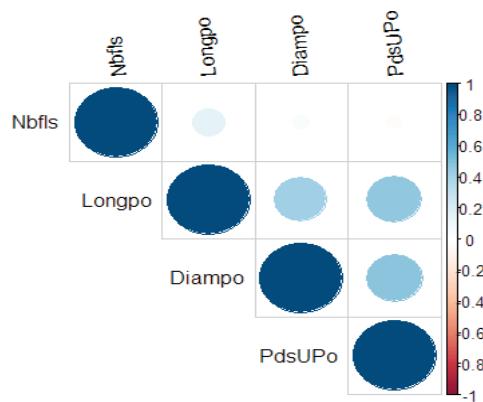
The cashew mother trees of the morphological group 3 have on average the best performance in apple production (large apples with high weights) (Table 4). Discriminant descriptors vary significantly between morphological groups ($P<0.01$).

Table 4- Morphological traits of the cashew mother trees groups

Descriptors	MORPHOLOGICAL GROUPS OF CASHEW MOTHER TREES						Prob	
	Group 1		Group 2		Group 3			
	m	se	m	se	m	se		
Diampo (cm)	4.28	0.06	4.63	0.05	5.26	0.07	0,000***	
Longpo (cm)	6.49	0.10	7.39	0.09	7.80	0.13	0,000***	
PdsUPo (g)	78.48	2.17	69.29	1.92	77.21	2.83	0,000***	
Txflh (%)	12.96	0.99	26.76	0.88	19.25	1.29	0,000***	
Nbfls	0.71	0.15	3.27	0.13	1.44	0.20	0,003**	

*** Significant at the 0.1% threshold; ** Significant at the 1% threshold. m = average; se = standard error

The Fig 5 shows the correlogram of the five discriminant quantitative descriptors of cashew mother trees. This correlogram reveals the relationships existing between these descriptors. Apples length (Longpo) was positively correlated with apples diameter (Diampo) ($r=0.41$) and apples weight (PdsUPo) ($r=0.46$). Positive associations were also observed between apples diameter (Diampo) and apples weight (PdsUPo) ($r=0.47$) in one hand, and between apples length (Longpo) and number of abnormal flowers (Nbfls) ($r=0.16$) in second hand. The correlations between number of abnormal flowers (Nbfls) and apples diameter (Diampo) and between number of abnormal flowers (Nbfls) and apples weight (PdsUPo) are not significant (P value>0.05).



Legend: Positive correlations are displayed in blue and negative correlations in red. The intensity of the color and the size of the circles are proportional to the correlation coefficients. On the right of the correlogram, the color legend shows the correlation coefficients and the corresponding colors.

Fig 5 - Correlogram of cashew mother trees descriptors

Distribution of cashew morpho-metric groups' mother trees

The Pearson's Chi-squared test showed that the cashew mother trees from two morpho-metric groups were linked to the agro-ecological zones studied ($P < 0.0001$). Indeed, the cashew mother trees from the morpho-metric group G1 were uniformly distributed in the three zones. Nevertheless, the cashew mother trees from the morpho-metric group G2 were predominantly concentrated in a single zone (Z V). Those from the morpho-metric group G3 were totally absent in one zone (Z IV) and strongly present in one of the other two zones (Z III) (Table 5).

Table 5 - Distribution of cashew mother trees' morpho-metric groups in agro-ecological zones

MORPHO-METRIC GROUP	AGRO-ECOLOGICAL ZONES			TOTAL
	Z III	Z IV	Z V	
G1	35	61	41	137
G2	5	4	167	176
G3	71	0	10	81
Total	111	65	218	394
X-squared value = 335.13			P-value < 0.0001	

Discussion

Knowledge of genetic diversity is an important step in the process of plant breeding and conservation of plant genetic resources (Adoukonou-Sagbadja *et al.*, 2007). According to Djekota *et al.* (2014), knowledge of population structure in plant genetic resources management is the first step in any selection process. The results of the present study show a great variability between cashew mother trees population, particularly relative to leaves, apples, nuts, flowers and inflorescences as observed in previous studies carried out in Malawi, Brazil and Benin (Chipojola *et al.*, 2009; Castro *et al.*, 2011; Chabi Sika *et al.*, 2015). These variations can be explained by adaptations to ecological conditions. However, these results show that quantitative agronomic parameters of cashew mother trees related to nut are not discriminating. This means that the three morphological groups of cashew mother trees are not significantly different in terms of their nut yields as well as the quality of the produced nuts. These results could be explained by the fact that these trees come from a selection using the same main criteria including the nut characteristics.

Chabi Sika *et al.* (2015) who studied Benin cashew accessions randomly selected in three production areas had obtained four (4) distinct morphological groups. In the characteristics of these 4 groups, there is a group having nuts unit weight equal to 5.88 g against 6.26 to 6.91 g for the other 3 groups. Among cashew mother trees selection criteria used in this study, one criterion requires that the average nuts unit weight or must be greater than 6 g. It can thus be deduced that the results of the two studies are well concordant since no cashew mother tree weighting less than 6 g (the fourth morphological group obtained by Chabi Sika *et al.*, 2015) was attended in the present study.

In this study, quantitative parameters discriminating the different morphological groups are only related to apples and inflorescences. Diverse morphological characterization carried out on cashew germplasm in other countries such as India, Venezuela and Tanzania (Sindoni *et al.*, 2005; Dasmohapatra *et al.*, 2014) supported the present findings. In brief, there is a considerable variation among the cashew mother tree populations in hermaphrodite flowers rate, apples unit weight, abnormal flowers number and nut basis shape suggesting high potential for selection.

The degree of association among traits is an important factor when dealing with a complex character such as apples weight, apples diameter and apples length that are controlled by many genes and highly influenced by the environment (Steel

and Torrie, 1980; Anyaoha *et al.*, 2018). Previous studies reported high positive correlations between apples weight and apples length (Chabi Sika *et al.*, 2015). The positive significant phenotypic correlations between apples traits are indications that selection in favor of these traits can lead to positive indirect selection for high apples sizes and weight that are useful traits in cashew breeding for juice industries (dos Santos *et al.*, 2011).

From this study, we observed that the cashew mother trees from two morphometric groups are linked to the agro-ecological zones. This can be explained by the cashew accession adaptation to similar agro-ecological zones conditions (Aliyu and Awopetu, 2007). Future studies will investigate about the variation of some morphological traits due to one or more environmental factors such as altitude, air temperature, wind, water availability or other factor.

Conclusion

The results obtained in this study showed a large variability between the cashew mother trees, particularly on leaves, apples, nuts, flowers and inflorescences levels. However, they show that the quantitative agronomic parameters of cashew mother trees related tonut are not discriminating because they come from a selection using the same criteria. These results are very important for the continuation of the ongoing varietal breeding program. They will be refined by molecular genetic characterization.

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