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Variation at isozyme loci in wild *Vigna unguiculata* (*Fabaceae, Phaseoleae*)

RÉMY S. PASQUET

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Abstract: An electrophoretic comparison of variation at 37 presumptive isozyme gene loci was performed for 55 wild *Vigna unguiculata* accessions. The analysis included seven subspecies, covering the whole range of variation available in wild *Vigna unguiculata*. The results of the isoenzymatic study broadly confirmed the previous infraspecific classification inferred from morphological data. Although the "southern taxa" (e.g., subsp. *tenuis*, subsp. *stenophylla*) are as yet too poorly sampled to discuss in depth, wild *Vigna unguiculata* can be divided into one mainly autogamous annual group (including subsp. *pubescens* and var. *spontanea*) and several widely divergent perennial subspecies.

Cowpea, *Vigna unguiculata* (L.) WALP. (*Fabaceae*), is one of the major pulses in the tropics. VERDCOURT (1970) and MARÉCHAL & al. (1978) classified *Vigna unguiculata* into *V. unguiculata* subsp. *unguiculata* (which included the cultivated forms), and six wild taxa (four subspecies and two varieties). This infraspecific classification was accepted until recently (NG & MARÉCHAL 1985, NG 1990).

However, at that time, VERDCOURT (1970) studied herbarium specimens and MARÉCHAL & al. (1978) a living collection of only seven wild *V. unguiculata* accessions. Furthermore, both authors were mainly interested in interspecific problems, and focused on characters (spurred stipules, symmetric lilac purple flowers, very short stigma beak, rather monomorphic pods, etc.) which illustrated the homogeneity of the wild *V. unguiculata*, in comparison to other species and sections.

Recently, we analyzed morphological data resulting from observations of more than 50 wild *V. unguiculata* accessions. Working with potted plants, we noted new traits, most of them hard to assess from herbarium material (primary leaf shape, shape of standard appendages, shape and size of coloured marks on standard, keel shape, ovule numbers). No less than eight infraspecific groups were characterized morphologically and geographically, and ranked as subspecies (PASQUET 1992).

The present isozyme study was undertaken with the intention of clarifying the relationships between the subspecies.

Material and methods

Plant material. The accessions studied for isoenzymes are listed in Table 1. Material sources were CIAT (C accessions), the IBPGR *Phaseolinae* collection initiated by the Faculté des

Table 1. *Vigna unguiculata* accessions studied

Subspecies	Accession No.		Country	Latitude	Longitude
spontanea	1 C	4901	Kenya	3° 37′ S	39° 51′ E
	2 C	4903	Kenya	2° 17′ N	40° 54′ E
	3 C	4904	Kenya	2° 17′ N	40° 54′ E
	4 MT	55	Zimbabwe	19° 03′ S	32° 44′ E
	5 MT	76	Zimbabwe	19° 57′ S	32° 05′ E
	6 MT	621	Botswana	22° 22′ S	26° 52′ E
	7 NI	198	Zaïre	4° 01′ N	19° 19′ E
	8 NI	301	Tanzania	10° 20′ S	40° 28′ E
	9 NI	319	Zaïre	6° 45′ S	23° 57′ E
	10 NI	320	Zaïre		
	11 NI	423	Zambia	13° 08′ S	28° 25′ E
	12 NI	437	Zaïre	6° 45′ S	23° 57′ E
	13 NI	817	Zimbabwe	18° 12′ S	31° 34′ E
	14 NI	874	Zimbabwe	18° 12′ S	31° 34′ E
	15 NI	945	Niger		
	16 NI	951	Nigeria	12° 00′ N	18° 30′ E
	17 NI	963	Sénégal	12° 32′ N	16° 45′ W
	18 NI	991	Niger	13° 29′ N	1° 57′ E
	19 NI	1048	Kenya	4° 20′ S	39° 33′ E
	20 NI	1167	South Africa		
	21 NI	1171	Zambia	11° 23′ S	29° 31′ E
	22 SP	3	Cameroon	11° 08′ N	14° 08′ E
	23 SP	5	Cameroon	11° 08′ N	14° 08′ E
	24 SP	46	Cameroon	11° 24′ N	14° 34′ E
	25 SP	52	Cameroon	5° 04′ N	14° 02′ E
	26 SP	66	Niger	11° 52′ N	3° 30′ E
pubescens	27 NI	856	Tanzania	6° 43′ S	38° 23′ E
	28 NI	910	Tanzania	6° 43′ S	38° 23′ E
	29 NI	947	?		
	30 NI	957	Tanzania	5° 14′ S	38° 47′ E
	31 NI	979	Kenya	3° 40′ S	39° 51′ E
	32 NI	989	Kenya	3° 55′ S	39° 46′ E
	33 NI	1029	Tanzania	6° 43′ S	38° 23′ E
tenuis	34 MT	4	Zimbabwe	17° 45′ S	31° 05′ E
stenophylla	35 MT	509	Botswana	24° 19′ S	25° 23′ E = Ni 1425
	36 MT	564	Botswana	22° 27′ S	22° 06′ E = Ni 1419
letouzeyi	37 SP	47	Cameroon	3° 49′ S	12° 01′ E = Ni 1424
	38 SP	48	Cameroon	3° 49′ S	12° 00′ E = Ni 1427
	39 SP	49	Cameroon	3° 49′ S	11° 59′ E = Ni 1426
	40 SP	51	Cameroon	3° 42′ S	11° 35′ E = Ni 1420
baoulensis	41 NI	794	Nigeria	7° 30′ N	3° 54′ E
	42 NI	933	Ghana	9° 24′ N	0° 59′ E
	43 NI	993	Nigeria		
	44 NI	1026	Nigeria	8° 30′ N	4° 33′ E
	45 NI	1034	Nigeria	9° 18′ N	5° 04′ E

Table 1 (continued)

Subspecies	Accession No.		Country	Latitude	Longitude
	46 SP	36	Cameroon	6° 24′ N	11° 34′ E = NI 1145
	47 SP	39	Cameroon	3° 51′ N	11° 28′ E
	48 SP	45	Cameroon	4° 14′ N	11° 02′ E
	49 SP	55	Cameroon	4° 18′ N	12° 15′ E
	50 SP	63	Cameroon	6° 41′ N	10° 43′ E
	51 SP	69	Ivory Coast	6° 43′ N	5° 50′ W
	52 SP	70	Ivory Coast	6° 43′ N	5° 50′ W
	53 SP	71	Ivory Coast	6° 14′ N	5° 05′ W
	54 SP	72	Ivory Coast	6° 14′ N	5° 05′ W
burundiensis	55 NI	456	Burundi		

Sciences Agronomiques de Gembloux and maintained at the Jardin Botanique National de Belgique in Meise (NI accessions), the IBPGR/University of Zimbabwe collection of RICHARD MITHEN (MT accessions), and that of ORSTOM Niger (SP accessions mostly collected by the author).

Fifty-five accessions representing seven subspecies were examined (Table 1). Several of the accessions listed as var. *spontanea* were morphologically intermediate between var. *spontanea* and different perennial taxa: NI 301 and NI 1167 (with subsp. *pubescens*), NI 1048 (with subsp. *dekindtiana*), NI 1171 (with subsp. *tenuis*). Therefore, we considered 21 accessions of var. *spontanea* sensu stricto, and 25 accessions of var. *spontanea* sensu lato. In addition, the fourth accession listed, MT 55, was attributed to var. *spontanea* by the morphological characterization, but it was too peculiar isozymically and was, therefore, considered on its own.

With regard to material collected by the author, each accession corresponds to the progeny of one plant. Of these accessions, SP 3 & 5, SP 47, 48 & 49, SP 69 & 70, SP 71 & 72 come from four populations.

For the other accessions, the sampling technique, and the number and method of regeneration are unknown. However, since their localities are identical, it is likely that C 4903 & C 4904, NI 319 & 437, NI 817 & 874, NI 856, 910 & 1029 belong to four other populations.

Two or three progenies from each non-ORSTOM accession were tested. In all cases, the progenies were electrophoretically identical, therefore, no further intra-accession variability was measured.

Biochemical methods. Individuals were screened by protein electrophoresis for a total of 22 enzyme systems, as Est and Amp display different isozymes in seeds and leaves (Table 2). Extracts for electrophoresis were obtained from imbibed seed or from the young leaves of adult plants. Several isozymes were detected in both seeds and leaves (i.e., Amp 1, Mdh, Pgm), but bands appeared stronger when using one of both organs.

Gels for horizontal starch gel electrophoresis were prepared as described by SECOND & TROUSLOT (1980). The histidine/citric acid gel at pH 6.0 was used for all enzymes. The gel mixture contained 14% starch. Staining procedures used were those of authors listed in Table 2. Amp was assayed using leucine- and alanine-beta-naphtylamide, Fle, αGal and βGlu using 4-methyl umbelliferyl derivatives.

Locus and allele designations were assigned as follows: loci were labelled sequentially with those migrating closest to the anodal end designated as number 1. The most common

Table 2. Enzyme systems studied in *Vigna unguiculata*

Enzyme	Abbreviation	E.C. No.	Organ studied	No. loci scored	Staining procedures
Alcohol dehydrogenase	Adh	1.1.1.1	seeds	2	SECOND & TROUSLOT (1980)
Malate dehydrogenase	Mdh	1.1.1.37	seeds	3	SECOND & TROUSLOT (1980)
Malic enzyme	Me	1.1.1.40	seeds	1	CARDY & al. (1983)
Shikimic dehydrogenase	Sdh	1.1.1.25	seeds	1	TANKSLEY & RICK (1980)
Isocitrate dehydrogenase	Idh	1.1.1.42	seeds	2	SECOND & TROUSLOT (1980)
Glucose-6-phosphate dehydrogenase	Gpd	1.1.1.49	seeds	1	VALLEJOS (1983)
Glutamate dehydrogenase	Gdh	1.4.1.2	seeds	1	SECOND & TROUSLOT (1980)
NADH Diaphorase	Dia	1.6.2.2	seeds	2	HARRIS & HOPKINSON (1978)
Glutathione reductase	Gr	1.6.4.2	seeds	1	HARRIS & HOPKINSON (1978)
Superoxyde dismutase	Sod	1.15.1.1	seeds	2	JAASKA & JAASKA (1988)
Glutamate oxyloacetate transaminase	Got	2.6.1.1	leaves	2	SECOND & TROUSLOT (1980)
Phosphoglucomutase	Pgm	2.7.5.1	seeds	2	SECOND & TROUSLOT (1980)
Esterase	Est	3.1.1.1	seeds	1	SECOND & TROUSLOT (1980)
			leaves	2	
Fluorescent esterase	Fle	3.1.1.2	seeds	2	HARRIS & HOPKINSON (1978)
Beta-glucosidase	βGlu	3.2.1.21	leaves	2	VALLEJOS (1983)
Alpha-galactosidase	αGal	3.2.1.22	leaves	1	VALLEJOS (1983)
Endopeptidase	Enp	3.4.–.–	seeds	1	CARDY & al. (1983)
Aminopeptidase	Amp	3.4.11.1	seeds	3	SECOND & TROUSLOT (1980)
			leaves	1	
Phosphoglucose isomerase	Pgi	5.3.1.9	seeds	3	SECOND & TROUSLOT (1980)
Mannose phosphate isomerase	Mpi	5.3.1.8	seeds	1	HARRIS & HOPKINSON (1978)

allele in var. *spontanea* was designated as 100 and all other allozymes were measured in millimeters from this standard (KOENIG & GEPTS 1989).

Numerical analysis. Genetic distances and genetic identities between individuals were estimated following NEI (1978). Genetic identity between several of the subspecies was also calculated by considering all the accessions of each subspecies as belonging to one population. The UPGMA procedure of SNEATH & SOKAL (1973) was used to produce a phenogram from the matrix of NEI's distance values (1978). Correspondence analysis, where the relationship between two individuals was measured by chi-square metric, was made using computer programme NDMS 1.01 (NOIROT & al. 1987).

Results

The 22 enzyme systems showed 37 putative loci products suitable for further analysis, 29 (78%) of which corresponded to polymorphic loci. Allozyme frequencies, mean gene diversity (H), proportion of polymorphic loci (L) and mean number of alleles at the polymorphic loci (A) are reported in Table 3.

Eight enzyme systems had a single well-resolved locus (Me, Sdh, Gpd, Gdh, Gr, αGal, Enp, Mpi), eight had two loci (Adh, Idh, Dia, Sod, Got, Pgm, Fle, βGlu), three had three loci (Mdh, Est, Pgi), and one had four (Amp).

Among these isozymes, Sdh, Dia 1, and Gpd appear as double bands, similar to Sdh in *Phaseolus vulgaris* (WEEDEN 1984). βGlu and Fle each show three isozymes, but βGlu 2 and Fle 2 were too weakly stained and too poorly resolved for further consideration. Similarly, the least anodal zone of enzyme activity of Mdh was too poorly resolved for interpretation (Fig. 1). Adh, Mdh 2 and Mdh 3, Pgi 2 and Pgi 3 displayed three-band patterns which are probably products of interacting loci.

Re Got, Got 2 was found in leaf extracts only and Got 1 appeared in both leaf and seed extracts (Fig. 1). Re Est, Est 1, and Est 2 appeared in leaves and Est 3 in seeds.

Among Amp isozymes, Amp 1 activity was stronger in leaf extracts, but the other isozymes were observed in seeds. Amp 4 stained stronger with alanine-beta-naphtylamide.

Numerical analysis. From Fig. 2, it can be seen that factors 1 and 2 of correspondence analysis separate subsp. *baoulensis*, subsp. *letouzeyi*, and NI 456 (subsp. *burundiensis*) from other accessions. Subsp. *stenophylla*, subsp. *pubescens*, and a few southern accessions, i.e., MT 55 (4), MT 76 (5), NI 301 (8), NI 817, and NI 874 (13 and 14), NI 1167 (20), MT 4 (34), are on the edge of the var. *spontanea* group.

Factors 3 and 4 of correspondence analysis separate subsp. *letouzeyi*, subsp. *pubescens*, subsp. *stenophylla*, subsp. *tenuis*, subsp. *burundiensis*, and almost the same southern accessions, with C 4903 (2), MT 621 (6), from a var. *spontanea*/subsp. *baoulensis* group (Fig. 3).

As shown in Fig. 4, UPGMA cluster analysis gave the same grouping: subsp. *burundiensis*, subsp. *baoulensis*, subsp. *stenophylla*, subsp. *letouzeyi*, MT 4 and MT 55 (4 and 33), subsp. *pubescens*, and accessions NI 301, MT 76, NI 1167, NI 817, and 874 (8, 5, 19, 13, and 14).

NEI's distances between individuals of the same or of different groups are given in Table 4, with the number of loci having no common alleles between subspecies.

Genetic distances between individuals, and between subspecies (considering each subspecies as one population), are reported in Table 5.

As some of the accessions belonged to the same populations, a few values of intrapopulation distances between accessions were calculated and found to be from 0 (NI 817–NI 874) to 0.114 (NI 910–NI 1029, SP 3–SP 5, SP 69–SP 70, SP 71–SP 72). These values were of the same order in the different subspecies, irrespective of longevity and breeding systems. For the same accessions genetic identities were from 0.891 to 1.

Discussion

Comparison with the previous morphological study (PASQUET 1993). There is general agreement between the morphological and isoenzymatic analyses. With the exception of subsp. *pubescens* and var. *spontanea*, the subspecies are very distinct. Distances between individuals of different subspecies are far more important than distances between individuals belonging to the same subspecies.

But in the morphological analysis all the perennial subspecies were at a similar distance from var. *spontanea*. In the isoenzymatic analysis the distances are not

Table 3. Electromorph frequencies, mean gene diversity index (H), proportion of polymorphic loci (L), and mean number of alleles at polymorphic loci (A), of the whole collection and for each *Vigna unguiculata* subspecies. For each column, the number of accessions studied is given in brackets

Enzyme		Total (55)	var. *spont.* (21)	var. *spont.* (25)	subsp. *baoul.* (14)	subsp. *letou.* (4)	subsp. *burun.* (1)	subsp. *pubes.* (7)	subsp. *steno.* (2)	subsp. *tenuis* (1)	MT 55	NI 301	NI 1048	NI 1167	NI 1171
Adh 1	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Adh 2	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Mdh 1	100	0.872	1.0	1.0	0.642	0.50	1	1.0	1.0	1	1	1	1	1	1
	95	0.127	0.0	0.0	0.357	0.50	0	0.0	0.0	0	0	0	0	0	0
Mdh 2	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Mdh 3	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Me	100	0.563	0.761	0.80	0.0	0.50	0	0.857	0.0	1	1	1	1	1	1
	95	0.436	0.238	0.20	1.0	0.50	1	0.142	1.0	0	0	0	0	0	0
Sdh	105	0.036	0.0	0.04	0.071	0.0	1	0.0	0.0	0	0	0	0	0	1
	100	0.781	0.761	0.72	0.928	1.0	0	1.0	0.0	0	1	1	1	0	0
	95	0.163	0.238	0.24	0.0	0.0	0	0.0	1.0	0	0	0	0	1	0
	90	0.018	0.0	0.0	0.0	0.0	0	0.0	0.0	1	0	0	0	0	0
Idh 1	105	0.018	0.0	0.0	0.0	0.0	0	0.0	0.5	0	0	0	0	0	0
	100	0.836	1.0	1.0	0.50	1.0	1	1.0	0.5	0	1	1	1	1	1
	90	0.145	0.0	0.0	0.50	0.0	0	0.0	0.0	1	0	0	0	0	0
Idh 2	105	0.018	0.047	0.04	0.0	0.0	0	0.0	0.0	0	0	0	0	0	0
	100	0.981	0.952	0.96	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Gpd	105	0.109	0.238	0.24	0.0	0.0	0	0.0	0.0	0	0	0	1	0	0
	100	0.509	0.761	0.72	0.071	0.25	0	1.0	0.5	0	0	1	0	0	1
	95	0.381	0.0	0.04	0.928	0.75	1	0.0	0.5	1	1	0	0	1	0
Gdh	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1

Dia 1	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Dia 2	112 B	0.109	0.0	0.0	0.428	0.0	0	0.0	0.0	0	0	0	0	0	0
	100 A	0.872	1.0	1.0	0.571	1.0	1	1.0	1.0	1	0	1	1	1	1
	88 C	0.018	0.0	0.0	0.0	0.0	0	0.0	0.0	0	1	0	0	0	0
Gr	100 A	0.727	1.0	1.0	0.0	1.0	0	1.0	1.0	1	1	1	1	1	1
	97 B	0.272	0.0	0.0	1.0	0.0	1	0.0	0.0	0	0	0	0	0	0
Sod 1	110	0.036	0.0	0.0	0.0	0.0	0	0.285	0.0	0	0	0	0	0	0
	100	0.927	1.0	1.0	1.0	1.0	1	0.428	1.0	1	1	1	1	1	1
	90	0.036	0.0	0.0	0.0	0.0	0	0.285	0.0	0	0	0	0	0	0
Sod 2	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Got 1	108 C	0.018	0.0	0.0	0.0	0.0	0	0.0	0.5	0	0	0	0	0	0
	104 E	0.018	0.0	0.0	0.0	0.0	0	0.0	0.0	0	1	0	0	0	0
	100 A	0.927	0.952	0.96	1.0	1.0	0	1.0	0.5	1	0	1	1	1	1
	96 D	0.018	0.047	0.04	0.0	0.0	0	0.0	0.0	0	0	0	0	0	0
	93 B	0.018	0.0	0.0	0.0	0.0	1	0.0	0.0	0	0	0	0	0	0
Got 2	110 B	0.109	0.190	0.24	0.0	0.0	0	0.0	0.0	0	0	0	1	0	1
	100 A	0.745	0.666	0.60	1.0	1.0	1	0.428	1.0	1	1	0	0	1	0
	0	0.145	0.142	0.16	0.0	0.0	0	0.571	0.0	0	0	1	0	0	0
Pgm 1	A 100 A	0.927	0.904	0.92	0.857	1.0	1	1.0	1.0	1	1	1	1	1	1
	96 B	0.054	0.047	0.04	0.142	0.0	0	0.0	0.0	0	0	0	0	0	0
	92 C	0.018	0.047	0.04	0.0	0.0	0	0.0	0.0	0	0	0	0	0	0
Pgm 2	105 C	0.018	0.0	0.0	0.0	0.0	1	0.0	0.0	0	0	0	0	0	0
	100 A	0.963	0.952	0.96	1.0	1.0	0	1.0	1.0	1	1	1	1	1	1
	95 B	0.018	0.047	0.04	0.0	0.0	0	0.0	0.0	0	0	0	0	0	0
Est 1	110 C	0.090	0.0	0.0	0.0	1.0	1	0.0	0.0	0	0	0	0	0	0
	105 B	0.109	0.190	0.16	0.0	0.0	0	0.0	1.0	0	0	0	0	0	0
	100 A	0.800	0.809	0.84	1.0	0.0	0	1.0	0.0	1	1	1	1	1	1
Est 2	103 A	0.081	0.190	0.24	0.928	0.0	1	0.0	0.0	0	0	1	0	1	0
	100 D	0.163	0.428	0.40	0.0	0.0	0	0.0	0.0	0	0	0	0	0	1
	94 C	0.181	0.333	0.32	0.071	0.0	0	0.0	0.5	0	0	0	1	0	0
	91 B	0.072	0.047	0.04	0.0	1.0	0	1.0	0.5	1	1	0	0	0	0

Table 3 (continued)

Enzyme		Total (55)	var. *spont.* (21)	var. *spont.* (25)	subsp. *baoul.* (14)	subsp. *letou.* (4)	subsp. *burun.* (1)	subsp. *pubes.* (7)	subsp. *steno.* (2)	subsp. *tenuis* (1)	MT 55	NI 301	NI 1048	NI 1167	NI 1171
Est 3	109 C	0.127	0.095	0.08	0.142	0.0	1	0.0	1.0	0	0	0	0	0	0
	103 B	0.327	0.047	0.04	0.857	0.75	0	0.0	0.0	1	1	0	0	0	0
	100 A	0.545	0.857	0.88	0.0	0.25	0	1.0	0.0	0	0	1	1	1	1
Fle 1	106 B	0.072	0.095	0.08	0.071	0.0	1	0.0	0.0	0	0	0	0	0	0
	100 A	0.927	0.904	0.92	0.928	1.0	0	1.0	1.0	1	1	1	1	1	1
Fle 3	100 A	0.890	0.809	0.80	1.0	1.0	1	1.0	1.0	0	0	1	0	1	1
	96 C	0.109	0.190	0.20	0.0	0.0	0	0.0	0.0	1	1	0	1	0	0
βGlu1	112 C	0.054	0.0	0.0	0.0	0.75	0	0.0	0.0	0	0	0	0	0	0
	106 D	0.109	0.047	0.04	0.0	0.25	1	0.0	0.5	1	1	0	0	0	0
	100 A	0.618	0.952	0.96	0.142	0.0	0	1.0	0.5	0	0	1	1	1	1
	94 B	0.218	0.0	0.0	0.857	0.0	0	0.0	0.0	0	0	0	0	0	0
βGlu3	100 A	0.127	0.0	0.0	0.142	1.0	1	0.0	0.0	0	0	0	0	0	0
	98 B	0.200	0.0	0.0	0.785	0.0	0	0.0	0.0	0	0	0	0	0	0
	0	0.672	1.0	1.0	0.071	0.0	0	1.0	1.0	1	1	1	1	1	1
αGal	111	0.072	0.047	0.08	0.0	0.0	0	0.285	0.0	0	0	1	0	0	0
	100	0.600	0.857	0.80	0.0	1.0	1	0.571	1.0	1	1	0	1	1	0
	89	0.327	0.095	0.12	1.0	0.0	0	0.142	0.0	0	0	0	0	0	1
Enp	107 E	0.036	0.0	0.0	0.142	0.0	0	0.0	0.0	0	0	0	0	0	0
	105 B	0.490	0.142	0.20	0.857	1.0	0	0.857	0.0	0	0	1	0	1	0
	103 F	0.036	0.0	0.0	0.0	0.0	0	0.0	1.0	0	0	0	0	0	0
	100 A	0.418	0.857	0.80	0.0	0.0	0	0.142	0.0	1	1	0	1	0	1
	95 D	0.018	0.0	0.0	0.0	0.0	1	0.0	0.0	0	0	0	0	0	0
Amp 1	100 A	0.490	0.809	0.76	0.0	0.0	0	0.714	1.0	1	0	1	0	1	0
	97 B	0.109	0.095	0.12	0.0	0.0	1	0.285	0.0	0	0	0	0	0	1
	95 C	0.272	0.047	0.08	0.928	0.0	0	0.0	0.0	0	0	0	1	0	0
	90 D	0.018	0.0	0.0	0.0	0.0	0	0.0	0.0	0	1	0	0	0	0
	0	0.109	0.047	0.04	0.071	1.0	0	0.0	0.0	0	0	0	0	0	0

Amp 2	102 C	0.018	0.047	0.04	0.0	0.0	0	0.0	0.0	0	0	0	0	0	0
	100 A	0.400	0.904	0.84	0.0	0.0	0	0.142	0.0	0	0	0	1	0	1
	98 D	0.309	0.047	0.12	0.285	0.5	1	0.857	0.0	0	1	1	0	1	0
	97 B	0.236	0.0	0.0	0.714	0.0	0	0.0	1.0	1	0	0	0	0	0
	93 E	0.036	0.0	0.0	0.0	0.5	0	0.0	0.0	0	0	0	0	0	0
Amp 3	100 A	0.654	0.904	0.88	0.285	0.0	1	1.0	0.0	1	1	1	1	1	0
	97 B	0.236	0.047	0.04	0.714	0.0	0	0.0	1.0	0	0	0	0	0	0
	94 C	0.036	0.047	0.08	0.0	0.0	0	0.0	0.0	0	0	0	0	0	1
	0	0.072	0.0	0.0	0.0	1.0	0	0.0	0.0	0	0	0	0	0	0
Amp 4	104 D	0.018	0.0	0.0	0.0	0.0	1	0.0	0.0	0	0	0	0	0	0
	100 A	0.600	0.571	0.60	0.857	1.0	0	0.0	0.5	1	0	0	1	1	1
	96 B	0.345	0.428	0.36	0.142	0.0	0	0.857	0.5	0	1	0	0	0	0
	93 C	0.018	0.0	0.0	0.0	0.0	0	0.142	0.0	0	0	0	0	0	0
	89 E	0.018	0.0	0.04	0.0	0.0	0	0.0	0.0	0	0	1	0	0	0
Pgi 1	100	1.0	1.0	1.0	1.0	1.0	1	1.0	1.0	1	1	1	1	1	1
Pgi 2	115 D	0.036	0.095	0.12	0.0	0.0	0	0.0	0.0	0	0	0	0	0	0
	107 C	0.090	0.0	0.0	0.0	0.0	0	0.714	0.0	0	0	0	0	0	0
	100 A	0.618	0.904	0.88	0.0	1.0	1	0.285	1.0	1	1	1	1	1	1
	92 B	0.254	0.0	0.0	1.0	0.0	0	0.0	0.0	0	0	0	0	0	0
Pgi 3	108 B	0.345	0.047	0.08	1.0	0.0	1	0.142	0.0	1	0	0	0	0	1
	100 A	0.654	0.952	0.92	0.0	1.0	0	0.857	1.0	0	1	1	1	1	0
Mpi	100 A	0.927	1.0	1.0	1.0	1.0	1	0.714	0.0	1	1	1	1	1	1
	91 B	0.072	0.0	0.0	0.0	0.0	0	0.285	1.0	0	0	0	0	0	0
H		0.307	0.157	0.168	0.122	0.071		0.113	0.081						
L		0.78	0.59	0.59	0.43	0.16		0.30	0.16						
A		3.24	2.45	2.59	2.06	2.0		2.18	2.0						

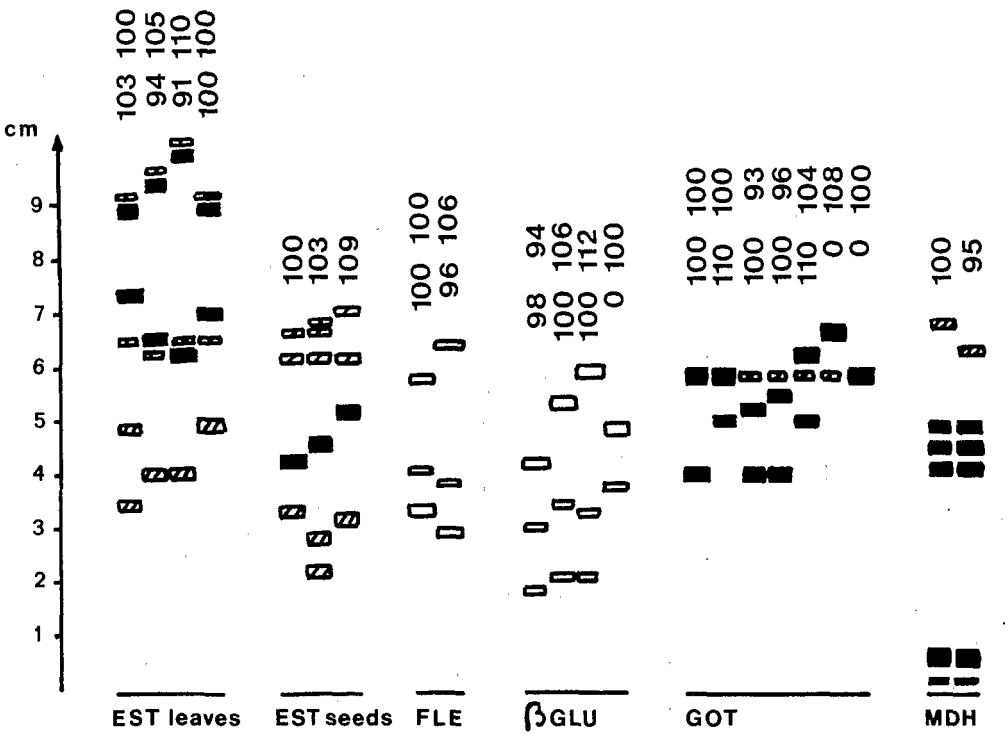

Fig. 1. Electrophoretic patterns of some enzymes in *Vigna unguiculata*. Scale on the left axis is in cm. Arrow indicates direction of anode

the same. Subsp. *baoulensis* and NI 456 (subsp. *burundiensis*) are very much isolated, and subsp. *letouzeyi*, subsp. *stenophylla*, and MT 4 (subsp. *tenuis*) are still clearly separated from other subspecies. However, subsp. *pubescens* accessions, which were widely separated from other subspecies and tightly grouped along factor 2 of the correspondence analysis of morphological data, are loosely grouped and barely separated from var. *spontanea* in the isoenzymatic analysis. Yet, if we consider only the isoenzymatic data, accession NI 989 would appear to belong to var. *spontanea* (Figs. 2, 3, 4, and Table 4).

Among "southern taxa", subsp. *tenuis* and subsp. *stenophylla* appear as two opposite poles, as was also shown by the morphological study (PASQUET 1993). However, the ambiguous location of accession MT 55 (4) indicates that the situation for "southern taxa" is not that simple. In the morphological study, MT 55 was considered as a var. *spontanea* accession, but in the isoenzymatic study it cannot be merged with either subsp. *stenophylla* or subsp. *tenuis*. Its nearest accession is MT 4, with which it is (poorly) associated by cluster analysis (Fig. 4).

Analysis of the patterns of variation within the subspecies remains outside the scope of this study, since sample sizes for most of the subspecies are limited. Only var. *spontanea*, with 21 accessions from most of Africa, and subsp. *baoulensis*, with 14 accessions from Ivory coast, Ghana, Nigeria, and Cameroon, can be considered as widely sampled.

However, the variability in var. *spontanea* is greater than that found in other subspecies, even the widely sampled subsp. *baoulensis* (Tables 4 and 5). This, and

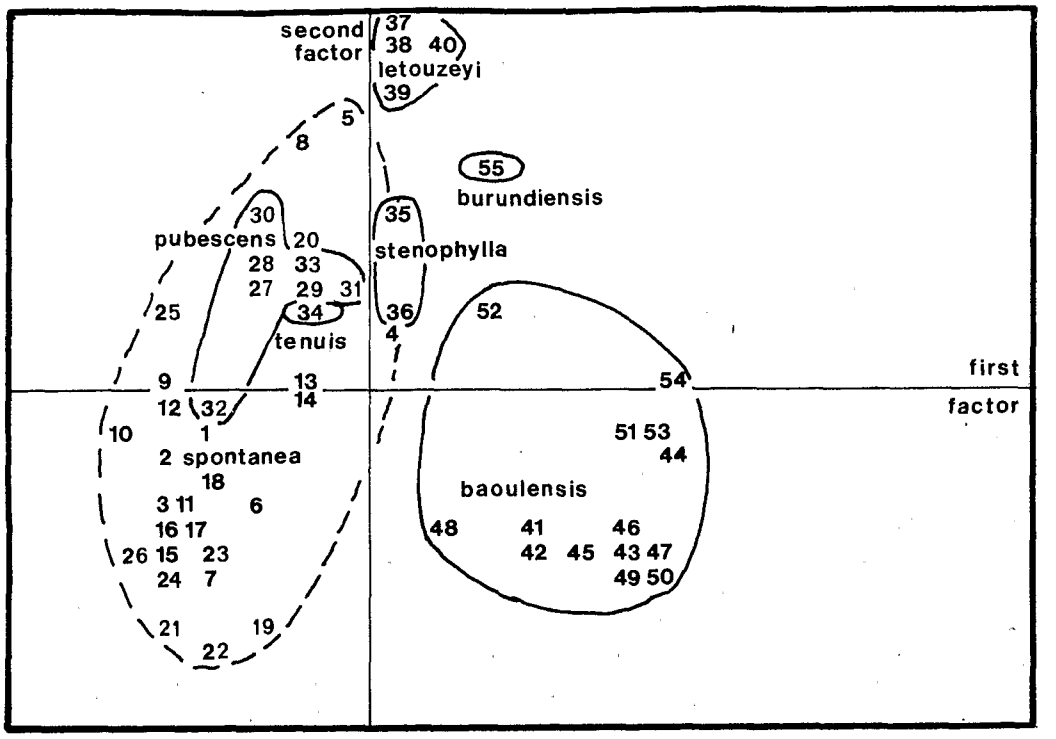

Fig. 2. First and second factors of correspondence analysis

the occurrence of intermediate accessions, would seem to be mainly due to introgressions between var. *spontanea* and perennial subspecies. But introgressed forms would appear to be rarer between perennial subspecies. No introgression between subsp. *baoulensis* and subsp. *letouzeyi* was observed in Cameroon, which was our most intensively sampled country and where distribution of the two subspecies overlaps.

However, introgression is probably very important in the "southern taxa", as is reflected by herbarium material (PASQUET 1993). Since there appears to be general agreement between morphological and isoenzymatic data, we can assume that introgression would have been detected, using isoenzymatic studies, if the southern part of Africa had been more widely sampled.

Comparison with similar works on related species from *Phaseolinae*, i.e., *Phaseolus acutifolius* A. GRAY (SCHINKEL & GEPTS 1989) and *Phaseolus vulgaris* L. (KOENIG & GEPTS 1989). These authors studied large numbers of wild accessions (respectively 55 and 83), collected from the whole dispersal area of these taxa.

Compared to these studies on *Phaseolus*, *V. unguiculata* shows greater variability. Total gene diversity (H) was 0.247 for *P. acutifolius* wild forms, and 0.132 for *P. vulgaris*, L was 0.63 and 0.45, A was 2.75 and 2.22. The explanation could be that these *Phaseolus* species are self-pollinating annuals, just as is var. *spontanea*. This range in diversity (observed in the wild forms of these two species) corresponds only to that found among var. *spontanea*.

In the studies on *Phaseolus*, relatively strong geographic distinctions in both species were noted by the authors, but these are not as sharp as those found by

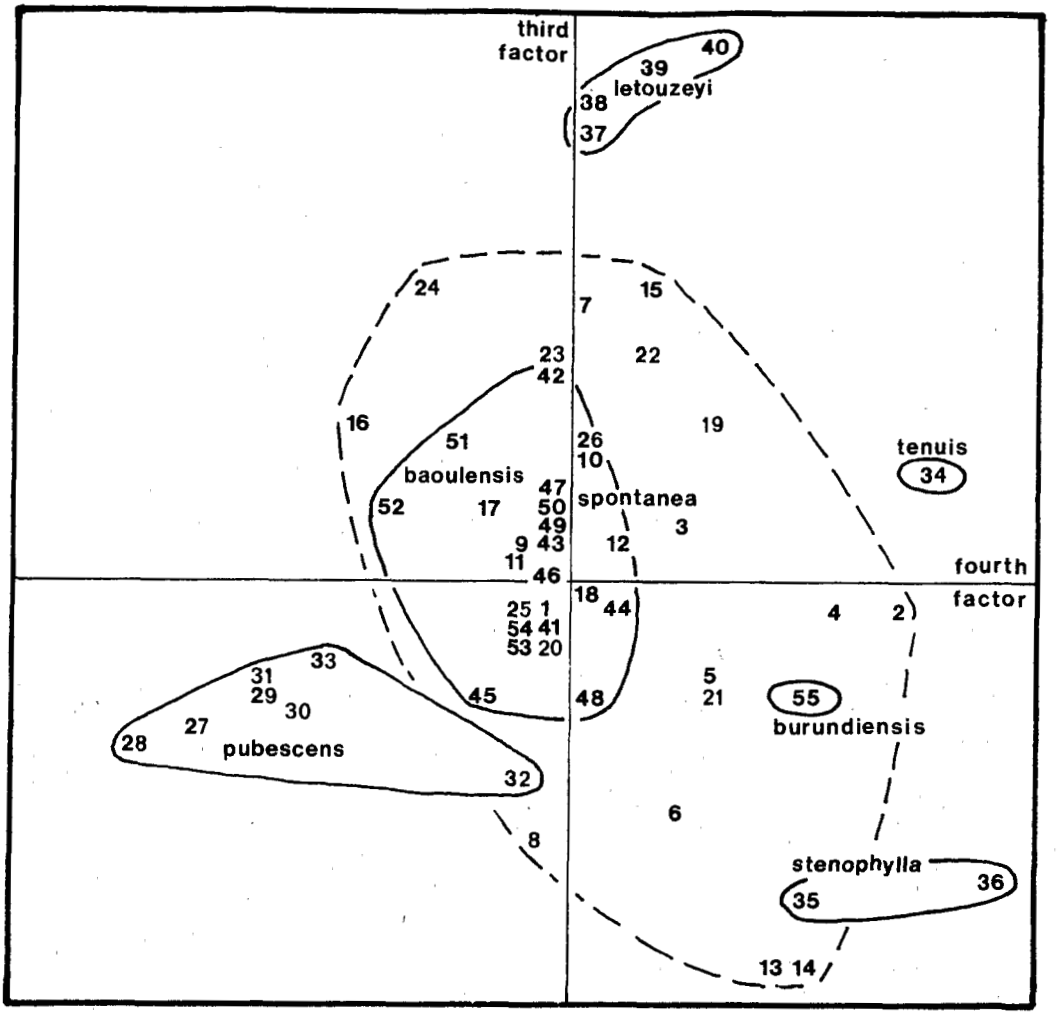

Fig. 3. Third and fourth factors of correspondence analysis

the present study between *V. unguiculata* subspecies. But no similar phenomenon was observed within var. *spontanea* (which presents more or less the same amount of diversity). The difference between both *P. vulgaris* groups corresponds to that between subsp. *pubescens* and var. *spontanea*.

Comparison with other taxonomic studies that used isoenzymatic analysis. A direct comparison of genetic identities with other studies is difficult to make since loci numbers usually differ and also because interpopulation identities are generally used, which was not possible in the present study due to lack of adequate sampling.

In Table 5, averages of genetic identity among individual plants (upper triangle values) give an inferior limit for genetic identity (in missing intrapopulation variability, which does exist), and genetic identities between subspecies – considering each subspecies as one population (lower triangle values) – give a superior limit (by exaggerating intrapopulation variability, as the subspecies comprise several panmictic units).

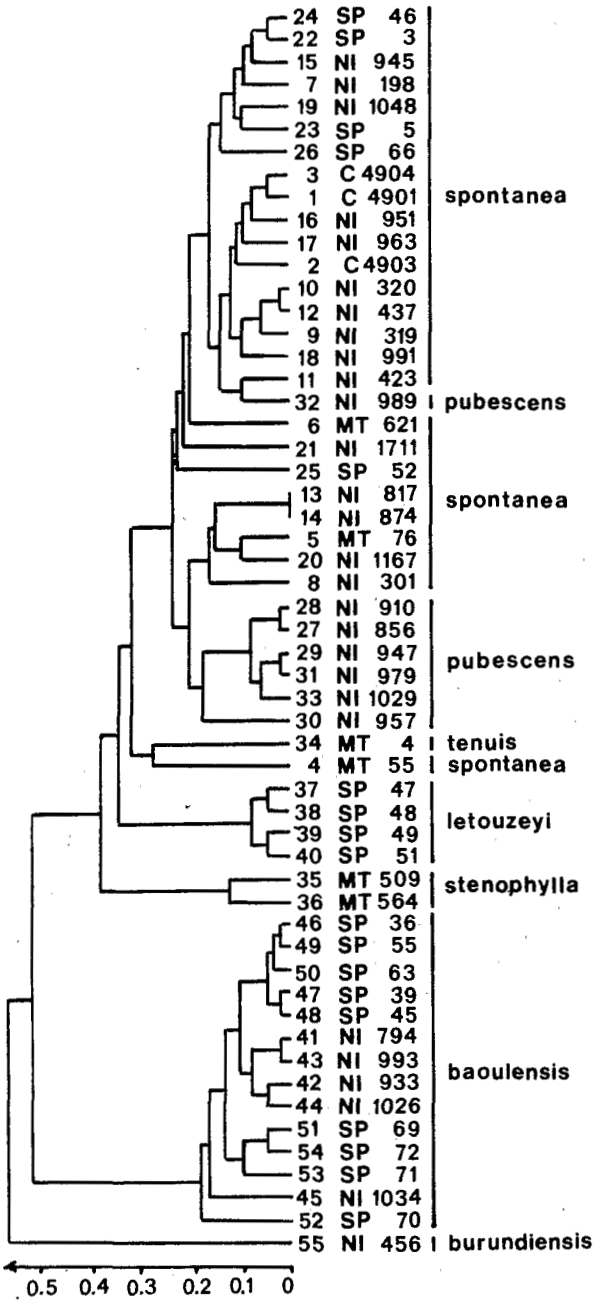

Fig. 4. UPGMA cluster phenogram based on NEI's distances

Thus, with the exception of the values between var. *spontanea* and subsp. *pubescens* (in the range of 0.77–0.93), our values are much lower than the limit usually recognized for genetic identities between species of 0.85–0.90 (CRAWFORD 1983). This leads to the hypothesis of a very ancient geographic separation between *V. unguiculata* subspecies.

Conclusion

The present study has emphasized the general agreement between morphological and isoenzymatic data (with the exception of subsp. *pubescens* which is now placed

Table 4. Distribution of NEI's genetic distances between individuals within or between groups: upper line is minimum distance, middle line is median distance (in bold), lower line is maximum distance (upper triangle). Proportion of loci with no electromorphs in common (lower triangle). For each column, the number of *Vigna unguiculata* accessions studied is given in brackets

	Var. *sponta.* (21)	Var. *sponta.* (25)	Subsp. *baoul.* (14)	Subsp. *letou.* (4)	Subsp. *burun.* (1)	Subsp. *pubes.* (7)	Subsp. *steno.* (2)	Subsp. *tenuis* (1)	MT 55	NI 301	NI 1048	NI 1167	NI 1171
Var. *sponta.* (21)	0.000 **0.177** 0.392	–	0.353 **0.566** 0.838	0.279 **0.392** 0.520	0.475 **0.615** 0.721	0.114 **0.244** 0.433	0.315 **0.392** 0.615	0.210 **0.315** 0.433	0.353 **0.372** 0.433	0.145 **0.227** 0.315	0.085 **0.210** 0.353	0.114 **0.210** 0.315	0.145 **0.244** 0.392
Var. *sponta.* (25)	–	0.000 **0.210** 0.566	0.315 **0.520** 0.838	0.244 **0.392** 0.520	0.475 **0.615** 0.721	0.114 **0.244** 0.433	0.244 **0.433** 0.615	0.210 **0.315** 0.433	0.353 **0.372** 0.433	–	–	–	–
Subsp. *baoul.* (14)	0.08	0.05	0.027 **0.145** 0.279	0.315 **0.520** 0.666	0.433 **0.566** 0.666	0.392 **0.566** 0.778	0.392 **0.566** 0.778	0.433 **0.520** 0.566	0.392 **0.543** 0.666	0.392 **0.520** 0.615	0.475 **0.566** 0.666	0.315 **0.454** 0.520	0.475 **0.520** 0.615
Subsp. *letou.* (4)	0.11	0.08	0.22	0.056 **0.085** 0.145	0.392 **0.497** 0.615	0.279 **0.353** 0.475	0.315 **0.433** 0.520	0.353 **0.412** 0.433	0.315 **0.372** 0.433	0.353 **0.353** 0.392	0.353 **0.392** 0.433	0.244 **0.261** 0.315	0.433 **0.454** 0.520
Subsp. *burun.* (1)	0.24	0.19	0.24	0.32	–	0.566 **0.615** 0.838	0.566 **0.590** 0.615	**0.566**	**0.566**	**0.615**	**0.721**	**0.475**	**0.615**
Subsp. *pubes.* (7)	0.0	0.0	0.14	0.19	0.35	0.027 **0.145** 0.244	0.353 **0.433** 0.520	0.279 **0.392** 0.475	0.244 **0.315** 0.392	0.145 **0.177** 0.177	0.279 **0.279** 0.392	0.145 **0.244** 0.279	0.279 **0.353** 0.433
Subsp. *steno.* (2)	0.08	0.08	0.27	0.24	0.41	0.16	**0.145**	0.353 **0.393** 0.433	0.475 **0.475** 0.475	0.433 **0.476** 0.520	0.433 **0.524** 0.615	0.244 **0.244** 0.244	0.475 **0.545** 0.615
Subsp. *tenuis* (1)	0.08	0.08	0.27	0.27	0.43	0.22	0.27	–	**0.244**	**0.432**	**0.315**	**0.279**	**0.392**
MT 55	0.11	0.08	0.32	0.24	0.43	0.35	0.32	0.22	–	**0.392**	**0.315**	**0.315**	**0.520**
NI 301	0.03	0.0	0.27	0.24	0.46	0.05	0.32	0.35	0.32	–	**0.279**	**0.145**	**0.315**
NI 1048	0.0	0.0	0.32	0.27	0.51	0.16	0.32	0.27	0.27	0.24	–	**0.244**	**0.244**
NI 1167	0.03	0.0	0.24	0.19	0.38	0.11	0.22	0.24	0.27	0.14	0.22	–	**0.315**
NI 1171	0.03	0.0	0.27	0.32	0.46	0.14	0.35	0.32	0.41	0.27	0.22	0.27	–

Table 5. Averages of genetic identities between individuals, within and between groups (upper triangle), and genetic identities between *Vigna unguiculata* subspecies – with each subspecies considered as a single population – (lower triangle). For each column, the number of accessions studied is given in brackets

	Var. *sponta.* (21)	Var. *sponta.* (25)	Subsp. *baoul.* (14)	Subsp. *letou.* (4)	Subsp. *burun.* (1)	Subsp. *pubes.* (7)	Subsp. *steno.* (2)	Subsp. *tenuis* (1)	MT 55	NI 301	NI 1048	NI 1167	NI 1171
Var. *sponta.* (21)	0.827	–	0.577	0.685	0.538	0.774	0.669	0.727	0.679	0.793	0.826	0.810	0.775
Var. *sponta.* (25)	–	0.818	0.580	0.686	0.540	0.773	0.662	0.728	0.679	–	–	–	–
Subsp. *baoul.* (14)	0.663	0.673	0.869	0.610	0.578	0.571	0.555	0.605	0.576	0.597	0.562	0.647	0.585
Subsp. *letou.* (4)	0.760	0.764	0.695	0.919	0.609	0.698	0.652	0.676	0.690	0.696	0.676	0.764	0.629
Subsp. *burun.* (1)	–	–	–	–	–	0.510	0.567	0.567	0.567	0.513	0.486	0.622	0.513
Subsp. *pubes.* (7)	0.898	0.903	0.646	0.746	–	0.869	0.656	0.684	0.722	0.850	0.730	0.799	0.707
Subsp. *steno.* (2)	0.769	0.766	0.617	0.691	–	0.708	0.864	0.676	0.622	0.622	0.595	0.716	0.567
Subsp. *tenuis* (1)									0.784	0.649	0.730	0.757	0.676
MT 55										0.676	0.730	0.730	0.595
NI 301											0.757	0.865	0.730
NI 1048												0.784	0.784
NI 1167													0.730

closer to var. *spontanea*), and broadly supports the taxonomic conclusions inferred from the morphological study. Strong divergences between the perennial subspecies has also been shown. This leads to confirmation of the division of wild *Vigna unguiculata* into several subspecies with introgressions between taxa in some cases. To be precise, we can see one more or less annual and autogamous group, comprising var. *spontanea* and subsp. *pubescens*, and several perennial and more or less allogamous taxa, among them the so-called "southern taxa", i.e., subsp. *stenophylla*, subsp. *tenuis*, etc., which deserve, as also shown by the previous morphological study, a rank equal to that of taxa which have been well characterized through living material (PASQUET 1993), i.e., subsp. *baoulensis*, subsp. *letouzeyi*, subsp. *burundiensis*.

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Address of the author: RÉMY S. PASQUET, ORSTOM (Institut Français de Recherche Scientifique pour le Développement en Coopération), BP 11416, Niamey, Niger.

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