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Isozyme polymorphism in some yellow- and blue-flowered *Vigna* species complexes (*Fabaceae, Phaseoleae*)

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Abstract: An electrophoretic comparison of variation at 28 isozyme loci was performed for 58 *Vigna* accessions belonging to the *V. luteola*, *V. ambacensis*, and *V. racemosa* groups of species. In all three groups, strong divergence is noted between results and actual nomenclature.

The genus *Vigna* Savi includes several species of economic importance: the cowpea *V. unguiculata* (L.) Walp., the bambara groundnut *V. subterranea* (L.) Verdc. and many other Asian species belonging to subg. *Ceratotropis* (Piper) Verdc.: the moth bean *V. aconitifolia* (Jacq.) Maréchal, the rice bean *V. umbellata* (Thunb.) Ohwi & H. Ohashi, the adzuki bean *V. angularis* (Willd.) Ohwi & H. Ohashi, the black gram or urb bean *V. mungo* (L.) Hepper, the green gram or mung bean *V. radiata* (L.) R. Wilczek.

The last comprehensive studies concerning the genus *Vigna* were those of *Verdcourt* (1970, 1971) and Maréchal & al. (1978). However, these were not general revisions of the genus. Verdcourt (1970, 1971) based his studies on herbarium specimens and was concerned primarily with the taxa represented in Eastern Africa (sensu Kew), whereas Maréchal & al. (1978) embraced the whole *Phaseolastreae* Baudet & Maréchal and based their studies largely on living materials available at that time. Nevertheless, Maréchal & al. (1978) were mainly interested in the delimitation of the genera *Vigna* and *Phaseolus* L., and their material was not rich enough to study closely related taxa relationships.

The concept of the genus *Vigna* which emerged from these studies (Maréchal 1982) remained stable. The only change was made by Jaaska & Jaaska (1988) who elevated sect. *Catiang* (DC.) Verdc. of subg. *Vigna* to subgeneric rank. Nevertheless, the most recent works tend to suggest important modifications in the future, particularly the separation of the new world *Vigna* from the rest of the genus (Delgado-Salinas & al. 1993, Vaillancourt & al. 1993a). In these works, the old world *Vigna* appear to be split into several groups: subg. *Haydonia* (R. Wilczek) Verdc., subg. *Ceratotropis*, the yellow- and blue-flowered species of subg. *Vigna*

(most of sect. *Vigna*), and a fourth group including subg. *Plectrotropis* (SCHUMACH.) BAK. and the pink- or purple-flowered species of subg. *Vigna*.

At the specific level, studies of *V. unguiculata* (PANELLA & GEPTS 1992, MITHEN & KIBBLEWHITE 1993, PADULOSI 1993, PASQUET 1993a, VAILLANCOURT & al. 1993b) *V. vexillata* (L.) A. RICH. (PIENAAR & KOK 1991; GARBA & PASQUET, unpubl.), *V. frutescens* A. RICH. and *V. membranacea* A. RICH. (PASQUET & VANDERBORGHT, unpubl.) have renovated the concept of these gene pools. However, no study was undertaken within the yellow- and blue-flowered species. These species include two morphologically well characterized species, i.e. *V. subterranea*, *V. multinervis* HUTCH. & DALZ., and three groups of closely related species.

The first group contains *V. luteola* (JACQ.) BENTH., a pantropical species, and approximately ten morphologically similar taxa: *V. fischeri* HARMS, *V. marina* (BURM.) MERR., *V. bequaertii* R. WILCZEK (no available accessions), *V. oblongifolia* A. RICH. with its two varieties *oblongifolia* and *parviflora* (BAK.) VERDC., *V. lanceolata* BENTH., *V. filicaulis* HEPPER with its var. *filicaulis* and var. *pseudovenulosa* MARÉCHAL, MASCHERPA & STAINIER. All these species show yellow flowers and a chromosome number of $2n = 22$ when chromosomes were counted (MARÉCHAL & al. 1978).

The second group is characterized by a chromosome number of $2n = 20$ (MARÉCHAL & al. 1978). It includes blue- and yellow-flowered taxa: *V. ambacensis* BAK. with its var. *ambacensis* an var. *pubigera* (BAK.) MARÉCHAL, MASCHERPA & STAINIER, *V. heterophylla* A. RICH., *V. benuensis* PASQUET & MARÉCHAL and, although morphologically more distant, *V. hosei* (CRAIB) BACKER, with its var. *hosei* and var. *pubescens* MARÉCHAL, MASCHERPA & STAINIER.

The third group includes blue-flowered species with a chromosome number of $2n = 22$ when chromosomes were counted (MARÉCHAL & al. 1978): *V. racemosa* (G. DON) HUTCH. & DALZIEL, *V. gazensis* BAK., *V. gracilis* (GUILL. & PERR.) HOOK f., *V. desmodioides* R. WILCZEK (no available accessions), *V. parkeri* BAK. with its subsp. *parkeri* (no available accessions) and subsp. *maranguensis* (TAUB.) VERDC. and *V. laurentii* DEWILD.

Therefore, the objective of this research was to assess the genetic distances between taxa within each of these three groups using isozymes, and to study systematic relationships between taxa within each of these three groups of yellow- and blue-flowered *Vigna* species. The possible conspecificity of very closely related species, i.e. *V. luteola* and *V. fischeri*, *V. ambacensis* and *V. benuensis*, *V. parkeri* and *V. laurentii*, was tested with special care. Thus the three groups were studied independently as focus was on low genetic distances.

Materials and methods

Plant material. The 58 accessions used in this study are presented in Table 1. The accessions X and NI are from the IPGRI base collection of *Phaseoleae* maintained at the National Botanic Garden of Belgium, Meise. The accessions V are from the ORSTOM collection which is now being duplicated at Meise, the accessions AC from the CSIRO, Santa Lucia. Each accession is made of one to three autogamous lines, and maintained as such, each of these lines coming from one seed of the original stock. For each accession, vouchers and photographs of flowers are deposited in Meise.

Table 1. Accessions of *Vigna* species studied

Accession number(s)	Country	Latitude and longitude	Locality
Vigna luteola			
AC 403	Australia	27°33′S 152°48′E	Colledges crossing, Brisbane River
V 53	Cameroun	4°02′N 9°05′E	Market place
NI 200	Tchad		
NI 409	Congo	4°30′S 11°40′E	Kwilu mouth
NI 414	Rwanda	2°10′S 30°20′E	Mugesera lake
NI 419	Rwanda		Kilimbi
NI 420	Rwanda		Karama
NI 858	Brazil	8°45′S 63°55′W	near Paso Balsa
NI 891	Argentina		
NI 949	Cuba		
NI 961	Colombia		I. de S. Andrés
NI 1018	Kenya	0°38′S 34°32′E	Rogor to Homa bay
V. marina			
AC 802	Australia	21°07′S 149°13′E	Lamberts Beach, Slade Point
X 2050	Thailand		Pattong Beach, Phuket Island
V. fischeri			
V 64	Cameroun	6°13′N 10°25′E	Anyajua
V 133	Cameroun	6°13′N 10°26′E	Tikijem to Anyajua
NI 316	Zambia	8°56′S 31°42′E	Lumi river
V. oblongifolia var. *oblongifolia*			
V 20	Cameroun	10°41′ N 13°36′ E	Roumzou
NI 123	Kenya		
NI 959	Malawi		
V. oblongifolia var. *parviflora*			
NI 282	Tanzania	7°37′ S 31°33′ E	Soda Locust Camp
NI 387	Rwanda	2°20′ S 30°10′ E	Bugesera province
NI 974	Zambia		
V. lanceolata			
AC 207 (NI 1437)	Australia	27°21′ S 151°10′ E	Nandy, Dalby
V. filicaulis var. *filicaulis*			
V 182	Ivory Coast	6°13′ N 5°00′ W	Lamto
V 207	Cameroun	8°35′ N 13°02′ E	km 1 Fignolé to Tchamba
NI 421	Senegal		
V. filicaulis var. *pseudovenulosa*			
NI 410	Senegal		Tambacounda
V. benuensis			
V 44 (NI 1206)	Cameroun	8°44′ N 13°32′ E	Mayo Boki
V 244 (NI 1472)	Cameroun	9°28′ N 13°20′ E	km 10 Gashiga to Demsa
V. ambacensis var. *ambacensis*			
V 129	Cameroun	5°29′ N 10°31′ E	km 8 Noun to Bafoussam
NI 449	Zaire	6°45′ S 23°57′ E	Gandajika
V. ambacensis var. *pubigera*			
V 187	Ivory Coast	6°13′ N 5°00′ W	Lamto
V 211	Cameroun	9°32′ N 13°24′ E	Ngutchumi to Garoua

Table 1 (continued)

Accession number(s)	Country	Latitude and longitude	Locality
V 230	Cameroun	7°18′ N 13°46′ E	km 2 Mbalang Djalingo to Ngang Ha
V 241	Congo	1°11′ S 15°58′ E	Komi bridge
NI 236	Zaire	6°45′ S 23°57′ E	Gandajika
NI 1147	Ghana		Tamale
V. heterophylla			
NI 122	Kenya		
V. hosei var. *hosei*			
NI 260	Indonesia		
NI 1175	Malaysia		Pahang province
V. hosei var. *pubescens*			
NI 321	Rwanda	1°20′ S 30°20′ E	Mutara, Bimba province
V. racemosa			
V 109	Cameroun	6°45′ N 11°52′ E	Labaré to Banyo
V 131	Cameroun	10°28′ N 13°41′ E	Liri to Gova
V 188 (NI 1462)	Ivory Coast	6°13′ N 5°00′ W	Lamto
V 223	Cameroun	5°34′ N 14°06′ E	km 7 Ndokayo to Bétaré Oya
NI 239	Zaire	6°45′ S 23°57′ E	INEAC Gandajika
NI 1245	Burundi	4°09′ S 29°32′ E	Kigwena
V. gracilis			
V 101	Ivory Coast	7°37′ N 8°06′ W	Kata
V 135 (NI 1373)	Cameroun	3°51′ N 11°18′ E	km 29 Nkolbisson to Matomb
V 219 (NI 1445)	Cameroun	6°51′ N 12°57′ E	Sabal Haléo
NI 177	Ivory Coast		
V. parkeri subsp. *maranguensis*			
V 220 (NI 1434)	Cameroun	6°52′ N 12°57′ E	Sabal Haléo
NI 121	Kenya		
NI 1315	Zaire	0°10′ S 29°14′ E	N'Yondo near Lubero
V. laurentii			
NI 322	Burundi	4°02′ S 30°07′ E	Kiofi
V. gazensis			
MT 264	Zimbabwe	18°29′ S 32°47′ E	
MT 381	Malawi	15°59′ S 35°32′ E	

Biochemical methods. The extracts are taken from seeds soaked for 24 hours and then ground in a drop of water. The gels are prepared according to the protocol described by SECOND & TROUSLOT (1980). The histidine/citrate systems at pH 6.0 with a starch concentration of 14% were used for all the enzymatic systems. The enzymatic systems and the staining procedures which were used are indicated in Table 2. AMP was stained with leucine- or alanine-β-naphtylamide, FLE and βGAL with derivatives of 4-methyl-umbelliferyl compounds.

Homologous gene products in different species were recognized in the following manner. For enzyme systems with only one isozyme (ENP, FDH, FLE, βGAL, GDH, GPD, ME, MPI, SDH) the enzymes in different species were assumed to be coded by homologous genes. For enzymes with more than one isozyme (ADH, AMP, DIA, EST,

Table 2. Enzyme systems studied in *Vigna species*

Enzyme	Abbreviation	E. C. number	No. of loci scored	Staining protocols
Alcohol dehydrogenase	ADH	1.1.1.1	2	SECOND & TROUSLOT (1980)
Formate dehydrogenase	FDH	1.2.1.2	1	WENDEL & WEEDEN (1989)
Malate dehydrogenase	MDH	1.1.1.37	3–4	SECOND & TROUSLOT (1980)
Shikimate dehydrogenase	SDH	1.1.1.25	1	SECOND & TROUSLOT (1980)
Malic enzyme	ME	1.1.1.40	1	WENDEL & WEEDEN (1989)
Isocitrate dehydrogenase	IDH	1.1.1.42	1–2	SECOND & TROUSLOT (1980)
Phosphogluconate dehydrogenase	PGD	1.1.1.43	2	SECOND & TROUSLOT (1980)
Glucose-6-phosphate dehydrogenase	GPD	1.1.1.49	1	VALLEJOS (1983)
Glutamate dehydrogenase	GDH	1.4.1.2	1	SECOND & TROUSLOT (1980)
NADH diaphorase	DIA	1.6.2.2	1–2	HARRIS & HOPKINSON (1978)
Superoxyde dismutase	SOD	1.15.1.1	1	WENDEL & WEEDEN (1989)
Phosphoglucomutase	PGM	2.7.5.1	2	SECOND & TROUSLOT (1980)
Esterase	EST	3.1.1.-	0–1	SECOND & TROUSLOT (1980)
Fluorescent esterase	FLE	3.1.1.-	1	HARRIS & HOPKINSON (1978)
β-Galactosidase	βGAL	3.2.1.23	1	VALLEJOS (1983)
Endopeptidase	ENP	3.4.-.-.	1	CARDY & al. (1981)
Aminopeptidase	AMP	3.4.11.1	1–2	SECOND & TROUSLOT (1980)
Phosphoglucose isomérase	PGI	5.3.1.9	3	SECOND & TROUSLOT (1980)
Mannose phosphate isomérase	MPI	5.3.1.8	1	HARRIS & HOPKINSON (1978)

IDH, MDH, PGD, PGI, PGM, SOD) homology could be supported by band intensity (VAILLANCOURT & WEEDEN 1993).

For each enzymatic system, the presumed loci are numbered by increasing distance from the anode, with three exceptions. For IDH, PGD, and PGI, the presumed loci are numbered according to the isozyme phenotype patterns observed in *V. unguiculata* (PASQUET 1993b). PGI2 designates the band presenting the strongest activity, whether it is faster or slower than PGI3. Similarly, PGD1 and IDH2 designate the band presenting the strongest activity.

For each isozyme in each group of species the most common allozyme and respective allele has been designated as 100 (as for *V. luteola*, *V. ambacensis* and *V. racemosa*) and the other allozymes have been measured in millimetres in relation to that standard. The procedure is the same one utilized by KOENIG and GEPTS (1989) with Phaseolus vulgaris L.

The data from the enzymatic analysis has allowed the calculation of Nei distances (NEI 1972). The UPGMAs (SNEATH & SOKAL 1973) have been computed using the BIOSYS software version 1.7 (SWOFFORD & SELANDER 1981).

Considering the small number of accessions studied in most taxa, the mean gene diversity index (H), the proportion of polymorphic loci (L) and the mean number of alleles at polymorphic loci (A) have not been calculated.

Results

The 19 enzymatic systems enable the scoring of 26 to 28 loci depending on the groups of species.

FDH, ME, GDH, βGAL and ENP appear as single bands. SOD is expressed as one strong band which corresponds to SOD2 in *V. unguiculata* (PASQUET 1993b). EST has not been satisfactorily stained in the *V. racemosa* and *V. ambacensis* groups, but in the *V. luteola* group, it appears as a single pink band, which correspond to Est3 in *V. unguiculata*. In *V. filicaulis*, EST was not detected and was considered as null. FLE produces a single band which is supposed to correspond to Fle3 in *V. unguiculata*. MPI appears as a single band. It does not exist in *V. filicaulis* var. *filicaulis*. It was weakly stained and not scored in the *V. racemosa* group.

SDH is expressed as a double band, except in some accessions of *V. ambacensis* where it appears as a single band with a stronger activity (coded as 100′). GPD also appears as a double band. It does not exist in *V. filicaulis* var. *filicaulis*.

IDH reveals two isozymes. The isozyme with the strongest activity (IDH2) is the slower in *V. hosei* (as in *V. unguiculata*) but this does not occur in the other species. IDH1 has not been considered in the *V. racemosa* group because it stains too weakly. PGD also reveals two isozymes. In the *V. racemosa* and *V. luteola* groups, in *V. hosei*, the slow enzyme shows the strongest band whereas in *V. ambacensis* the fast enzyme is the strongest band. In *V. filicaulis*, a single band (PGD2) is observed. PGM reveals two isozymes. The fast isozyme has the strongest activity, as in *V. unguiculata*. In *V. filicaulis*, the activity of PGM2 was so weak that it could not be scored.

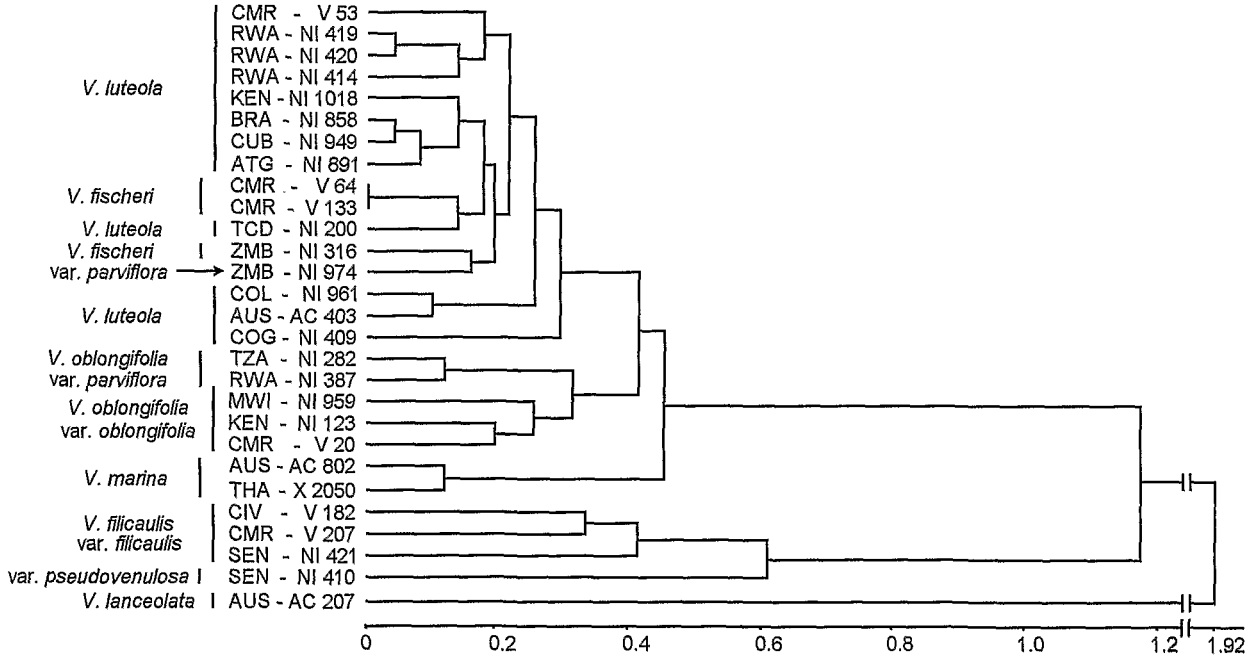

Fig. 1. *Vigna luteola* group. Nei distance UPGMA. Within the *V. luteola* cluster, V 53 and NI 409 are "atlantic *V. marina*"

Table 3. *Vigna luteola* group. Distribution of Nei distances between individuals within and between taxa. Minimum, mean (in bold) and maximum distances are given in upper, middle and lower line, respectively. For each column, the number of accessions studied is given in brackets

	V. luteola (incl. *V. fischeri*) (15)	*V. marina* (2)	var. *oblongifolia* (3)	var. *parviflora* (3)	var. *filicaulis* (3)	var. *pseudovenulosa* (2)	*V. lanceolata* (1)
Vigna luteola	0.000						
incl.	**0.217**						
V. fischeri	0.481						
	0.303						
V. marina	**0.439**	**0.125**					
	0.624						
	0.288	0.442	0.197				
V. oblongifolia	**0.452**	**0.615**	**0.236**				
var. *oblongifolia*	0.624	0.693	0.255				
	0.154	0.303	0.210	0.125			
V. oblongifolia	**0.346**	**0.409**	**0.294**	**0.163**			
var. *parviflora*	0.526	0.499	0.405	0.210			
	0.934	1.135	1.126	1.030	0.336		
V. filicaulis	**1.290**	**1.370**	**1.604**	**1.451**	**0.391**		
var *filicaulis*	1.705	1.723	1.946	1.937	0.499		
V. filicaulis	0.622	0.829	0.894	0.721	0.615		
var. *pseudovenulosa*	**0.737**	**0.855**	**0.946**	**0.827**	**0.615**		
	0.863	0.881	0.972	0.963	0.615		
	1.522	1.819	1.714	1.377	1.030		
V. lanceolata	**2.199**	**1.883**	**1.720**	**1.547**	**1.139**	**1.937**	
	3.332	1.946	1.723	1.723	1.253		

Table 4. *Vigna luteola* group. Allelic frequencies. For each column, the number of accessions studied is given in brackets. *Vigna fischeri* and the "atlantic *V. marina*" are included in *V. luteola*

Enzyme		*V. luteola* (15)	*V. marina* (2)	var. *oblongifolia* (3)	var. *parviflora* (3)	var. *filicaulis* (3)	var. *pseudovenulasa* (1)	*V. lanceolata* (1)
Adh1	100	1	1	1	1	0	0	0
	95	0	0	0	0	1	1	0
	90	0	0	0	0	0	0	1
Adh2	100	1	1	1	1	0	0	0
	97	0	0	0	0	1	1	1
Fdh	109	0	0	0	0	0.333	0	0
	106	0	0	0	0	0	0	1
	103	0.367	0	0.666	0.833	0.333	1	0
	100	0.566	0.5	0	0	0.333	0	0
	97	0.067	0.5	0.333	0.167	0	0	0
Mdh1	105	0	0	0	0	1	1	0
	0	1	1	1	1	0	0	1
Mdh2	100	0.933	1	1	1	1	1	0
	90	0.007	0	0	0	0	0	1
Mdh3	100	0.933	1	1	1	1	1	0
	90	0.007	0	0	0	0	0	1
Mdh4	100	1	0.75	1	1	1	1	0
	80	0	0.25	0	0	0	0	1
Sdh	100	1	1	1	1	0	0	0
	93	0	0	0	0	1	1	1
Me	100	0.8	1	1	1	0.333	1	0
	97	0	0	0	0	0.666	0	1
	94	0.2	0	0	0	0	0	0
Idh2	103	0.067	1	0	0	0.666	1	1
	100	0.933	0	1	1	0.333	0	0
Idh1	100	1	1	1	1	0	1	0
	93	0	0	0	0	1	0	1
Pgd1	100	1	1	1	1	1	1	0
	96	0	0	0	0	0	0	1
Pgd2	102	0.067	0	0	0	0	0	0
	100	0.933	1	0.333	1	0	0	0
	96	0	0	0	0	0	0	1
	92	0	0	0.666	0	0	0	0
	0	0	0	0	0	1	1	0
Gpd	108	0	1	0	0	0	0	0
	100	0.933	0	1	0.666	0	1	0
	96	0.067	0	0	0.333	0	0	1
	0	0	0	0	0	1	0	0
Gdh	103	0	0	1	1	0	0	1
	100	1	1	0	0	1	1	0
Dia1	100	0.866	1	1	1	0	1	1
	95	0.133	0	0	0	1	0	0

Table 4 (continued)

Enzyme		*V. luteola* (15)	*V. marina* (2)	var. *oblongifolia* (3)	var. *parviflora* (3)	var. *filicaulis* (3)	var. *pseudovenulasa* (1)	*V. lanceolata* (1)
Sod2	110	0.133	0	0	0	0	0	0
	104	0	0	1	0	0	0	0
	100	0.867	1	0	1	0.333	1	0
	92	0	0	0	0	0.666	0	1
Pgm1	100	0.667	0.5	0.666	1	0.333	1	0
	96	0.133	0.5	0.333	0	0	0	0
	90	0.200	0	0	0	0.666	0	1
Pgm2	104	0.133	0	1	1	0	1	0
	100	0.867	1	0	0	0	0	0
	96	0	0	0	0	0	0	1
	0	0	0	0	0	1	0	0
Fle3	116	0	0	0	0	0.666	0	0
	106	0	0	0	0	0.333	1	0
	103	0	0	0	0	0	0	1
	100	1	1	1	1	0	0	0
Est 3	100	1	0	0	0.333	0	0	0
	96	0	1	0.666	0.666	0	0	0
	92	0	0	0.333	0	0	0	0
	88	0	0	0	0	0	0	1
	0	0	0	0	0	1	1	0
βGal	106	0	0	0.333	0	0	0	0
	104	0	0	0.333	0.333	0	0	0
	102	0.133	1	0.333	0.666	0	0	1
	100	0.467	0	0	0	0.666	1	0
	98	0.400	0	0	0	0.333	0	0
Enp	115	0	0	0	0	0.333	0	0
	113	0	0	0	0	0	0	1
	110	0	0	0	0	0	1	0
	107	0	0	0	0	0.666	0	0
	103	0.133	0	1	0	0	0	0
	100	0.667	1	0	1	0	0	0
	98	0.067	0	0	0	0	0	0
	95	0.133	0	0	0	0	0	0
Amp2	102	0.067	1	0	0	0	0	0
	100	0.933	0	0.333	0.666	0.333	1	0
	98	0	0	0	0	0.333	0	0
	93	0	0	0.666	0.333	0.333	0	1
Pgi1	103	0.067	1	0	0.333	0.666	1	0
	100	0.933	0	1	0.666	0.333	0	1
Pgi3	104	0.133	0	0	0	0	0	0
	100	0.733	0.500	0.333	1	0	0	0
	92	0.133	0.500	0.666	0	0	0	0
	88	0	0	0	0	0	1	0
	82	0	0	0	0	0.666	0	1
	78	0	0	0	0	0.333	0	0

Table 4 (continued)

Enzyme		*V. luteola* (15)	*V. marina* (2)	var. *oblongifolia* (3)	var. *parviflora* (3)	var. *filicaulis* (3)	var. *pseudovenulasa* (1)	*V. lanceolata* (1)
Pgi2	115	0	0	0	0	0.333	0	0
	110	0.033	0	0.167	0	0	0	0
	108	0	0	0	0	0.333	0	0
	105	0.033	0	0	0	0.333	0	1
	100	0.667	1	0.833	1	0	0.500	0
	98	0.067	0	0	0	0	0	0
	94	0.200	0	0	0	0	0.500	0
Mpi	102	0	0	0.333	0.666	0	0	1
	100	0.933	0	0.666	0.333	0	0	0
	98	0.067	0	0	0	0	0	0
	96	0	0	0	0	0	1	0
	0	0	1	0	0	1	0	0

ADH appears in the form of a three-banded heterodimer and reveals two loci, Adh1 and Adh2. As in *V. unguiculata*, the fast homodimer presents a weaker activity, except in *V. gracilis* where the opposite is found. V 220 (*V. parkeri*) and NI 322 (*V. laurentii*) are characterized by null activity of products of both loci.

MDH presents the same isozyme pattern as in *V. unguiculata*. The most anodal, MDH1, appears as a weak band, except in *V. hosei* and several species of the *V. luteola* group, where it is lacking. In the *V. racemosa* group, MDH1 is very weak or lacking. Next bands are the heterodimer formed between MDH2 and MDH3,

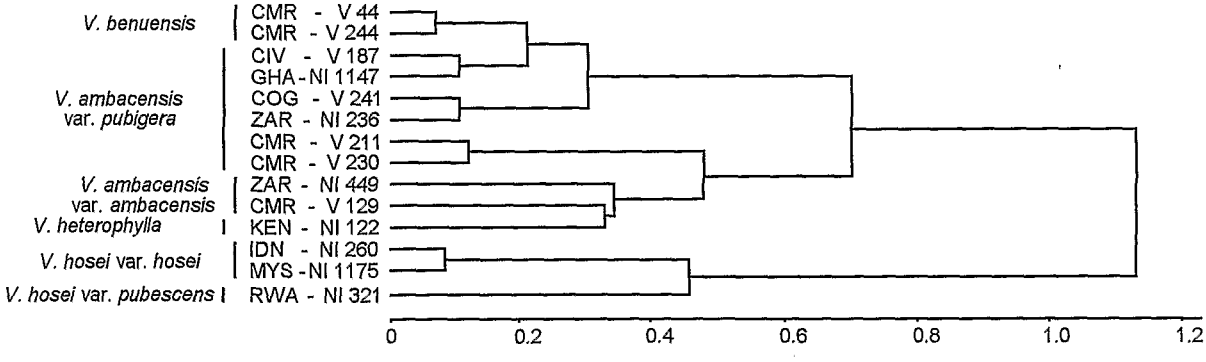

Fig. 2. *Vigna ambacensis* group. Nei distance UPGMA

Table 5. *Vigna ambacensis* group. Distribution of Nei distances between individuals within or between upper taxa. Minimum, mean (in bold) and maximum distances are given in upper, middle and lower line, respectively. For each column, the number of accessions studied is given in brackets

	V. benuensis (2)	var. *pubigera* (6)	var. *ambacensis* (2)	*V. hetero-phylla* (1)	var. *hosei* (2)	var. *pubescens* (1)
Vigna benuensis	**0.074**					
	0.154	0.113				
V. ambacensis	**0.473**	**0.573**				
var. *pubigera*	0.881	0.972				
	0.560	0.405				
V. ambacensis	**0.629**	**0.549**	**0.353**			
var. *ambacensis*	0.684	0.649				
		0.303	0.333			
V heterophylla	**0.693**	**0.597**	**0.346**			
		0.767	0.353			
	1.021	0.847	1.244	1.253		
V. hosei	**1.091**	**1.117**	**1.327**	**1.281**	**0.085**	
var. *hosei*	1.135	1.451	1.386	1.308		
	0.972	1.021	1.072		0.405	
V. hosei var.	**0.996**	**1.189**	**1.186**	**1.183**	**0.466**	
pubescens	1.021	1.368	1.299		0.526	

except in *V. racemosa* where it is expressed as a unique band (coded by Mdh2-100 and Mdh3-100). MDH4 appears as a strong band which migrates significantly, faster than in *V. unguiculata* where its migration is negligible.

PGI1 is supposed to be chloroplastic by analogy with the situation in *V. unguiculata* (VAILLANCOURT & al. 1993b). PGI2 and PGI3 form an heterodimer. Except in *V. gazensis*, in NI 421 (*V. filicaulis*) and in NI 1147 (*V. ambacensis* var. *pubigera*) which present a similar pattern as in the cowpea, the strongest activity (PGI2) is that of the slow isozyme.

DIA1 is expressed as a double band, as in *V. unguiculata*. DIA2 is a single band, only scored within the *V. racemosa* group.

For AMP, with the exception of the *V. racemosa* group where AMP4 was scored, only the isozyme corresponding to AMP2 in *V. unguiculata* was scored. The other bands were too weak to be included in the study.

The group of *V. luteola* In this group 28 loci were investigated.

Vigna lanceolata and *V. filicaulis* appear very distant from the other taxa. The genetic distance between the two subspecies of *V. filicaulis* is also notable (Table 3), similar to the highest Nei distances found within *V. unguiculata* gene pool (PASQUET 1993b). The polymorphism in *V. filicaulis* is important: 60% of the

Table 6. *V. ambacensis* group. Allelic frequencies. For each column, the number of accessions studied is given in brackets. Sdh 100' is the allele represented by a single band, compared to Sdh represented by a double band

		V. benuensis (2)	var. *pubigera* (6)	var. *ambacensis* (2)	*V. heterophylla* (1)	var. *hosei* (2)	var. *pubescens* (1)	Group V 44 (6)	Group V 211 (2)	Group V 129 (3)
Adh1	114	0	0	0.5	0	0	0	0	0	0
	110	0	0.666	0	0	0	0	0	0.5	0.333
	105	0	0.167	0.5	1	0	0	0	0.5	0.666
	100	1	0.167	0	0	1	1	1	0	0
Adh2	100	1	1	1	1	0	0	1	1	1
	93	0	0	0	0	1	0	0	0	0
	90	0	0	0	0	0	1	0	0	0
Fdh	104	0	0	0	0	1	1	0	0	0
	100	0	0.333	1	1	0	0	0	1	1
	96	1	0	0	0	0	0	0.333	0	0
	94	0	0.666	0	0	0	0	0.666	0	0
Mdh1	100	1	1	1	1	0	0	1	1	1
	0	0	0	0	0	1	1	0	0	0
Mdh2	100	1	1	1	1	1	1	1	1	1
Mdh3	100	1	1	1	1	1	1	1	1	1
Mdh4	112	0	0	0	0	1	1	0	0	0
	106	0	0.333	0	0	0	0	0	1	0
	100	1	0.666	1	1	0	0	1	0	1
Sdh	104	1	0	0	0	0	1	0.333	0	0
	100	0	0.500	0	0	1	0	0.500	0	0
	100	0	0.333	0	1	0	0	0.167	1	0.333
	96	0	0.166	0.5	0	0	0	0	0	0.333
	88	0		0.5	0	0	0	0	0	0.333
Me	100	0	0.5	1	1	0	1	0.167	1	1
	97	1	0.5	0	0	1	0	0.833	0	0
Idh2	104	0	0.333	0	0	0	0	0	1	0
	102	0	0	0.5	0	0	0	0	0	0.333
	100	1	0.666	0.5	0	0	0	1	0	0.333
	90	0	0	0	1	0	0	0	0	0.333
	82	0	0	0	0	1	1	0	0	0
Idh1	100	1	1	1	1	1	1	1	1	1
Pgd1	100	1	1	1	1	0	0	1	1	1
	95	0	0	0	0	1	1	0	0	0
Pgd2	104	0	0	0	0	1	1	0	0	0
	100	1	1	1	1	0	0	1	1	1
Gpd	100	1	0.666	1	1	0	0	1	0	1
	97	0	0.333	0	0	0	0	0	1	0
	94	0	0	0	0	1	1	0	0	0
Gdh	104	0	0	0	0	1	1	0	0	0
	100	0	0.333	1	1	0	0	0	1	1
	96	1	0	0	0	0	0	0.333	0	0
	93	0	0.666	0	0	0	0	0.666	0	0
Dia1	104	0	0	0	0	1	1	0	0	0
	100	1	0.666	0	0	0	0	1	0	0
	96	0	0.333	0.5	1	0	0	0	1	0.666
	90	0	0	0.5	0	0	0	0	0	0.333
Sod2	100	1	1	1	1	1	1	1	1	1

Table 6 (continued)

		V. benuensis (2)	var. *pubigera* (6)	var. *ambacensis* (2)	*V. heterophylla* (1)	var. *hosei* (2)	var. *pubescens* (1)	Group V 44 (6)	Group V 211 (2)	Group V 129 (3)
Pgm1	104	1	0.333	0	0	0	1	0.666	0	0
	100	0	0.666	1	1	1	0	0.333	1	1
Pgm2	108	0	0.333	0	0	0	0	0	1	0
	104	0	0	0.5	0	0	1	0	0	0.333
	100	1	0.666	0.5	1	1	0	1	0	0.666
Fle3	105	0.5	0.333	0.5	0	0	0.5	0.167	1	0.333
	100	0.5	0.500	0	0	0.25	0	0.666	0	0.333
	97	0	0.167	0.5	1	0.75	0.5	0.167	0	0.333
ßGal	100	1	0.666	1	0	0	0	1	0	0.666
	96	0	0.333	0	0	0	0	0	1	0
	92	0	0	0	1	0	1	0	0	0.333
	90	0	0	0	0	1	0	0	0	0
Enp	100	0	0.333	0.75	1	0	0	0.833	1	0.833
	96	0.5	0.666	0.25	0	0	0	0	0	0.167
	92	0.5	0	0	0	0.5	0	0.167	0	0
	88	0	0	0	0	0.5	1	0	0	0
Amp2	100	1	0.666	0	0	0.5	1	1	0	0
	98	0	0	1	1	0.5	0	0	0	1
	95	0	0.333	0	0	0	0	0	1	0
Pgi1	100	1	1	1	1	0	0	1	1	1
	98	0	0	0	0	1	0	0	0	0
	96	0	0	0	0	0	1	0	0	0
Pgi2	108	0	0	0.5	0	0	0	0	0	0.333
	104	0	0.083	0	1	0	0	0	0.25	0.333
	100	1	0.583	0.5	0	1	1	0.666	0.75	0.333
	94	0	0.333	0	0	0	0	0.333	0	0
Pgi3	110	0	0	1	0	0	0	0	0	0.666
	106	0	0.167	0	0	0	0	0.167	0	0
	102	0	0.167	0	1	0	0	0	0.5	0.333
	100	1	0.333	0	0	0	0	0.500	0.5	0
	96	0	0.167	0	0	1	1	0.167	0	0
	88	0	0.167	0	0	0	0	0.167	0	0
Mpi	114	0	0	0	0	0	1	0	0	0
	112	0	0	0	0	1	0	0	0	0
	100	0	0.500	0.5	0	0	0	0.500	0	0.333
	98	0	0.167	0	0	0	0	0	0.5	0
	95	1	0.167	0	1	0	0	0.333	0.5	0.333
	90	0	0.167	0.5	0	0	0	0.167	0	0.333

loci are polymorphic (as calculated with 27 loci, without Est3) although only four accessions were studied. Only Adh2, Mdh1, Mdh2, Mdh3, Mdh4, Pgd1, Pgd2, and Gdh are monomorphic within *V. filicaulis* (Table 4).

If we leave these three taxa (*V. lanceolata* and the two varieties of *V. filicaulis*) aside, the remainder of the group appears more homogeneous (Table 3), with a mean NEI distance of 0.287. 75% of the loci are polymorphic within the 23

Table 7. *Vigna ambacensis* group. Distribution of Nei distances between individuals within or between taxa. Minimum, mean (in bold) and maximum distances are given in upper, middle and lower line, respectively. For each column, the number of accessions studied is given in brackets

	V. benuensis group (6)	V 211, V230 (2)	*V. ambacensis* var. *ambacensis* group (3)
Vigna benuensis group	0.074		
V 44, V 187, V 241,	**0.243**		
V 244, NI 236, NI 1147	0.442		
V. ambacensis	0.767		
var. *pubigera*	**0.880**	**0.125**	
V 211, V 230	0.972		
V. ambacensis	0.499	0.303	0.336
var. *ambacensis*	**0.619**	**0.490**	**0.347**
V 129, NI 122, NI 449	0.767	0.649	0.353

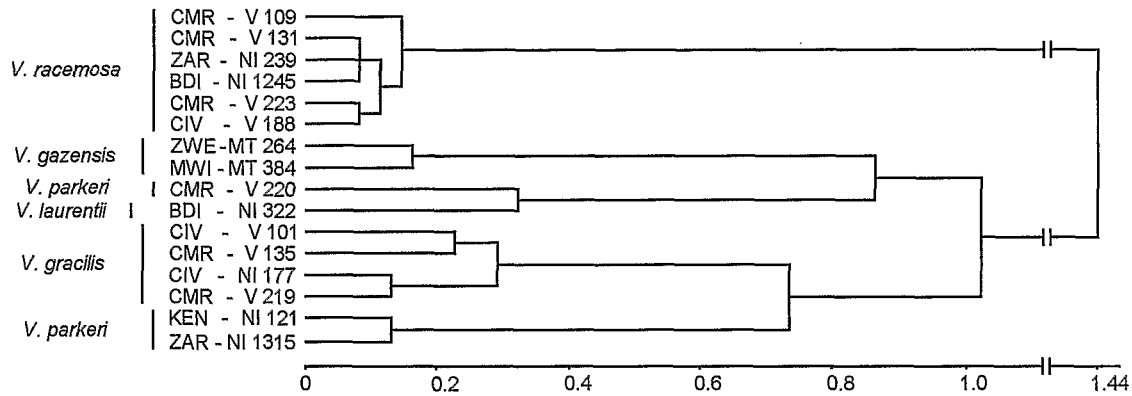

Fig. 3. *Vigna racemosa* group. Nei distance UPGMA

accessions studied. Adh1, Adh2, Mdh1, Sdh1, Idh1, Pgd1 and Fle are monomorphic within these 23 accessions (Table 4).

UPGMA of Nei distances shows three nearly equivalent groups: *V. luteola*, *V. oblongifolia* and *V. marina* (Fig. 1). One accession of *V. oblongifolia* var. *parviflora* (NI 947), however, is found inserted in the *V. luteola* cluster. The two varieties of *V. oblongifolia*, var. *oblongifolia* and var. *parviflora*, are well separated despite that the NEI distances between them are small.

According to Fig. 1, all three accessions of *V. fischeri* appear within the *V. luteola* cluster. Similarly, the accessions from the Atlantic coast (V 53 from Cameroon and NI 409 from Congo), assigned by MARÉCHAL & al. (1978) to *V. marina* and by PADULOSI & NG (1993) as belonging to *V. marina* subsp. *oblonga* (BENTH.) PADULOSI, appear scattered among the *V. luteola* cluster. The American accessions of *V. luteola* are no more distinct from the African accessions. Within *V. luteola*, only those accessions from the Pacific appear distinct due to alleles shared with *V. marina* (βgal-102, Pgi3-92).

The group of *V. ambacensis*. Twenty seven loci were studied in this group.

Vigna hosei is very distant from the other taxa (Table 5, Fig. 2). The Nei distances between the two varieties of this species correspond to those expected between accessions of a same species (PASQUET 1993b). The polymorphism appears less important than in *V. filicaulis*: 40% of the loci are polymorphic within the species. Adh1, Fdh, Mdh1, Mdh2, Mdh3, Mdh4, Idh2, Idh1, Pgd1, Pgd2, Gpd, Gdh, Dia1, Sod, Pgi2 and Pgi3 are monomorphic within the species (Table 6).

Vigna benuensis appears close to *V. ambacensis* var. *pubigera*, differing only in alleles of Fdh, Sdh, Gdh, and Enp (Table 5, Fig. 2). *V. heterophylla* accession is distinct from *V. ambacensis* in alleles of Idh2 and βGal but, as *V. benuensis* accessions, it clusters with *V. ambacensis* accessions. On the other hand, the diversity inside the whole group (*V. benuensis-V. ambacensis-V. heterophylla*) is

Table 8. *Vigna racemosa* group. Distribution of Nei distances between individuals within or between taxa. Minimum, mean (in bold) and maximum distances are given in upper, middle and lower line, respectively. For each column, the number of accessions studied is given in brackets

	V. racemosa (6)	*V. gazensis* (2)	*V. gracilis* (4)	*V. parkeri* NI 121, NI 1315 (2)	*V. parkeri* (V 220), *V. laurentii* (NI 322) (2)
	0.112				
V. racemosa	**0.080**				
	0.214				
	1.061				
V. gazensis	**1.376**	**0.167**			
	1.649				
	1.061	1.179	0.135		
V. gracilis	**1.529**	**1.315**	**0.248**		
	2.159	1.457	0.331		
	1.179	0.860	0.619		
V. parkeri	**1.505**	**0.908**	**0.744**	**0.123**	
NI 121, NI 1315	1.649	0.956	0.860		
V. parkeri (V220),	1.061	0.773	0.693	0.619	
V. laurentii (NI 322)	**1.286**	**0.862**	**0.905**	**0.808**	**0.314**
	1.649	0.956	1.061	1.061	

Table 9. *Vigna racemosa* group. Allelic frequencies. For each column, the number of accessions studied is given in brackets

		V. racemosa (6)	*V. gazensis* (2)	*V. gracilis* (4)	*V. parkeri* (3)	*V. laurentii* (1)	NI 121, NI 1315 (2)	V 220, NI 322 (2)
Adh1	105	0	0	1	0	0	0	0
	100	1	0	0	0	0	0	0
	97	0	1	0	0.666	0	1	0
	92	0	0	0	0	0	0	0
	0	0	0	0	0.333	1	0	1
Adh2	105	0	1	0	0.666	0	1	0
	100	1	0	0	0	0	0	0
	97	0	0	1	0	0	0	0
	0	0	0	0	0.333	1	0	1
Fdh	103	0	1	0	0	0	0	0
	100	1	0	0	0.333	0	0	0.5
	97	0	0	0.25	0.333	1	0.5	0.5
	92	0	0	0.75	0.333	0	0.5	0
Mdh2	112	0	1	1	1	1	1	1
	100	1	0	0	0	0	0	0
Mdh3	106	0	1	1	1	1	1	1
	100	1	0	0	0	0	0	0
Mdh4	100	1	1	1	1	0	1	0.5
	93	0	0	0	0	1	0	0.5
Sdh	100	1	0	0	0.333	1	0	1
	93	0	1	0	0	0	0	0
	90	0	0	0	0.666	0	1	0
	87	0	0	0.25	0	0	0	0
	75	0	0	0.75	0	0	0	0
Me	103	0	1	0	1	1	1	1
	100	1	0	0.5	0	0	0	0
	94	0	0	0.5	0	0	0	0
Idh2	108	0	0.5	0	0	0	0	0
	100	1	0.5	0	0	0	0	0
	96	0	0	1	1	1	1	1
Pgd1	108	0	1	0	0	0	0	0
	103	0	0	1	1	1	1	1
	100	1	0	0	0	0	0	0
Pgd2	100	1	0	0	0	0	0	0
	98	0	0.5	0	0	0	0	0
	96	0	0	0	0.333	1	0	1
	93	0	0	0.5	0.666	0	1	0
	90	0	0	0.5	0	0	0	0
	84	0	0.5	0	0	0	0	0
Gpd	108	0	0.5	0	0	0	0	0
	104	0	0.5	0	0	0	0	0
	100	1	0	1	0.333	1	0	1
	94	0	0	0	0.666	0	1	0
Gdh	100	0.5	1	0	0.333	1	0	1
	95	0.5	0	1	0.666	0	1	0

Table 9 (continued)

		V. racemosa (6)	*V. gazensis* (2)	*V. gracilis* (4)	*V. parkeri* (3)	*V. laurentii* (1)	NI 121, NI 1315 (2)	V 220, NI 322 (2)
Dai1	104	0	0	1	0	0	0	0
	100	1	1	0	0.666	1	0.5	1
	90	0	0	0	0.333	0	0.5	0
Dai2	110	0	1	1	1	1	1	1
	100	1	0	0	0	0	0	0
Sod2	100	1	0	0	0	0	0	0
	93	0	1	1	1	1	1	1
Pgm1	104	0.666	0	0	0	0	0	0
	100	0.333	0	0	1	1	1	1
	96	0	1	1	0	0	0	0
Pgm2	100	1	1	0	0	0	0	0
	93	0	0	0	0.333	0	0.5	0
	91	0	0	1	0.333	0	0.5	0
	89	0	0	0	0.333	1	0	1
Fle3	100	1	1	0.75	1	1	1	1
	97	0	0	0.25	0	0	0	0
βGal	100	0.833	0	0.5	0.666	0	1	0
	97	0.167	0	0.5	0	0	0	0
	93	0	0.5	0	0.333	1	0	1
	87	0	0.5	0	0	0	0	0
Enp	106	0	0	0.75	0	0	0	0
	103	0.167	1	0.25	1	0	1	0.5
	100	0.833	0	0	0	1	0	0.5

very high even if 66% only of the loci are polymorphic. Very high Nei distances are observed between accessions of *V. ambacensis* var. *pubigera*, up to 0.972 *Vigna ambacensis* appears split in two very distinct groups (Table 7, Fig. 2). Adh2, Mdh1, Mdh2, Mdh3, Idh1, Pgd1, Pgd2, Sod and Pgi1 are monomorphic within the group *V. ambacensis-V. benuensis-V. heterophylla* (Table 6).

The group of *V. racemosa*. Twenty six loci were studied in this group.

In this group, *V. racemosa*, *V. gracilis* and *V. gazensis* appear to be clearly distinct (Table 8, Fig. 3). The polymorphism within the different species is fairly low: only 19% of the loci are polymorphic in *V. racemosa*, and 38% in *V. gracilis*. Four loci (βGal, Gpd, Idh1, Pgd1) separate the two accessions of *V. gazensis* (Table 9).

On the other hand, the situation with *V. parkeri* and *V. laurentii* seems more complex. V 220 and NI 322 are differentiated by seven loci (Fdh, Enp, Pep2, Pep3, Mdh4, Pgi2 and Pgi3), NI 121 and NI 1315 by three loci (Dia1, Fdh and Pgm2) (Table 9). But the two pairs of species are separated by Nei distances from 0.6 to 1.0 (Table 8).

Discussion

The results obtained for the *V. luteola* group should have an important influence on the nomenclature. *Vigna lanceolata* and *V. filicaulis* may be considered as distinct species. However, the very high polymorphism within *V. filicaulis* and the important Nei distances observed between the accessions of the two varieties could eventually justify considering var. *filicaulis* and var. *pseudovenulosa* as distinct species. It would, however, be desirable to study a larger number of accessions.

On the other hand, *V. luteola*, *V. fischeri* (and probably *V. bequaertii*), *V. marina* and *V. oblongifolia* would appear to form a single species. The Nei distance observed within this group are similar to those found by VAILLANCOURT & WEEDEN (1993) with 26 loci, though this study was based on a different set of enzyme systems. These genetic distances are mostly lower than the distances observed between perennial subspecies of *V. unguiculata* (PASQUET 1993b), between the two varieties of *V. hosei*, and between the two varieties of *V. filicaulis*. The highest Nei distances inside this group are not much higher than the mean of the Nei distances between accessions from different species (0.4) as given by CRAWFORD (1989). *Vigna luteola* and *V. oblongifolia* var. *parviflora* appear particularly close.

The incorporation of *V. fischeri* into *V. luteola* at an infraspecific level seems justified and strengthened by the fact that MARÉCHAL (unpubl. pers. comm.) claims to have obtained a fertile hybrid between the two taxa, using *V. luteola* as female parent. This study, that of VAILLANCOURT & al. (1993a), and that of VAILLANCOURT & WEEDEN (1993) brought strong arguments to merge *V. luteola* into *V. oblongifolia*, particularly because several authors reported hybridization of the two taxa (FARIS 1963, MURDOCK 1992, NG 1992). However, both taxa are clearly separated by several morphological characters (pubescence, flower size and color, and presence versus absence of an eccentric aril).

Within the group of *V. ambacensis*, *V. hosei* appears as a very distinct species including two fairly distinct infraspecific taxa, even though only three accessions have been studied.

Regarding *V. ambacensis*, the situation does not correspond with the pattern given by the accepted nomenclature. *Vigna* benuensis seems included in *V. ambacensis* var. *pubigera* and could deserve a varietal rank because of its very peculiar morphological characters (PASQUET & MARÉCHAL 1989). *Vigna heterophylla* appears to be included in *V. ambacensis* var. *ambacensis*, the only difference being the pubescence (VERDCOURT 1971, MARÉCHAL & al. 1978). On the other hand, there seems to exist within the group two well differentiated entities, perhaps two distinct species. But the flower color, used to separate var. *ambacensis* and var. *pubigera*, does not seem to be a useful character. Similarly, the number of ovules per pod, which clearly separates *V. benuensis* from *V. ambacensis*, does not help in differentiating either *V. ambacensis* clusters. The number of nodes in the inflorescence could be a more useful morphological character.

The east-west cline noted by VERDCOURT (1970) and MARÉCHAL & al. (1978) seems to be invalidated by the present results (NI 236 is given in the "western" cluster). A similar observation of an apparent altitudinal cline, particularly in

Cameroon (with blue flowering accessions encountered only in highland areas), also does not fit the present results (V 230 is from the Adamawa Range, but V 211 comes from the Bénoué plain).

In the *V. racemosa* group, *V. racemosa*, *V. gracilis* and *V. gazensis* appear as homogeneous and distinct species. In *V. gazensis*, the distinction between the forms from Zimbabwe and Malawi, as suggested by MARÉCHAL & al. (1978) on the basis of the shape of the keel and of the appendages of the standard, find no confirmation with the allozyme data used here.

On the other hand, the situation of *V. parkeri* is much more problematic. Examination of herbarium material suggests *V. laurentii* as very close to *V. parkeri* subsp. *maranguensis* from which it is distinct by lanceolate leaflets instead of round ones, a higher number of nodes per inflorescence, a seed with an eccentric aril, and a larger number of ovules per pod, with the flower and pod morphology being identical.

Many additional accessions (as the taxa extend from Cameroon to Zimbabwe and Madagascar, through eastern Africa) are required to elucidate the true relationships among these taxa.

Conclusion

The present study validates some previous results based on morphology and justifies the specific rank for the following taxa: *V. lanceolata*, *V. filicaulis*, *V. hosei*, *V. racemosa*, *V. gazensis* and *V. gracilis*.

On the other hand, *V. luteola*, *V. fischeri* and *V. marina* (and probably *V. bequaertii*) should, in light of the present results, be merged into a single species. Low Nei distances between accessions of *V. luteola* and *V. oblongifolia* could also lead to placement of both taxa into *V. oblongifolia* s. l., even if some morphological characters clearly separate these taxa.

It appears that *V. benuensis* and *V. heterophylla* should not be maintained at the specific rank.

The situation with *V. ambacensis* and *V. parkeri* seems to be more complex, and some divisions appear that were not considered in the current classification. For the two cases, the present study justifies further investigations which would include morphology, isoenzyme and cpDNA patterns based on a larger number of accessions which, primarily concerning *V. parkeri*, have yet to be collected.

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