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AFLP analysis of the phenetic organization and genetic diversity of *Vigna unguiculata* L. Walp. reveals extensive gene flow between wild and domesticated types

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Abstract Amplified fragment length polymorphisms (AFLPs) were used to evaluate genetic relationships within cowpea [*Vigna unguiculata* (L.) Walp.] and to assess the organization of its genetic diversity. Nei's genetic distances were estimated for a total of 117 accessions including 47 domesticated cowpea (ssp. *unguiculata* var. *unguiculata*), 52 wild and weedy annuals (ssp. *unguiculata* var. *spontanea*), as well as 18 perennial accessions of the wild subspecies *pubescens*, *tenuis* and *alba*. AFLP variation was also used to study genetic variation among and within domesticated and wild accessions based on their geographical origin (western, eastern and southern Africa). Wild annual cowpea (var. *spontanea*) (H_T=0.175) was more diverse than domesticated cowpea (H_T=0.108). Wild cowpea was more diverse in eastern (H_S=0.168) than in western Africa (H_S=0.129), suggesting an eastern African origin for the wild taxon. The AFLP data were consistent with earlier findings of a unique domestication event in cowpea in the northern part of the continent and suggested that domestication in eastern or southern Africa was unlikely. It did not allow a more precise localization of domestication due to extensive gene flow between wild and domesticated forms that has led to a large crop-weed complex distributed over the entire African continent. In addition, wild materials from northeastern Africa are still lacking. Overall, the superiority of the AFLP technique over isozymes resided in its ability to uncover variation both within domesticated and wild cowpea, and should be a powerful tool once additional wild material becomes available.

Keywords *Vigna unguiculata* · Cowpea · Domestication · AFLP · Genetic diversity

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Introduction

Cowpea, *Vigna unguiculata* (L.) Walpers (2n=2*x*=22), is an essential crop in less-developed countries of the tropics and subtropics, especially in sub-Saharan Africa, Asia, and Central and South America (Singh et al. 1997). As a legume, cowpea is valued for the high protein content of its grains, but also for the vitamins and minerals present in the young leaves, pods and peas (Nielsen et al. 1997). *V. unguiculata* includes annual cowpeas (ssp. *unguiculata*) and ten wild perennial subspecies. Subspecies *unguiculata* includes all the domesticated (var. *unguiculata*), as well as wild and weedy, forms [var. *spontanea* (Schweinf.) Pasquet] (Pasquet 1993). Among the perennial forms, subspecies *pubescens* (R. Wilczek) Pasquet, *tenuis* (E. Mey.) Maréchal, Mascherpa and Stainier, and *alba* (G. Don) Pasquet are genetically the closest to ssp. *unguiculata* (Pasquet 1999). A widely used classification subdivided all domesticated forms into four cultivar-groups based essentially on seed and pod characters (Westphal 1974; Ng and Maréchal 1985). The cultivar-groups include Unguiculata grown as a pulse, Biflora (catjang) used mainly as a forage, Sesquipedalis (yardlong or asparagus bean) grown as a vegetable, and Textilis cultivated for the fibers of its long floral peduncles. Recently, Pasquet (1998) proposed the addition of another cultivar-group, namely Melanophthalmus (blackeyed pea).

The African origin of cowpea has never been a point of contention since wild forms are endemic to Africa. Moreover, of the wild forms, var. *spontanea* (Schweinf.) Pasquet (also referred to as var. *dekindtiana* sensu Verdcourt by some authors) is accepted as the most likely progenitor of domesticated cowpea (Padulosi and Ng 1997; Pasquet 1999). Its morphology and growth habit are very similar to that of cowpea landraces, but it also possesses wild-like attributes such as shattering pods with small seeds. Despite the wide distribution of var. *spontanea* throughout sub-Saharan Africa, molecular studies point to a unique domestication event (Panella and Gepts 1992; Pasquet 1999). A disagreement exists, however, as to where cowpea was domesticated. Two domestication areas have been proposed, in western and northeastern Africa, respectively (Baudoin and Maréchal 1985; Ng and Maréchal 1985; Vaillancourt and Weeden 1992; Ng 1995; Pasquet 2000). A western African center of domestication has been proposed based on: (1) the highest level of morphological diversity for cultivated cowpea, (2) the existence of weedy intermediates between wild and cultivated cowpeas, (3) the oldest archeological evidence for cowpea in Ghana, and (4) the identification of a wild and a cultivated accession with an identical cpDNA in Nigeria (Ng and Maréchal 1985; Vaillancourt and Weeden 1992; Ng 1995). An alternative center of domestication of cowpea has been proposed in northeastern Africa based on: (1) the absence of true ecologically wild cowpea in West Africa, (2) the high level of morphological diversity of wild cowpea in the region from Ethiopia to South Africa (Baudoin and Maréchal 1985), and (3) results from ethnobotanic, linguistic, and isozyme studies (Pasquet 1996). Isozyme studies have revealed the absence of a center of diversity in West Africa (Vaillancourt et al. 1993) and a higher level of genetic diversity in cultivars of Ethiopian origin (Pasquet 2000).

Some important shortcomings of previous studies include the limited size and representativity of the samples used. In particular, of the 26 wild accessions surveyed by Vaillancourt and Weeden (1992), only 12 correspond to the most-probable progenitor of cowpea, var. *dekindtiana sensu* Verdcourt. They include accessions from just five countries (Nigeria, Burundi, Tanzania, Malawi and Zimbabwe). Among the domesticated accessions, the cultivar-group Textilis was missing. Another caveat shared by studies of proponents of both scenarios is the limited level of polymorphism uncovered by isozymes and cpDNA markers. For example, only two cpDNA patterns were identified among 32 domesticated accessions (Vaillancourt and Weeden 1992). In the study by Panella and Gepts (1992), 14 of the 24 putative isozyme loci were polymorphic within the wild accessions, and only one in the domesticated group. Finally, even in the more exhaustive study by Pasquet (2000), including 271 domesticated accessions from 30 countries, 36 isozyme loci were scored, of which more than half were monomorphic. Moreover, the most abundant allele had frequencies above 0.9 for all but five loci.

The objectives of the present study were to assay AFLP variation in a representative sample of the primary gene pool of cowpea to: (1) confirm the occurrence of a single domestication event and investigate a more-precise origin of domestication for cowpea; (2) assess the importance of gene flow in the current organization of domesticated cowpea; and (3) compare the AFLP and isozyme-derived organization of genetic diversity in cowpea.

Materials and methods

Plant materials

A total of 117 accessions, reflecting the geographical distribution and current taxonomy of *V. unguiculata* ssp. *unguiculata* and closely related taxa, were evaluated (Table 1). The accessions included 47 domesticated annuals, 52 wild and weedy annuals, and 18 perennial forms. Domesticated materials (D) were divided into three groups based on their geographical origin: western-central Africa (DW), eastern Africa (DE), and southern Africa (DS). Likewise, wild accessions (W) were divided into western, eastern, and southern African groups (WW, WE, and WS). The perennial accessions belonged to ssp. *pubescens*, ssp. *tenuis*, and ssp. *alba*. For comparative purposes, all accessions used in the AFLP work had previously been surveyed with 36 isozyme loci (Pasquet 1999, 2000).

AFLP analysis

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). The amplified fragment length polymorphism (AFLP) protocol followed was that described by Vos et al. (1995). All adaptors and primers are listed in Table 2. AFLP banding patterns were revealed by exposure of X-ray films (Kodak X-Omat, Sigma Chemical Co., St. Louis, Mo.) for 1–2 days. Only the bands that could be read unambiguously on each autoradiograph were considered for analysis.

Genetic distance estimates and cluster analysis

Each band was treated as a separate putative locus, and scored as present (1) or absent (0) in each accession. Estimates of similarities were based on three different measures: (1) Nei and Li's similarity index (1979) developed for endonuclease restriction-generated fragments, also known as the Dice coefficient; (2) Jaccard's (1908) coefficient; and (3) Nei's (1972) similarity.

Cluster analysis was based on the unweighted paired-group method using arithmetic averages (UPGMA) of the NTSYS-pc software (Rohlf 1992). The dendrogram was created with the TREE option of NTSYS and the goodness of fit of the clustering to the data was calculated using the COPH and MXCOMP procedures (Rohlf 1992). Similarly, the correspondence between isozyme and AFLP-based clustering of the accessions surveyed was tested. In order to identify major cluster groups, a principal coordinate analysis was conducted based on dissimilarity measures (1-S*ij*) using the DCENTER and EIGEN procedures in NTSYS (Rohlf 1992). Genetic relationships were also investigated among wild and domesticated forms according to their geographic origin. Unbiased estimates of between-groups genetic distances based on the frequency of the presence of each band in each group were computed using Nei's (1972) distance. Calculations of group genetic distances were carried out with Popgene software version 1.21 (Yeh et al. 1997).

Table 1 List of 117 accessions of *V. unguiculata* (L.) Walp. assayed for AFLP variation and their origin. Countries of origin are indicated by their United Nations codes

Taxonomic group		Country	Geographic Group (African ssp. *unguiculata* only)	Accessions
ssp. *unguiculata* var. *unguiculata*	cultivar-group Biflora	CMR	DW	NO 89, 106, 3113
		ZAR	DW	ZR7
		EGY	DE	EG 1
		ETH	DE	ET 1, 2, 14, 15, 25, 28, 31
		IND		EX 35
		LAO		EX 37
		PAK		EX 51
		YEM		ET 33, 39
	cultivar-group Melanophthalmus	CMR	DW	O 574, 1387
		DZA		AG 1
		NGA	DW	IT84S2049 (breeding line from IITA)
		USA		524B (breeding line from Riverside)
	cultivar-group Sesquipedalis	NCL		EX 38
		PHL		EX 43
	cultivar-group Textilis	CMR	DW	NO 27, 198, 274, 275, 577, 1769, 2292, 2297, 2418, 2300, 3076
	cultivar-group Unguiculata	CMR	DW	NO 74, 90, OU 65, 100, CS 5, 15, 53B, 56B
		ETH	DE	ET 35
		UGA	DE	UG 8
		ZAF	DS	AS 3E, 10C
ssp. *unguiculata* var. *spontanea*		BDI	WE	NI 1232
		BWA	WS	MT 623, NI 1379, 1381, 1383, 1384
		CMR	WW	SP 37, 46, 52, 93
		COG	WW	NI 1390, 1391, SP 148, 149, 151
		GNB	WW	SP 140
		KEN	WE	SP 87
		MWI	WS	NI 1392, SP 84
		NAM	WS	SP 154, 160
		NER	WW	NI 991, SP 80, GAC25, TOE8
		NGA	WW	NI 951
		SEN	WW	NI 963
		SDN	WE	SP 86
		TZA	WE	NI 1385, 1386, 1405, SP75, HM 2250, 2255, 2265, 2275, 2306, TV 297, 298, 301, 503
		YEM		SP 171
		ZAF	WS	NI 1167, SP 159, 192, TV 1343, 1351
		ZMB	WS	MT 651, NI 1171, 1387
		ZWE	WS	MT 99
ssp. *pubescens*		BDI		NI 1417
		KEN		NI 979, 1186, 1408
		TZA		SP 83, 142, NI 947, 1406, TV 110, 505
ssp. *tenuis*		MOZ		TV 1446
		MWI		MT 363
		ZAF		SP 162, 194
		ZMB		MT 206
		ZWE		MT 4, 38
ssp. *alba*		COG		NI 1388, SP 150

Gene-diversity estimates

Genetic variability was assessed using the proportion of polymorphic fragments (P), and the total diversity (H_t) (Nei 1973). Total diversity was partitioned into the weighted average diversity within cultivar-groups (H_s) and the between cultivar-groups gene diversity (D_{st}). The proportion of total allelic diversity found among cultivar-groups (G_{st}) was calculated as the ratio D_{st}/H_t (Nei 1973). The NPAR1WAY and GLM procedures of SAS V7.0 (SAS Institute 1999) were used to analyze the effect of grouping (domesticated vs wild; western, eastern, and southern Africa) on gene diversity and to detect significant differences among ranked means. The degree of AFLP polymorphism was also quantified using Shannon's information index for phenotypic diversity (Bowman et al. 1971). The phenotypic diversity values were calculated and compared for different groups as described by Wachira et al. (1995).

Table 2 Oligonucleotide adaptors and primers used for AFLP analysis

	Restriction enzyme	Sequence
Adaptor	*Eco*RI	5′- GACTGCGTACC-3′ 3′- CTGACGCATGGTTAA-5′
	*Mse*I	5′- GACGATGAGTCCTGAG-3′ 3′- TGCTACTCAGGACTCAT-5′
Primers	*Eco*RI+1	5′-AGACTGCGTACCAATT+C-3′
	*Mse*I+1	5′-GACGATGAGTCCTGAGTAA+A-3′
	*Eco*RI+3	5′-AGACTGCGTACCAATTC+AAC-3′[a] 5′-AGACTGCGTACCAATTC+ACA-3′ 5′-AGACTGCGTACCAATTC+ACC-3′ 5′-AGACTGCGTACCAATTC+AGT-3′
	*Mse*I+3	5′-GACGATGAGTCCTGAGTAA+AGG-3′[a] 5′-GACGATGAGTCCTGAGTAA+ACA-3′ 5′-GACGATGAGTCCTGAGTAA+ATG-3′ 5′-GACGATGAGTCCTGAGTAA+AGA-3′

[a] Primer combinations are A02P11: *Eco*RI+AAC/*Mse*I+AGG, A05P05: *Eco*RI+ACA/*Mse*I+ACA, A06P15: *Eco*RI+ACC/*Mse*I+ATG, A12P09: *Eco*RI+AGT/*Mse*I+AGA

Table 3 Gene diversity statistics estimated from AFLP data with accessions grouped according to type (domesticated vs wild) and geographic location (western, eastern, and southern Africa). Within-group estimates of gene diversity (H_S) followed by the same letter are not significantly different with the S-N-K test (P<0.05)

Geographic groups (# accessions)[a]	Number of polymorphic loci (%)	H_S[b]	H_T[c]	D_m[d] (D_{ST})[d]	G_{ST}[f]
DW (25)	63 (34)	0.105[B]			
DE (10)	54 (29)	0.101[B]			
DS (2)	13 (7)	0.029 [C]			
All domesticated (37)	**69 (37)**	**0.078**	**0.108**	**0.045 (0.030)**	**0.278**
WW (16)	74 (39)	0.129[B]			
WE (16)	91 (48)	0.168[A]			
WS (18)	98 (52)	0.165[A]			
All var. *spontanea* (50)	**110 (59)**	**0.154**	**0.175**	**0.032 (0.021)**	**0.120**
Ssp. *pubescens* (10)	70 (37)	0.124[B]			
Ssp. *tenuis* (7)	51 (27)	0.097[B]			
Ssp. *alba* (2)	7 (4)	0.015[C]			
All perennials (19)	**83 (44)**	**0.079**	**0.130**	**0.077 (0.051)**	**0.392**
Entire population (106)[g]	**114 (61)**	**0.104**	**0.173**	**0.078 (0.069)**	**0.400**

[a] DW, DE, DS=domesticated accessions from western, eastern and southern Africa, respectively. WW, WE, and WS=var. *spontanea* (wild annual) accessions from western, eastern and southern Africa, respectively
[b] H_S: within-populations gene diversity estimates
[c] H_T: total gene diversity estimates
[d] D_m: standardized measure of among populations gene diversity
[e] D_{ST}: among populations gene diversity estimates
[f] G_{ST}: relative magnitude of gene differentiation among populations
[g] Excluding AG 1 and ten non-African accessions

Results

Gene diversity and group genetic distances

A total of 188 loci were scored, of which 114 (61%) were polymorphic. Twelve fragments were present only in wild annual and perennial accessions, of which three were wild annual-specific and one was perennial-specific. No fragments were found that were present only in domesticated accessions. However, some fragments were much more frequent in domesticated than wild accessions, and vice versa. These included *A12P09–46* (0.79 in domesticated vs 0.06 in wild accessions), *A06P15–50* (0.87 vs 0.19), *A05P05–39* (0.62 vs 0.20), *A05P05–38* (0.00 vs 0.61), *A05P05–09* (0.09 vs 0.60) and *A06P15–13* (0.21 vs 0.71). On average, the domesticated group (H_T=0.108) was significantly less diverse than var. *spontanea* (H_T= 0.175) and all the wild accessions combined (H_T=0.174). Among domesticated types, primitive cultivars, i.e., cultivar-groups Textilis and northeastern Biflora (H_T=0.097), were as diverse as more evolved cultivars (H_T=0.094). Within var. *spontanea*, gene diversity was higher in wild accessions from eastern Africa (H_S=0.168) than in wild accessions from western Africa (H_S=0.129) (Table 3). The shortest distance between wild and domesticated forms of a region was found in western Africa, although the domesticated forms of northeastern African origin (DE) were consistently closer to all wild forms (Table 4).

Table 4 Nei's 1978 unbiased measures of genetic identity (above the diagonal) and genetic distance (below the diagonal) among groups

Group	DW	DE	DS	WW	WE	WS	Ssp. *pubescens*	Ssp. *tenuis*	Ssp. *alba*
DW		0.9913	0.9423	0.9737	0.9393	0.9314	0.9162	0.9014	0.8244
DE	0.0880		0.9403	0.9763	0.9443	0.9407	0.9272	0.9099	0.8481
DS	0.0594	0.0616		0.9092	0.8823	0.8728	0.8623	0.8538	0.7837
WW	0.0267	0.0240	0.0952		0.9696	0.9616	0.9494	0.9206	0.8634
WE	0.0626	0.0573	0.1252	0.0308		0.9745	0.9705	0.9588	0.8943
WS	0.0710	0.0612	0.1360	0.0391	0.0259		0.9615	0.9654	0.9150
Ssp. *pubescens*	0.0875	0.0755	0.1482	0.0519	0.0300	0.0393		0.9417	0.9018
Ssp. *tenuis*	0.1038	0.0945	0.1580	0.0827	0.0421	0.0353	0.0600		0.9276
Ssp. *alba*	0.1932	0.1648	0.2437	0.1468	0.1118	0.0889	0.1033	0.0752	

Total genetic diversity in all wild accessions, including perennial ones (H_T =0.174, 69 accessions) or just in var. *spontanea* (H_T=0.175, 50 accessions), was comparable to those inferred earlier by Vaillancourt et al. (1993) and Pasquet (1999) based on isozymes. But the most striking difference between both marker types was in the number of polymorphic markers: 111 AFLP markers against a maximum of 30 isozyme loci (Pasquet 1999). As in the isozyme studies, AFLPs clustered ssp. *pubescens*, ssp. *tenuis* and ssp. *alba* accessions fairly well, but barely distinguished them from ssp. *unguiculata* var. *spontanea* accessions. Based on AFLP data, these subspecies could be genetically closer to each other than suggested by isozymes, morphological and biogeographical data.

Within ssp. *unguiculata* var. *spontanea*, AFLP analyses showed the WS and WE groups to be more diverse than the WW group, as did previous isozyme analyses. Higher values of WS diversity could be explained by introgression with perennial subspecies, and lower values for the WW group by introgression with the low diversity domesticated group or escape from the latter group. Higher diversity of the WE group could be due to introgression with a perennial subspecies. Introgression between the WE group and ssp. *pubescens* does occur and is shown by cpDNA data since a few var. *spontanea* accessions show a ssp. *pubescens* cpDNA profile and a reciprocal one (R. Pasquet and P. Gepts, unpublished). However, if introgressions between the WE group and ssp. *pubescens* do occur, AFLP data indicate that introgression with ssp. *pubescens* alone can hardly account for the higher diversity of the WE group. As some of the accessions from East Africa show a rootstock and an allogamous floral morphology, these higher values for the WE group could be explained by the East African origin of ssp. *unguiculata* var. *spontanea*.

Total genetic diversity in the domesticated gene pool (var. *unguiculata*: H_T=0.108, 37 accessions) was higher than those obtained by Vaillancourt et al. (1993) (H_T= 0.029, 112 accessions), Panella and Gepts (1992) (H_T= 0.018, 34 accessions), and Pasquet (2000) (H_T=0.061, 271 accessions), based on isozymes. Here again, the most striking difference between both marker types lies in the number of polymorphic markers: 76 AFLP marker loci against a maximum of 15 isozyme loci (Pasquet 2000). Although there is a center of maximum morphological diversity in West Africa for domesticated cowpea (Ng and Maréchal 1985; Ng 1995), AFLPs did not reveal such a center. Diversity levels in domesticated cowpea were similar in western (H_S=0.105) and northeastern Africa (H_S=0.101).

Cluster analysis

The cluster analysis based on the 117 accessions generated a unique dendrogram with two main clusters (Fig. 1). All domesticated accessions belonged to the same subcluster. Seven wild accessions were found amidst the domesticated cluster (NI 951, NI 991, NI 1383, SP37, SP 80, GAC25 and TV 1343). These wild accessions were from western, southern, and eastern Africa. Additional wild accessions (TOE8 to NI 1171) that were included in the same main cluster differed from the two previous groups in allele frequencies at 25/114 loci (difference=0.22). These accessions combine loci with alleles characteristic of the first and second main clusters. The second main cluster (MT 623 to NI 1387) differed from the domesticated groups in allele frequencies at 52/114 loci (difference=0.46). It included all perennial accessions (except NI 1417) and most var. *spontanea* accessions from southern and eastern Africa (WE and WS groups), but no var. *spontanea* accession from western Africa (WW group). Within this main group, most WS accessions (including those from the isoenzymatic BWA group, Pasquet 1999) clustered with ssp. *alba* and ssp. *tenuis* accessions. Most ssp. *pubescens* accessions fell into a single cluster, neighboring a cluster of WE var. *spontanea* accessions.

The distance parameters used had limited influence on the composition of the clusters. The Jaccard (1908) and Nei and Li (1979) indices yielded the same tree, while Nei's (1972) distance changed the location of just four domesticated accessions, i.e., ET 28, ET 33, ET 39, and NO 3113.

Principal coordinate analysis

The principal coordinate analysis (Fig. 2) yielded a picture similar to the one given by the cluster analysis. Up

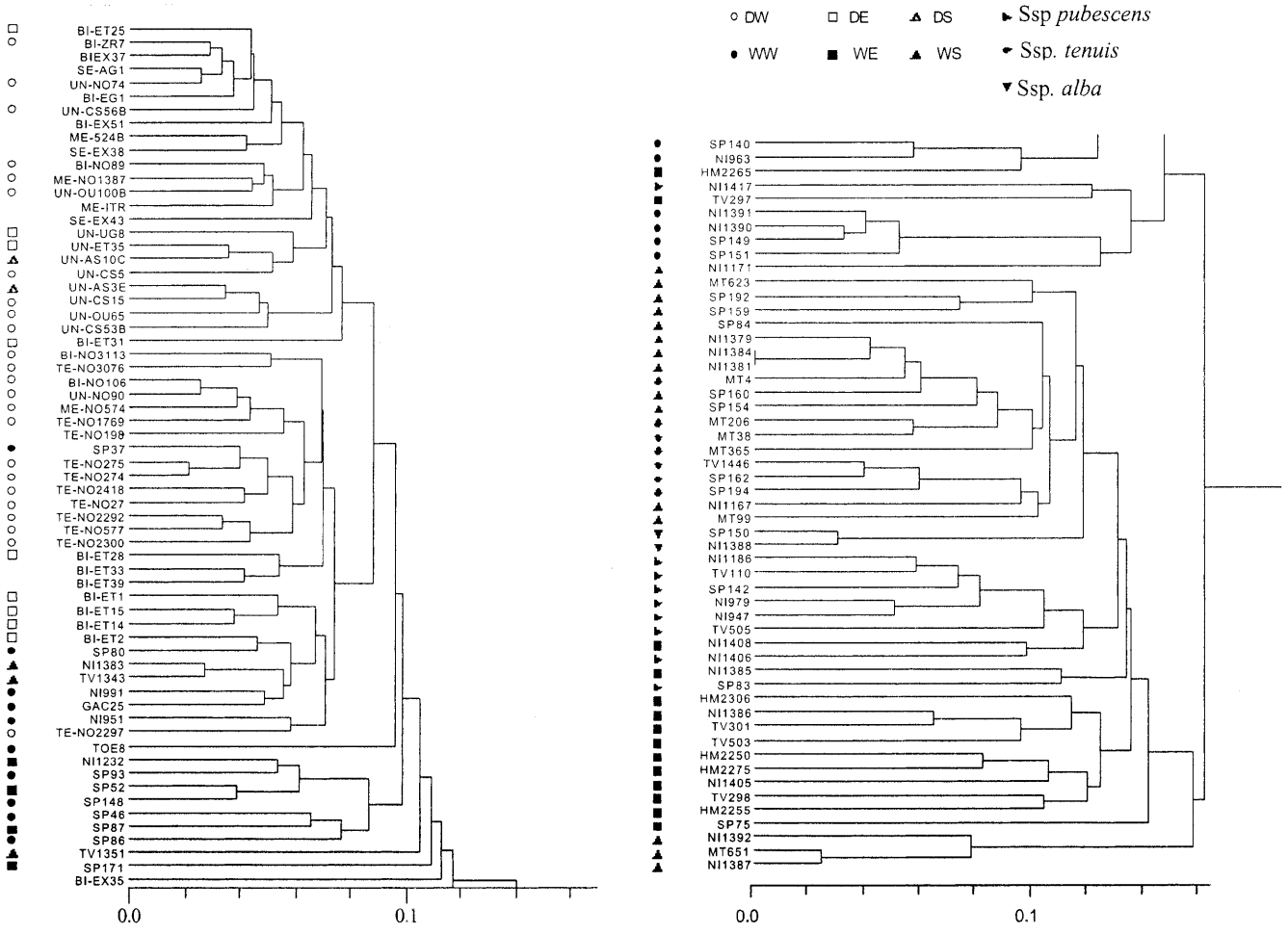

Fig. 1 UPGMA dendrogram of 117 accessions of the genus *Vigna*, essentially *V. unguiculata*, based on AFLPs. Phenetic relationships were derived from Nei and Li (1979) pairwise genetic distance estimates. *DW*, *DE*, and *DS*=domesticated accessions from western, eastern and southern Africa, respectively. *WW*, *WE* and *WS*=wild var. *spontanea* from western, eastern and southern Africa, respectively. For domesticated accessions, the cultivar-group is indicated: *BI*: Biflora; *ME*: Melanophtalmus; *SE*: Sesquipedalis; *TE*: Textilis; *UN*: Unguiculata. Accessions without a symbol originated outside Africa

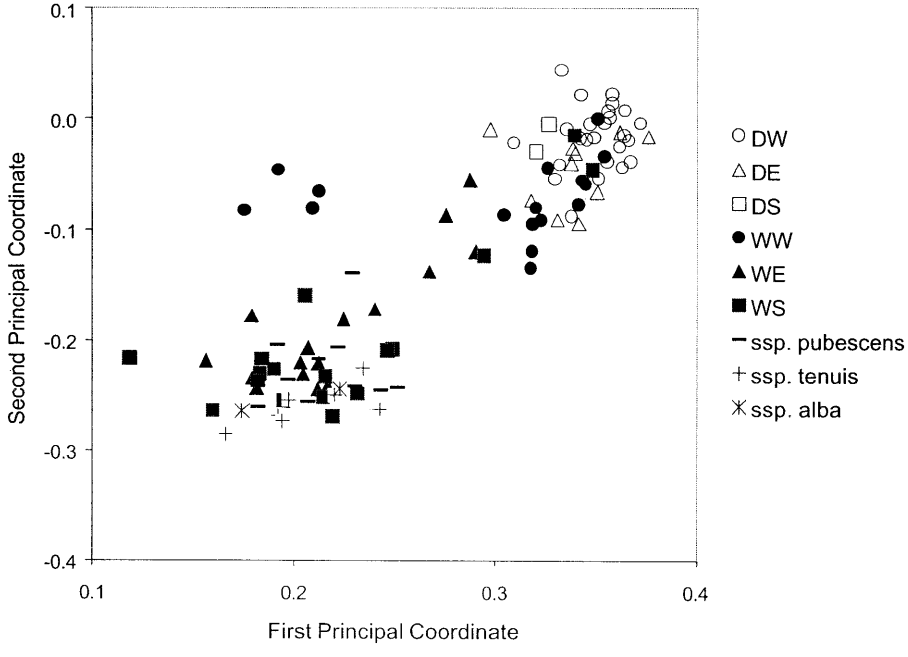

Fig. 2 Principal coordinate analysis based on the distance matrix inferred from the Dice (or Nei and Li) similarity index

to 57% of the total variation was explained by the first three axes, which accounted for respectively 32%, 17% and 9% of the observed variation. The two main groups from the cluster analysis appeared well separated. Domesticated accessions and WW accessions appeared in the right, upper quadrant. Perennial accessions appeared in the left, lower quadrant, and were mixed with most WS and WE group accessions. Although ssp. *pubescens* and WE accessions were concentrated towards the upper part of the bottom group, and ssp. *tenuis*, ssp. *alba* and WS accessions were concentrated towards the lower part, the two ensembles overlapped widely.

Isozyme-AFLP comparison

Initially, cophenetic correlation coefficients were used to test the extent to which the clustering of the genotypes depicted in the AFLP and isozyme dendrograms consistently represented the estimates of genetic distances between accessions, for each marker type. This goodness-of-fit test resulted in correlation scores of r=0.83 (P<0.001) and 0.85 (P<0.001) for isozymes and AFLPs, respectively. When isozyme and AFLP data were compared using the Mantel matrix correspondence test between their cophenetic matrices, the correlation was 0.44 (P<0.001).

Both isozyme and AFLP clustering analyses revealed a single cluster for all domesticated forms, with two major groups. However, cluster membership and the order of accessions within the cluster differed between methods. With isozymes, the ”domesticated” cluster included more wild accessions than the AFLP-based cluster and contained two main groups: one composed essentially of wild accessions with some Biflora and Textilis forms interspersed among them, and another with mostly domesticated accessions. In the latter group, no clear separation of Textilis and Ethiopian Biflora, or Unguiculata was observed. Overall, isozyme analysis resulted in a poorer resolution of the domesticated gene pool than AFLPs.

Discussion

Nei’s genetic distances between wild and domesticated forms fell between 0.02 and 0.09 (Table 4), a range expected between crops and their presumed progenitors. The bottleneck between var. *spontanea* and var. *unguiculata*, i.e. H_T=0.175 to H_T=0.108 (62%), is much-less important than those inferred from the data of Vaillancourt et al. (1993) (H_T=0.153 to H_T=0.029, 19%), Panella and Gepts (1992) (H_T=0.090 to H_T=0.018, 20%), and Pasquet (1999, 2000) (H_T=0.199 to H_T=0.061, 31%), based on isozymes. However, in the dendrogram (Fig. 1) as well as in the PCA (Fig. 2), domesticated accessions are grouped, still suggesting a single domestication event leading to domesticated cowpea. As used here, the expression ”single domestication” refers to a domestication that took place in a circumscribed area. This observation confirmed previous observations made by Panella and Gepts (1992), Panella et al. (1993), and Pasquet (1999).

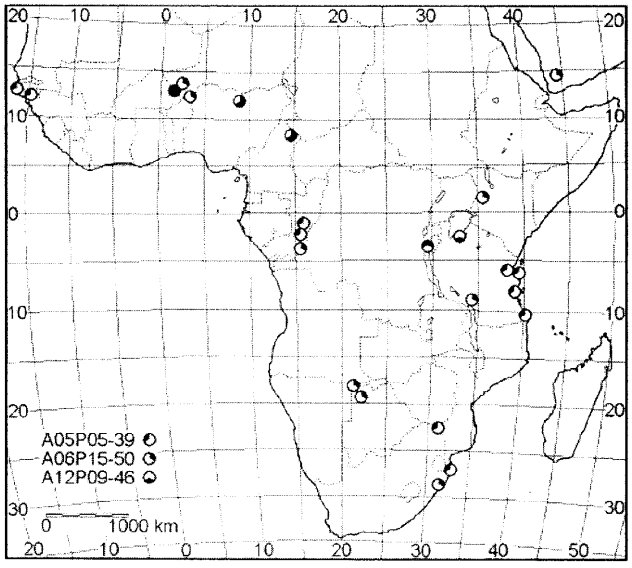

Fig. 3 Geographic distribution map of the three markers frequent in domesticated groups and rare within wild groups (see figure legend). Each wild accession is represented by a pie. Each third of the pie (top left, top right, bottom) represents one of the three markers, and is *black* if the marker is present or *white* if the marker is absent

AFLP studies of crop and wild progenitor diversities are not numerous, especially within legumes. Sharma et al. (1996) reported a similar level of diversity in domesticated lentil, *Lens culinaris* (H_T=0.096), although the observed gene diversity in the presumed progenitor of lentil, ssp. *orientalis*, (H_T=0.105), was slightly higher and the bottleneck less marked. Compared to wild and domesticated soybean (Maughan et al. 1996), diversity is higher in both cowpea and its wild progenitor. The soybean bottleneck (reductions of P from 31.2 to 16.8 and of H_{SH} from 2.32 to 2.13) is less marked than in cowpea (H_{SH} from 6.44 to 3.89).

Is it possible to pinpoint a more specific center of origin for domesticated cowpea? All the data (Figs. 1, 2 and Table 4) show that the WW group is closer to the domesticated types than the WE and WS groups. However, the cluster and PCA analyses reveal accessions that form a transition between domesticated and wild forms of various origins (Figs. 2, 3). Several wild eastern (NI 1232, SP 87) and even southern (NI 1383, TV 1343, TV 1351) accessions occur near the northern ones within the domesticated cluster (Fig. 1) or the domesticated area (Fig. 2). The few markers with low frequencies in var. *spontanea* and high frequencies in var. *unguiculata*, i.e., which could have indicated a narrow center of origin [*e.g.*, *A05P05–39* (0.31 versus 0.62), *A12P09–46* (0.23 versus 0.79) and *A06P15–50* (0.46 versus 0.87)], are distributed from Senegal to South Africa through the Congo, Kenya and Yemen (Fig. 3). Therefore, these markers demonstrate that there is a widely distributed

cowpea crop-weed complex all over Africa, more convincingly so than did the isozyme marker *Amp2*^{102} (Pasquet 1999). 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. This may be particularly true for the wild accessions from western Africa, which are not found in natural undisturbed habitats. Instead, they are found usually in disturbed habitats such as field margins. This type of habitat seems to preclude domestication in that part of the continent, unless the actual progenitor became extinct or only survived as weedy types in that region.

Could domestication then have taken place from the WE or WS groups? This would be unlikely as markers *A05P05–42* and *A12P09–47*, with frequencies as high as 0.60 and 0.62 in domesticated types, are absent in the WE group, and marker *A12P09–46* (0.79 in domesticated) is absent from the WS group (Fig. 3). Of course, such extreme situations do not occur with the WW group. Although markers absent in WW and present in domesticated types are numerous, domesticated frequencies for these markers are usually below 0.1 and do not reach beyond 0.30. Therefore, cowpea domestication from a progenitor from eastern or southern Africa is also unlikely. This is a clear answer that isozyme studies were unable to give (Pasquet 1999). A more conclusive answer regarding the actual domestication center in western or northeastern Africa awaits further data. Wild accessions need to be collected in northeastern Africa, specifically north of 4°N and east of 21°E (Fig. 3). In addition, markers near or at genes for the domestication syndrome need to be identified; analysis of genetic diversity in crops and their wild progenitors using these markers provides a more consistent separation between domesticated and wild types, which in turn may help identify the wild populations most closely allied to the crop (R. Papa and P. Gepts, unpublished data).

In conclusion, AFLP data, along with unpublished morphological data and field observations, showed that var. *spontanea* originated in East Africa and spread westward and southward. This migration was accompanied by a change toward a more annual lifespan and a more selfing breeding system. AFLP data showed that within var. *spontanea*, domestication could not have occurred in southern or eastern Africa. These data also showed that gene flow appears to have had a considerable impact on the organization of genetic diversity within *V. unguiculata*, resulting in a large number of weedy forms. This genetic exchange blurs the distinction between the ancestral state and post-domestication evolution, hence the difficulty in inferring where domestication actually occurred, i.e. in western versus northeastern Africa.

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