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Morphological traits of control-pollinated fruits in African plum (*Dacryodes edulis* (G.Don).Lam.) using multivariate statistical techniques

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Abstract

Phenotypic variation on 26 well-known accessions of African plum collected from four provenances established as genebanks was assessed under controlled-field conditions using a full nested mating design. Data were recorded for 12 agro-morphological fruit traits using multivariate statistical techniques. Descriptive statistics for each studied trait were calculated. In addition, patterns of morphological variation were assessed using principal component analysis (PCA). Studied accessions showed considerable variation in fruit length, fruit width, fruit and pulp weight, pulp thickness and fruit:kernel weight ratio. Clustering of accessions into similarity groups was performed using Ward's hierarchical algorithm based on squared Euclidean distances. The accessions based on studied traits were classified in 03 groups. Results showed that, fruits from accessions within Boumnyebel and Kekem provenances constitute cluster 1. Accessions in this cluster had better fruits traits and could be selected as raw material for breeding purposes or clonal multiplication. Principal component analysis (PCA) revealed that the first two principal components (fruit length, fruit width) accounted for 87.01% of the total variation. Among the studied traits, fruit length, fruit width, fruit and pulp weight, pulp thickness and fruit:kernel weight ratio showed strong and high positive link with the first component (PC1) whereas kernel weight and fruit length:width ratio showed positive link with the second component (PC2). These results suggest that fruit weight is a good predictor of pulp yield, although its predicting power differed among clusters.

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Introduction

Success in breeding for yield superiority of indigenous fruit trees is still constrained by the lack of availability of improved germplasm (Akinnifesi et al., 2007). Improvement of indigenous fruit tree cultivars is drawing great attention from breeders. In some cases, a participatory approach to cultivar development is implemented with success (Leakey et al., 2003). In the fulfilment of cultivars development for priority tree species, two key elements are (1) the identification of "plus trees" in natural populations and (2) their propagation by vegetative techniques. Prior to "plus trees" identification, quantitative characterization of fruit, nut and kernel variation (Leakey et al., 2005a), variation in nutritive value and other food properties (Leakey et al., 2005b) have to be studied and an understanding of the interactions between different traits for multi-trait selection is needed (Leakey et al., 2004). *Dacryodes edulis* (G.Don.) H.J.Lam.) or African plum, an oleaginous fruit tree belonging to the family Burseraceae in the Gulf of Guinea (Bourdeaut, 1971), is one of a number of indigenous fruits under domestication (Tchoundjeu et al, 2002; 2006) and widely commercialized in Cameroon, Gabon, Democratic Republic of Congo, Ghana and Nigeria (Awono et al., 2002). The species contributes to rural incomes, supplements the local diet and is used in traditional and modern therapies (Schreckenberg et al., 2002; 2006). The value of the fruit (Safou or African plum) lies in its edible pulp which is a good source of proteins, fats and carbohydrates that could be used to alleviate malnutrition in children (Kinkela et al., 2006; Ajayi and Adesanwo, 2009). The fruits could also provide vegetable oils for food (Kengni et al., 2004; Ikhuoria and Maliki, 2007), pharmaceutical (Koudou et al., 2008) and cosmetic (Dawodu, 2009; Ajibesin, 2011) industries. In efforts to enhance the species' genetic conservation and utilization, it was identified as one of the top ten agroforestry tree species for future crop diversification in West and Central Africa (Franzel et al., 2007). Works on African plum especially in Nigeria (Anegbeh et al., 2005) and Cameroon (Leakey et al., 2002a; Waruhiu et al., 2004) have revealed considerable phenotypic and genotypic variation and allowed selection of superior trees based on fruit and pulp weight, fruit width, pulp taste and pulp colour. The species has been reported to be amenable to vegetative propagation methods like air-layering and stem-cutting (Mialoundama et al., 2002). In addition, the rooting system of vegetative propagate trees of African plum have been reported to be stable (Asaah et al., 2010) and apparently less competitive for below ground resource compare to trees of seed origin (Asaah et al., in press.). Despite the advantages of vegetative propagation techniques: which are hastening sexual maturity period and giving the exact replicate of the mother-tree desired characters, it is feared that they could severely narrow the genetic diversity and increase inbreeding within the species at farm level leading to a decline in future production. By contrast, controlled-cross-pollination can help to combine some desired characters in the fruits of one tree and increase the high inter-tree variability between selected superior genotypes with high-viability.

To our knowledge, no study has documented morphological and genetic characterization of African plum fruits obtained through controlled-cross-pollination. Such bottom-up approach may help to develop and characterize "control-pollinated fruits", improve raw material for breeding and clonal development while allowing the species to better express its potential (i.e. fruit size and yield, pulp productivity, pulp taste, pulp oil content, etc.) and identify links between traits. The current study aims at (1) matching the assessment of control-pollinated African plum morphological fruit traits in order to maximize the availability of improved African plum germplasm, (2) analyzing its relationship with intra-provenances performance and, (3) analyzing the implications to further breeding improvement leading to the expression of the genetic gain in F_2 and F_3 generations. Thus, the following questions were addressed: (i) Do control-pollination increase the quality of the quantitative fruit traits of well-known African plum accessions?

(ii) Which African plum's ecological provenance posses best fruits traits for selection through control-pollination? Information presented herein, would help breeders to develop high yielding and good quality African plum hybrids for breeding, clonal selection and cultivar development.

Materials and methods

Floral biology of the study species

Kengue (2002) defined the breeding system of this species as allogamous. Cross-pollination is effectuated by insects notably honeybees of the species *Meliponula erythra* with possibilities of self-pollination in hermaphrodite flower, but an earlier controlled-crossing study suggests that out crossing is a frequent mode of reproduction Kengue (1990). The fact that African plum flowers are very small (Fig. 1 and 2) and are grouped into inflorescences presents problems in the investigation of reproductive biology. The species is gynodioecious with homogamous trees (carry only female flowers) and heterogamous or mixed trees (carry male and hermaphrodite flowers). The inflorescence is axial and made up of a panicle with a biparous cyme on leafy branches. The male-hermaphrodite (up to 40 cm long) inflorescences have a pyramidal shape (Fig. 3) with a bright yellow colour bearing about 300-500 flowers among which only 75-120 will rich anthesis. The female inflorescence (Fig. 4) is smaller (5-30 cm long) and bears only about 90 flowers due to its small size. Flowers with 3-8 mm long and 3-4 mm diameter in size are reddish-brown and open sequentially from basal to distal positions (acropetal blooming). Morphologically, the hermaphrodite flowers resemble the male flowers, but bear potent female reproductive organs. The lifetime of a flower is three to four days. Both flower sexes produce copious amounts of sugar-rich nectar (melliferous tree) and have 3-5 free sepals and 2-4 petals. The androecium has 6 stamens that are about 3 mm long and the peri-ovarien disk has 6 lobes. The ovary is divided into two loges by a median placenta that is limited by a one-layered membrane. For both sexes, the blooming period is about 40 days.

Study sites

The present study was carried out from January 2010 to September 2011 at two experimental field trials established by World Agroforestry Centre (ICRAF) technical staff with the participation of farmers and at the ICRAF's central nursery at Nkolbison (3°51'N Lat., 11°27'E Long.) for nursery activities (Fig. 5). This site lies at an altitude of 760 m.a.s.l. The first living gene bank is situated at Minkoa-Meyos near Yaounde, Cameroon (3°51'N Lat., 11°25'E Long.), which lies at an altitude of 813 m.a.s.l., with a mean annual rainfall of approx.1400 mm with bimodal distribution and a mean annual temperature of 25°C. The soils are moderately acid i.e pH 1:1 soil:water 5 to 6 and Al saturation 20. The second one is situated at 65 Km from Yaounde in the Mbalmayo division (3°10'N Lat., 11°00'E Long.), which lies at an altitude of 650 m a.s.l., with a mean annual rainfall of approx.1802 mm and a mean annual temperature of 24°C.The soils are deep ferralitic (Ambassa-Kiki, 2000).

Plant materials and agro-morphological traits

The experimental plant material comprised of 26 (18 females and 08 males) selected superior genotypes accessions of African plum (9 years old) submitted to controlled-cross-hand-pollination using a full nested mating design (Zobel and Talbert, 1984). In established provenances, pollens harvested from 02 male trees were used to fertilize ovules on 05 female trees. Characteristics selected for accessions include size and fruit flavour, colour and thickness of the pulp, pulp oil content, the fruiting season, disease resistance and pest, the frequency and regularity of fruiting performance (yield). The collected germplasm were accessions of well-known and appropriate origin from home gardens, crop fields, forest fallow, cocoa and coffee farms. The trees were also located using a Global Positioning System for further sites mapping (Table 1). The four provenances (Makenene, Kekem, Boumnyebel and Limbe) established as living gene banks in two experimental field trials represented site type widely available for *D. edulis* in Cameroon. Each provenance-plot was surrounded by external perimeter rows of unimproved and unknown *D. edulis* provenances. Trials were hand-weeded twice per year for at least the first 7 years. The data were recorded for 12 morphological and agronomic fruit traits listed in tables 2, 3 and 4.

Pollination experiments

The pollination experiments were performed between January 20 and March 3, 2010 at the Minkoa-Meyos locality, whereas at Mbalmayo site they were carried out between March 17 and April 13, 2010 using a full nested mating design. The experimental design included provenance as a fixed factor, treatment as within-subject (i.e. repeated measures) fixed factor and plant individual as a random factor (subject). In each provenance we marked, labelled and bagged ten healthy and vigorous panicles on five female individuals and two male-hermaphrodites one week prior to anthesis. Panicles were bagged on both sexes with mesh (1 mm^2) in size of 30 cm x 15 cm that allowed the passage of light and air but not insects to avoid insect visitation and possible pollen removal. The bags were removed at anthesis when the first open flowers were in the female stage. The average number of 18 flowers was used and any unopened buds were removed. Pollen was collected with a pair of pliers and a fine paint brush with black hairs against which the pollen could be seen, and kept in a Petri dish (Fig. 6 and 7). On each selected female tree, bags were remove every day at 9 a.m., recently opened flowers were pollinated once by pollen previously harvested on two male-hermaphrodite between 6 and 8 a.m., located at least 10 m away from the target female. The panicles were then rebagged immediately after hand-pollination. Flowers were monitored every three to four days recording the number of fruit set per panicle and per female tree and bags were removed after eight days Makueti et al. (in press).

Experimental details and parameters measured

Upon physiological maturity (up to 17-21 weeks after hand-cross-pollination), fruits were collected in open-weave collection bags, labelled and transported to the ICRAF office at Nkolbison for characterization as soon as possible (2-3 days later). Fruit length, fruit width and pulp thickness were measured using 0.1 mm digital calipers, while fruit weight and kernel weight were determined using a 0.1 g electronic balance (Ohaus HP-320), based on the methods of Leakey et al. (2002a) and Waruhiu et al. (2004) for the same species. Pulp weight (fruit weight-kernel weight) was derived by difference. Fruit length:width ratio, fruit:kernel weight ratio, fruit:pulp weight ratio were assessed and the number of kernel per fruit recorded. To improve accuracy, fruit's width was measured at the first, the second and the third quarter of each fruit and the arithmetic means were considered as the fruit's width. Pulp thickness was measured at four points on a longitudinal split half. The mean value gave the pulp thickness. Epicarp and mesocarp colours were assessed using a Home base colour chart; values were recorded on characterization forms developed by ICRAF.

Statistical analyses

Analysis of variance was performed to determine the descriptive statistics such as mean, standard error, standard deviation and variance for each one of nine quantitative traits over twelve studied traits. Pearson test was used to assess correlation among variables. Hierarchical clustering analysis (dendrogram) using Ward's hierarchical algorithm based on squared Euclidean distances was performed to study selected accessions with some promising crosses out of which those with high performing traits could be selected. Prior to squared Euclidean distance calculation, the data were standardized by variable to have a mean of zero and a variance of one. In addition, patterns of morphological variation were assessed using principal component analysis (PCA). Those PCs with eigen values >1.0 were selected, as proposed by Jeffers (1967). Multivariate ANOVA Tests were used to confirm the accuracy of grouping that produced by cluster analysis. Student-Newman-Keuls Test was used to identify the discriminative traits within and between clusters.

As the pulp is the principal trait of commercial importance we also carried out a linear regression to identify predictors of pulp yield per fruit and to test if predicting power of the explanatory variables differs between crosses. We built a linear regression for pulp weight per fruit, with four independent variables measured on fruits (length, width, thickness and weight). Pearson's correlation was performed between the independent variables to test multicollinearity. Since there were significant strong correlations between pairs of variables (r > 0.60, P < 0.001) only one independent variable (fruit weight) was finally used in the regression model. We insert crosses classified in 3 clusters in the model as dummy variable (Kutner et al., 2005). Three models shown in the equations below were tested:

$Pulp\ weight = \beta_0 + \beta_1(fruit\ weight) + \varepsilon$ ....EQ₁

$Pulp\ weight = \beta_0 + \beta_1(fruit\ weight) + \beta_2(cluster) + \varepsilon$ ....EQ₂.

$Pulp\ weight = \beta_0 + \beta_1(fruit\ weight) + \beta_2(cluster) + \beta_3(fruit\ weight \times cluster) + \varepsilon$ ......EQ₃

β_0 indicates the intercept, β_1, β_2 and β_3 the partial regression slopes and ε the unexplained error associated to the model. The residuals normality plot, the residual vs. fitted plot and the residuals vs. leverage plots with Cook distance were used to diagnose the regressions models (Quinn and Keough, 2005). Data were processed under SPSS version 17.0.0 (Aug 23, 2008).

Results

Description of fruit traits from controlled pollinated accessions of African plum

Fruit, pulp and kernel weight displayed continuous and highly significant (P<0.05) tree-to tree variation in all crosses combined (Table 2). Mean fruit weight was 68.49 g. The heaviest fruits were registered from crosses from Boumnyebel, Makenene and Kekem accessions respectively. Mean pulp weight was 56.92 g. The heaviest pulp was also recorded in Boumnyebel, Makenene and Kekem accessions respectively. Mean kernel weight was 11.57 g. Mean kernel weight differ significantly (P<0.05) between crosses. Variation was between fruits within each crossing and between tree provenances. Fruit:kernel weight ratio range from 1.67 to 14.80 with a mean of 5.93 ± 0.05 g whereas fruit:pulp weight ratio range from 1.00 to 2.50 with a mean of 1.25 ± 0.00 g.

Mean fruit length and width differed significantly (P<0.05) between crosses with continuous tree-to-tree variation (Table 2). Mean fruit length was 78.83 mm and the longest fruits were registered from Boumnyebel (88.15 mm) and Makenene (76.54 mm) crosses. Mean fruit width was 40.15 mm and the largest fruits were recorded from Boumnyebel (42.51 mm) crosses. Mean fruit width differed significantly (P<0.05) between crosses. Fruit length:width ratio range from 0.83 to 4.02 with a mean of 1.97 ± 0.00 mm.

Mean pulp thickness differed significantly (P<0.05) between trees with continuous tree-to-tree variation (Table 2). Mean pulp thickness was 6.80 mm. The pulp thickness differ significantly (P<0.05) between crosses and the highest pulp thickness was registered from Makenene (7.01 mm) crosses.

The number of kernel per fruit varied from zero (00) to one (01). Occasionally, crossing from the four provenances had fruits with no kernels and sometimes many fruits of a particular crossing had no kernels (C_106*104 and C_106*116 within Makenene provenance). Among the 1261 fruits obtained after controlled pollination, 42 appear with no kernel making a frequency of 3.3 %.

Epicarp and mesocarp colours of studied fruits varied from one tree to another. Five different epicarp colours and seven different mesocarp colours were registered. The most common epicarp colour was Hereford heather found in 72.6 % of the fruits. Green pastures and Eucalyptus were the most common mesocarp colours (34.9 % and 33.0 % respectively).

Table 1. List of the 26 studied African plum accessions collected from the two agro-ecologic zones in favour with growth and development in Cameroon.

Acc. no.	Code in gene bank	Collection sites	Accession sex	Latitude	Longitude	Altitude (m)
AC-01	BUM/DE/26 Seedling 015	Boumnyebel	Female	3°52'58.34''N	10°50'57.62"E	358
AC-02	BUM/DE/25 Seedling 026	Boumnyebel	Female	3°52'58.34''N	10°50'57.62"E	358
AC-03	BUM/DE/37 Seedling 111	Boumnyebel	Female	3°52'58.34''N	10°50'57.62"E	358
AC-04	BUM/DE/25 Seedling 114	Boumnyebel	Female	3°52'58.34''N	10°50'57.62"E	358
AC-05	BUM/DE/26 Seedling 122	Boumnyebel	Female	3°52'58.34''N	10°50'57.62"E	358
AC-06	MAK/DE/04 Seedling 078	Makenene	Female	4°53'03.84''N	10°47'41.44"E	696
AC-07	MAK/DE/28 Seedling 104	Makenene	Female	4°53'03.84''N	10°47'41.44"E	696
AC-08	MAK/DE/01 Seedling 116	Makenene	Female	4°53'03.84''N	10°47'41.44"E	696
AC-09	MAK/DE/04 Seedling 144	Makenene	Female	4°53'03.84''N	10°47'41.44"E	696
AC-10	KEK/DE/18 Seedling 050	Kekem	Female	5°09'05.91''N	10°01'16.07"E	715
AC-11	KEK/DE/18 Seedling 070	Kekem	Female	5°09'05.91''N	10°01'16.07"E	715
AC-12	KEK/DE/07 Seedling 074	Kekem	Female	5°09'05.91''N	10°01'16.07"E	715
AC-13	KEK/DE/13 Seedling 079	Kekem	Female	5°09'05.91''N	10°01'16.07"E	715
AC-14	KEK/DE/07 Seedling 142	Kekem	Female	5°09'05.91''N	10°01'16.07"E	715
AC-15	MAK/DE/35 Seedling1B1	Makenene	Female	4°53'03.84''N	10°47'41.44"E	696
AC-16	BUM/DE/14/99 Seedling5B3	Boumnyebel	Female	3°52'58.34''N	10°50'57.62"E	358
AC-17	MAK/DE/94 Seedling13B3	Makenene	Female	4°53'03.84''N	10°47'41.44"E	696
AC-18	LIMBE/DE/106Seedling 22B1	Limbe	Female	4°01'20.62''N	9°11'43.71"E	36
AC-19	BUM/DE/29 Seedling 070	Boumnyebel	Pure male	3°52'58.34''N	10°50'57.62"E	358
AC-20	BUM/DE/29 Seedling 050	Boumnyebel	Male-hermaphrodite	3°52'58.34''N	10°50'57.62"E	358
AC-21	MAK/DE/33 Seedling 106	Makenene	Pure male	4°53'03.84''N	10°47'41.44"E	696
AC-22	MAK/DE/33 Seedling 126	Makenene	Male-hermaphrodite	4°53'03.84''N	10°47'41.44"E	696
AC-23	KEK/DE/02 Seedling 088	Kekem	Pure male	5°09'05.91''N	10°01'16.07"E	715
AC-24	KEK/DE/02 Seedling 102	Kekem	Male-hermaphrodite	5°09'05.91''N	10°01'16.07"E	715
AC-25	BUM/DE/09/98 Seedling 12B1	Boumnyebel	Pure male	3°52'58.34''N	10°50'57.62"E	358
AC-26	BUM/DE/14/99 Seedling 16B4	Boumnyebel	Male-hermaphrodite	3°52'58.34''N	10°50'57.62"E	358

Table 2. Basic statistics for nine quantitative agro-morphologic traits in 18 African plum studied accessions (n = 1261).

Code	Descriptors	Minimum	Maximum	Mean	SE	SD	Variance
T01	Fruit length (mm)	33.90	112.00	78.33	0.38	13.83	191.27
T02	Fruit width (mm)	18.35	57.75	40.15	0.17	6.17	38.11
T03	Pulp thickness (mm)	4.08	9.38	6.80	0.02	0.81	0.66
T04	Fruit weight (g)	10.00	118.00	68.49	0.66	23.66	559.84
T05	Pulp weight (g)	6.60	99.00	56.92	0.63	22.70	515.63
T06	Kernel weight (g)	0.00	25.00	11.57	0.10	3.66	13.40
T07	Fruit length:width ratio (fruit form)	0.83	4.02	1.97	0.00	0.26	0.06
T08	Fruit:pulp weight ratio	1.00	2.50	1.25	0.00	0.17	0.03
T09	Fruit:kernel weight ratio	1.67	14.80	5.93	0.05	2.05	4.24

SE: Standard Error; SD: Standard Deviation

Table 3. Frequency of epicrap fruit color appearance in 18 African plum studied accessions (n = 1261).

Code	Qualitative descriptor	Frequency	Percent (%)
EC01	Hereford heather	916	72.6
EC02	Malvern blue	56	4.4
EC03	Mulberry	61	4.8
EC04	Royal blue	81	6.4
EC05	Viola	147	11.6
	Total	1261	100

Table 4. Frequency of mesocrap fruit color appearance in 18 African plum studied accessions (n = 1261).

Code	Qualitative descriptor	Frequency	Percent (%)
MC01	Eucalyptus	440	34.9
MC02	Green pastures	416	33.0
MC03	Lime	81	6.4
MC04	Misty green	82	6.5
MC05	Spring green	125	9.9
MC06	Summer rose	56	4.4
MC07	White wine	61	4.8
	Total	1261	100

Table 5: Correlation between quantitative morphological traits of control-pollinated African plum fruits and Rotated Component Matrix.

Code	Quantitative descriptors	PC1	PC2
T01	Fruit:kernel weight ratio	0.973	-0.071
T02	Fruit width	0.971	0.167
T03	Fruit:pulp weight ratio	-0.968	0.182
T04	Pulp weight	0.964	0.173
T05	Fruit weight	0.949	0.234
T06	Fruit length	0.838	0.499
T07	Pulp thickness	0.731	0.311
T08	Kernel weight	0.081	0.876
T09	Fruit length:width ratio	0.076	0.815
Eigen value		6.181	1.651
Proportion		68.677	18.339
Cumulative		68.677	87.016

Table 6. Means and standard errors of quantitative morphological traits of control-pollinated African plum fruits.

Code	Quantitative descriptors	Cluster1	Cluster2	Cluster3	*P*
T01	Fruit length (mm)	90.339 ± 1.098[a]	63.423 ± 1.644[b]	72.213 ± 1.324[c]	0.000
T02	Fruit width (mm)	43.838 ± 0.412[a]	32.351 ± 0.550[b]	39.985 ± 0.497[c]	0.000
T03	Pulp thickness (mm)	7.239 ± 0.068[a]	6.083 ± 0.09[b]	6.178 ± 0.082[c]	0.000
T04	Fruit weight (g)	83.688 ± 2.76[a]	38.249 ± 3.691[b]	67.291 ± 3.33[c]	0.000
T05	Pulp weight (g)	71.264 ± 2.642[a]	26.573 ± 3.523[b]	57.215 ± 3.187[c]	0.000
T06	Kernel weight (g)	12.424 ± 0.310[a]	11.706 ± 0.414[a]	10.076 ± 0.374[b]	0.000
T07	Fruit length:width ratio (fruit form)	2.071 ± 0.022[a]	1.999 ± 0.029[a]	1.818 ± 0.026[b]	0.000
T08	Fruit:pulp weight ratio	1.177 ± 0.011[a]	1.443 ± 0.015[b]	1.186 ± 0.014[a]	0.000
T09	Fruit:kernel weight ratio	6.0874 ± 0.210[a]	3.374 ± 0.280[b]	6.393 ± 0.253[a]	0.000

Means followed by the same letter within a column are not significantly different at P< 0.05 (Student-Newman-Keuls test).

Table 7. Calculated Correlations Coefficients

	Fruit length	Fruit width	Fruit lenght:width ratio	Pulp Thickness	Fruit weight	Kernel weight	Fruit :kernel weight ratio	Pulp weight
Fruit length	1							
Fruit width	0.760**	1						
Fruit lenght:width ratio	0.396	-0.267	1					
Pulp Thickness	0.590**	0.646**	-0.025	1				
Fruit weight	0.800**	0.797**	-0.090	0.544**	1			
Kernel weight	0.316	0.248	0.120	0.321	0.333	1		
Fruit:kernel weight ratio	0.558**	0.623**	-0.018	0.324	0.716**	-0.353	1	
Pulp weight	0.782**	0.791**	0.074	0.515**	0.988**	0.185	0.791**	1

**: Very significant at 5%.

Principal component analysis

The principal component analysis (PCA) performed on nine agro-morphological fruit traits of 18 African plum accessions showed that the first two principal components (fruit length, fruit width) had eigen values more than one and accounted for 87.01 % of the total variation. Table 5 presents the correlation between the axes and quantitative traits. The first component (PC1), which explained 65,6 % of the total variation showed a strong and positive link with and between fruit length, fruit width, fruit and pulp weight, pulp thickness, fruit:kernel weight ratio, whereas it was negatively correlated with fruit:pulp weight ratio. This result mean that fruits from crosses with high PC1 values have greater fruit length, fruit width, fruit and pulp weight and fruit:kernel weight ration whereas they have lower kernel weight and fruit length:width ratio. Figure 8 shows the projection of the individuals from the four crossed provenances onto axes 1 and 2 and the crosses' position on the scatter plot. PC2 explained 21.41 % of the total variation and was positively influenced by kernel weight and fruit length:width ratio. This means that crossing with high PC2 values have high kernel weight and fruit length:width ratio. Nevertheless, this second component was negatively correlated with fruit:kernel weight ratio. From this plot (Fig. 8) and table 5 it can be deduced that all crosses located in the upper positive part of the axis 1 outclassed the others for most of the quantitative traits, but showed low values for the fruit:kernel weight ratio. In contrast, the other crosses had high values for the ratio fruit weight/kernel weight.


Fig. 1. *D. edulis* male flower


Fig. 2. *D. edulis* female


Fig. 3. *D. edulis* male inflorescence


Fig. 4. *D. edulis* female inflorescence


Fig. 6. *D. edulis* male flowers in a Petri dish

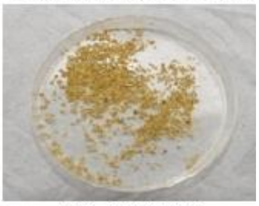
Fig. 7. *D. edulis* pollen

Cluster analysis

The 26 African plum accessions grouped in 36 crosses based on 9 over 12 morphological traits were classified in 03 groups using Ward's hierarchical algorithm as shown in Figure 9. Cluster 1 contained sixteen accessions from Boumnyebel (10) and Makenene (06). Crosses from these provenances are characterized by greater values of eight over nine quantitative fruit traits studied, except fruit:pulp weight ratio. Cluster 2 comprises nine accessions from Boumnyebel (02), Kekem (02), Makenene (03) and Limbe (02). In this cluster, except in fruit:pulp weight ratio, crosses are characterize by lower values in all studied traits. The third cluster included eleven accessions from Kekem (08) and Makenene (03) and is intermediate between clusters 1 and 2. Accuracy of produced groups by cluster analysis was done using multivariate ANOVA (Table 6).

Modelling pulp yield per fruit

Regression equations were used to build predictive models for pulp yield (the principal trait for commercial importance) based on fruit weight (Table 5). There were highly significant and strong relationships (Table 7) between fruit weight and pulp weight ($R^2 = 0.988$). However, fruit weight was a strong predictor of pulp weight (Fig.10). Results showed that only the first model tested was significant, thus the standard linear regression for African plum pulp yield per fruit was:

Pulp weight = -8.040 (0.004) + 0.948 (0.299) x fruit weight....................EQ4

Fig. 5. Localization of studied sites (Source: National Institute of Cartography 2006 (Redrawn by Priscilla Ngaukam).

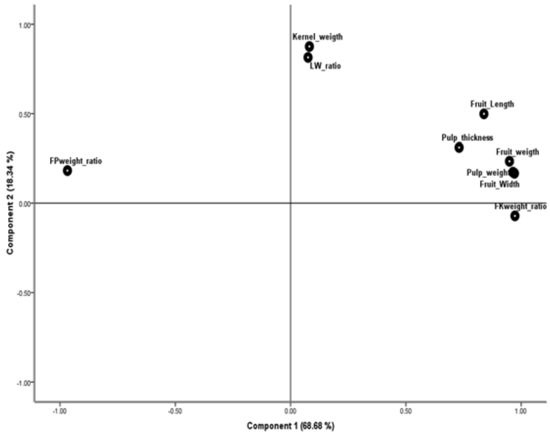

Fig. 8. Scatter plot of the PCA analysis showing links between African plum control-pollinated fruit traits.

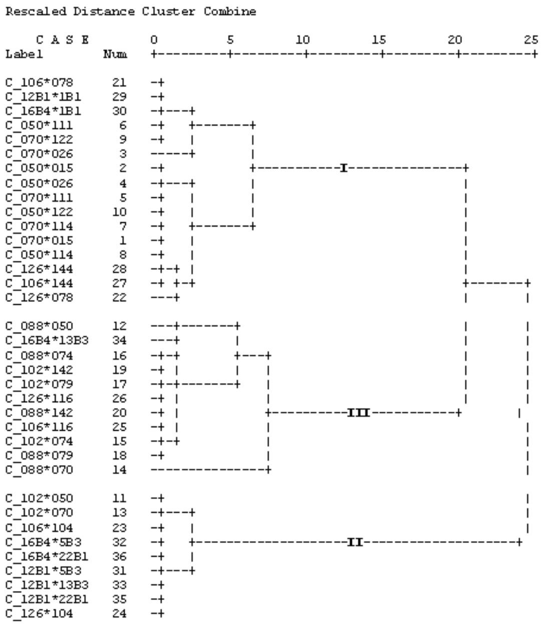

Fig. 9. Ward's dendrogram of 36 crosses on 18 female accessions studied for African plum control-cross-pollinated fruit morphological traits in Cameroon.

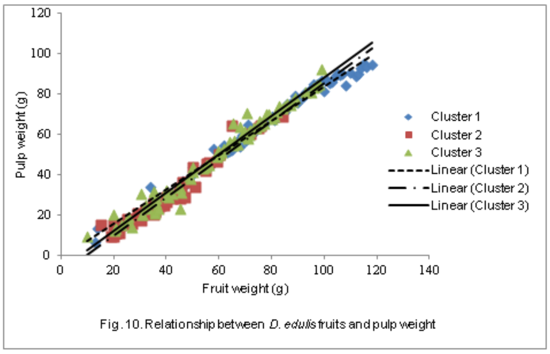

Fig. 10. Relationship between D. edulis fruits and pulp weight.

Discussion

This pioneer study quantifies variation in fruits traits from control-cross-pollinated accessions of African plum and provides basic knowledge on the range of variation of several morphological fruit traits within and between well-known provenance accessions. The studied accessions displayed considerable variation in fruit and pulp weight traits as well as moderate variation for fruit length, fruit width, kernel weight and fruit:kernel weight ratio whereas they showed lower variation in pulp thickness, fruit length:width ratio and fruit:pulp weight ratio. These results suggest that fruits smaller in size and lower in weight have lower fruit:pulp weight ratio. This may imply that in superior trees, increase in pulp weight is greater than that of the kernel weight. The very extensive variation found irrespective of the studied traits is consistent with previous studies on the same species respectively in Cameroon and Nigeria (Leakey et al., 2002a; Waruhiu et al., 2004 and Anegbeh et al., 2005) and other indigenous fruit trees such as *Irvingia gabonenis (*Aubry-Lecompte ex O'Rorke) Baill. ex Lanen (Atangana et al., 2001, 2002), Sclerocarya *birrea* (A. Rich) Hochst. subsp. caffra (Leakey et al., 2002b), *Detarium macrocarpum* Guill. & Perr. (Kouyaté and Van Damme, 2002); *Ziziphus mauritiana* Lam. (Koné et al., 2009), *Allanblackia floribunda* Oliver (Atangana, 2010), *Adansonia digitata* L. (Kouyaté et al., 2011). The relatively strong relationships between fruit weight and pulp weight suggested by the predictive models indicate that selection for pulp can be based on fruit weight. The variability of the relation between fruit weight and pulp weight confirms the moderate differences between clusters and may have been driven by both ecological and genetic variation (Kouyaté, 2005; Sanou et al., 2006; Diallo et al., 2008; Fandohan et al., 2011 and Assogbadjo et al., 2011). Thus, further use of the obtained models should be made with respect to the provenances. This study indicates that based on the quantitative traits, most of the observed variation is held within provenances. Nevertheless, the between provenances variation was found to relatively high, particularly for fruit length, fruit width, fruit weight, pulp weight and pulp thickness. These results are in line with the results reported by Leakey et al., 2004; Waruhiu et al., 2004 and Anegbeh et al., 2005 in the same species respectively in West and Central Africa and by Thiong'o et al., 2002; Kadzere et al., 2006 and Assogbadjo et al., 2011, respectively on Sclerocarya *birrea*, *Uapaka kirkiana* Muell. Arg. in South Africa and *Adansonia digitata* in West Africa. The evidence of continuous intraspecific variation found in the open-pollinated fruit and nut traits of *D. edulis* and other indigenous fruit trees such as *Irvingia gabonensis*, *Vitellaria paradoxa* C.F. Gaertn.., *Adansonia digitata*, *Detarium* *macrocarpum* represents the normal variability arising from out-breeding. The reported results are in line with results of the present study which was focused on controlled pollination of well-known accessions. Nevertheless, this occurrence of continuous variation also questions the validity of Okafor (1983) who postulated the existence of two varieties with different sizes and Youmbi et al., 1989; Ndoye et al., 1997 and Silou et al., 2000. These authors' categorized *D. edulis* fruits into size classes for market studies. In addition, the results of the present study suggest that fruit size is not necessarily related to other fruit traits and they are in fairly agreement with those reported by Leakey et al., 2002a and Waruhiu, 2004 in the same species.

The variation in fruit:kernel weight ratio reported here supports earlier evidence from Kengue (2002) that a few trees produce kernel-less fruits, especially those trees that produce late in the season (Kengue, 1990). In fact, it was observed in this study that, all crosses occasionally had fruits with no kernels and sometimes many fruits of a particular crossing had no kernels. In general, kernel number per fruit is one in this species, thus the variation within individual tree fruit samples from 0 to 1 kernel per fruit may suggest that this trait is affected by some environmental factors in addition to genetics. In fruits obtained through opened-cross-pollination, a possible explanation for this variability in kernel number per fruit could be that not all ovules were successfully pollinated, perhaps as a result of lack of pollinators, adverse hormonal levels or competition for carbohydrate and/or nutrients, excessive distances between trees, or inappropriate weather for pollination activity and even predation on developing seeds (Diallo et al., 2008). One of the urgent actions to address this problem is the setting of beehives in a male tree for dioecious species such as marula (*Sclerocarya birrea*), *Allanblackia floribunda*, African canarium (*Canarium schweinfurthii* Engl.) and masuku (*Uapaca kirkiana*), or in a hermaphrodite tree for monoieciuos species such as baobab (*Adansonia digitata*), ber (*Ziziphus mauritiana* Lam.), tamarind (*Tamarindus indica* L.), *Detarium macrocarpum*, she butter tree (*Vitellaria paradoxa*) and nere (*Parkia biglobosa*) [Jacq.] Benth.

Our study was focused on well-known accessions submitted to controlled-cross-pollination, a process which permits the breeder to deposit pollen exactly onto the stigma of an open flower. The existence of fruit with no kernel in this present work can also be explained by parthenocarpy, a phenomenon which is the natural or artificially induced production of fruit without fertilization of ovules, the fruit is therefore seedless (Parent, 1990). Fruits with a high proportion of zero kernels were among those with highest pulp weight and lowest mean kernel weight, while those with 1 kernel were among those with highest mean kernel weight and lowest pulp weight. Studies on avocado (*Persea americana* L.) Alcaraz et al. (2009), mango (*Mangifera indica* L.) Anila and Rhada (2003) and citrus (Muhammad et al., 2011) species confirmed this hypothesis. Moreover, some "plus trees" from Makenene provenance reputed as off-season tree have many fruits with no kernel (Pers. comm.). As the kernel is of obvious importance for sexual regeneration, it would be of little importance in vegetatively regenerated cultivars and consequently seedless cultivars could be a desirable market trait.

Results showed that crosses which lead to high PC1 values have better fruits traits and can be selected for breeding and cultivar development. Otherwise, crosses with high PC2 values have high kernel weight thus low pulp weight and high fruit length:with ratio. Kernel weight and fruit length:width ratio traits did not have significant importance as selected traits. According to breeding goal, African plum breeders can select accessions by considering appropriate PCs values. Principal component analysis has been widely used in studying agro-morphological fruit characterization in germplasm collections of many exotic crops such as sorghum (*Sorghum bicolor* (L.) Moench. (Mujaju et al., 2008), *Helianthus annuus* L. (Nooryazdan et al., 2010), groundnut (*Vigna subterranea* L.)

Onwubiko et al. (2011), castor bean (*Ricinus communis* L.) Goodarzi et al. (2012) and native indigenous fruit trees such as *Uapaca kirkiana* (Mwase et al. (2006); tamarind (*Tamarindus indica*) Fandohan et al. (2011) and Baobab (*Adansonia digitata*) Assogbadjo et al. (2011). The high variability indicates great potential for further improvement through the development of cultivars from elite trees using horticultural techniques. Speedy benefits may be obtained by selecting superior hybrids from control-cross-pollination, establishing them as seed orchards and propagating such stocks as clones. Since crosses belonging to clusters 1 and 3 from Boumnyebel, Makenene and Kekem provenances portrayed the highest values for most of the investigated traits, especially pulp weight per fruit, they may be of particular interest for breeding with the purpose of improving pulp yield per fruit. Such candidate trees could later in seed orchard be cloned via air-layering and/or stem-cutting (Mialoundama et al., 2002). Crosses from Limbe provenance belong to cluster 2 and showed intermediate or low fruits size, low fruit and pulp weight but high fruit:kernel weight ratio.

In addition, the perceived qualitative variation (pulp oil content, pulp taste, epicarp and mesocarp colours) may be genetically determined and should not be neglected. Our studied showed that the most common epicarp colour was Hereford heather whereas the most common mesocarp colours were Green pastures and Eucalyptus. These results are in line with the results reported by Leakey et al. (2002a), and Waruhiu et al. (2004), on the same species. The higher the amount of variation presents for a character in the breeding materials, the greater the scope for its improvement through selection (De Smedt et al., 2011). From these results, it is clear that controlled-cross-pollination did not increase African plum fruit size as observed in citrus species (Iqbal and Karacali, 2004; Basharat et al, 2008; Al-Naggar et al., 2009). Nevertheless, the qualitative genetic gain (pulp taste, pulp oil content) will be tested from the obtained F_1 hybrids (already set on a field trial) during the first production. As cultivars are developed for their morphological attributes (Waruhiu, 2004), it is clear from this study and previous that detailed study of tree-to-tree variation in taste, nutritional qualities and oil content in African plum control-pollinated fruits is required to meet the food and industrial markets, respectively. Morphological characters have traditionally been used to obtain information on variation within plant species. These characters are usually controlled by many loci and may be affected by environment, which can complicate the evaluation of genetic diversity. By contrast, molecular markers are not generally influenced by environment; they are often but not always selectively neutral and, if chosen carefully throughout the genome, supposedly unbiased. Random Amplified Polymorphic DNA (RAPD) is one of these methods which does not require previous knowledge of DNA sequences is easy to perform and is one of the most cost-effective methods for obtaining polymorphic markers in many plant genera (Ngo-Mpeck, 2004; Atangana, 2010). For the on-going African plum scaled-up breeding program, we suggested that studies on molecular markers should be investigated for further genetic diversity for fruit traits improvement.

Conclusion

This study has highlighted required information for African plum improvement breeding program based on crosses on well-known accessions. The studied accessions showed high variability in fruit and pulp weight traits as well as moderate variation for fruit length, fruit width, kernel weight and fruit:kernel weight ratio whereas they showed low variability in pulp thickness, fruit length:width ratio and fruit:pulp weight ratio. The 18 females accessions based on studied traits were classified in 03 groups. Results showed that the most studied accessions have been clustered together in groups 1 and 3 indicating high genetic variability in African plum germplasm. Principal component analysis (PCA) revealed that the first two principal components accounted for 87.01% of the total variation. Among the studied traits, fruit length, fruit width, fruit and pulp weight, pulp thickness and fruit:kernel weight ratio showed strong and high positive link with the first component (PC1) whereas kernel weight and fruit length:width ratio showed positive link with the second component (PC2). According to breeding goal, breeders can chose accessions by considering appropriate PCs values.

Moreover, the developed predictive models could allow researchers and policy makers in partnership with local people to make more quantitative assessment of the pulp potential of African plum trees established in traditional agroforestry systems. In fact, phenotypically superior candidate African plum individuals will be coppiced, brought into clonal propagation via air-layering or stem-cuttings, followed by clonal testing of those clones that are easy to propagate. From this study, it is clear that controlled-cross-pollination did not increase African plum fruit size; otherwise, the perceived qualitative variation (pulp oil content, pulp taste, epicarp and mesocarp colours) should not be neglected and would be tested in the F_2 and F_3 generations (genetic gain) in further investigations. In addition, as the tree-to-tree variation is higher within provenances, this result suggests that the improvement of indigenous fruit trees germplasm through vegetative propagation does not narrow the genetic diversity in the studied species. For the on-going scaled-up African plum breeding program, we suggested that tree-to-tree variation in taste, nutritional qualities and oil content in African plum control-pollinated fruits must be taken to meet the food and industrial markets. Studies on molecular markers should be investigated for further genetic diversity for fruit traits improvement.

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