Marshall, D.R., and R.W. Allard. 1970. Isozyme polymorphism in natural populations of *Avena Fatua* and *A. barbata*. J. Hered. 25:373–382.
National Research Council. 1993. The science of collecting genetic resources. p. 153–172. *In* B.J. Rice and J.L. Overton (ed.). Managing global genetic resources: Agricultural issues and policies. Natl. Acad. Press, Washington, DC.
Putman, D.H., P.R. Miller, and P. Hucl. 1996. Potential for production and utilization of annual canarygrass. Cereal Foods World 41(2):75–83.
Rohlf, F.J. 1993. NTSYS-pc: Numerical taxonomy and multivariate analysis system. Version 1.80. Applied Biostatistics Inc., Setauket, NY.
Shields, C.R., T.J. Orton, and C.W. Stuber. 1983. Isozymes in plant genetics and breeding. Part A. Elsevier, Amsterdam.
Stuber, C.W., M.M. Goodman, and F.M. Johnson. 1977. Genetic control and racial variation of β-glucosidase isozymes in maize (*Zea mays* L.). Biochem. Genet. 15:383–394.
Vaillancourt, R. 1989. Inheritance and linkage of morphological markers and isozymes in lentil. Ph.D. Diss. Univ. of Saskatchewan, Saskatoon, SK.
Villarroya, M., E. Cadahia, and J.M. Garcia-Baudin. 1983. Isozyme variation in *Phalaris minor* Retz. and *P. brachystachys* Link. (In Spanish.) Anales. Inst. Nac. Invest. Agra. Serie Agricola 23:85–95.
Weeden, N.F. 1984. Linkage between the gene coding the small subunit of ribulose bisphosphate carboxylase and the gene coding for malic enzyme in *Phaseolus vulgaris*. Ann. Rep. Bean Imp. Coop. 27:123–124.
Weeden, N.F. 1989. Applications of isozymes in plant breeding. Reviews. Vol. 6. Timber Press. Portland, OR.
Wendel, J.F., and N.F. Weeden. 1989. Visualization and interpretation of plant isozymes. p. 5–45. *In* D.E. Soltis and P.S. Soltis (ed.) Isozymes in plant biology. Dioscorides Press, Portland, OR.
Yndgaard, F., and A. Hoskuldsson. 1985. Electrophoresis: A tool for genebanks. Plant Genet. Res. Newsl. 63:34–40.
Zhang, X., and J.A. Mosjidis. 1998. Rapid detection of mating system of *Vicia* species. Crop Sci. 38:872–875.

Isozyme Diversity in Bambara Groundnut

Rémy S. Pasquet, Sonya Schwedes, and Paul Gepts*

ABSTRACT

Bambara groundnut [*Vigna subterranea* (L.) Verdc.] is an important crop in many countries of tropical Africa. Compared with other leguminous crops, little is known about the organization of its genetic diversity. Our goal was to investigate its population structure and the partitioning of genetic diversity between domesticated and wild accessions. Seventy-nine accessions of domesticated and 21 wild Bambara groundnut populations were evaluated for genetic diversity at 41 isozyme loci, representing 23 enzyme systems. Domesticated accessions were characterized by very low genetic diversity (Ht = 0.052) with only 7 polymorphic loci. Wild populations were characterized by higher genetic diversity (Ht = 0.087), with 14 polymorphic. This suggests a marked bottleneck between wild and domesticated forms. Intrapopulation diversity was comparatively high (Hs = 0.033 for domesticated and Hs = 0.025 for wild populations) despite the near absence of heterozygous individuals, which suggests a predominantly selfing mode of pollination in both wild and domesticated Bambara groundnut. High genetic identity between wild and domesticated forms suggests that wild Bambara groundnut is the true progenitor of domesticated Bambara groundnut. Both morphological and isozyme data show a gradient between true wild and domesticated Bambara groundnut through weedy populations. The improved understanding of the organization of genetic diversity of Bambara groundnut provided by our data will allow a better conservation of its genetic resources.

Bambara groundnut is an important leguminous crop in Tropical Africa, although in many countries it has been replaced as a major crop by the groundnut, *Arachis hypogaea* L. Nevertheless, Bambara groundnut cultivation still has a wide distribution from Senegal to the Ethiopian lowlands, as well as in South Africa and Madagascar (Jacques-Felix, 1950). In the seventeenth century, it was introduced into South America, as Linnaeus (1763) described it from a plant from Surinam. Later it was carried to the Philippines and Indonesia (Brouk, 1975).

Within domesticated forms, authors used to distinguish bunch, semi-bunch, and open cultivars, categorized on the basis of the ratio of petiole length/internode length (with ranges of 8.1–11, 7.0–8.0, 4.4–6.5, respectively) (Doku, 1969; Karikari, 1972) or the canopy diameter 100 d after planting (less than 40 cm, 40–80 cm, and greater than 80 cm, respectively) (Ezedinma and Maneke, 1985). Begemann (1988) reached a similar conclusion after an exhaustive multivariate morphological analysis: internode length was the main component of morphological variation. A survey of Cameroonian landraces (Pasquet and Fotso, 1997), however, pointed to a new morphological character. In Cameroon, domesticated accessions could be split into two distinct groups: a northern group characterized by one-seeded pods and a southern group characterized by two- to four-seeded pods. This latter group was apparently not studied by Begemann (1988) but fits the description of Kwango (Zaire) landraces given by Rassel (1960). This finding was especially interesting as a similar phenomenon has been observed with Cameroonian cowpea [*Vigna unguiculata* (L.) Walp.] landraces. In the northern area, the

R.S. Pasquet, ORSTOM, Univ. of California, Dep. of Agronomy and Range Science, One Shields Ave., Davis, CA 95616-8515 and ORSTOM, ICIPE, P.O. Box 30772, Nairobi, Kenya; R.S. Schwedes and P. Gepts, Univ. of California, Dep. of Agronomy and Range Science, One Shields Ave., Davis, CA 95616-8515. Received 9 March 1998. *Corresponding author (plgepts@ucdavis.edu).

Published in Crop Sci. 39:1228–1236 (1999).

Abbreviations: AAT, aspartate aminotransferase; ADH, alcohol dehydrogenase; Amp, aminopeptidase; CMR, Cameroon; DIA, NADH Diaphorase; ENP, endopeptidase; EST, esterase; FDH, formate dehydrogenase; FLE, fluorescent esterase; βGAL, β-galactosidase; GDH, glutamate dehydrogenase; GHA, Ghana; βGLU, β-glucosidase; G6PD, glucose-6-phosphate dehydrogenase; GR, glutathione reductase; HVO, Burkina Faso; Idh, isocitrate dehydrogenase; MDH, malate dehydrogenase; ME, malic enzyme; MNR, menadione reductase; MPI, mannose phosphate isomerase; PGD, phosphogluconate dehydrogenase; PGI, phosphoglucose isomerase; PGM, phosphoglucomutase; SDH, shikimate dehydrogenase; SOD, superoxide dismutase; TZA, Tanzania; UPGMA, unweighted pair group method analysis; ZMB, Zambia.

cultigroups Biflora and Melanophthalmus display a low ovule number and in the southern area, the cultigroup Unguiculata displays a high ovule number (Pasquet and Fotso 1994).

Wild Bambara groundnut [var. *spontanea* (Harms) Hepper] was collected first in 1909 by Dalziel in Nigeria and by Ledermann in North Cameroon (Harms, 1912; Stapf, 1913). Wild Bambara groundnut was later collected by Jacques-Felix in Central Cameroon, by Tisserant in Central African Republic (Jacques-Felix, 1950), and in Nigeria again by Hepper (1963) who carefully studied morphological differences between wild and domesticated forms. Domesticated types have a compact growth, whereas wild types produce long runners. The freshly dug domesticated fruits possess thick fleshy pods which wrinkle on drying, but the wild fruits have thin pods which do not wrinkle upon drying. Wild seeds are small, quite uniform in size, between 9 and 11 mm long, while domesticated seeds appear to vary in size from 11 to 15 mm in length. Petioles of leaves (and petiolules of terminal leaflets) of wild plants are much shorter and not as erect or as closely tufted, and more slender than those of domesticated types (Hepper, 1963).

Recently, wild Bambara groundnut accessions were collected in 29 localities of Cameroon (Pasquet and Fotso, 1997). These collections showed that wild Bambara groundnut, while not uncommon between 9° and 21° longitude E, 6° and 11° latitude N, is rather difficult to find. During this collection trip, it was noticed that local people differentiated between really wild Bambara groundnut and what could be called weedy Bambara groundnut. These weedy plants still had a trailing growth habit with long internodes like wild plants but had larger seeds and thicker pods that were similar to those of domesticated plants. Weedy plants also showed long leaf petioles akin to those of domesticated forms.

The objective of this study was to characterize population structure and genetic diversity in *V. subterranea* by isozymes and to answer the following questions.

1. Is wild Bambara groundnut the progenitor of domesticated Bambara groundnut?
2. Has there been a loss of genetic variation as a result of domestication?
3. Is there a center of genetic diversity for domesticated Bambara groundnut?
4. Are there relationships between morphological and isozyme variation, first, between the domesticated vs. wild or weedy gene pools, and, second, between the wild vs. weedy gene pools?
5. What are the breeding systems of wild, weedy, and domesticated Bambara groundnut?

MATERIALS AND METHODS

Plant Materials

Six to eight plants from each of 21 wild-weedy populations and 114 plants from 79 domesticated accessions were used in this study (Table 1). Because of their low genetic variability, domesticated accessions from individual locations were pooled into larger domesticated populations according to geographic origins and morphological characteristics of accessions (Table 2). Accessions from Cameroon were collected by the first author. Original seeds from the explorations were obtained from the ORSTOM collection held at Montpellier (Pasquet and Fotso, 1997). Seeds of accessions from Burkina Faso, Ghana, Tanzania, and Zambia were provided by IITA, Ibadan, and were derived from one or more gene bank multiplications.

Isozyme Analyses

Seeds were imbibed overnight in water at room temperature (about 20°C). Tips of cotyledons were removed by slicing with a razor blade and ground for enzyme extraction. The remainder of the seed was retained for morphological characterization of the plant and leaf isozyme analysis (see below). Imbibed seed tissue was ground in water while young leaf tissue was ground in 0.1 *M* TRIS-HCl, pH 7.5, 5% (w/v) sucrose, 0.1 % (v/v) mercaptoethanol (Wendel and Weeden, 1989). Seed tissue was used to assay ADH (E.C. 1.1.1.1), AMP (E.C. 3.4.11.1), ENP (E.C. 3.4.-.-), FDH (E.C. 1.2.1.2), FLE (E.C. 3.1.1.2), βGAL (3.2.1.23), GDH (E.C. 1.4.1.2), βGLU (E.C. 3.2.1.21), G6PD (E.C. 1.1.1.49), GR (E.C. 1.6.4.2), and MPI (E.C. 5.3.1.8) while leaf tissue was used for AAT (E.C. 2.6.1.1), EST (E.C. 3.1.1.1), ME (E.C. 1.1.1.40), PGD (1.1.1.

Table 1. Ecogeographical data for wild and weedy populations of Bambara groundnut studied.

Population	Country	Number of plants	Latitude	Longitude	Locality
W 2	CMR	6	10°36′ N	13°59′ E	Goudoul
W 6	CMR	6	10°36′ N	13°59′ E	Goudoul
W 10	CMR	6	10°17′ N	13°08′ E	Bourrah Wango
W 11	CMR	6	10°39′ N	14°18′ E	Hadoua, Boulore Mt
W 13	CMR	6	7°18′ N	13°16′ E	Likok, Tchabal Mangari
W 15	CMR	6	10°34′ N	13°56′ E	Sabongari
W 22	CMR	6	10°38′ N	14°06′ E	Meftek, Wazang
W 26	CMR	8	10°21′ N	14°14′ E	Maho lopé
W 33	CMR	6	9°57′ N	13°42′ E	SW of Bossoum
W 34	CMR	6	10°42′ N	13°36′ E	Roumzou
W 37	CMR	6	9°41′ N	13°33′ E	Dembo
W 38	CMR	6	7°22′ N	13°05′ E	Lewa
W 45	CMR	6	8°24′ N	12°57′ E	Voko
W 46	CMR	6	9°59′ N	13°31′ E	Dourbey
W 48	CMR	6	8°54′ N	14°13′ E	Badjari
W 50	CMR	6	8°32′ N	13°10′ E	Toukté
W 51	CMR	6	6°35′ N	12°15′ E	Tongo
W 54	CMR	6	7°26′ N	13°56′ E	Ngang Ha
W 57	CMR	6	8°22′ N	13°57′ E	Bassodjé
W 58	CMR	6	8°29′ N	13°19′ E	Hoy
W 60	CMR	6	8°08′ N	13°35′ E	Nigba

Table 2. Ecogeographical data for domesticated populations of Bambara groundnut studies.

Population	Country	Number of plants	Latitude	Longitude	Morphological features (growth habit; pod; seed size or color)
C 1	CMR	10	5–6° N	10–11° E	open; 2–3 seeded; black, cream, or speckled
C 2	CMR	10	2–5° N	9–15° E	open; 2–3 seeded; black or cream
C 3	CMR	6	6–7° N	12–15° E	open; 2–3 seeded; black or cream
C 4	CMR	6	6–9° N	13–15° E	semi-bunch; 2–3 seeded; black & striped
C 5	CMR	8	8–10° N	13–14° E	semi-bunch; 1-seeded; pink
C 6	CMR	6	8–11° N	12–15° E	bunch; 1-seeded; large, striped
C 7	CMR	10	8–11° N	12–15° E	bunch; 1-seeded; black, cream, or speckled
C 8	CMR	20	7–11° N	12–15° E	bunch; 1-seeded pod; not wild-colored seed
C 9	GHA-HVO	16	NA†	NA	bunch/semi-bunch; 1-seeded pod
C 10	TZA	4	NA	NA	bunch/semi-bunch; 1-seeded pod
C 11	ZMB	18	NA	NA	bunch/semi-bunch; 1-seeded pod

† NA: not available.

44), and SOD (E.C. 1.15.1.1). Either seed or leaf tissue was used for DIA (E.C. 1.6.2.2), IDH (E.C. 1.1.1.42), MDH (E.C. 1.1.1.37), MNR (E.C. 1.6.99.2), PGI (E.C. 5.3.1.9), PGM (E.C. 2.7.5.1), and SDH (E.C. 1.1.1.25). All enzymes were assayed in a histidine-citric acid pH 6.0 buffer system. The gel mixture contained 14% (w/v) starch as described by Second and Trouslot (1980) but enzymes were run for a shorter distance in the gels (5–6 cm) than in the original protocol (10–12 cm). Enzyme-specific staining was done according to Wendel and Weeden (1989) with either leucine-β-naphtylamide or alanine-β-naphtylamide for AMP, and 4-methyl-umbelliferyl compounds for FLE, βGAL, and βGLU. GR was stained according to Harris and Hopkinson (1978).

Although no formal segregation analyses were conducted to determine the inheritance of the banding patterns observed, inferences were made on the number of loci involved on the basis of similar analyses made in related *Vigna* and *Phaseolus* species (e.g., Panella and Gepts, 1992; Vaillancourt et al., 1993a; Koenig and Gepts, 1989). For each enzymatic system, the presumed loci were numbered in ascending order from the anode. For each isozyme, the most common allele was designated as 100 and the other allozymes alleles were designated according to their migration distance in millimetres relative to that standard (Koenig and Gepts, 1989).

Allelic compositions of each population were determined at 41 presumed loci. Genetic distances between populations (Nei, 1972) were calculated and UPGMA dendrograms (Sneath and Sokal, 1973) were computed by the BIOSYS software version 1.7 (Swofford and Selander, 1981). Total gene diversity (Ht), within population gene diversity (Hs), between population gene diversity (Dst), and gene differentiation (Gst) were calculated following Nei (1973).

Morphological Characterizations

Morphological characterizations were made in a greenhouse in Davis, CA, from July to September (maximum temperature 35°C, minimum temperature 25°C). After removel of the the tip of the cotyledons for isozyme analyses, the seeds were sown in pots (15 by 15 cm, one seed per pot). Pots were distributed at random within the greenhouse. All plants started flowering before September but fruit set was almost nonexistent, probably because of lack of adaptation. The low number of plants was due to the utilization of original seed obtained during the field explorations instead of seeds obtained from a gene bank. Few plants died before the completion of morphological characterizations. Nevertheless, entries with an incomplete data set were not included in the analyses. Therefore, only fully healthy and well-growing plants were considered in the morphological analyses. Each plant was characterized for the following traits: dry seed weight, fourth internode length, fourth leaf petiole length, fourth terminal leaflet length and width, and flower standard petal width. Unfortunately, fruit shells were not available for most of the accessions. Except for seed weight, two measures per plant were made and data were averaged across accessions for each population. The average population data were used in a principal component analysis.

RESULTS

Morphology

Morphological data averages (Table 3) and a principal component analysis (Fig. 1) showed a marked difference between wild and domesticated Bambara groundnuts, with the possible exception of populations W6 and W15. The first two principal components accounted for 72.9 and 13.5%, respectively, of total variation. The traits responsible for separation along the principal components included (with loadings in first and second components respectively in parentheses) seed weight (0.825, 0.503), internode length (−0.882, −0.239), petiole length (0.963, 0.022), leaflet length (0.843, 0.179), leaflet width (0.828, −0.300), and standard petal width (0.758, −0.525). Populations W6 and W15 appeared to be weedy populations that resembled domesticated types. They showed large seeds and tough, wrinkled pods but also had smaller leaves and much longer internodes than the domesticated populations (Table 2). Wild populations to the left of the diagram showed thin and smooth pod testa, small seeds (0.23–0.39 g), small leaves (4.5–6.5 by 1.9–2.8 cm), small leaf petioles (5.1–7.3 cm), and long internodes (6.5–10.0 cm), compared with domesticated populations. The latter showed thick and wrinkled pod testa, large seeds (0.56–1.37 g), large leaves (7.5–9.4 by 2.8–3.6 cm), long leaf petioles (12.4–14.5 cm), and short internodes (1.3–3.4 cm). In the middle of Fig. 1, populations like W38 or W50 were intermediate between true wild populations and weedy populations like W6 and W15 (Table 3).

Enzyme Variation and Diversity

The 23 enzyme systems allowed us to score 41 loci. One band of activity was noted when staining for AMP, DIA, ENP, FDH, GDH, βGAL, GDH, G6PD, GR, MPI, and SDH. Therefore one locus was inferred and scored in each of these enzyme systems. Two bands of activity were found for FLE, βGLU, IDH, ME, MNR, PGM, and EST in seed extracts, but both the faster

Table 3. Means and standard deviations of morphological traits for Bambara groundnut plant populations studied.

Population	Seed weight	Internode length	Leaf petiole length	Leaflet length	Leaflet width	Standard width
	mg	mm				
			Wild populations			
W 2	364 ± 84	81.3 ± 5.8	97.6 ± 8.7	62.3 ± 6.7	27.8 ± 3.1	8.8 ± 0.3
W 6	697 ± 49	50.3 ± 5.9	90.1 ± 8.0	54.6 ± 4.5	21.1 ± 1.8	9.0 ± 0.1
W 10	346 ± 40	50.4 ± 5.1	49.6 ± 7.2	54.4 ± 9.5	24.1 ± 3.4	9.0 ± 0.2
W 11	396 ± 38	90.6 ± 8.4	62.6 ± 8.6	50.2 ± 7.9	21.6 ± 2.9	7.7 ± 0.3
W 13	218 ± 27	68.7 ± 10.4	81.6 ± 10.9	55.2 ± 8.7	26.3 ± 3.2	8.1 ± 0.3
W 15	582 ± 31	44.8 ± 3.5	124.8 ± 11.6	72.4 ± 2.0	29.1 ± 1.4	8.5 ± 0.4
W 22	369 ± 32	98.3 ± 19.9	68.3 ± 11.3	60.5 ± 10.4	23.3 ± 3.3	8.0 ± 0.1
W 26	272 ± 21	100.1 ± 23.5	70.9 ± 9.6	45.2 ± 10.3	21.2 ± 4.0	8.0 ± 0.2
W 33	230 ± 21	89.6 ± 12.5	73.3 ± 11.0	65.3 ± 9.0	27.8 ± 2.7	7.0 ± 0.4
W 34	246 ± 41	75.3 ± 5.4	51.5 ± 9.3	65.6 ± 3.8	19.0 ± 2.2	7.3 ± 0.4
W 37	375 ± 63	75.1 ± 10.6	66.6 ± 7.5	60.5 ± 7.7	28.1 ± 3.9	7.5 ± 0.3
W 38	393 ± 95	66.8 ± 18.0	99.4 ± 10.5	65.8 ± 7.1	30.2 ± 2.9	8.7 ± 0.6
W 45	383 ± 92	105.2 ± 29.8	88.3 ± 17.9	64.7 ± 9.3	28.8 ± 3.4	8.0 ± 0.1
W 46	316 ± 82	65.5 ± 14.9	59.6 ± 16.6	47.8 ± 8.1	26.0 ± 3.7	8.2 ± 0.2
W 48	284 ± 60	95.3 ± 18.0	65.4 ± 9.8	58.1 ± 8.8	24.8 ± 3.7	8.1 ± 0.2
W 50	398 ± 72	82.1 ± 7.8	99.1 ± 13.5	63.3 ± 8.3	32.6 ± 3.2	9.0 ± 0.2
W 51	315 ± 56	105.8 ± 21.8	84.0 ± 14.6	64.2 ± 10.6	27.0 ± 2.5	8.1 ± 0.7
W 54	378 ± 70	73.3 ± 10.4	85.8 ± 11.8	63.6 ± 8.1	27.1 ± 2.9	8.0 ± 0.4
W 57	357 ± 55	87.1 ± 19.8	75.6 ± 17.4	60.8 ± 9.3	25.0 ± 2.8	8.9 ± 0.4
W 58	478 ± 72	84.8 ± 14.9	95.0 ± 16.8	64.1 ± 11.0	31.1 ± 3.0	9.2 ± 0.5
W 60	394 ± 57	73.2 ± 14.1	65.3 ± 11.3	64.0 ± 5.7	26.2 ± 3.6	8.0 ± 0.6
			Domesticated populations			
C 1	973 ± 78	34.0 ± 11.3	138.8 ± 24.4	74.7 ± 8.3	32.0 ± 4.5	8.8 ± 0.2
C 2	689 ± 129	32.4 ± 4.5	144.6 ± 20.7	80.6 ± 8.0	36.2 ± 3.6	9.9 ± 0.3
C 3	704 ± 109	32.6 ± 3.9	140.8 ± 10.9	78.3 ± 4.5	35.3 ± 2.4	9.6 ± 0.6
C 4	1267 ± 116	16.0 ± 4.7	145.0 ± 20.5	76.1 ± 8.7	34.0 ± 4.3	8.5 ± 0.4
C 5	791 ± 74	16.1 ± 6.4	124.1 ± 9.1	82.0 ± 9.9	27.8 ± 2.0	9.1 ± 0.3
C 6	1367 ± 238	14.9 ± 2.9	144.5 ± 14.8	94.4 ± 8.8	30.1 ± 2.3	8.1 ± 0.3
C 7	742 ± 88	13.1 ± 3.2	129.2 ± 15.1	81.1 ± 11.5	31.2 ± 3.5	8.9 ± 0.4
C 8	698 ± 105	12.9 ± 3.5	132.3 ± 21.2	80.3 ± 12.6	29.9 ± 4.5	9.0 ± 0.5
C 9	573 ± 60	17.7 ± 5.8	138.4 ± 18.4	77.8 ± 8.4	32.0 ± 6.4	9.5 ± 0.5
C 10	704 ± 33	23.5 ± 3.0	134.8 ± 12.7	82.3 ± 3.9	31.5 ± 2.2	9.5 ± 0.4
C 11	558 ± 90	24.6 ± 6.2	137.6 ± 13.1	79.5 ± 11.5	32.8 ± 4.4	9.4 ± 0.5

αEST band and the slower βEST band were too weakly stained to be scored. Three bands of activity were noted when staining for ADH, the middle zone being a heterodimer between products of *Adh1* and *Adh2*. Three bands of activity also were noted when staining for AAT, PGD, and SOD. Three bands of activity were observed for αEST in leaf extracts but only the middle one was stained strongly enough to be scored. Four

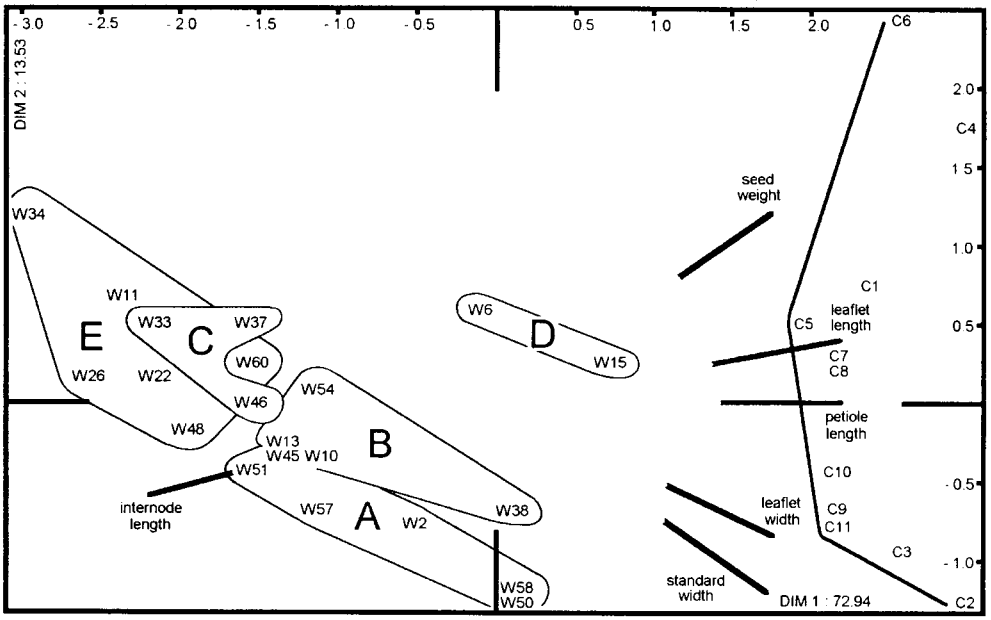

Fig. 1. Principal component analysis of diversity for morphological traits in Bambara groundnut data. Biplot with loadings × 2. Domesticated populations are grouped together, wild and weedy populations are grouped according to the isozyme cluster analysis results in Fig. 2. The first two principal components account for 72 and 11%, respectively, of total variation. Straight lines represent the loading of individual morphological variables on the first and second principal components (see text).

Table 4. Gene diversity calculated according to Nei (1973) in domesticated and wild Bambara groundnut.†

	Population number	Number of plants	Ht	Hs	Dst	Gst	L	A
Domesticated	**11**	**114**	**0.052**	**0.033**	**0.019**	**0.365**	**0.17**	**2.14**
Wild A‡	**6**	**36**	**0.049**	**0.022**	**0.027**	**0.551**	**0.19**	**2.12**
Wild B	**4**	**24**	**0.058**	**0.031**	**0.027**	**0.466**	**0.24**	**2.00**
Wild C	**3**	**18**	**0.049**	**0.017**	**0.032**	**0.653**	**0.19**	**2.00**
Wild D	**2**	**12**	**0.012**	**0.003**	**0.009**	**0.750**	**0.02**	**2.00**
Wild E	**6**	**38**	**0.068**	**0.034**	**0.034**	**0.500**	**0.24**	**2.00**
Wild total	**21**	**128**	**0.087**	**0.025**	**0.062**	**0.712**	**0.34**	**2.07**

† Ht is total gene diversity, Hs is mean diversity within populations, Dst is Ht − Hs, and Gst is Dst/Ht.
‡ Wild A–Wild E correspond to clusters of the dendrogram in Fig. 2.

bands were observed with PGI. The fastest band (PGI1) was assumed to be chloroplastic as in cowpea (Vaillancourt et al., 1993a); the next three were assumed to be the homo- and heterodimer products of *Pgi2* and *Pgi3*. Five bands of activity were visible when staining for MDH. The most anodal band (MDH1), although weakly stained, could be scored. The second band was formed by the homodimer from *Mdh2*, the third by a heterodimer between products from *Mdh2* and *Mdh3*, and the fourth by the homodimer from *Mdh3*. The fifth band (MDH4) migrated over a longer distance (1–1.5 cm) from the origin.

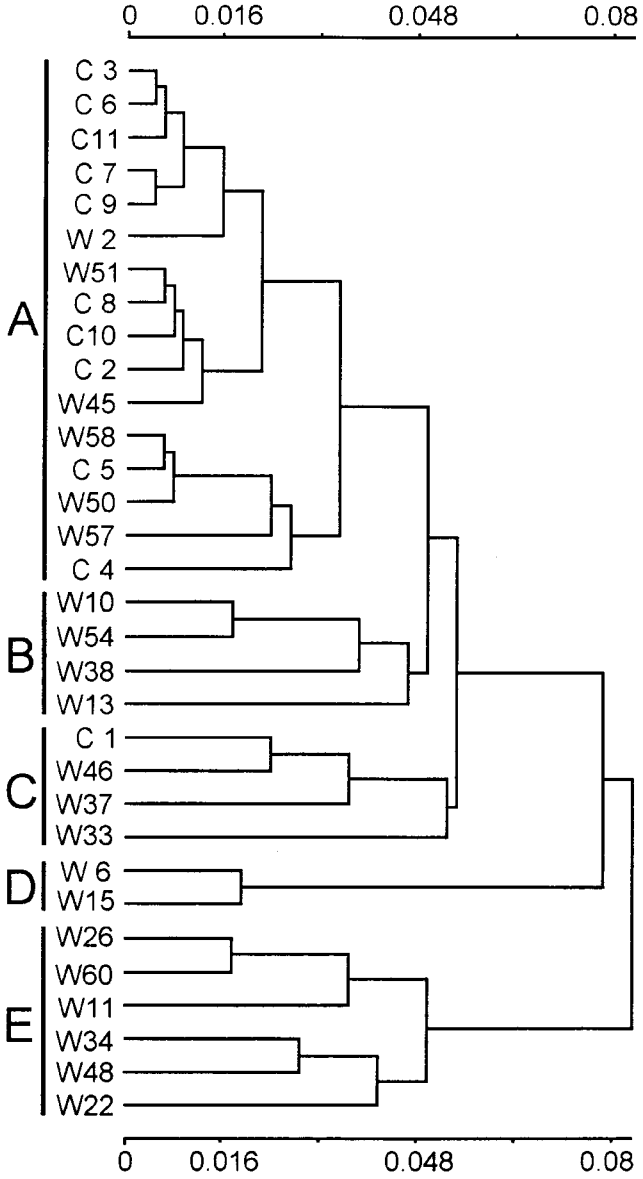

Fig. 2. UPGMA cluster analysis of Nei distances. Uppercase letters to the left of the dendrogram indicate clusters of wild accessions.

Among domesticated populations, seven of 41 (17%) loci showed polymorphism, with a total gene diversity of 0.052. With one exception ($Pgi1^{98}$), all the domesticated alleles were encountered in wild Bambara groundnut. Wild populations showed more diversity as fourteen loci (34%) were polymorphic and total gene diversity was 0.087 (Table 4). Within domesticated populations, diversity of 2- to 3-seeded pod accessions (Ht = 0.060 with 32 plants) from southern Cameroon was higher than that of 1-seeded pod accessions (Ht = 0.047 with 82 plants) from the rest of the continent but comparison of genetic diversity between accessions from Cameroon (Ht = 0.056, L = 0.14 with 76 plants), Ghana (Ht = 0.046, L = 0.17 with 16 plants), Tanzania and Zambia (Ht = 0.026, L = 0.12 with 22 plants) does not show a geographical organization of the diversity. Within wild and weedy populations, diversity of populations from Cluster C and E (wild; Ht = 0.075) was similar to those from Cluster A, B, and D (weedy; Ht = 0.069) (Fig. 1).

Both wild and domesticated Bambara groundnut showed a similar within- population diversity, which accounts for most of the domesticated total diversity (Table 4). However, heterozygotes were very rare. Only 10 wild plants (of the128 plants studied) and 5 domesticated plants (of the 114 plants studied) showed heterozygosity for at least one locus, despite the existence of within-population variability. If we disregard the *Mdh1* locus, where the heterozygote phenotype could not be clearly distinguished from the $Mdh1^{100}$ phenotype, 11 wild and 7 domesticated populations showed within-population diversity without showing a single heterozygous phenotype.

Phenetic Isozyme Analysis

UPGMA analysis lead to five clusters (Fig. 2). Cluster A included both domesticated and wild populations from the center of the morphological analysis biplot (Fig. 1). Cluster B also included wild populations from the center of the morphological analysis biplot. Cluster C included wild populations from the left of the morphological analysis biplot and one domesticated population. Cluster D included the two weedy populations (W6 and

Table 5. Nei distances (minimum, average in italics, and maximum) between and within groups of populations identified during the cluster analysis.

Populations	Wild A† (36, 6)‡	Wild B (24, 4)	Wild C (18, 3)	Wild D (12, 2)	Wild E (38, 6)	Domesticated (114, 11)
Wild A	0.006 *0.033* 0.060					
Wild B	0.024 *0.057* 0.097	0.017 *0.038* 0.054				
Wild C	0.023 *0.059* 0.104	0.026 *0.068* 0.096	0.025 *0.050* 0.075			
Wild D	0.049 *0.070* 0.121	0.047 *0.075* 0.114	0.102 *0.122* 0.151	0.017 *0.017* 0.017		
Wild E	0.057 *0.093* 0.128	0.030 *0.069* 0.127	0.037 *0.065* 0.101	0.082 *0.110* 0.134	0.016 *0.042* 0.070	
Domesticated	0.005 *0.027* 0.058	0.024 *0.045* 0.087	0.022 *0.051* 0.093	0.036 *0.070* 0.104	0.039 *0.082* 0.122	0.003 *0.021* 0.060

† Wild A–Wild E, Domesticated correspond to clusters of the dendrogram in Fig. 2.
‡ The two numbers under each group indicate the number of individuals and populations, respectively.

W15), which are morphologically closest to domesticated types. Cluster E included populations from the left of the morphological analysis biplot.

The principal component analysis of Nei's (1972) distance matrix gave a slightly different image of the variability and emphasized the close relationship between domesticated and wild Clusters A, B, and C (Table 5) (Fig. 3). For example, W13 is as close to Cluster B (0.045) as to domesticated and wild populations from Cluster A (0.048). Population W33 is as close to its cluster (0.052) as to domesticated and wild populations from Cluster A (0.051). Population C1 is as close to its Cluster C (0.033) as to other domesticated populations (0.037). Populations C4 and C5 are slightly closer to populations W50, W57, and W58 (0.020) than to populations W2, W45, and W51 (0.035) and to other domesticated populations (0.032). Pooling wild populations from Cluster A, B, and C would not modify Table 5 in a major way as the average of pairwise Nei distances between wild populations and Clusters A, B, and C is 0.053 only. Nei distances between wild and domesticated populations fell within the range 0.005-0.122 with an average of 0.055 (Table 5). Nei identities between wild and domesticated populations fell in the range 0.886-0.995 with an average of 0.948.

As expected from the low total genetic diversity of wild and domesticated populations and the comparatively high within-population diversity, distances between populations were very low. Cluster analysis separated groups of populations only according to changes in the frequency of the most common alleles. Population C1 was in Cluster C because of its Amp^{100} frequency, i.e., 0.9 instead of 0 to 0.125 in the other domesticated populations. Populations C4 and C5 were clustered with populations W50, W57, and W58 because of their $Aat2^{100}$ frequences, i.e., 0.12 and 0.16 instead of 0.6 to 1.0 in W2, W45, W51, and domesticated populations. No allele was exclusive to a particular cluster, with the exception of Clusters D and E, which showed contrasting allelic frequencies compared to allelic frequencies among domesticated populations (Table 6).

Rare alleles (frequencies lower than 0.1) were encountered in a few populations: $Idh1^{97}$ *and* $Idh2^{97}$ *in W26,* $Adh2^{98}$ and Dia^{103} in W13, $Pgd3^{98}$ in W13 and W38, $Sod1^{97}$ in W51 and four domesticated populations, and $Pgi1^{98}$ only in three domesticated populations.

DISCUSSION

Comparison of Bambara Groundnut Isozyme Patterns with Those of Other *Vigna* Species

Isozyme patterns were very similar to those observed in other species from the genus *Vigna*, i.e., *V. unguiculata* (Panella and Gepts, 1992; Pasquet, 1993; Vaillancourt et al., 1993a), *V. vexillata* (L.) A. Rich (Garba and Pasquet, 1999), *V. reticulata* Hook. f. (Garba and Pasquet, 1998), *V. frutescens* A. Rich., and *V. membranacea* A. Rich. (Pasquet and Vanderborght, 1999), and especially those observed within the blue- and yellow-flowered *Vigna* (Pasquet and Vanderborght, 1999). DIA did not yield numerous bands, and patterns were more similar to those encountered in *V. unguiculata*, *V. ambacensis* Baker, and *V. gracilis* (Guill. & Perr.) Hook. f. than to those of *V. reticulata* and *V. vexillata*. G6PD and SDH did not show a double band profile in Bambara groundnut as in the other species but this could be due to the shorter migration conditions used in this study preventing separation of the two bands. The faster ADH homodimer (*Adh1* product) was weakly stained as in *V. unguiculata*, *V. ambacensis*, *V. luteola* (Jacq.) Benth., and *V. racemosa* (G. Don) Hutch. & Dalziel, and unlike *V. gracilis*, *V. frutescens*, *V. membranacea*, *V. reticulata*, and *V. vexillata* where it is strongly stained. The fast IDH isozyme (*Idh1* product) was strongly stained as in *V. gracilis*, *V. ambacensis*, *V. luteola*, *V. membranacea*, and *V. frutescens* and unlike *V. unguiculata* and *V. reticulata* where it is weakly stained. The faster PGI homodimer (*Pgi2*) was weakly stained as in *V. luteola*,

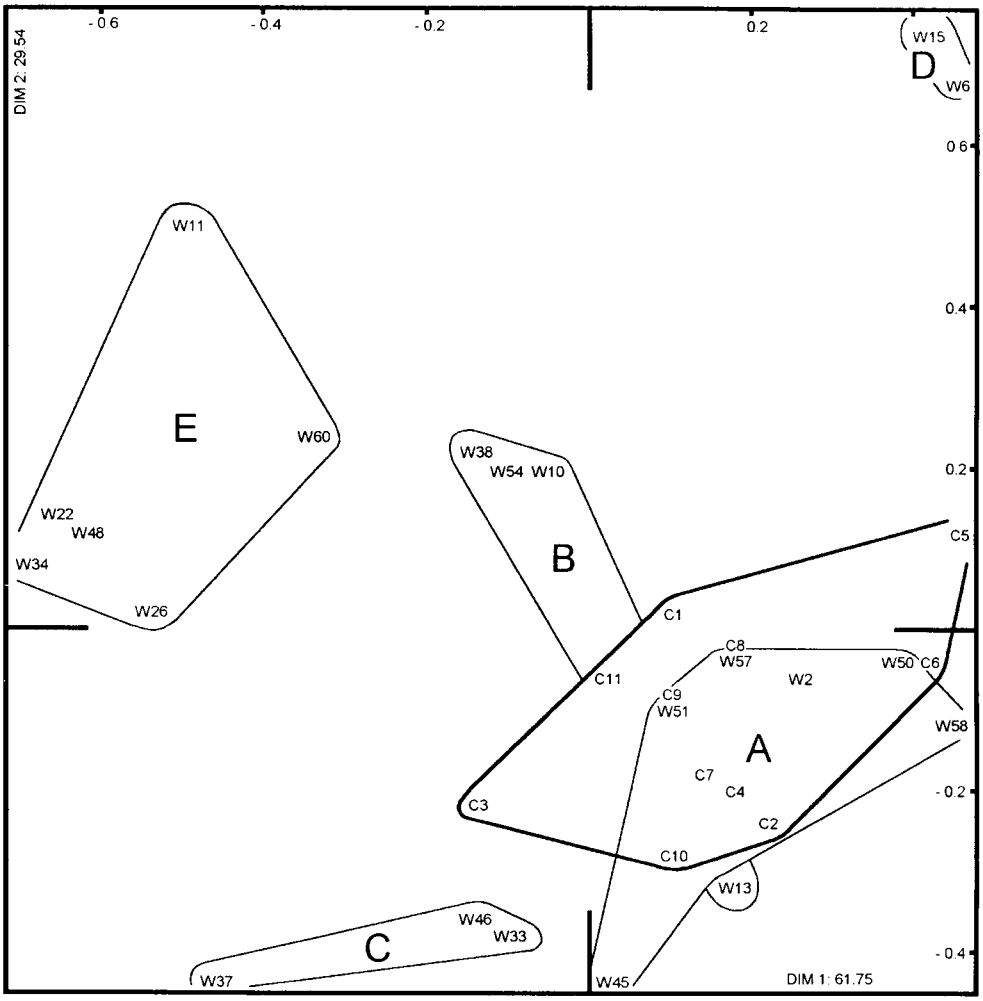

Fig. 3. Principal component analysis of Nei distances matrix. Domesticated populations are grouped together, wild and weedy populations are grouped according to the isozyme cluster analysis results in Fig. 2. The first two principal components account for 62 and 30%, respectively, of total variation.

V. ambacensis, and *V. gracilis*, and not strongly stained as in *V. unguiculata*, *V. vexillata*, *V. reticulata*, *V. frutescens*, and *V. membranacea*. Finally, MDH4 migrated far from the origin as in *V. luteola*, *V. ambacensis*, and *V. gracilis* and much further from the origin than in *V. unguiculata*, *V. vexillata*, *V. reticulata*, *V. frutescens*, and *V. membranacea*. All these results indicate that the Bambara groundnut, *Vigna subterranea*, is more closely related to the blue- and yellow-flowered *Vigna* (*V. luteola*, *V. ambacensis*, and *V. gracilis*) than to the pink-flowered Old World *Vigna* (*V. unguiculata*, *V. vexillata*, *V. reticulata*, *V. frutescens*, and *V. membranacea*), which agrees with previous isozyme and cpDNA results (Vaillancourt and Weeden, 1993; Vaillancourt et al., 1993b) and with the taxonomic position of *V. subterranea* within the section *Vigna* as proposed by Maréchal et al. (1978).

Genetic Diversity Analysis

The best estimate of genetic diversity is obtained with a large number of accessions, collected from the whole range of the species, and screened for a large number of loci (Ayala, 1982; Nei, 1978). In this study we attempted to fulfill all of these conditions for both wild and domesticated Bambara groundnut. Wild Bambara groundnut is distributed from eastern Nigeria to the western part of the Central African Republic (Jacques-Felix, 1950). Therefore, the populations studied here were representative of only half of the longitudinal extension of the taxon but were representative of the whole of its latitudinal extension. The morphological data also showed that we studied both weedy and wild populations. With regard to domestication Bambara groundnut, we studied accessions representing the entire range of morphological variability, including entries with spreading to bunch growth habits, and one-seeded to two- to three-seeded pods. We focused not only on the area close to the supposed domestication center (from Nigeria to Central African Republic where wild Bambara groundnuts are encountered), but also on areas in West Africa, East Africa, and South Africa.

We surveyed 41 loci although 20 loci are generally regarded as a reasonable number of loci for a survey of our type. Usually such a type of study is made with accessions multiplied ex situ. Here most of the populations were studied with original seeds. Measurement of

Table 6. Isozyme† allele frequencies in the different allozyme groups from the cluster analysis.

Isozyme loci and alleles	Wild A‡ (36, 6)§	Wild B (24, 4)	Wild C (18, 3)	Wild D (12, 2)	Wild E (38, 6)	Wild total (128, 21)	Domesticated (114, 11)
Aat2^{100}	0.444	0.896	0.944	0	0.868	0.683	0.763
Aat2^{98}	0.555	0.104	0.055	1	0.131	0.316	0.237
Adh2^{100}	1	0.875	1	1	1	0.977	1
Adh2^{98}	0	0.125	0	0	0	0.023	0
Amp^{100}	0.125	0.167	0.889	0	0.776	0.422	0.127
Amp^{99}	0.875	0.833	0.111	1	0.224	0.578	0.873
Dia^{103}	0	0.125	0	0	0	0.023	0
Dia^{100}	1	0.875	1	1	1	0.977	1
Enp^{100}	0.055	0	0.333	0	0.263	0.140	0.026
Enp^{99}	0.944	1	0.667	1	0.737	0.859	0.974
Est^{103}	0	0.083	0.333	0	0.789	0.297	0
Est^{100}	1	0.917	0.667	1	0.210	0.703	1
Idh1^{100}	1	1	1	1	0.974	0.992	1
Idh1^{97}	0	0	0	0	0.026	0.008	0
Idh2^{100}	1	1	1	1	0.974	0.992	1
Idh2^{97}	0	0	0	0	0.026	0.008	0
Mdh1^{100}	0.833	0.917	0.889	1	0.579	0.797	0.671
Mdh1^{98}	0.166	0.083	0.111	0	0.421	0.203	0.329
Me1^{00}	0.555	0.146	0.278	0.417	0.631	0.340	0.386
Me1^{97}	0.444	0.854	0.722	0.583	0.368	0.660	0.614
Pgd3^{100}	1	0.583	1	1	1	0.922	1
Pgd3^{98}	0	0.417	0	0	0	0.078	0
Pgi1^{100}	0.916	1	1	0	1	0.883	0.807
Pgi1^{99}	0.083	0	0	1	0	0.117	0.131
Pgi1^{98}	0	0	0	0	0	0	0.061
Pgi2^{100}	0.916	0.958	0.944	1	0.684	0.867	1
Pgi2^{98}	0.083	0.042	0.055	0	0.315	0.132	0
Sod1^{108}	0.028	0.75	0.055	1	0.947	0.531	0.083
Sod1^{100}	0.944	0.25	0.944	0	0.053	0.461	0.859
Sod1^{97}	0.028	0	0	0	0	0.008	0.057

† Monomorphic isozymes were omitted (*Adh1*, *Fdh*, *Fle1*, *Fle2*, β*Gal*, *Gdh*, β*Glu1*, β*Glu2*, *Aat1*, *Aat3*, *G6pd*, *Gr*, *Mdh2*, *Mdh3*, *Mdh4*, *Me2*, *Mnr1*, *Mnr2*, *Mpi*, *Pgd1*, *Pgd2*, *Pgi3*, *Pgm1*, *Pgm2*, *Sdh*, *Sod2*, *Sod3*).
‡ Wild A–Wild E, Domesticated corresponds to clusters of the dendrogram in Fig. 2.
§ The two numbers under each group indicate the number of individuals and populations, respectively.

within population diversity parameters was therefore possible.

Overall levels of isozyme diversity observed in our study were quite low, confirming a previous study by Howell (1990). However, compared with that study, we analyzed a larger number of loci (14 polymorphic loci out of 41 vs. 2 polymorphic loci out of 9) and a broader cross-section of germplasm. For example, AMP was polymorphic in our study but monomorphic in the Howell (1990) study. Nevertheless, diversity in Bambara groundnut is quite low. Gene diversity parameters (Table 3) were lower than those for various domesticated species compiled by Doebley (1989) for crops and their wild relatives. This low diversity could be due to a strongly autogamous breeding system in both wild and domesticated Bambara groundnut. Very few heterozygous phenotypes were observed despite the high within-population diversity observed (compared with total gene diversity) and the use of originally collected seeds in all wild populations and most of the domesticated populations.

Despite the low genetic diversity of both wild and domesticated Bambara groundnuts, a bottleneck between wild and domesticated types is obvious as shown by total gene diversity (Ht) or percentage of polymorphic loci (L) data (Table 6; Fig. 3). Seven alleles (*Adh2*^{98}, *Dia*^{103}, *Est*^{103}, *Idh1*^{97}, *Idh2*^{97}, *Pgd3*^{98}, and *Pgi2*^{98}) were only encountered in wild populations. This low diversity within domesticated populations may explain the lack of correlation between morphological (either habit or seed per pod) or geographical origin and isozyme diversity, even if such a correlation has been documented in other crops (Doebley, 1989). Comparison of genetic diversity levels among accessions from Ghana, Tanzania and Zambia, and Cameroon does not show a clear center of diversity.

Phenetic Analysis

The morphological gradient between truly wild (left), slightly weedy (left center), strongly weedy (right center), and domesticated (right) (Fig. 1) is correlated to a certain extent with isozyme data. Clusters C and E, Clusters A and B, and Cluster D represent wild and weedy populations, respectively. The wild populations that were most distantly related to the domesticated types were the same in both morphological and isozyme analyses (Fig. 1 and Table 5). However, the low genetic diversity in Bambara groundnut must be emphasized. Cluster analysis accounts mainly for changes in allele frequencies. As a matter of fact, domesticated populations and wild populations from Clusters A, B, and C are genetically close (Table 5).

The absence of the *Pgi1*^{98} allele within wild populations studied could be explained by a lack in wild population sampling especially westward (Bauchi Plateau, Ni-

geria) or eastward (Central African Republic). On the other hand, the case of populations W6 and W15 is more surprising. The two populations are genetically very close, which could be due to a close geographical origin (north-east and south-west slopes of the same small mountain). Both populations are morphologically very close to domesticated forms and are genetically most closely related to the wild and domesticated populations from Cluster A (Table 5). Their originality lies in their very low within-populations diversity (Table 6), the high frequencies of alleles that are rare within domesticated accessions ($Aat2^{98}$, $Pgi1^{99}$), and the high frequency of another allele which characterizes wild populations ($Sod1^{108}$).

The high genetic identities between wild and domesticated Bambara groundnut, which almost reach 0.9, clearly show that wild Bambara groundnut is the progenitor of domesticated Bambara groundnut. With the exception of $Pgi1^{98}$, all domesticated alleles were encountered within wild populations. Unlike sorghum [*Sorghum bicolor* (L.) Moench; Ollitrault et al., 1989; Aldrich et al. 1992], pearl millet [*Pennisetum glaucum* (L.) R.Br.; Tostain, 1992], and cowpea (Pasquet, 1996) no shift in allelic composition is observed between wild and domesticated Bambara groundnuts (Fig. 3).

In summary, both wild and domesticated Bambara groundnuts are characterized by a low total genetic diversity and a comparatively high intrapopulation diversity, which suggest a predominantly selfing mode of pollination. However, the marked bottleneck between wild and domesticated forms emphasizes the probable usefulness of wild Bambara groundnut germplasm for Bambara groundnut breeding.

ACKNOWLEDGMENTS

We thank Dr. N.Q. Ng (IITA, Ibadan) and S. Dussert (ORSTOM, Montpellier) for providing seeds used in this study.

REFERENCES

Aldrich, P.R., J. Doebley, K.F. Schertz, and A. Stec. 1992. Patterns of allozyme variation in domesticated and wild *Sorghum bicolor*. Theor. Appl. Genet. 85:451–460.

Ayala, F.J. 1982. Population and evolutionary genetics: a primer. The Benjamin-Cummings Publ. Co., Menlo Park, CA.

Begemann, F. 1988. Ecogeographic differentiation of Bambara groundnut (*Vigna subterranea*) in the collection of the International Institute of Tropical Agriculture (I.I.T.A.). Wissenschaftlicher Fachverlag, Giessen, Germany.

Brouk, B. 1975. Plants consumed by man. Academic Press, London.

Doebley, J. 1989. Isozymic evidence and the evolution of crop plants. p. 165–191. *In* D.E. Soltis and P.S. Soltis (ed.) Isozymes in plant biology. Chapman and Hall, London.

Doku, E.V. 1969. Growth habit and pod production in Bambara groundnut (*Voandzeia subterranea*). Ghana J. Agric. Sci. 2:91–95.

Ezedinma, F.O.C., and F.O. Maneke. 1985. Preliminary studies on Bambara groundnut (*Voandzeia subterranea* Thouars) in the derived Savanna Belt of Nigeria. Trop. Grain Legume Bull. 31:39–44.

Garba, M., and R.S. Pasquet. 1998. Isozyme polymorphism in section Reticulatae of genus *Vigna* (Tribe Phaseoleae: Fabaceae). Biochem. Syst. Ecol. 26:297–308.

Garba, M., and R.S. Pasquet. n.d. Isozyme diversity in *Vigna vexillata* (L.) A. Rich. (Fabaceae) complex. S. Afr. J. Bot. 64:163–175.

Harms, H. 1912. Über die Heimat der Erderbse, *Voandzeia subterranea* (L.) Thou. Notzbl. Bot. Gart. Berlin 5:253–258.

Harris, H., and D.A. Hopkinson. 1978. Handbook of enzyme electrophoresis in human genetics. North Holland Publishing Company, Amsterdam.

Hepper, F.N. 1963. The Bambara groundnut (*Voandzeia subterranea*) and Kersting's groundnut (*Kerstingiella geocarpa*) wild in west Africa. Kew Bull. 16:395–407.

Howell, J.A. 1990. Variation and evolution of Bambara groundnut (*Vigna subterranea* (L.) Verdc.; Fabaceae). PhD dissertation, Miami University, OH (Diss Abstr. AAT9029376).

Jacques-Felix, H. 1950. Pour une enquête sur le voandzou (*Voandzeia subterranea* Thou). Agron. Trop. 5:62–73.

Karikari, S.K. 1972. Correlation studies between yield and some agronomic characters in Bambara groundnut (*Voandzeia subterranea* Thouars). Ghana J. Agric. Sci. 5:79–83.

Koenig, R., and P. Gepts. 1989. Segregation and linkage of genes for seed proteins, isozymes, and morphological traits in common bean (*Phaseolus vulgaris*). J. Hered. 80:455–459.

Linnaeus, C. 1763. Species plantarum, ed. 2. Laurent Salvi, Stockholm.

Maréchal, R., J.M. Masherpa, and F. Stainier. 1978. Etude taxonomique d'un groupe complexe d'espèces des genres *Phaseolus* et *Vigna* (Papilionaceae) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. Boissiera 28:1–273.

Nei, M. 1972. Genetic distance between populations. Am. Nat. 106: 283–292.

Nei, M. 1973. Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. (USA) 70:3321–3323.

Nei, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89:583–590.

Ollitrault, P., M. Arnaud, and J. Chanterau. 1989. Polymorphisme enzymatique des sorghos. II. Organisation génétique et évolutive des sorghos cultivés. Agron. Trop. 44:211–222.

Panella, L., and P. Gepts. 1992. Genetic relationships within *Vigna unguiculata* (L.) Walp. based on isozyme analyses. Genet. Resources Crop Evol. 39:71–88.

Pasquet, R.S. 1993. Variation at isozyme loci in wild *Vigna unguiculata* (L.) Walp. (Fabaceae, Phaseoleae). Plant Syst. Evol. 186:157–173.

Pasquet, R.S. 1996. Domesticated cowpea (*Vigna unguiculata*) evolution. p. 101–108. *In* B. Pickersgill and J.M. Lock (ed.). Advances in legume systematics 8: Legumes of economic importance. Royal Botanic Gardens, Kew, England.

Pasquet, R.S., and M. Fotso. 1994. Répartition des cultivars de niébé (*Vigna unguiculata* (L.) Walp.) du Cameroun: Influence du milieu et des facteurs humains. J. Agric. Trad. Bot. Appl. 36:93–143.

Pasquet, R.S., and M. Fotso. 1997. The ORSTOM Bambara groundnut collection. p. 119–123. *In* J. Heller et al. (ed.) Bambara groundnut *Vigna subterranea* (L.) Verdc. IPGRI, Rome.

Pasquet, R.S., and T. Vanderborght. 1999. Isozyme polymorphism in some yellow- and blue-flowered *Vigna* species complexes (Fabaceae-Phaseoleae). Plant System. Evol. 215:163–175.

Pasquet, R.S., and T. Vanderborght. 1999. Isozyme polymorphism in the *Vigna frutescens - V. membranacea* complex (Tribe Phaseoleae, Fabaceae). Biochem. Syst. Ecol. 27 (in press).

Rassel, A. 1960. Le voandzou *Voandzeia subterranea* Thouars et sa culture au Kwango. Bull. Agr. Congo Belge Ruanda-Urundi 51:1–26.

Second, G., and P. Trouslot. 1980. Electrophorèse d'enzymes de riz (*Oryza* sp.). Travaux et Documents de l'ORSTOM no. 120. ORSTOM, Paris.

Sneath, P.H.A, and R.R. Sokal. 1973. Numerical taxonomy. W.H. Freeman, San Francisco.

Stapf, O. 1913. Discovery of *Voandzeia subterranea* in the wild state. Bull. Misc. Inf. Kew 1913:213.

Swofford, D.L., and R.K. Selander. 1981. BIOSYS-1. A FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. J. Hered. 72:281–283.

Tostain, S. 1992. Enzyme diversity in pearl millet (*Pennisetum glaucum*). 3. Wild millet. Theor. Appl. Genet. 83:733–742.

Vaillancourt, R.E., and N.F. Weeden 1993. Lack of isozyme similarity between *Vigna unguiculata* and other species of subgenus *Vigna* (Leguminosae). Can. J. Bot. 71:586–591.

Vaillancourt, R.E., N.F. Weeden, and J. Barnard. 1993a. Isozyme diversity in the cowpea species complex. Crop Sci. 33:606–613.

Vaillancourt, R.E., N.F. Weeden, A. Bruneau, and J.J. Doyle. 1993b. Chloroplast DNA phylogeny of old world *Vigna* (Leguminosae). Syst. Bot. 18:642–651.

Wendel, J.F., and Weeden, N.F. 1989. Visualization and interpretation of plant isozymes. p. 5–45. *In* D.E. Soltis and P.S. Soltis (ed.) Isozymes in plant biology. Chapman and Hall, London.