Phenetic analysis of wild populations of Momordica charantia L. (Cucurbitaceae) in West Africa and inference of the definition of the new subspecies macroloba Achigan-Dako & Blattner

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Abstract

ACHIGAN-DAKO, E., S. NDANIKOU, A. AHANCHEDE, J. GANGLO & F. BLATTNER (2008). Phenetic analysis of wild populations of Momordica charantia L. (Cucurbitaceae) in West Africa and inference of the definition of the new subspecies macroloba Achigan-Dako & Blattner. *Candollea* 63: 153-167. In English, English and French abstracts.

Momordica charantia L. *(Cucurbitaceae)* is a paleotropical species used as medicinal plant or vegetable in West Africa. This paper examines the morphological variation within some wild populations of *Momordica charantia* in the West African phytogeographical regions. The results point out that ecological conditions affect leaf size in that species, as well as the size of male and female bracts and peduncle lengths of that species. However, characters linked to the fruit size are uniform across regions except for pericarp thickness and tubercle height. The location of the bract on the peduncle is independent of environmental conditions. Multivariate analyses clarifies also the long-standing ambiguity for the identification of *Momordica charantia* observed in Benin by clearly discriminating two taxonomical groups based on the leaf lobe characters, the common subspecies *charantia* and the new subspecies *Momordica charantia* subsp. *macroloba* Achigan-Dako & Blattner endemic to the Dahomey gap and the Sudano-Guinean phytoregion of Benin and Togo.

Key-words

CUCURBITACEAE – *Momordica* – West Africa – Morphological characters – Multivariate analysis – Taxonomy

Résumé

ACHIGAN-DAKO, E., S. NDANIKOU, A. AHANCHEDE, J. GANGLO & F. BLATTNER (2008). Analyse phénétique de populations sauvages de Momordica charantia L. (Cucurbitaceae) en Afrique de l'Ouest et inférence de la définition de la nouvelle sous-espèce macroloba Achigan-Dako & Blattner. *Candollea* 63: 153-167. En anglais, résumés anglais et français.

Momordica charantia L. *(Cucurbitaceae)* est une espèce paléotropicale utilisée en médecine traditionnelle ou comme légume dans les régions de l'Afrique de l'Ouest. Ce travail décrit la variation morphologique observée au niveau de populations sauvages de *Momordica charantia* des régions phytogéographiques de l'Afrique de l'Ouest. Les résultats montrent que les conditions écologiques affectent la taille des feuilles et de la bractée florale, de même que la longueur des pédoncules mâles et femelles de cette espèce. Cependant, les caractères liés à la taille du fruit sont uniformes d'une région à une autre à l'exception de l'épaisseur du péricarpe et de la hauteur des tubercules. La position de la bractée sur le pédoncule est indépendante des conditions environnementales. Les analyses multivariées suggèrent deux groupes taxonomiques d'individus discriminés sur la base des caractères des lobes, la sous-espèce commune *charantia* et la nouvelle sous-espèce *Momordica charantia* subsp. *macroloba* Achigan-Dako & Blattner endémique au couloir sec dahoméen et à la zone Soudano-Guinéenne du Bénin et du Togo.

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Introduction

Momordica charantia L. (tribe *Joliffeae* Schrad.; subtribe *Thladiantinae* Pax) is a paleotropical cucurbit species, which is cultivated in many regions of Asia (MARR & al., 2004; NJOROGE & VAN LUIJK, 2004) but appears to occur wild in almost all countries of Africa. It is a medicinal plant primarily used to treat diabetes and malaria (ADJANOHOUN & al., 1985; BURKILL, 1985; JEFFREY, 2001). In West Africa, the plant is also used as febrifuge either by washing or drinking (NJOROGE & VAN LUIJK, 2004). In Togo *M. charantia* is one of the most important local medicinal plants both for ritual and ethnomedicinal practices (BELOIN & al., 2005). The plant is also used as vegetable in many West African communities (E. Achigan-Dako, *pers. comm.),* and in Zimbabwe, young non-bitter fruits are eaten as salad (NJOROGE & VAN LUIJK, 2004).

Momordica has approximately 45 species (JEFFREY & DE WILDE, 2006) with many representatives in Africa. For *M. charantia,* no formal infraspecific system of classification exists (WILLIAMS & NG, 1976; MARR & al., 2004), although YANG & WALTERS (1992) presented a classification of three Chinese «horticultural types» based upon fruit colour and size, and pericarp thickness. Small-fruited wild types have often been considered to form a distinct taxon at varietal (var. *abbreviata* Ser.) or subspecific (subsp. *abbreviata* (Ser.) Greb.) level, but the phylogenetic integrity of such a taxon is yet to be established, and was therefore not formally recognized by JEFFREY (2001). The species is a monoecious annual climber or trailing herb with stems up to 5 m long, bearing yellow axillary flowers that are subtended by a bract on the peduncle. The leaves are alternate, simple, deeply palmately lobed to about beyond the middle with the margin of the lobes more or less sinuate. The fruits are muricate when young, with irregular lines of warts (KEAY, 1954: 211). The species is similar to *M. balsamina* L. (BURKILL, 1985: 597) which occurs in drier regions (BERHAUT, 1975), while *M. charantia* occurs in lowland rain forest and

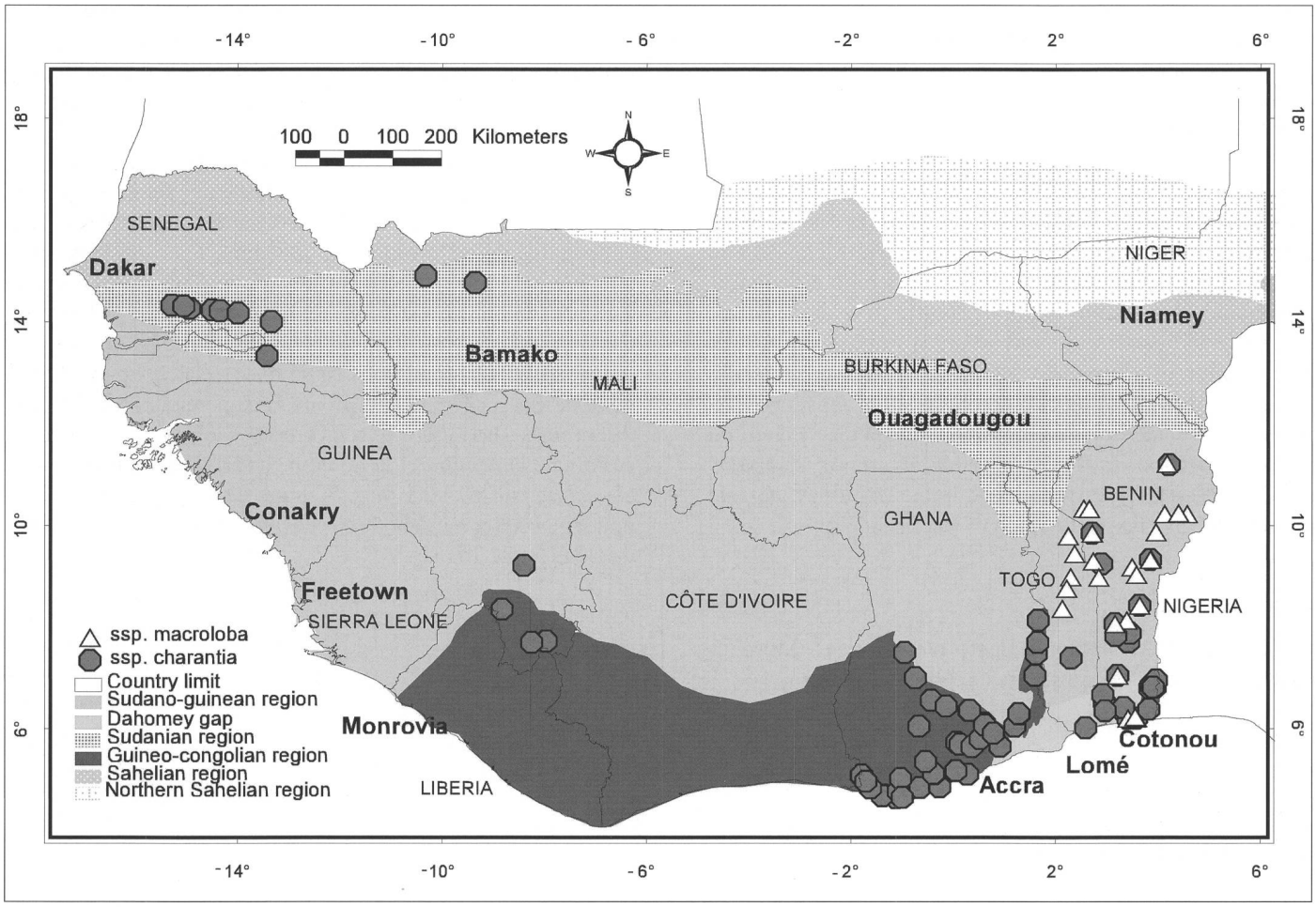


Fig. 1. – Populations sampling sites in West Africa (○: *Momordica charantia* L. subsp. *charantia*; △: *M. charantia* subsp. *macroloba* Achigan-Dako & Blattner).

wooded areas with higher rainfall (BURKILL, 1985). Very few studies on morphological variation in both species have been carried out in West Africa and the confusion between the two species is exacerbated by the presence of plants in Benin showing different characters.

In this study, we thoroughly examine morphological variation in wild populations of *M. charantia* from 31 locations in Senegal, Mali, Ghana, Guinea, Togo and Benin covering four important phyto-geographic regions of West Africa. We use *in situ* numerical characterization of samples based on leaf, inflorescence, fruit, and seed characters, and identify conspicuous characters within the West African *M. charantia* populations. Thereby, we provide an analytical key for identification of two intraspecific taxa in the region.

Material and Methods

Sampling areas, plant material and measurements

Plant populations were investigated in four ecological regions in West Africa from the moister areas to the drier ones. These are:

- the evergreen and semi-deciduous forest zone in Ghana, Guinea and in southeastern Benin known as the Guineo-Congolian zone;
- the forest/savannah mosaic in the Dahomey gap of southern Benin and Togo (WHITE, 1983; SALZMANN & HOELZMANN, 2005);
- the Sudano-Guinean zone in central and northern Benin and Togo;
- the Sudanian zone in southern Senegal and northwestern Mali (Fig. 1).

We analysed 102 individuals of *M. charantia* from 31 locations (Fig. 1). The sampled populations were selected based on a mega transect with 50-100 km distances between collection sites or according to habitat changes. At each station a radial transect helped find wild plants of *M. charantia* from which we randomly selected and marked three plants. Each plant was analysed for 24 morphometric characters (WILLIAMS & NG, 1976; MARR & al., 2004) (see Table 1). Metric characters were obtained using a Vernier calliper (0.02 mm default) and fruits were weighted with a weighting scale (50 g maximum).

Only 48 plants from 17 locations were found bearing mature fruits. Those plants were taken into account for fruit and seed measurements. Fruit and seed characters were measured only on mature fruits. Leaf characters were exclusively measured on the tenth leaf counted from the stem apex. At this position the plant bears full blooming flowers and the leaf has reached full development.

Table 1. – Descriptors list of leaf, inflorescence, fruit and seed, and their abbreviations.

	Descriptors	Abbreviations
Leaf	leaf length [mm]	*laml*
	lamina width [mm]	*lamw*
	central lobe length [mm]	*clbl*
	secondary lobe length [mm]	*slbl*
	basal width of the central lobe [mm]	*clbw*
	distance from the vein basis to the lobe sinus [mm]	*dvli*
	midvein's angle with secondary veins [°]	*ivag*
Inflorescence	male peduncle length [mm]	*mpdl*
	female peduncle length [mm]	*fpdl*
	distance from axil to male bract [mm]	*dmpb*
	distance from axil to female bract [mm]	*dfpb*
	male bract length [mm]	*mbrl*
	male bract width [mm]	*mbrw*
	female bract length [mm]	*fbrl*
	female bract width [mm]	*fbrw*
Fruit	fruit weight [g]	*frwt*
	fruit length [mm]	*frlg*
	fruit diameter [mm]	*frwd*
	tubercle height [mm]	*tbht*
	pericarp thickness [mm]	*peth*
	number of seeds per fruit	*senu*
Seed	seed length [mm]	*selg*
	seed width [mm]	*sewd*
	seed thickness [mm]	*seth*

Statistical analysis

For every single variable the frequency distribution on 102 individuals was graphically examined and normality was tested using the Kolmogorov-Smirnov test. Mean values and standard deviations were calculated, and a multivariate analysis of variance (MANOVA) was performed to compare characters of samples from different phytogeographical regions. This was followed by univariate analysis of variance (ANOVA) to identify the specific variables that contributed to the significant overall effect. A deviation to the homogeneity of variances and covariances was tested using the Box's M test (Hill & Lewicki, 2006).

To compare characters between male and female flowers the paired t-test was performed. Independent t-test was used to compare traits between two independent groups. The Pearson correlation helps measure the strength of relation between characters (Sokal & Rohlf, 1995) when it exists.

Principal component analysis (PCA) was carried out to describe the variation of the morphological measurements in terms of a set of uncorrelated variables, each of which being a particular linear combination of the original variables (Everitt & Dunn, 2001). For this analysis we used the correlation matrix to give equal importance to the characters (Stuessy, 1990).

Canonical discriminant analysis (CDA) (using Wilk's criterion) was performed to pinpoint morphological characters that best discriminate populations from different phytogeographic regions and to reveal populations which were most similar to each other. The relative contribution of each variable to phenetic differentiation was examined. The smallest Mahalanobis distance was used to decide on the appropriate membership of individuals to groups (Hill & Lewicki, 2006). Quantitative relationships within the species were assessed using agglomerative hierarchical clusteringtechniques(Stuessy, 1990) using Ward's algorithm of the squared Euclidean distance. This method produces partitions by a series of successive fusions of the 102 individuals into groups in a way that the degree of association between two individuals is maximal if they are most similar and minimal otherwise.

Results

Variability of fruit and seed characters

The mean values and ranges of fruit and seed characters are presented in Table 2 according to different geographical location. These characters showed homogeneity of variances and covariances (Box's M test, $p = 0.29$). MANOVA of fruit and seed characters indicated a significant variation between

Table 2. – Summary statistics (Mean ± SD, range in parentheses) for fruit and seed characters of *Momordica charantia* L. from three bioclimatic areas in West Africa.

	Guineo-Congolean (n = 23)	**Dahomey gap** (n = 9)	**Sudano-Guinean** (n = 16)
Fruit weight [g]	8.89 ± 3.75 (4-20)	7.5 ± 1.97 (5-10)	11.28 ± 4.54 (5-18)
Fruit length [mm]	44.72 ± 7.27 (34.4-60.8)	42.92 ± 4.80 (39.3-52.9)	48.75 ± 9.39 (34.0-63.2)
Fruit diameter [mm]	26.04 ± 4.0 (20.8-33.9)	25.71 ± 3.68 (21.0-30.7)	27.59 ± 4.01 (22.3-34.6)
Pericarp thickness [mm]	3.37 ± 0.64 (2.1-4.8)	4.33 ± 0.56 (3.4-5.0)	4.34 ± 0.93 (2.9-6.0)
Tubercle height [mm]	2.03 ± 1.08 (0.5-5.8)	2.08 ± 0.62 (0.8-2.6)	3.1 ± 0.98 (1.0-4.8)
Number of seed per fruit	11.47 ± 3.23 (6-18)	14.85 ± 3.67 (10-20)	13 ± 4.8 (5-19)
Seed length [mm]	9.62 ± 0.64 (8.4-10.8)	9.05 ± 0.70 (8.4-10.4)	9.6 ± 0.71 (8.4-10.9)
Seed width [mm]	5.42 ± 0.51 (4.1-6.6)	5.12 ± 0.43 (4.7-6.0)	6.04 ± 0.38 (5.2-6.9)
Seed thickness [mm]	3.05 ± 0.26 (2.5-3.5)	2.82 ± 0.21 (2.4-3.1)	2.99 ± 0.30 (2.4-3.5)

phytogeographical regions ($p < 0.01$). However, univariate analysis of variances indicated significant differences for only few characters. For instance, the fruit weight varies between 4 and 20 g according to samples. The average weight of a *M. charantia* fruit is 9.74 g ± 4.14 (mean ± SD) and there is no significant difference among phytoregions ($p = 0.07$). The fruit measures on average 46.5 mm ± 8.39 in length with a diameter of 26.7 mm ± 3.96. None of these two characters varies from one region to another according to the univariate analysis. The fruit is broadly ovoid with eight (rarely nine) longitudinal ribs, each rib being a succession of tubercles. The height of tubercles (on average 2.41 mm ± 1.08) varies from one phytoregion to another with a significant difference ($p = 0.006$). Samples from the Sudano-Guinean region showed greater tubercle height (mean = 3.1 mm ± 0.98), and also a thicker pericarp than those collected in forest areas ($p < 0.001$). On average the pericarp is 3.86 mm ± 0.86 thick, while in savannah areas (in the Sudano-Guinean region and the Dahomey gap) the pericarp reaches 4.33 mm ± 0.36 and 4.34 mm ± 0.93, respectively. The fruit contains on average 13 seeds. The number of seeds per fruit does not vary significantly among phytoregions ($p = 0.12$). The seed size is described by the seed length, width, and the seed thickness. The mean seed length is 9.56 mm ± 0.7 and similar across the phytoregions ($p = 0.14$). The seed thickness shows the same trend and measures on average 2.98 mm ± 0.29. Only the seed width (5.61 mm ± 0.56) is significantly greater in the Sudano-Guinean region ($p < 0.001$) where it reaches 6.04 mm ± 0.38. The magnitude of this variation is however low and hence not perceptible.

Phenetic relationships based on fruit and seed characters

The analysis of relationships between characters indicated several positive high correlations (Table 3). Fruit and seed size characters vary in the same direction. For instance, the fruit weight showed positive medium and high correlation with the other characters. The seed thickness, however, showed low correlation with most variables but significant negative correlation with the number of seed per fruit.

In PCA of the fruit and seed characters of 48 individuals of *M. charantia* L. from 17 locations, the first component explains 48.73% of the variability and the second component explains 17.74% of the variability (Fig. 2). The first axis is highly correlated to fruit and seed size and the second axis opposes the seed thickness (negative side) to the number of seeds per fruit (positive side). The scatterplot of the individuals formed by the two principal components (figure not shown) did not show any clear separation of populations.

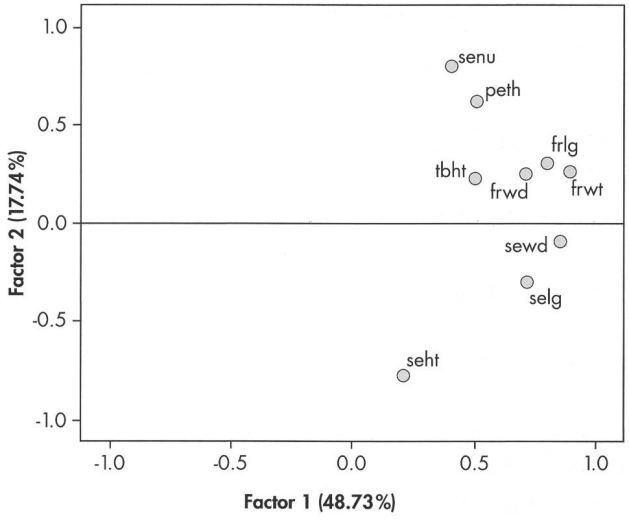


Fig. 2. – PCA of the fruit and seed characters showing the first two principal components axes (66.47% of the total variation). For the abbreviations used, see Table 1.

Table 3. – Correlation matrix of nine fruit and seed characters of the 48 plants of *Momordica charantia* from 17 locations (*: $p < 0.05$; **: $p < 0.01$). For the abbreviations used, see Table 1.

	frwt	frlg	selg	sewd	seth	senu	peth	frwd	tbht
frwt	1								
frlg	0.83**	1							
selg	0.51**	0.55**	1						
sewd	0.66**	0.56**	0.55**	1					
seth	0.06	-0.10	0.13	0.18	1				
senu	0.62**	0.61**	0.1	0.2	-0.42**	1			
peth	0.6**	0.49**	0.06	0.47**	-0.2	0.67**	1		
frwd	0.74**	0.53**	0.36*	0.54**	-0.01	0.49**	0.49**	1	
tbht	0.44**	0.41**	0.2	0.53**	-0.17	0.23	0.37*	0.24	1

Variability of leaf and inflorescence characters

The Box's M test for homogeneity of variance and covariance matrices for the leaf and flower characters is significant ($p < 0.01$) maybe due to the very high sensitivity of this test and the presence of dichotomous variables (HILL & LEWICKI, 2006). However, the log determinants appeared to be nearly equal, indicating that the covariance matrices of the dependant variables are equal across regions. MANOVA of leaf and inflorescence characters indicated significant differences across regions ($p < 0.01$). In contrast to fruit and seed characters, most leaf and inflorescence characters showed significant variation. The mean values and ranges of leaf and inflorescence characters are given in Table 4 according to geographical locations.

The leaf length reaches sometimes 140 mm but on average fluctuates around 84.11 mm ± 17.13. ANOVA results show that it is not similar across ecological regions ($p < 0.01$). Samples from the Sudanian region in Senegal and Mali showed greater leaf length (102.14 mm ± 18.8). The lamina width is on average 61.97 mm ± 14.26. It varies also according to ecological regions ($p < 0.01$). The small lamina width values were recorded for the Dahomey gap samples (58.03 mm ± 9.97) in Benin and high lamina width values were recorded in the

Table 4. – Summary statistics (mean ± SD, range in parentheses) for leaf and flower characters of *Momordica charantia* L. individuals from four bioclimatic areas in West Africa.

	Guineo-Congolian (n = 36)	**Dahomey gap** (n = 21)	**Sudano-Guinean** (n = 24)	**Sudanian** (n = 21)
Leaves length [mm]	79.55 ± 18.35 (49-105)	74.76 ± 10.94 (59-99)	83.33 ± 7.75 (67-98)	102.14 ± 18.84 (76-137)
Lamina width [mm]	58.02 ± 9.97 (41-89)	51.23 ± 10.68 (35-76)	62.58 ± 8.21 (48-75)	78.76 ± 14.72 (37-101)
Central lobe length [mm]	49.44 ± 9.41 (34-69)	42.42 ± 6.34 (34-54)	51.25 ± 6.46 (37-62)	67.85 ± 9.86 (54-85)
Secondary lobe length [mm]	39.61 ± 7.94 (25-56)	34.23 ± 4.62 (28-42)	42.12 ± 5.95 (32-54)	52.23 ± 9.72 (40-70)
Angle between central and secondary veins [°]	54.52 ± 10.95 (33-82)	49 ± 12.25 (32-79)	47.66 ± 6 (36-59)	46.85 ± 6.25 (38-62)
Distance from veins basis to the lobes sinus [mm]	5.60 ± 1.54 (3.1-8.8)	12.48 ± 8 (3.7-25)	21.58 ± 9.79 (6.1-34)	9.95 ± 2.27 (6.8-14.9)
Basal width of the central lobe [mm]	4.37 ± 1.06 (2.3-6.2)	11.97 ± 8.89 (3.7-25.2)	21.10 ± 10 (3.7-33.1)	6.85 ± 2.15 (3.7-11.8)
Male bract length [mm]	7.21 ± 1.92 (4.3-11)	6.57 ± 0.81 (5.6-8.1)	8.37 ± 1.5 (6.6-12.7)	5.06 ± 2.21 (2.3-9.8)
Male bract width [mm]	8.71 ± 2.93 (4.9-16)	7.92 ± 1.52 (5.5-10)	10.15 ± 2.15 (7.3-16.5)	7.18 ± 3.96 (2.4-15.8)
Female bract length [mm]	7.13 ± 2.29 (3.5-11)	7.58 ± 1.85 (4.8-10.6)	7.38 ± 2.11 (4.7-12.9)	4.18 ± 1.28 (2.36-6)
Female bract width [mm]	8.53 ± 3.14 (3.8-14)	8.43 ± 2.06 (6 -11.9)	9.01 ± 2.56 (5.7-16.4)	5.75 ± 3.07 (1.5-8.8)
Male peduncle length [mm]	69.25 ± 21.42 (33-102)	49 ± 7.78 (38-59)	55.33 ± 9.32 (44-70)	89.14 ± 30.23 (65-141)
Female peduncle length [mm]	67.58 ± 15.35 (40-90)	60.57 ± 11.6 (43-74)	67.4 ± 18.1 (46-109)	75.85 ± 14.2 (60-99)
Distance from the axil to the male bract [mm]	21.91 ± 10.73 (10-50)	22.42 ± 4.3 (17-29)	20.7 ± 8.79 (9-33)	19.16 ± 7.83 (5.7-32)
Distance from the axil to the female bract [mm]	11.87 ± 8.46 (3.7-33)	10 ± 2.44 (6-13)	11.12 ± 4.61 (5-19)	10-16 ± 7.7 (3-21.8)

Sudanian populations (78.76 mm ± 14.72). The central lobe length follows the same trend. Samples from the Dahomey gap showed shorter central lobe length (42.43 mm ± 6.34). The mean value of that character is 52.22 mm ± 11.88 with a significant difference between ecological regions ($p < 0.01$). In general the secondary lobe length (41.76 mm ± 9.67) is shorter than the central lobe length. The variation of this character between ecological regions follows the same trend as the lamina width and the central lobe length. In the Dahomey gap region, leaves have a smaller size while in the Sudanian region they are bigger. The angle between the central and secondary lobes (interveins angle) is on average 50.2° ± 9.9 and is not similar across ecological regions ($p = 0.009$). The forest region samples have a wider interveins angle (54.53° ± 10.95) in comparison with others. The distance from the veins basis to the lobes sinus and the basal width of the central lobe both do not follow a normal distribution. This is supported by the Kolmogorov-Smirnov test ($p < 0.001$). The frequency distribution curves of the 102 individuals of *M. charantia* L. are flatted and left skewed in both cases indicating a bimodal distribution (Fig. 3).

The inflorescence characters analyzed are male and female peduncle length, the bract location on the peduncle and the bract size. The average length of the male peduncle is 65.9 mm ± 23.96 but the peduncle length of some rare individuals recorded in the Guineo-Congolian and in the Sudanian have more than 100 mm ($p < 0.01$). Likewise, the female peduncle length varies significantly between regions ($p = 0.02$) with 67.79 mm ± 15.72 on average. The female peduncle length is shorter in the Dahomey gap (60.57 mm ± 11.61) and longer in the Sudanian (75.86 mm ± 14.24). No significant variation is observed between male and female peduncle length (paired t-test, $p = 0.36$). The location of the bract on the peduncle is defined by the distance from the axil to the bract. This average distance is 21.17 mm ± 8.6 for male peduncle and did not vary between ecological regions ($p = 0.59$). Likewise, the location of the female bract (10.96 mm ± 6.59) did not logarithm-transformed values of the distance from the axil to the female bract did not indicated any significant difference ($p = 0.35$) between regions. However, the distance to the male bract is almost every time greater than the distance to the female bract ($p < 0.01$) as

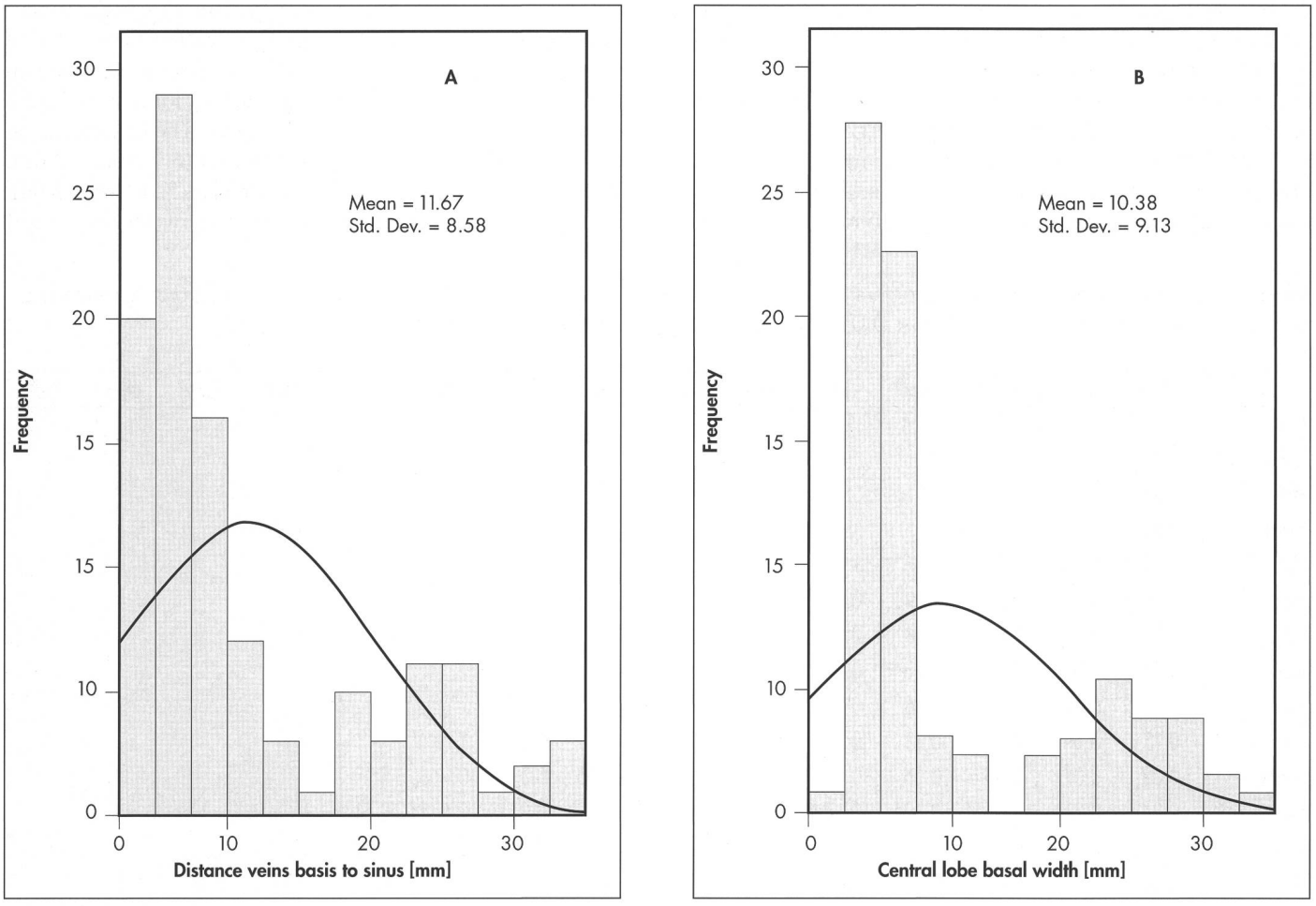


Fig. 3. – Frequency distribution. **A.** the distance from veins basis to the lobes sinus; **B.** the basal width of the central lobe.

shown by a paired t-test, as well this latter also shows that the variation in bract size is not linked to sex ($p = 0.29$ for bract length; $p = 0.05$ for bract width). On average the bract length is 6.91 mm ± 2.04 for male inflorescence and 6.7 mm ± 2.42 for female one. The bract length varied significantly between ecological region for male inflorescence ($p < 0.01$) and for female inflorescence as well ($p < 0.01$). The bract width is on average 8.57 mm ± 2.94 for male inflorescence and 8.05 mm ± 3.01 for female inflorescence. It follows a similar trend with the bract length. In all cases, small bract sizes were observed in few populations from Senegal.

Phenetic relationships based on leaf and inflorescence characters

The analysis of relationships between characters indicates positive and negative high correlations (Table 5). The leaf length showed significant high and positive correlation with the lamina width, the central lobe length, the secondary lobe length and the male peduncle length. The correlation between the leaf length and the female peduncle length is also significant ($r = 0.32$, $p = 0.001$). The male peduncle length and the female peduncle length evolve in the same direction and are strongly linked ($r = 0.51$, $p < 0.01$). However, these two characters are weakly correlated to the bract size. The location of the bract is not correlated to peduncle length for male inflorescence ($r = 0.16$) but for female flowers it is ($r = 0.56$, $p < 0.01$). The distance from the axil to the male bract (male bract location) and the distance from the axil to the female bract (female bract location) are positively correlated ($r = 0.47$, $p < 0.01$). The male and female bract length, and the male and female bract width are also positively correlated. The basal width of the central lobe showed high positive correlation with the distance from the veins basis to the lobes sinus ($r = 0.98$, $p < 0.01$) and negative correlation with the interveins angle ($r = -0.34$, $p < 0.01$).

The correlation circles from the principal components analysis on the 102 individuals of *M. charantia* are shown in Fig. 4 A-D. The first four components explained 78.28% of the total variability. The scatterplot of the first two components indicate the correlation of the leaf size characters (leaves length, lamina width, central lobe length, etc.) and the male peduncle length to the axis 1, which explained 28.67% of the variability (Fig. 4A and 4B). Axis 2 explained 20.11% of the variability (Fig. 4A-D) and is correlated to female and male bract size. Axis 3 is correlated to lobe characteristics such as the central lobe basal width and the distance from veins basis to sinus and explained 18.14% of the variability (Fig. 4B and 4D). The location of the bract on the female and male peduncle and the female peduncle length are correlated to axis 4 (Fig. 4C) which explained 11.15% of the variability. The scatterplot of the first three component scores revealed two distinct groups (called Group 1 and Group 2) without intermediates (Fig. 5). Group 1 is made up of *M. charantia* sensu stricto with deeply lobed leaves, the central lobe being narrowed at the basis. The distance from the veins basis to the lobe sinus is shorter (6.98 mm ± 2.62). The central lobe basal width is also smaller (5.16 mm ± 1.84). Group 2 is made up of samples that show larger central

Table 5. – Correlation matrix of 15 characters measured on flowers and leaves of the 102 individuals of *Momordica charantia* L. from 31 locations (*: $p < 0.05$; **: $p < 0.01$). For the abbreviations used, see Table 1.

	laml	lamw	clbl	mpdl	slbl	ivag	dvli	clbw	dmpb	mbrl	mbrw	fbrl	fbrw	dfpb	fpdl
laml	1														
lamw	0.78**	1													
clbl	0.86**	0.88**	1												
mpdl	0.68**	0.56**	0.65**	1											
slbl	0.83**	0.85**	0.93**	0.65**	1										
ivag	-0.29**	0.01	-0.25*	-0.19	-0.31**	1									
dvli	0.03	0.02	0.05	-0.18	0.14	-0.4**	1								
clbw	-0.06	-0.06	-0.04	-0.26**	0.04	-0.34**	0.98**	1							
dmpb	0.07	-0.15	-0.06	0.16	-0.04	-0.20*	-0.07	-0.07	1						
mbrl	-0.15	-0.09	-0.2*	-0.13	-0.14	0.17	0.10	0.13	0.21*	1					
mbrw	0.03	0.02	-0.06	0.06	0.03	-0.01	0.06	0.06	0.31**	0.9**	1				
fbrl	-0.2*	-0.21*	-0.26**	-0.22*	-0.17	0.08	0.07	0.08	-0.06	0.63**	0.51**	1			
fbrw	-0.09	-0.14	-0.17	-0.05	-0.07	0.04	0.06	0.05	0.13	0.61**	0.62**	0.86**			
dfpb	0.08	-0.06	0.04	0.14	0.03	-0.14	0.02	0.01	0.47**	-0.01	0.06	0.02	0.24*	1	
fpdl	0.32**	0.24*	0.34**	0.51**	0.37**	-0.19	0.09	0.04	0.3**	0.07	0.18	-0.03	0.15	0.56**	1

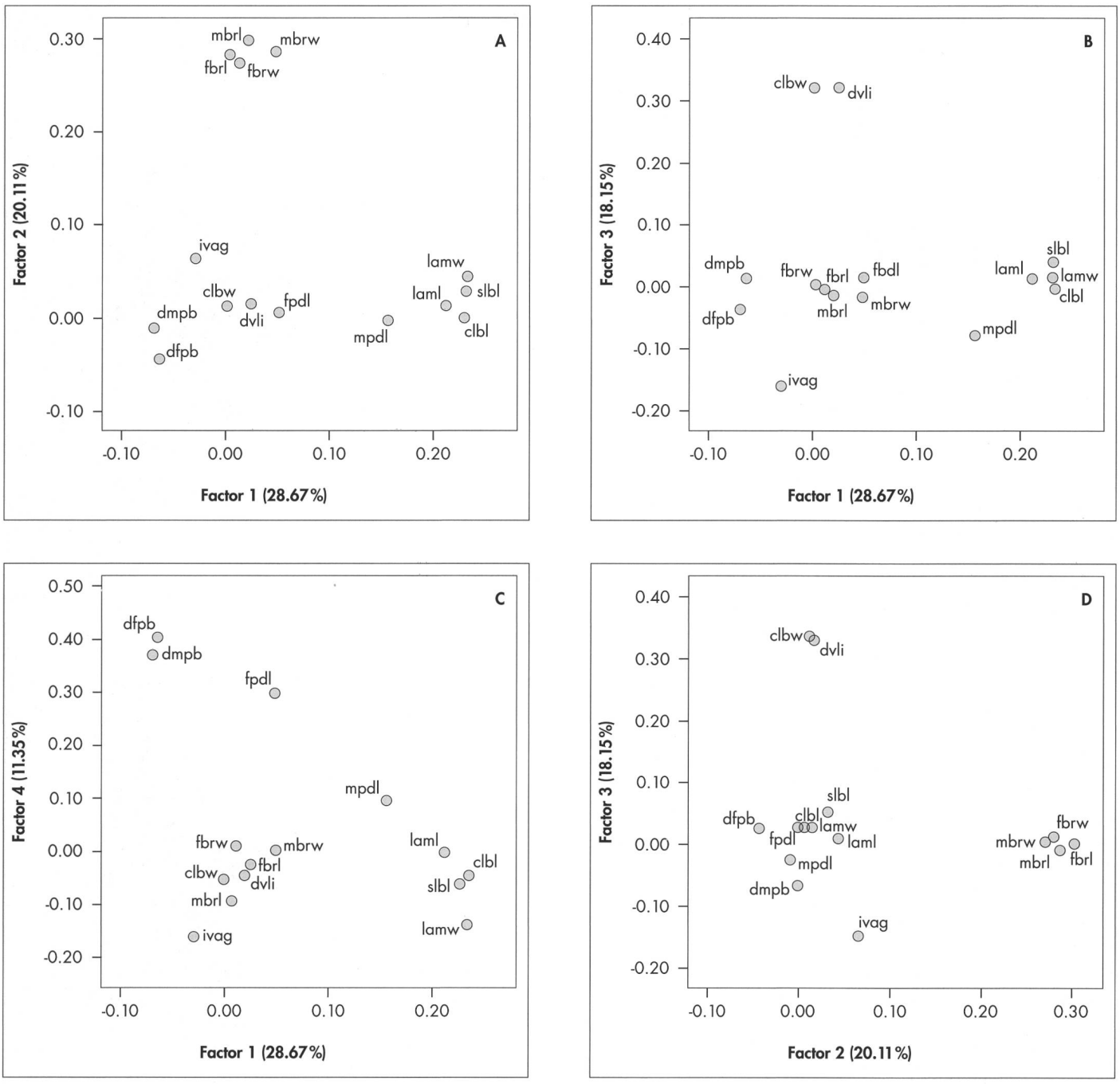


Fig. 4. – Correlation circle of the first four principal components from leaf and flower characters. **A.** Factors 1 and 2; **B.** Factors 1 and 3; **C.** Factors 1 and 4; **D.** Factors 2 and 4 (four principal components explained altogether 78.28 % of the total variation). For the abbreviations used, see Table 1.

lobe basal width (24.9 mm ± 4.08). The distance from the veins basis to the lobes sinus is also longer for these plants (24.71 mm ± 5.13).

CDA, using the phytoregions as grouping variable, allocated correctly 78.4% of individuals to their respective region groups (Fig. 6A-B). Deviations occurred mainly for some individuals from the Dahomey gap which showed a Mahalanobis distance closer to the centroid of the Sudano-Guinean group or the reverse (Fig. 6A). The first two canonical axes accounted for 94.7% of the discriminatory power among regions. The first canonical axe explained 70.1% of the total variance and was strongly correlated to leaf and peduncle size characters (canonical correlation coefficient, $Rc = 0.88$) such as the central lobe length, the male flower peduncle length and

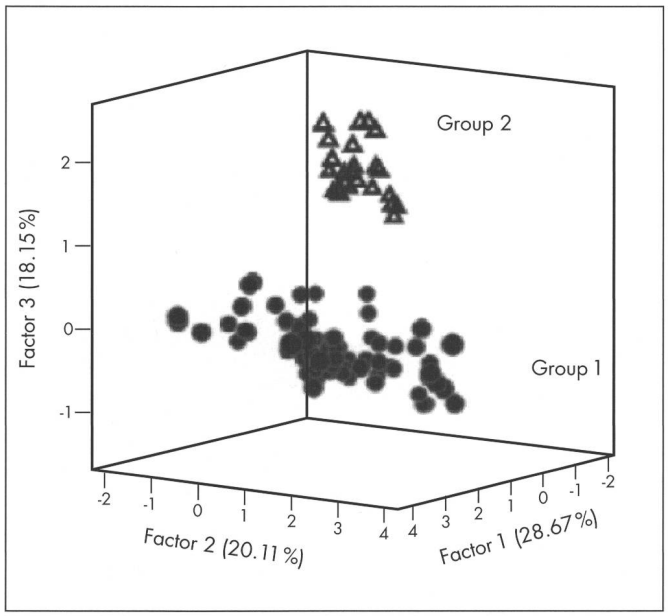
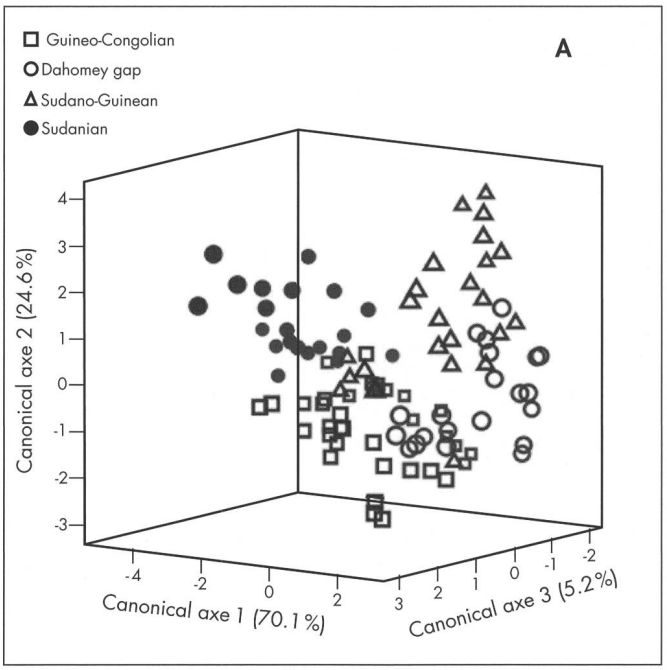


Fig. 5. – Scatterplot of the first three component scores from PCA on leaf and flower characters showing two groups based on leaf and flower characters.



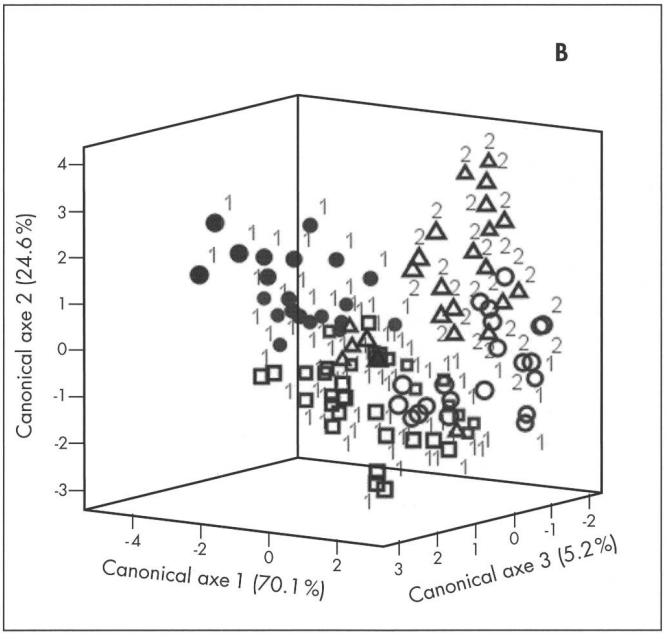


Fig. 6. – **A.** Three-dimensional plot of the canonical axes scores from leaf and flower characters; **B.** Plot of the canonical axes shown with superimposition of Group 1 and 2 from the principal components analysis.

the female bract length as indicated by the canonical structure coefficients matrix (Table 6). The second canonical axe accounted for 24.6% of the discrimination between groups and was weighted heavily (canonical correlation coefficient, Rc = 0.74) by lobe characters such as the basal width of the central obe and the distance from the veins basis to the lobes sinus. Individuals tend to be separated by size across the first canonical variate while the second canonical variate accounted heavily for lobe characters. This trend is described from the principal component analysis from which we inferred two possible groups. A superimposition of the two groups revealed by the principal component analysis in the three dimensional plot using the first three canonical axe scores indicates a clear-cut discrimination of both groups regardless of the geographical origin of their members (Fig. 6B). The canonical discriminant analysis based on these two groups indicates 100% of correct classification. Individuals of Group 2 occur basically in the Dahomey gap and the Sudano-guinean regions but are mostly present in the latter. The dendrogram resulting from agglomerative hierarchical cluster analysis divides the populations in four sets regardless of the geographical origin. Importantly, individuals of Group 2 are again clustered together (Fig. 7).

Analytical key for subspecies identification

A comparison between the two identified subsets for fruit and seed characters revealed significant differences for the pericarp thickness only (p = 0.002). For Group 1, the pericarp thickness is on average 3.55 mm ± 0.80 while for Group 2, the mean value is 4.30 mm ± 0.76. From the multivariate analyses we infer that the two subgroups identified represent two subspecies of the species *Momordica charantia.*

The identification key and characters for the identification of these two subspecies is given as follows:

Table 6. – Structure coefficients matrix of the flower and leaf characters for the first three canonical variates used in the phenetic analysis of populations of *Momordica charantia* L. from four phytogeographical regions of West Africa. For the abbreviations used, see Table 1.

Characters (abbreviations)	Canonical axe 1	Canonical axe 2	Canonical axe 3
clbl	-0.491	0.467	0.184
mpdl	-0.389	0.038	0.307
lamw	-0.375	0.312	0.259
fbrl	0.333	-0.192	0.194
fbrw	0.238	-0.149	0.133
dmpb	-0.165	0.106	0.124
dfpb	-0.113	0.058	-0.098
dvli	0.295	0.773	-0.192
clbw	0.364	0.720	-0.165
slbl	-0.457	0.465	0.087
laml	-0.311	0.336	-0.024
ivag	0.178	-0.324	0.258
mbrl	0.298	0.071	0.717
mbrw	0.146	0.111	0.464
fpdl	-0.046	0.124	0.202
Variation explained [%]	70.1	24.6	5.2

1. Leaves palmately 5-7-lobed beyond the middle, central lobe basis narrowed, 5 ± 2 mm wide, distance from vein basis to central sinus 7 ± 3 mm. Pericarp 2-5 mm thick ..... subsp. ***charantia*** (Fig. 8)

1a. Leaves 3-5-lobed not beyond the middle, central lobe basis large, 25 ± 4 mm wide, distance from vein basis to central sinus 25 ± 5 mm. Pericarp 3-6 mm thick ..... subsp. ***macroloba*** (Fig. 9)

Momordica charantia subsp. ***macroloba*** Achigan-Dako & Blattner, **subspec. nova** (Fig. 9)

> **Typus: Benin: Tchaourou, Koko**, 253 m, 9°03'59"N 2°21'40"E, 29.VIII.2005, *Achigan-Dako 927 IS 5* (holo-: GAT).
> *Folia lobata non ultra medium longitudinis, 3 usque ad 5 lobi, basis lobi centralis ampla 25 ± 4 mm, distantia de basi venae usque ad sinum centralem 25 ± 5 mm; pericarpium latus 3 usque ad 6 mm.*

Leaves are 3 to 5 lobed. The lobes are not divided beyond the middle of the leaf with a wider central lobe basis (25 mm ± 4). The distance from vein basis to central sinus is about 25 mm ± 5. The pericarp is 3-6 mm thick.

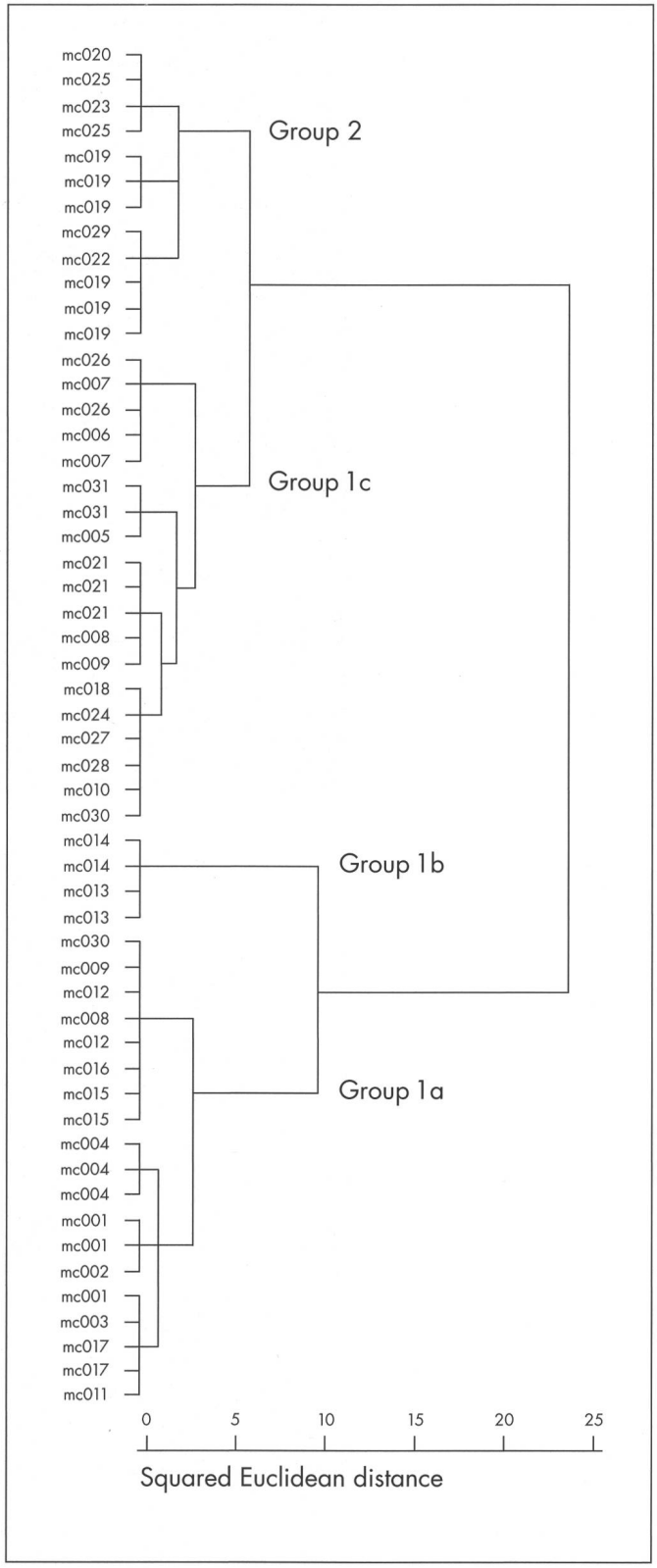


Fig. 7. – Dendrogram obtained from agglomerative cluster analysis using Ward algorithm of the squared Euclidean distance. Numbers *(mc001* to *mc 031)* represent individuals from a given location.

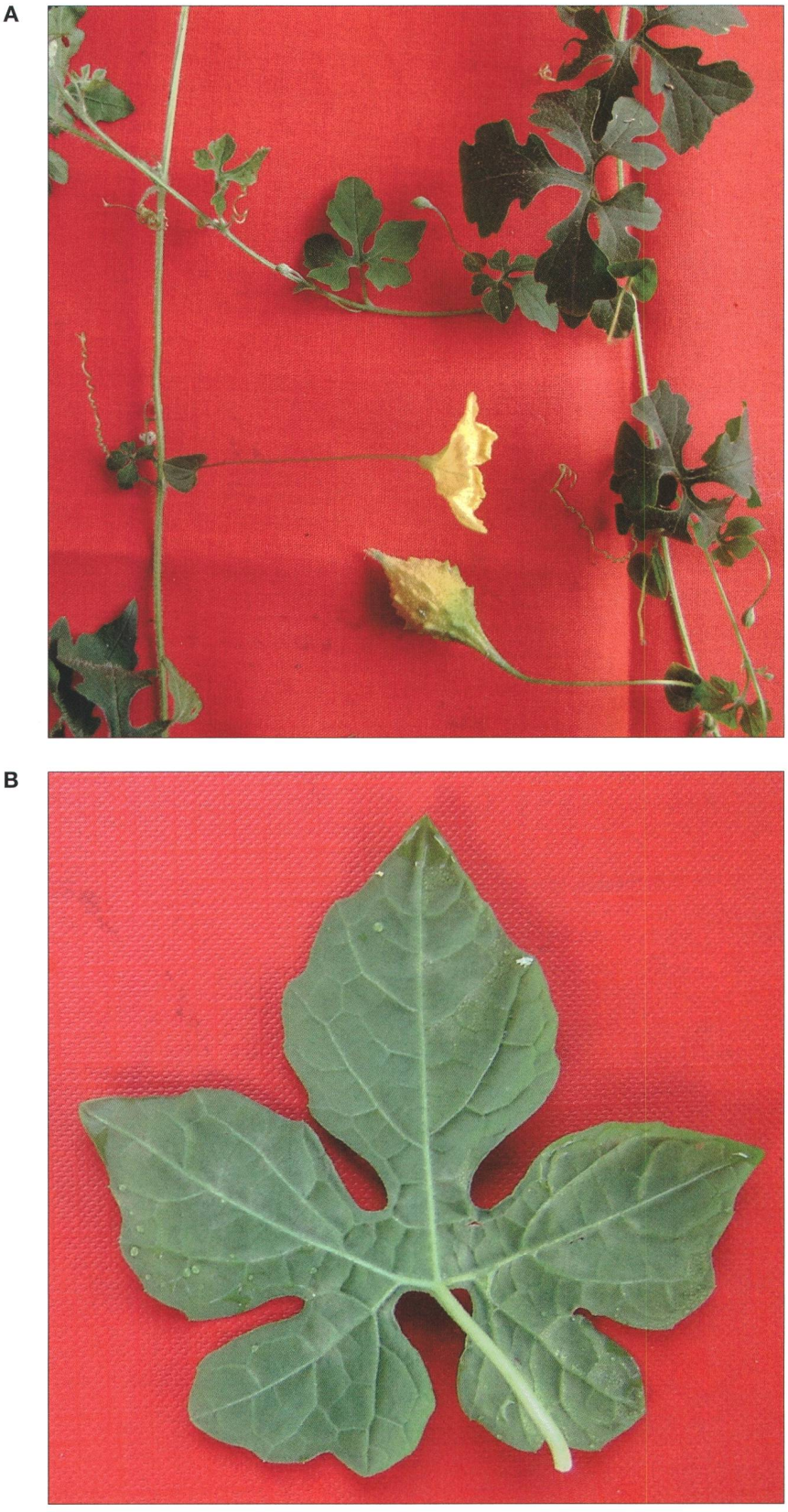


Fig. 8. – A. *Momordica charantia* L. subsp. *charantia*; **B.** Leaf of *M. charantia* L. subsp. *charantia*.

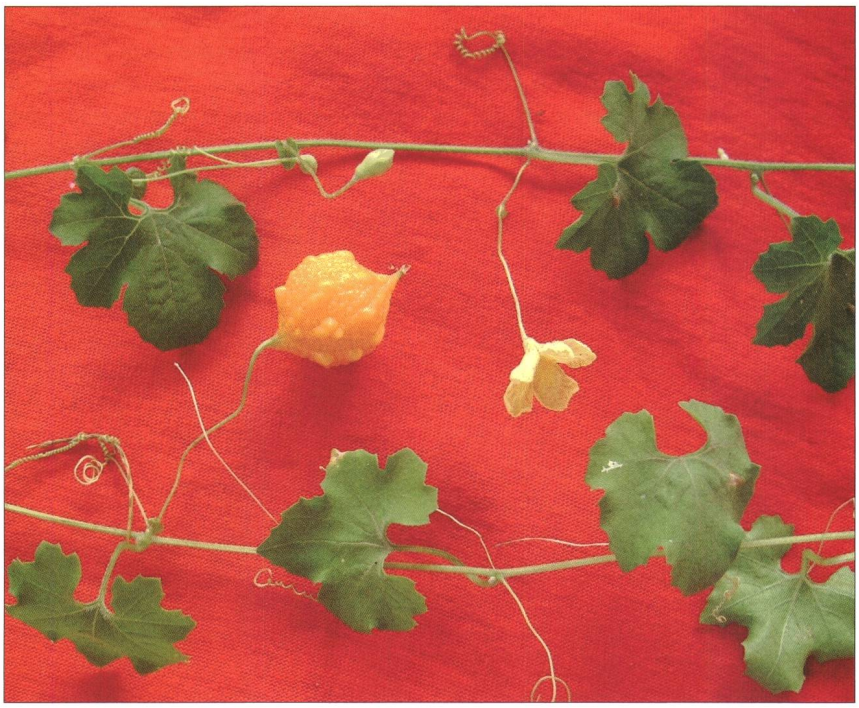


Fig. 9. – *Momordica charantia* subsp. *macroloba* Achigan-Dako & Blattner.

Distribution, ecology and phenology. – *Momordica charantia* subsp. *macroloba* occurs in the Dahomey gap and in the Sudano-Guinean region of Benin and Togo where it shares the same habitat with *M. charantia* subsp *charantia* (Fig. 1). It was sampled only in the Dahomey gap and the Sudano-Guinean regions in Benin and Togo. Its distribution area is small, as it was neither found in any of the other visited forests, nor in the Sudanian regions in Senegal and Mali. Thus, we assume that *M. charantia* subsp. *macroloba* is endemic to the Savannah habitats of Benin and Togo. It shares the same habitat with *Momordica charantia* subsp *charantia* and the same (macro-) ecological niche, and it flowers at the same time of the year. Like *M. charantia* subsp. *charantia, M. charantia* subsp. *macroloba* bears flowers throughout the year but more importantly in raining seasons (April to July and/or September to November). Fruit development and maturation occur toward the end of the raining seasons (one to three months after flowering) although some individuals bear fruit longer

Other specimens examined. – We examined several materials of *M. charantia* subsp. *macroloba.* Many samples (herbarium specimen, young leaves for DNA extraction and seeds) have been collected within the distribution range. Several samples were multiplied (from seeds) in greenhouse at the Leibniz Institute of Plant Genetics and Crop Sciences (Gatersleben, Germany) in 2006 and 2007 and kept in the Herbarium of the institute. We carefully examined the sample *Houngnon 3715* (BENIN) wrongly classified as *M. balsamina* – it is classified as *M. charantia* subsp. *macroloba* – while the same type (vouchers *AV 4281* and *AA3813)* collected by other authors was classified as *M. charantia* (ADJAKIDJÈ, 2006). The list of *specimina visa* is:

BENIN. Copargo: 9°50'10"N 1°32'29"E, 10.VIII.2006, *Enoch Achigan-Dako 06NIA502* (GAT); **Copargo:** 9°50'10" N 1°32'29"E, 10.VIII.2006, *Enoch Achigan-Dako 06NIA504* (GAT); **Abomey:** 7°11'03"N 2°00'56"E, 11.VIII.2006, *Enoch Achigan-Dako 06NIA518* (GAT); **Abomey-Calavi:**6°24'49"N 2°20'10"E, 30.VIII.2006, *Enoch Achigan-Dako 06NIA603* (GAT); **Mareguinta:** 10°11'45"N 3°17'52"E, 8.IX.2006, *Enoch Achigan-Dako 06NIA545* (GAT); **Ndali:** 9°51'35"N 2°43'07"E, 9.IX.2006, *Enoch Achigan-Dako 06NIA550* (GAT).

TOGO. Niamtougou: 9°47'03"N 1°05'59"E, 14.V.2007, *Enoch Achigan-Dako & Ndanikou Sognigbe 07NIA974* (GAT).

Note. – The subspecies *macroloba* is very similar to subspecies *charantia* regarding fruit characters and gross morphology that means there is a possibility for cross-pollination between the two groups. Inter-taxon gene flow seems however limited, as we found no intermediate forms in our analyses. This

calls for thorough studies on the reproductive and population biology of *M. charantia* to estimate the degree of reproductive isolation between both taxa. Until reproductive isolation is proved, we prefer to consider them as subspecies instead of giving them species rank.

Discussion and conclusions

The few attempts for an infraspecific classification in *M. charantia* were focused on fruit size or pericarp thickness (Williams & Ng, 1976; Yang & Walters, 1992). Small-fruited wild forms have often been considered to form a distinct taxon at varietal (var. *abbreviata)* or subspecific (subsp. *abbreviata)* level, but this classification was not formally recognized by Jeffrey (2001). Njoroge & van Luijk (2004) indicated also in their literature review that there is yet no good infraspecific classification system. Yang & Walters (1992) differentiated subgroups as «horticultural types» within Asian cultivars. Cultivation practices may, however, have introduced increase in fruit size in accessions of Asia. However, fruit size is uniform across habitats and ecological zones in West Africa and is, thus, not a good character for classification in wild populations. The classification inferred in this study is not based on fruit characteristics but rather on leaf lobe characters. Focusing infraspecific classification on leaf morphology is challenging due to the plasticity of this organ in flowering plants. However, we analyzed carefully the variation in these characters among the West African populations and found the observed differences between *M. charantia* subsp. *charantia* and subsp. *macroloba* consistent among different ecological regions. Thus, lobe characters discriminate very well the two taxa.

The phenetic analysis of wild populations of *M. charantia* provides useful information on the morphological structure of the populations in the West African phytoregions. Previous studies from Marr & al. (2004) revealed that wild accessions of *M. charantia* from Laos, China, Thailand and Nepal exhibited low morphological diversity. This is in agreement with our results, which revealed that many characters are similar within the West African *M. charantia* populations. For instance the fruit weight and size characters vary not much. Fruit size data are similar to those obtained by Marr & al. (2004) for wild accessions. However, characters such as pericarp thickness and tubercle height vary according to ecological conditions. Marr & al. (2004) found variation of this character between domesticated and wild accessions. However, whether the character varies within wild populations was not clear due to the small sample size of wild accessions in their study. Although the pericarp thickness and tubercle height vary according to ecological regions, it should be recognized that the magnitude of this variation is low and not visually perceptible and admittedly, this variation is due to the presence of the two subspecies. Additional varying characters are the leaf size and the peduncle length. Bigger leaves occur in the drier regions. For instance, samples from Ghana showed smaller leaf size in comparison with samples from Senegal. Although the peduncle size varies among ecological regions, the location of the bract for both sexes is constant, the location of the female bract being always closer to the axil than the one of the male bract. Many authors interpreted this observation differently and reported that the bract might either be positioned close to the axil or toward the middle of the peduncle, but did not take into account the flower sex (Keay, 1954; Jeffrey, 1964; Keraudren, 1967; Berhaut, 1975; Adjakidjè, 2006).

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