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Genetic diversity in cowpea [*Vigna unguiculata* (L.) Walp.] as revealed by RAPD markers

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Abstract

The present study, using RAPD analysis, was undertaken to characterize genetic variation in domesticated cowpea and its wild progenitor, as well as their relationships. The materials used consisted of 26 domesticated accessions, including accessions from each of the five cultivar-group, and 30 wild/weedy accessions, including accessions from West, East and southern Africa. A total of 28 primers generated 202 RAPD bands. One hundred and eight bands were polymorphic among the domesticated compared to 181 among wild/weedy cowpea accessions. Wild accessions were more diverse in East Africa, which is the likely area of origin of *V. unguiculata* var. *spontanea*. Var. *spontanea* is supposed to have spread westward and southward, with a loss of variability, loss counterbalanced in southern Africa by introgressions with local perennial subspecies. Although the variabilty of domesticated cowpea was the highest ever recorded, cultivar-groups were poorly resolved, and several results obtained with isozyme data were not confirmed here. However primitive cultivars were more diverse than evolved cultivars, which still suggests two consecutive bottlenecks within domesticated cowpea evolution. As isozymes and AFLP markers, although with a larger number of markers, RAPD data confirmed the single domestication hypothesis, the gap between wild and domesticated cowpea, and the widespread introgression phenomena between wild and domesticated cowpea.

Abbreviations: bp – base pair, BWA – Botswana, cpDNA – chloroplast DNA, RAPD – Random Amplified Polymorphic DNA.

Introduction

The genus *Vigna* currently includes around 80 species distributed throughout the tropics (Pasquet 2001). It comprises seven domesticated species, five of which are Asiatic and two are of African origin. The Asiatic group consists of green gram/mung bean (*V. radiata* (L.) Wilczeck), black gram/urd bean (*V. mungo* (L.) Hepper), moth bean (*V. aconitifolia* (Jacq.) Maréchal), adzuki bean (*V. angularis* (Willd.) Ohwi et Ohashi), and rice bean (*V. umbellata* (Thunb.) Ohwi et Ohashi). The African group comprises Bambara groundnut (*V. subterranea* (L.) Verdc.) and cowpea (*V. unguiculata* (L.) Walp.).

The cowpea is cultivated in all tropical areas, as well as under more temperate climates like in California's Central Valley or in the Mediterranean basin. Cultivated on at least 12.5 million hectares, with an annual production of over 3 million tons world-wide (Singh et al. 1997), cowpea is one of the main grain legumes. However, Africa is the main area of production, where the crop is very important for low input agriculture, which characterizes most of the continent. The seeds are most often consumed but

young leaves, fresh or dry, and young pods are also used. Cowpea is also cultivated as a fodder, in the Sahelian area of West Africa as well as in the dry areas of Asia, and also for the fiber of its floral peduncles (Pasquet and Baudoin 2001).

The species *V. unguiculata* includes domesticated forms, i.e., *V. unguiculata* ssp. *unguiculata* var. *unguiculata*, wild annual forms, i.e., ssp. *unguiculata* var. *spontanea* (Schweinf.) Pasquet, and 10 wild perennial subspecies (Pasquet 1993a, 1993b, 1997). This classification is based on results from morphological (Pasquet 1993a; Padulosi 1993), allozyme (Panella and Gepts 1992; Vaillancourt et al. 1993; Pasquet 1993c, 1999), and cpDNA studies (Vaillancourt and Weeden 1992). *V. unguiculata* ssp. *unguiculata* var. *spontanea* (some authors are using the name ssp. *dekindtiana sensu* Verdc.) is the likely progenitor of the domesticated cowpea (Padulosi and Ng 1997; Pasquet 1999).

Domesticated cowpea includes five cultivar-groups. Morphological analysis contrasted evolved cultivars and more primitive cultivars according to seed size, on the one hand, early and late flowering (under inductive conditions) on the other hand. The latter character was markedly correlated with ovule number. Photosensitive and early-flowering photo independent cultivars had 11–17 ovules per pod and late flowering photoperiod-independent cultivars had 16–25 ovules per pod (Pasquet 1998). However, this organization of the domesticated gene-pool was poorly correlated with isozyme data, which only showed more diversity in primitive cultivars (Pasquet 2000).

The African origin of cowpea was suggested as early as 1847 (Richard 1847), and since Piper (1913), no one is contesting it since wild cowpea plants are found only in tropical Africa and Madagascar, but not in Asia (Steele 1976). However, where the crop was first domesticated is still uncertain and different centers of diversity and origin of the cowpea have been proposed, i.e., Ethiopia (Vavilov 1926; Steele 1972; Pasquet 2000), West Africa (Murdock 1959; Faris 1963; Rawal 1975; Maréchal et al. 1978; Vaillancourt and Weeden 1992; Ng 1995), and Eastern and Southern Africa (Baudoin and Maréchal 1985), while a 'diffuse' domestication in the savanna after the dispersal of cereals was also hypothesized (Chevalier 1944; Steele 1976; Garba and Pasquet 1998). The latter hypothesis was supported by Harlan (1971) who considered that the cowpea was domesticated in his African Non-Center.

However, with the exception of a recent AFLP work (Coulibaly et al. 2002), all the studies involving molecular markers, including storage proteins (Panella et al. 1993; Fotso et al. 1994; Odeigah and Osanyinpeju 1996), were hampered by the lack of representativity of the samples of the domesticated cowpea and its progenitor.

RAPD (Random Amplified Polymorphic DNA) (Williams et al. 1990) analysis can be used to characterize DNA variation patterns within species and among closely related taxa. Within grain legume crops alone, RAPD markers have been widely used for the identification of genetic relationships among cultivars (Haley et al. 1994; Paiva et al. 1994; Beebe et al. 1995; Link et al. 1995; Mienie et al. 1995; Nienhuis et al. 1995; Samec and Nasinec 1995; Skroch and Nienhuis 1995; Mignouna et al. 1998; Zhang et al. 1996; Doldi et al. 1997; Johns et al. 1997; Briand et al. 1998; Ferguson et al. 1998; Thompson et al. 1998; Duarte et al. 1999; Yee et al. 1999; Beebe et al. 2000; Brown-Guedira et al. 2000; Subramanian et al. 2000; Amadou et al. 2001; Dwivedi et al. 2001; Galvan et al. 2001; Li and Nelson 2001; Maciel et al. 2001; Sonnante and Pignone 2001; Tosti and Negri 2002), among wild forms (Freyre et al. 1996; Cattan-Toupance et al. 1998), or between cultivars and wild forms (Lanham et al. 1992; Abo-Elwafa et al. 1995; Hilu and Stalker 1995; Ratnaparkhe et al. 1995; Sharma et al. 1995; Ahmad et al. 1996; Liu 1996; Ratnaparkhe et al. 1995; Sharma et al. 1995; Ahmad et al. 1996; Liu 1996; Vasconcelos et al. 1996; Fofana et al. 1997, 1997; Santalla et al. 1998; Ahmad 1999; Banerjee et al. 1999; Gimenes et al. 2000; Xu et al. 2000; Mimura et al. 2000; Raina et al. 2001).

To date, few studies have been performed in cowpea using RAPDs (Akundabweni 1995; Menéndez et al. 1997; Mignouna et al. 1998; Tosti and Negri 2002) and none of them has studied the organization of genetic diversity in wild and domesticated forms. The present research had the following objectives: to determine if RAPD markers could be used to estimate genetic diversity among wild forms and landraces of cowpea, to investigate the genetic relationships between wild forms and landraces of this species, and to determine whether the main groups of cultivars could be distinguished using RAPD marker data.

Materials and Methods

Plant material

Fifty-six accessions (26 domesticated and 30 wild/

weedy accessions) were used in this study. With the exception of IT84S2049 and 524B studied by Menéndez et al. (1997), all wild and domesticated accessions were previously studied for isozyme polymorphism. Details about the origin of the various accessions were previously published (Pasquet 1999, 2000).

The domesticated accessions belonged to the five cultivar-groups, i.e., cv.-gr. Melanophthalmus (NO 574 and NO 1387 from Cameroon, IT84S2049, a breeding line from International Institute of Tropical Agriculture, 524B, a breeding line from University of California Riverside), cv.-gr. Biflora (ET 1, ET 2, ET 14, ET 25, ET 31, and ET 39 from Ethiopia, EX 35 from India, EX 37 from Laos, EX 51 from Pakistan, NO 106 and NO 3113 from Cameroon, ZR 7 from Zaïre), cv.-gr. Unguiculata (AS 10 F from South Africa, ET 35 from Ethiopia, NO 90 and OU 65 from Cameroon), cv.-gr. Sesquipedalis (EX 38 from New Caledonia and EX 43 from Philipinos), and cv.-gr. Textilis (NO 198, NO 274, NO 577, and NO 2300 from Cameroon). More accessions from the cultivar-groups Biflora and Textilis were included because these two cultigroups are more polymorphic than the other cultivar-groups (Pasquet 2000).

The wild/weedy accessions belonged to ssp. *unguiculata* var. *spontanea* (NI 951 from Nigeria, NI 963 from Senegal, SP 46 and SP 52 from Cameroon, SP 199 from Niger, NI 1386, SP 75, SP 182, SP 185, TVNU 503, and TVNU 1248 from Tanzania, SP 87 from Kenya, MT 55 and MT 76 from Zimbabwe, MT 131, MT 651 and NI 1171 from Zambia, NI 1167 and TVNU 1351from South Africa, NI 1383 from Botswana, NI 1392 from Malawi, SP 143 from Angola, SP 149 from Congo,), to the BWA group (Pasquet 1999) from var. *spontanea* (NI 817 from Zimbabwe, NI 1382 from Boswana, SP 154 and SP 160 from Namibia), and to a group intermediate between ssp. *pubescens* and var. *spontanea* (NI 979 from Kenya, NI 1417 from Burundi, and SP 83 from Tanzania). Within var. *spontanea* accessions, some do not show a var. *spontanea* cpDNA profile (Pasquet, unpublished) and are supposed to be introgressed by various perennial subspecies. They included the BWA group and accessions MT 76, MT 651, NI 1167, NI 1392, and SP 143). The wild accessions were obtained from the World *Phaseolinae* collection maintained at the Jardin Botanique National de Belgique (BR), Meise, Belgium (NI accessions); the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria (TVNU accessions); the International Plant Genetic Resources Institute (IPGRI), Harare, Zimbabwe (MT accessions); and the IRD collection maintained in Montpellier, France (SP accessions).

Each accession is made of two to three autogamous lines, and maintained as such, each of these lines derived from one seed of the original stock. Two or 3 plants were assayed per accession.

RAPD amplification procedures

Approximately 5 g of leaf tissue were harvested from plants grown in the greenhouse and used for extraction of total genomic DNA, based on the CTAB procedure described by Doyle and Doyle (1987). DNA samples were first suspended in 100 mL of Tris EDTA buffer and then diluted to 4 ng/μL with ddH_2O. The PCR reaction mixture, contained in a total reaction volume of 25 μL, included 32 ng of template DNA, $MgCl_2$ to a final concentration of 1.9 mM, 25 mM of each dNTP, and 33 ng of decamer primer (kits A, B, and E of Operon Technologies, Alameda, CA). DNA sequences were amplified using a 96-well Twinblock (Ericomp) thermal cycler. The parameters of the PCR cycle were as follows: 1 cycle at 94 °C for 2 min; 40 cycles of 94 °C for 1 min, 35 °C for 1 min, and 72 °C for 2 min; 1 cycle at 72 °C for 5 min; and 1 cycle at 30 °C for 30 min. After amplification, 5 μL of gel loading buffer were added to each sample. A sample of 15 μL of PCR product was separated by electrophoresis at 100 volts for 3 h on a 10% (30:0.8) polyacrylamide gel ($7.3 \times 10.2 \times 0.001$ cm) and stained for 10 min (0.5 μL ethidium bromide per mL of buffer) before visualizing the banding patterns under UV light with an IS-1000 Digital imaging system and scanning the images. A 123 bp DNA ladder was used as ladder for sizing the RAPD bands.

Primers were selected from among those chosen by Menéndez et al. (1997) in their development of a genetic linkage map of cowpea because they revealed polymorphism between the two domesticated parents of their mapping population. A total of 28 10-mer primers (OA1–OA4, OA9–OA10, OA13, OA16–OA19, OB5-OB6, OB9, OB11–OB16, OE6–OE7, OE10–OE12, OE14, OE18–OE19) from Operon Technologies, Alameda, CA, were used for amplification. An 'O' prefix and then the kit letter and primer number designate the primers.

Each reaction was repeated twice and only those bands that could be scored without ambiguity were scored (Figure 1). Variation in the intensity of bands was not taken into account. Bands were scored as either present (1) or absent (0). For each primer, the

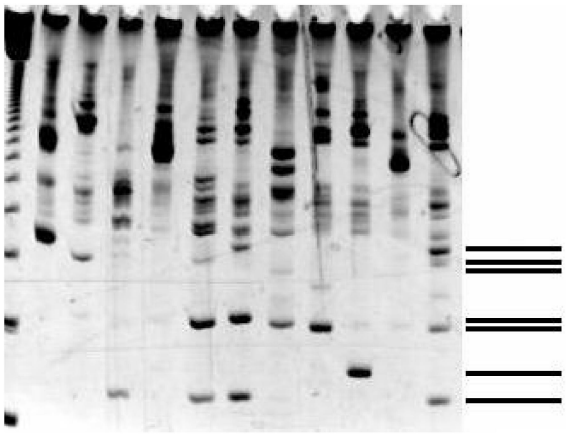

Figure 1. Primer A02. From top to bottom, fragments A02-1, A02-2, A02-3, A02-4, A02-5, A02-6, A02-7. Fragments A02-1 and A02-7 are present within most domesticated accessions and usually absent within wild accessions.

larger fragment scored was designated '1' with additional fragments numbered sequentially in order of decreasing size.

Data analysis

Data were analyzed using NTSYS version 1.80 (Rohlf 1993). Estimates of distances were based on two different measures: (1) Nei and Li's similarity index (1979) converted into distance by the relation $D_{ij} = 1\text{-}S_{ij}$ and (2) Nei (1972) distance. Nei and Li's similarity index (1979) is also known as the Dice (1945) coefficient. Cluster analysis was based on (1) the unweighted paired-group method using arithmetic averages (UPGMA), and (2) a phylogenetic reconstruction with the Neighbor Joining method of Saitou and Nei (1987). Multivariate relationships among accessions were revealed through principal coordinate (PCO) analysis of distances.

Genetic variability was assessed using the proportion of polymorphic fragments (P), total diversity (Ht) (Nei 1973). Total diversity was partitioned into the weighted average diversity within groups (H_S) and the between groups gene diversity (D_{st}). The proportion of total allelic diversity found among groups (G_{st}) was calculated as the ratio D_{st} / H_t (Nei 1973).

Results

RAPD profiles

A total of 202 scorable fragments were generated using the 28 oligonucleotide primers. The number of scorable fragments per primer ranged from 1 with OB16 to 19 with OA01, and the average was 7.2 fragments per primer. The size of the amplified fragments ranged from 200 bp to 1.5 kb. Of the 202 fragments, 187 (92.6%) were polymorphic in the accessions tested, with an average of 6.4 polymorphic fragments per primer.

Within wild accessions, 198 fragments were detected, 181 (91 %) being polymorphic. Within domesticated accessions, 146 fragments were detected, 108 (74 %) being polymorphic. One hundred and forty two fragments were common to both groups. Fifty six fragments were unique to wild accessions while four fragments only were unique to domesticated accessions. Three of these latter fragments were found in one or two accessions only but one fragment (A03-8) had a frequency of 0.42 and was equally distributed in almost all cultivar groups. In addition, some fragments were much more frequent in domesticated than wild accessions. These included A03–8 (0.42 in domesticated versus 0 in wild), A02–1 (0.88 versus 0.13), A02–7 (0.96 versus 0.20), A13–9 (0.81 versus 0.10), A16–1 (0.96 versus 0.20), A19–16 (0.81 versus 0.13), and B16 (0.81 versus 0.20). However, no fragment showed frequency markedly higher in wild. We can just highlight E18–4 (0.08 in domesticated versus 0.57 in wild), A01–6 (0 versus 0.37), and A18–4 (0.08 versus 0.43).

If we consider the differences between domesticated types and the various geographic groups of wild accessions, there are few strong differences between frequencies of domesticated and wild types from West Africa, with the exception of marker A19–6 (0.81 in domesticated versus 0 in wild from West Africa). However, these differences are more important in East Africa, as shown by markers A02–1 (0.89 in domesticated types versus 0.09 in wild types from East Africa), A02–7 (0.96 versus 0.09), A13–9 (0.81 versus 0.09), A16–1 (0.96 versus 0.18), A19–6 (0.81 versus 0.09), and E18–4 (0.08 versus 1), and in southern Africa, i.e., A01–6 (0 in domesticated types versus 0.71 in wild types from southern Africa, A02–1 (0.89 versus 0.14), A02–7 (0.96 versus 0.21), A13–9 (0.81 versus 0), A16–1 (0.96 versus 0.14), B06–4 (0.85 versus 0.07), E06–5 (0.92 versus 0.21), and E19–1 (1 versus 0.21), although the A02–1, A06–5, and E19–1 cases are mainly due to introgressed accessions (according to the cpDNA profile of these accessions).

Regarding wild accessions, five fragments were unique to accessions from West Africa (including 2

with a frequency higher than 0.25), 16 to accessions from East Africa (three with higher frequency), and 25 to accessions from southern Africa (five with higher frequency). If introgressed or intermediate accessions were not considered, the values would be 11 (one fragment only with higher frequency) in southern Africa and 14 in East Africa (still three with higher frequency). Usually these fragments have a low frequency or are absent within domesticated accessions, with the exception of A18–1 (0.39 in domesticated and 0.40 in wild from West Africa).

Within domesticated accessions, one fragment was unique to cv.-gr. Melanophthalmus, three to cv.-gr. Unguiculata, three to cv.-gr. Sesquipedalis, five to cv.-gr. Textilis, and 14 to cv.-gr. Biflora. However, the frequency of these rare alleles is always low. Partitioning domesticated accessions between evolved versus primitive cultivars does not show frequency differences between the two groups, while partitioning domesticated accessions between low ovule number versus high ovule number accessions show a frequency inversion in marker E06–7 (0.94 versus 0.37). Regarding the geographic distribution of the fragments, 15 fragments (including three with a frequency higher than 0.4) present in Asian cv.-gr. Biflora, cv.-gr. Unguiculata or Sesquipedalis and 10 fragments present in cv.-gr. Melanophthalmus and West African cv.-gr. Biflora are not present in north-eastern Africa (and cv.-gr. Textilis), while 10 fragments are shared by accessions from north-eastern Africa (and cv.-gr. Textilis) and either of the two other groups (and not both).

Cluster and Principal Coordinate analyses

Both cluster analyses, based on UPGMA using Nei's distance matrix and the Neighbor Joining Method using the Dice coefficient (Figure 2), generated a unique dendrogram. Both trees were very similar with domesticated accessions clustered at the top and accessions from the BWA group clustering at the bottom of the tree with other introgressed accessions from Southern Africa. In both trees, ssp. *pubescens*-related accessions were grouped in the middle of the var. *spontanea* accessions from East Africa. In both trees, domesticated accessions were not sorted by cultivar-groups. For example, cv.-gr. Biflora accessions were spread all over the upper part of the trees. In both trees, wild accessions from West Africa appeared close to or within domesticated accessions while wild accessions from East Africa appeared in the middle part of both trees. The two distance parameters used had limited influence on the composition of the clusters.

The principal coordinate analysis (Figure 3) yielded a picture similar to the one given by the cluster analyses, with domesticated accessions on the left side and wild accessions on the right side. The overlap between the two groups is due only to four out of the five wild accessions from West Africa. Regarding wild accessions, the nine accessions from the right side (i.e., from top to bottom, SP 154, MT 76, NI 817, SP 143, SP160, NI 1382, NI 1392, MT 651, and NI 1167) are the ones which may be introgressed based on their cpDNA pattern. Other accessions from East and southern Africa, i.e., SP 185 from Tanzania, NI 1171 from Zambia and NI 1383 from Botswana, are still closely related to domesticated accessions. Regarding domesticated accessions, the figure is more informative than the cluster analyses. With the exception of NO 3113, we see from left to right a first group with cultivar groups Sesquipedalis, Unguiculata, Melanophthalmus and accessions from cv.-gr. Biflora outside north-east Africa (plain circle and plain triangle), a second group with cv.-gr. Textilis (T), and a third group with accessions from cv.-gr. Biflora from north-east Africa (plain diamond).

Genetic distances between groups

The shortest distance between wild and domesticated forms was found in West Africa: 0.098 between NI 951 and NO 3113. However, NO 3113 is a very primitive cultivar from North Cameroon, almost a weedy type. If NO 3113 is not considered, the domesticated forms of North-eastern African origin would become slightly closer to all wild forms (mean 0.247, versus 0.251 for cv.-gr. Textilis, 0.256 for cv.-gr. Melanophthalmus and West African cv.-gr. Biflora, 0.266 for Asian cv.-gr. Biflora and 0.268 for cv.-gr. Unguiculata and cv.-gr. Sesquipedalis). In reality, as highlighted by the principal coordinate analysis, the morphologically primitive group including NO3113, cv.-gr. Textilis and cv.-gr. Biflora from North-east Africa, is closer to all wild form than the morphologically evolved group including the other accessions, especially those from cultivar-groups Melanophthalmus, Unguiculata and Sesquipedalis. Their Nei's distance averages 0.245 versus 0.262.

On the other hand, of all the wild forms, the accessions from West Africa were consistently closer to all domesticated forms: 0.098–0.252 (mean 0.174) versus 0.178–0.338 (mean 0.247) for wild forms from East Africa and 0.126–0.410 for wild forms from

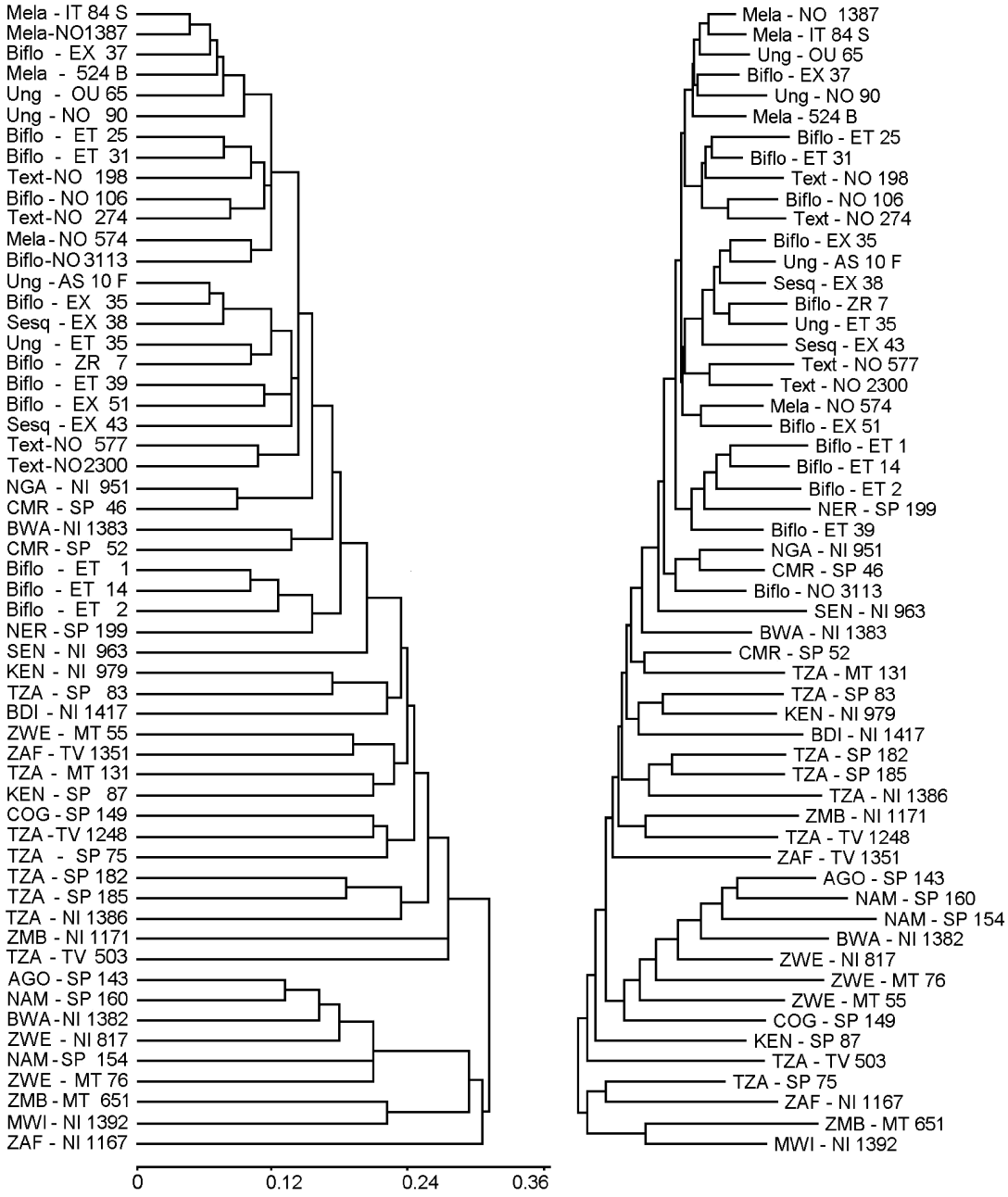

Figure 2. Cluster analyses: UPGMA from Nei's distance matrix (left) and Neighbor Joining Method from the Dice coefficient (right).

South Africa (mean 0.290). However, several accessions from southern Africa are introgressed by perennial subspecies. If these accessions are not considered, distances between domesticated accessions and accessions from southern Africa would be in the range 0.126–0.331 (mean 0.235).

Genetic diversity

The domesticated group (H_T = 0.123) was less diverse than the wild group (H_T = 0.223). Among domesticated types, it was not possible to compare the diversity of the various cultivar-groups because the

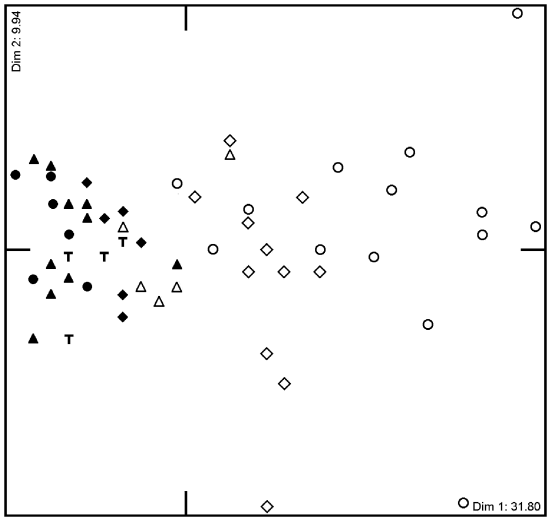

Figure 3. Principal coordinate analysis. Plain circle: cv.-gr. Unguiculata and cv.-gr. Sesquipedalis; plain triangle: cv.-gr. Melanophthalmus and cv.-gr. Biflora (except North-East Africa); plain diamond: cv.-gr. Biflora from North-East Africa; T: cv.-gr. Textilis; open circle: wild accessions from southern Africa; open diamond: wild accessions from East Africa; open triangle: wild accessions from West Africa. NO 3113 is the last domesticated spot toward the right.

number of accessions studied was too variable between cultivar-groups. However, various groupings of these domesticated accessions were tested. The groups identified by the cluster and principal coordinate analyses show low differences in their diversity level. Primitive cultivars, with wild-colored small seeds and slightly dehiscent pods, i.e., mainly cv.-gr. Textilis and north-eastern cv.-gr. Biflora (H_T = 0.123), were slightly more diverse than evolved cultivars (H_T = 0.105). In the same way, primitive cultivar-groups, i.e., cv.-gr. Biflora and cv.-gr. Textilis (H_T = 0.127) were also more diverse than evolved cultivar-groups, i.e., Melanophthalmus, Unguiculata and Textilis (H_T = 0.100). Ovule number, which separates the two major groups of domesticated forms, does not influence the level of variability: both groups, i.e., low ovule number and high ovule number, display similar variability levels.

Among wild accessions, West Africa (H_S = 0.124) was less diverse than East Africa (H_S = 0.203) or southern Africa (H_S = 0.215) (Table 1). However, if the introgressed accessions are disregarded, southern Africa diversity is reduced to H_S = 0.162, a value intermediate between those from West and East Africa. In the same way, if the accessions introgressed with ssp. *pubescens* are not considered, East Africa diversity is slightly reduced (H_S = 0.196).

Discussion

The wild gene-pool

Total genetic diversity in var. *spontanea* (H_T = 0.223, 30 accessions) was slightly higher than those inferred from isozyme data, i.e., from 0.090 to 0.199 (Vaillancourt et al. 1993; Panella and Gepts 1992; Pasquet 1999), and higher than the one reported by Coulibaly et al. (2002) with AFLP markers (H_T = 0.175, 50 accessions). However, RAPDs and AFLPs are more efficient in identifying markers than isozymes: 181 polymorphic RAPD markers and 110 polymorphic AFLP markers compared with 28 isozyme loci.

Within ssp. *unguiculata* var. *spontanea*, RAPD analyses showed southern Africa and especially East Africa to be more diverse than West Africa, as did AFLP and isozyme analyses. Using isozymes, Pasquet (unpublished) found diversity values of 0.115 for West Africa (107 accessions), 0.139 for East Africa (27 accessions), 0.175 for South Africa without the BWA group (46 accessions), and 0.237 for South Africa including the BWA group (total of 90 accessions). However, RAPD diversity values are higher in East Africa than in southern Africa, if introgressed and intermediate accessions are not considered. Then the values for East Africa (H_S = 0.196), southern Africa (H_S = 0.162), and West Africa (H_S = 0.124) show a real bottleneck. In the same way, if introgressed and intermediate accessions are not considered, there is also a reduction in the percentage of polymorphic loci, from 60.4 in East Africa to 41.6 in southern Africa and 32.2 in West Africa, and in the number of unique fragments, from 14 in East Africa to 11 in southern Africa and 5 in West Africa. Moreover, since fragment A18-1 has a frequency of 0.40 within wild types from West Africa, and 0.39 in domesticated types as well as 1.00 in cv.-gr. Melanophthalmus, this unique fragment could be considered as a consequence of introgression.

These results strengthen the hypothesis of the east-African origin of ssp. *unguiculata* var. *spontanea*. The spreading of var. *spontanea* westward and southward was linked to a reduction in genetic diversity,

Table 1. Gene diversity statistics (Nei 1973) estimated from RAPD data with accessions grouped according to type (domesticated vs. wild), cultivar-groups and geographic location. N: number of accessions, NPF: number of polymorphic fragments; P: percentage of polymorphic fragments, H_T: total diversity, D_{ST}: diversity between the corresponding groups; G_{ST}: diversity among the corresponding groups; BnonNEA: cv.-gr. Biflora except North-East Africa; BNEA: cv.-gr. Biflora from North-East Africa; BT: cultivar-groups Biflora and Textilis; MUS: cultivar-groups Melanophthalmus, Unguiculata and Sesquipedalis.

	N	NPB	P	H_T	D_{ST}	G_{ST}
BnonNEA	6	60	29.7	0.107		
BNEA	6	58	28.7	0.109	0.014	0.117
cv.-gr. Melanophthalmus	4	30	14.9	0.058		
cv.-gr. Biflora	12	82	40.6	0.122		
cv.-gr. Textilis	4	49	24.3	0.097		
cv.-gr. Unguiculata	4	41	20.3	0.086		
cv.-gr. Sesquipedalis	2	22	10.9	0.054	0.025	0.205
BT	16	95	47.0	0.127		
MUS	10	67	33.2	0.100	0.006	0.050
Low ovule number	18	94	46.5	0.119		
High ovule number	8	70	34.7	0.111	0.006	0.048
All Domesticated	*26*	*108*	*53.5*	*0.123*	*0.025*	*0.205*
West Africa	5	67	32.2	0.124		
East Africa	11	138	68.3	0.203		
Southern Africa	14	144	71.3	0.215	0.028	0.126
All wild	*30*	*181*	*89.6*	*0.223*	*0.028*	*0.126*
Entire population	*56*	*187*	*92.6*	*0.202*	*0.025*	*0.125*

counterbalanced in southern Africa by introgressions with perennial subspecies.

The domesticated gene-pool

Our study shows that RAPD markers were effective in the detection of polymorphism in domesticated cowpea. Total genetic diversity in var. *unguiculata* (H_T = 0.123, 26 accessions) was much higher than those obtained with isozymes, from 0.018 to 0.061 (Vaillancourt et al. 1993; Panella and Gepts 1992; Pasquet 2000), and slightly higher than the one reported by Coulibaly et al. (2002) with AFLP (H_T = 0.108, 47 accessions). Here again, the difference between markers is more obvious with the number of polymorphic markers: 108 RAPD markers and 69 AFLP markers against a maximum of 15 isozyme loci.

Diversity levels in domesticated cowpea were similar in West Africa (H_S = 0.107) and north-eastern Africa (H_S = 0.109). These values are very close to the ones reported by Coulibaly et al. (2002). This can be paralleled with the lack of difference in diversity between cv.-gr. Biflora from north-eastern Africa and other cv.-gr. Biflora accessions (Table 1), while isozymes showed North-East Africa to be much more diverse (Pasquet 2000). In the same way, as with isozymes, diversity of low ovule number accessions is only slightly higher than that of high ovule number accessions (Table 1).

An other isozyme result that is not confirmed with RAPD data is the intermediate position of north-eastern Africa (and cv.-gr. Textilis) between West African cv.-gr. Biflora and Melanophthalmus, on the one hand, and Asian cv.-gr. Biflora, cv.-gr. Unguiculata and cv.-gr. Sesquipedalis, on the other hand, the domesticated accessions from North-East Africa being considered a pivotal group in the center of the domesticated gene-pool (Pasquet 2000). Numerous fragments present eastward or westward are not present in North-East Africa (as well as within cv.-gr. Textilis).

However, as with isozyme markers, RAPD markers barely separated low ovule number and high ovule number accessions, as only one fragment show marked difference in frequency between the two groups. Of course, the same marker separates cv.-gr. Biflora and cv.-gr. Melanophthalmus from cv.-gr. Unguiculata and cv.-gr. Sesquipedalis, but overall the two morphophysiological groups (Pasquet 1998) are as poorly separated as they were with the use of isozymes (Pasquet 2000).

More interestingly, like isozyme data, evolved cultivar-groups (Melanophthalmus, Unguiculata, and Sesquipedalis) are less diverse than primitive cultivar-groups (Biflora and Textilis): H_S = 0.100 versus H_S

= 0.127), and the classification of the cultivar-groups according to their diversity (Table 1) is the same as with isozyme data. RAPD do support the hypothesis of two consecutive bottlenecks (Pasquet 2000).

Cowpea domestication

Nei's genetic distances between wild and domesticated forms ranged from 0.098 and 0.410. However, if the var. *spontanea* accessions that are intermediate and introgressed are not considered, the highest distance becomes 0.331, a value expected between crops and their presumed progenitors. The bottleneck between var. *spontanea* and var. *unguiculata*, i.e., from $H_T = 0.223$ (or $H_T = 0.202$ if introgressed accessions are not considered) to $H_T = 0.123$, therefore a reduction in diversity of 45% or 39%, is much less important than that inferred from isozyme data, between 69% and 81% (Vaillancourt et al. 1993; Panella and Gepts 1992; Pasquet 1999, 2000), but closer to the one reported by Coulibaly et al. (2002) with AFLP, i.e. from $H_T = 0.175$ to $H_T = 0.108$ (38%).

However, in the dendrograms (Figure 2) as well as in the principal coordinates analysis (Figure 3), domesticated accessions are grouped, still suggesting a single domestication leading to domesticated cowpea. This observation confirmed previous observations made by Panella and Gepts (1992), Panella et al. (1993), Pasquet (1999), and Coulibaly et al. (2002). In addition, the separation of wild and domesticated cowpea gene pools observed with isozyme and AFLP data (Pasquet 1999; Coulibaly et al. 2002) is further emphasized by RAPD data as several markers seem to separate both groups, especially A03–8 (0.42 in domesticated versus 0 in wild) and A19–6 (0.81 in domesticated versus 0 in wild from West Africa). All the data show that the wild types from West Africa are closer to the domesticated types than to the wild types from East or southern Africa. Therefore, wild populations giving rise to domesticated cowpea must be located in the northern part of Africa. Being more precise is still difficult due to the lack of available accessions from North-East Africa. However, RAPD results confirm that a domestication in East Africa or southern Africa is unlikely.

Nevertheless, the cluster and the principal coordinate analyses reveal accessions that form a transition between domesticated and wild forms of various origins, including SP 185 from Tanzania, NI 1171 from Zambia and NI 1383 from Botswana. Considering the few markers with low frequencies in var. *spontanea* and high frequencies in var. *unguiculata*, i.e., A02-1, A02-7, A13-9, A16-1, A19-16, and B16, they are distributed from Senegal to South Africa (Figure 4). Therefore, these markers, which could have indicated a narrow center of origin, demonstrate that there is a widely distributed cowpea crop-weed complex all over Africa, as do some isozyme (Pasquet 1999), cpDNA (Pasquet unpublished), and AFLP (Coulibaly et al. 2002) markers. Taking into account that there appears to have been a single domestication event, the genetic similarity of some of these wild accessions to the domesticated group would be the result of post-domestication gene flow between wild and domesticated forms due to their sympatric distribution. Such accessions would represent 'weedy' types.

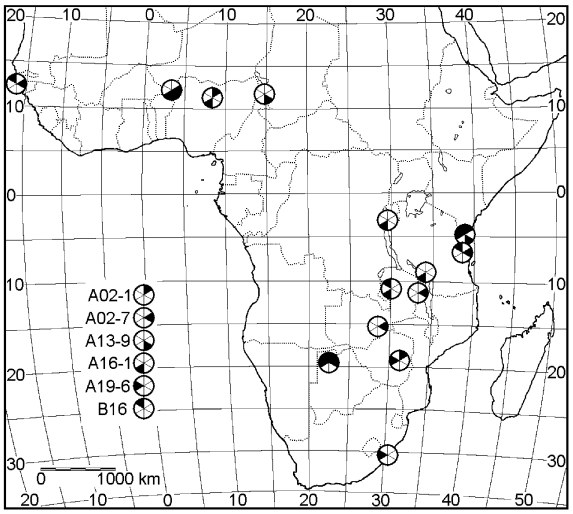

Figure 4. Geographic distribution of wild accessions characterized by the presence of the fragments frequent within domesticated accessions and rare within wild accessions. Each slice of the pie represent one fragment. The slice is black if the corresponding fragment is present.

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