PERGAMON

Biochemical Systematics and Ecology 28 (2000) 29–43

biochemical systematics and ecology

Isozyme polymorphism in the *Vigna frutescens–V. membranacea* complex (Tribe Phaseoleae, Fabaceae)

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Received 30 July 1998; accepted 18 January 1999

Abstract

An electrophoretic comparison of variation at 29 isozyme loci was performed for 21 *Vigna* accessions belonging to *V. frutescens*, *V. membranacea* and *V. friesiorum*. The results do not fit accepted nomenclature and the taxonomic ranks should be reassessed. *V. frutescens*, *V. friesiorum*, and various subspecies of *V. membranacea* should be considered as putative species, although hybridisation data are needed in order to obtain a clear picture of the organisation of this gene pool. However, the results do not support the existence of two sections, and section Liebrechtsia is merged with section Macrodontae in a newly enlarged section Macrodontae.

Keywords: *Vigna frutescens*; *Vigna membranacea*; Fabaceae; Isozyme polymorphism; Genetic distance

1. Introduction

The genus *Vigna* belongs to the family Fabaceae, subfamily Faboideae. Interest in this genus arises from the fact that it contains the cultivated cowpea (*V. unguiculata* (L.) Walp.). On a global basis, it can be estimated that cowpea is cultivated on at least

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0305-1978/99/$ - see front matter
PII: S0305-1978(99)00034-4

12.5 Mha, with an annual production of over 3 Mt worldwide (Singh et al., 1997). In addition to the cultivated cowpea, the genus contains 81 species (Maréchal et al., 1978). Recent cpDNA studies suggest that important taxonomic modifications to the genus are likely to be forthcoming, particularly the separation of the new world *Vigna* from the rest of the genus (Vaillancourt et al., 1993a; Delgado-Salinas et al., 1993) which should reduce *Vigna* to around 50 species, mostly from the old world. Old world *Vigna* should be rearranged into four main groups: subgenus *Haydonia* (R.Wilczek) Verdc., subgenus *Ceratotropis* (Piper) Verdc., the yellow and blue flowered species of subgenus *Vigna* (most of section *Vigna*), and a fourth group including the pink or purple flowered species of the subgenera *Vigna* and *Plectrotropis* (Schumach.) Bak.

The latter group contains Marechal's section Macrodontae (Harms), Reticulatae Verdc., *Liebrechtsia* (De Wild.) Bak. f., *Catiang* (DC.) Verdc., and subgenus *Plectrotropis* (Schumach.) Bak. Available living material from these groups was studied recently through isozyme polymorphism: cowpea (section Catiang) by Panella and Gepts (1992), Vaillancourt et al. (1993b), and Pasquet (1993b), section Reticulatae and subgenus *Plectrotropis* by Garba and Pasquet (1998a,b). The last species that remain to be studied within this group are *V. frutescens* A. Rich. from section Liebrechtsia, *V. friesiorum* Harms and *V. membranacea* A. Rich. from section Macrodontae.

Studying species from both these sections together is not irrational. Both *V. frutescens* and *V. membranacea* share the same chromosome number $2n = 20$ (Sen and Bhowal, 1960; Maréchal et al., 1978), and Maréchal et al. noted about section Macrodontae: "relativement bien isolée, cette section présente un maximum de similitude avec la section Liebrechtsia". Both sections are separated by their style beak, long in section Liebrechtsia, short in section Macrodontae, and by their flowering phenology, i.e. pyrophytic in section Liebrechtsia (Verdcourt, 1971; Maréchal et al., 1978).

Section Liebrechtsia includes a single species, *V. frutescens*, and section Macrodontae includes two species separated on the basis of their habit. *V. friesiorum* is an erect or decumbent herb, whereas *V. membranacea* is a climber (Verdcourt, 1970). Before Verdcourt's work, taxonomy of these groups was fairly complicated. More than 30 species were described between 1847 and 1947, most of these now in synonymy with *V. frutescens*, *V. friesiorum*, or *V. membranacea*. Similar situations were found in *V. unguiculata* (Pasquet, 1993a) and *V. vexillata* (Garba and Pasquet, 1998b).

V. frutescens is divided into three subspecies (subsp. *frutescens*, subsp. *incana* (Taub.) Verdc., subsp. *kotschyi* (Schweinf.) Verdc.) according to calyx-lobe length and standard pubescence, and *V. membranacea* is recognised as having four subspecies (subsp. *membranacea*, subsp. *caesia* (Chiov.) Verdc., subsp. *hapalantha* (Harms) Verdc., subsp. *macrodon* (Robyns and Boutique) Verdc.) according to flower size and calyx-lobe length (Verdcourt, 1970) (Table 1). However, the characters concerned did not include ovule number and keel shape variations (although Verdcourt, 1970, noticed keel shape variation) which are important for discriminating subspecies of *V. unguiculata* (Pasquet, 1993a). One can also note in Table 1 that *V. friesiorum* and *V. membranacea* subsp. *hapalantha* are morphologically very close if only flower sizes and calyx-lobe lengths are considered, as in Verdcourt (1970).

Table 1
Morphological characters of the different taxa

	Germination	Rootstock	Flower size in mm	Calyx-lobe length in mm	Keel shape	Ovule number	Seed size
V. frutescens							
subsp. *frutescens*	Hypogeal	Yes	15–27	1–10	Straight	11–23	Large
V. friesiorum	Hypogeal	Yes	9–15	1.5–3	Straight	16–23	Small
Northern Kenya specimens	?	Yes	10–14	2–3	Straight	10–13	?
V. membranacea							
subsp. *membranacea*	Epigeal	No	10–15	5–9	Straight	15–23	Large
subsp. *caesia*	Hypogeal/intermediate	Yes	17–24	1.5–7	Straight	20–28	Small
subsp. *hapalantha*	Epigeal	No	12–13	1–2	Straight	13–15	Small
Subsp. *macrodon*	?	No	15–25	8–17	Twisted	13–18	Small
Red Sea hills specimens	?	?	12	4–5	?	10	?

Regarding biogeography, *V. frutescens* is an almost panafrican taxon, encountered from Nigeria to Ethiopia and South Africa (Maréchal et al., 1978), while *V. membranacea* and *V. friesiorum* are restricted to northeast Africa, i.e. mainly Ethiopia and Kenya (Verdcourt, 1970).

With the above as an introduction, the objective of the present research is to assess the genetic distances between these taxa using isozymes, and to investigate their systematic relationships.

2. Material and methods

2.1. Plant material

The 21 accessions used in this study are presented in Table 2. These accessions represented all the available material at the time of the study. Accessions were from *V. frutescens* (7), *V. friesiorum* (1), *V. membranacea* subsp. *caesia* (4), subsp. *hapalantha* (4) and subsp. *membranacea* (4). Accession NI 1526 was not considered as a subsp. *caesia* accession since it displayed several *V. frutescens* (seed, germination, and general habit) and *V. membranacea* subsp. *caesia* (especially style beak) characters. Table 1 described some of the morphological features of the different taxa. Germination and rootstock were checked on the accessions studied. Flower sizes and calyx-lobe lengths were measured on a large number of herbarium specimens. Keel shapes, ovule numbers and seed sizes were assessed from the accessions noted here and from a small number of herbarium specimens.

The NI accessions were from the IPGRI base collection of wild *Phaseolus* and *Vigna* species maintained at the National Botanical Garden of Belgium, in Meise. Some of them were originally provided by ILRI, Addis Ababa, Ethiopia, and by CIAT, Cali, Columbia (FLG accessions). The MT accessions were collected by Mithen and are now duplicated in Meise. Each accession consisted of one to three autogamous lines, and were maintained as such, each of these lines originating from one seed of the original stock.

2.2. Procedure

Seeds were set out on water to imbibe for 24 h and then ground after removing the testa. The gels were prepared as described by Second and Trouslot (1980). The histidine/citrate system at pH 6.0 was used for all enzymes. The gel mixture contained 14% starch. The enzyme systems (ADH, AMP, ENP, EST, FLE, βGAL, FDH, GDH, G6PD, IDH, MDH, ME, MNR, MPI, PGD, PGI, PGM, SDH, and SOD) and the reference for staining procedures were those used with *V. reticulata* (Garba and Pasquet, 1998a). AMP was stained with leucine-β-naphtylamide (Amp2 and Amp3) or alanine-β-naphtylamide (Amp2 and Amp4), FLE and βGAL with derivatives of 4-methyl-umbelliferyl compounds.

For each enzymatic system, the presumed loci were numbered by increasing distance from the anode, with one exception. For IDH, however, following the isozyme patterns observed in *V. unguiculata* (Pasquet, 1993b), IDH2 designated the

Table 2
Accessions studied

Accession	Country	Latitude and longitude	Locality	Other accession number
V. membranacea subsp. *caesia*				
NI 918	KEN	3°37 S 39°51 E	km 1 Kilifi - > Tezo	
NI 919	KEN	3°36 S 39°51 E	km 2 Kilifi - > Malindi	
NI 925	KEN	3°53 S 39°47 E	km 30 Mombasa - > Malindi	
NI 1229	KEN	3°48 S 39°40 E	km 33 Mombasa - > North	
V. membranacea subsp. *hapalantha*				
NI 911	KEN	4°06 S 39°40 E	km 6 Mombasa - > North	
NI 915	KEN	4°06 S 39°40 E	km 6 Mombasa - > North	
NI 921	KEN	3°40 S 39°50 E	km 1 Kilifi - > Mombasa	
NI 953	KEN	3°25 S 39°54 E	km 28 Kilifi - > Malindi	
V. membranacea subsp. *membranacea*				
NI 1447	ETH	7°19 N 38°39 E	52 km S Abernossa Ranch	ILRI 7534
NI 1448	ETH	8°14 N 38 °29 E	Soddo area	ILRI 8780
NI 1222	ETH	12°31 N 37°02 E	km 4 Aykel - > West	
NI 1322	ZAR	2°25 N 28°55 E	Sake - Goma pass road	
Vigna friesiorum				
NI 1178				FLG 4573
Vigna frutescens				
NI 287	TZA	3°24 S 37°20 E	km 6 Moshi - > South	
NI 411	RWA		Bujesera distr, plateau Kyasa	
MT 195	ZMB	12°35 S 28°10 E		
MT 265	ZWE	17°45 S 31°08 E		
MT 267	ZMB	17°52 S 31°03 E		NI 1521
MT 269	ZWE	17°48 S 31°05 E		
MT 278	ZWE	18°18 S 30°53 E		
Not attributed to a particular taxon				
NI 1526	ETH	6°48 N 41°05 E	km 66 km Ghinir- > lmi	

band presenting the strongest activity. For each isozyme, the most common allele was designated as 100 and the other allozymes were measured in millimetres relative to that standard. This procedure was the same as utilised by Koenig and Gepts (1989) with *Phaseolus vulgaris* L.

The data from the enzymatic analysis has allowed the calculation of Nei's distances (1972). The UPGMA (Sneath and Sokal, 1973) was computed using the BIOSYS software version 1.7. The principal coordinate analysis was computed using the program ANADIS from ADDAD software version 89.1.

3. Results and discussion

3.1. Isozyme patterns

The 19 enzymatic systems enable the scoring of 29 loci. Isozyme patterns were very close to those observed in *V. reticulata* (Garba and Pasquet, 1998a), *V. unguiculata*

(Pasquet, 1993b; Vaillancourt et al., 1993b), and *V. vexillata* (Garba and Pasquet, 1998b).

FDH, ME, GDH, βGAL, EP and MPI appeared as unique bands; whereas Sdh and G6pd were expressed as double bands.

MNR pattern included two bands but MNR2 was not stained uniformly and was not scored. Similarly, SOD was expressed as one strong band, SOD2. SOD1 and SOD3 were stained poorly and not scored. Seed extracts had a single βEST band (EST3). A very faint fast αEST band was observed but not scored. Contrary to observations on *V. reticulata*, *V. unguiculata*, and *V. vexillata*, the β-EST band (EST3) is faster than the α-EST bands. FLE had two bands (FLE1 and FLE3), the slower lightly stained.

ADH was in the form of a heterodimer from two loci, Adh1 and Adh2. The fast monomer stained strongly, as it did in *V. reticulata* and *V. vexillata* and contrary to that in *V. unguiculata*. MDH presents four isozymes. The isozyme closest to the anode, MDH1, appeared as a weak band. Next, there were the homodimers and the heterodimer formed by Mdh2 and Mdh3 products. Then, MDH4, a strongly stained band did not migrate far from the cathode, as in *V. reticulata*, *V. unguiculata*, and *V. vexillata*, and contrary to the blue- and yellow-flowered *Vigna* (Pasquet and Vanderborght, 1999). IDH had three isozymes, but only the fast IDH2 was stained enough to be scored, as in *V. vexillata* and contrary to *V. reticulata* and *V. unguiculata*. Both PGD and PGM also had two isozymes and the PGM isozyme closer to the anode stained stronger. For AMP, AMP2, which cleaved both alanine- and leucine-β-naphtylamide showed greater staining. The substrate for AMP4 was alanine-β-naphtylamide. Pgi1 was supposed to be chloroplastic, as it is in cowpea (Vaillancourt et al., 1993b). Pgi2 and Pgi3 formed homodimers and an heterodimer. As in *V. unguiculata* and *V. vexillata*, and opposite to most blue- and yellow-flowered *Vigna* (Pasquet and Vanderborght, 1999), most of the staining occurred by the isozyme (PGI2) closest to the anode.

The *V. frutescens–V. friesiorum–V. membranacea* isozymes patterns which are close to those of *V. reticulata*, *V. unguiculata*, *V. vexillata* and remote from those of blue and yellow-flowered *Vigna* (Pasquet and Vanderborght, 1999) may be considered as one of the criteria for considering the opposition between pink or purple-flowered *Vigna* on the one hand and blue- and yellow flowered *Vigna* on the other hand.

3.2. Polymorphism

Mean gene diversity index (H), proportion of polymorphic loci (L), and number of alleles at polymorphic loci (A) are in Table 3 with allelic frequencies. Regarding all the accessions studied, total diversity ($H = 0.39$) was higher than that encountered in *V. reticulata* with 40 accessions ($H = 0.21$) (Garba and Pasquet, 1998a), in *V. vexillata* with 125 accessions ($H = 0.25$) (Garba and Pasquet, 1998b), and in wild *V. unguiculata* with 55 accessions ($H = 0.31$) (Pasquet, 1993b) or 186 accessions ($H = 0.29$) (Pasquet, 1999). Regarding each taxon, genetic diversities are in the range 0.06 (subsp. *caesia*) – 0.225 (subsp. *membranacea*). This could be an argument for considering several

species within this group of taxa. The low diversity within subsp. *caesia* is easily explained by the close geographic origin of the accessions.

3.3. Inter-taxa relationships

The UPGMA cluster phenogram based on Nei's distances (Fig. 1) and the Table 4 showed three sets of accessions: a first set including *V. frutescens*, *V. friesiorum*, subsp. *caesia*, and accession NI 1526, a second set including subsp. *hapalantha* accessions and a third set including subsp. *membranacea* accessions.

While accession NI 1178 (*V. friesiorum*) was close to subsp. *caesia* (Fig. 1, Table 4) and characterised by only two alleles not encountered elsewhere (Est3-103, Amp4-104) (Table 3), Nei's distances observed between taxa (Table 4) showed that subsp. *hapalantha* and subsp. *membranacea* were well isolated from the other taxa. Nei's distances were in the range of 0.329–0.916 between these three groups. Both subsp. *hapalantha* and subsp. *membranacea* were characterised by alleles not encountered elsewhere: Adh1-103, Sdh-99, Me-102, Pgd1-96, G6pd-106 for subsp. *membranacea*, Pgd2-96, G6pd-94, Pgi2-96 for subsp. *hapalantha*.

Accession NI 1526 was closer to *V. friesiorum* (0.414) than to *V. frutescens* (0.363–0.618) and subsp. *caesia* (0.546–0.609). It showed three alleles not encountered elsewhere (Mnr-98, Pgd2-98, and Pgi1-96), and, among alleles which separated the taxa, had more alleles in common with *V. membranacea* (Me-106, β-Gal-99, Pgi3-100, Mpi-100) and *V. friesiorum* (Gdh-96, Sdh-101), than with *V. frutescens* (G6pd-100, Est3-100, Amp2-103). Accession NI 1322, while merged with other subsp. *membranacea* accession through cluster analysis, was distant from all other taxa (0.517–0.684 with *V. frutescens*, 0.547 with *V. friesiorum*, 0.437–0.523 with subsp. *caesia*, 0.618–0.916 with subsp. *hapalantha* and 0.496–0.684 with subsp. *membranacea*). NI 1322 had several alleles not encountered elsewhere (Adh1-97, Me-96, Est3-89, β-Gal-97) and few alleles which usually characterise other taxa (G6pd-100, Gdh-102, Pgm2-100, Pgi3-100). NI 1526 and NI 1322 situations were better illustrated in the Principal Coordinate Analysis of Nei distances (Fig. 2). In this diagram, NI 1322 appeared closer to subsp. *membranacea* while NI 1526 appeared close to either *V. frutescens*, *V. friesiorum*, and subsp. *caesia*.

These results show inconsistencies with those of Vaillancourt et al. (1993a) and Vaillancourt and Weeden (1993), but this is mainly due to mistakes in the identification of the accessions they studied.

First, it appeared that some disturbances occurred during field collection. We studied accession MT 165 from seeds received from Harare but MT 165 was not a *V. frutescens* accession but one of *Macroptilium atropurpureum* (DC.) Urban.

Next, some misidentifications could have occurred during multiplication of part of the Mithen collection in Florida. As we received some accessions from both Harare and Florida, we were able to notice some of these errors. MT 265 seeds received from Harare were true *V. frutescens* seeds, while MT 265 seeds received from Florida belonged to *Macroptilium atropurpureum*. However, this could be due to a mix within original Zimbabwean seed lots and a better seed production of *M. atropurpureum* under Florida conditions.

Table 3
Allelic frequencies, mean gene diversity index (H), proportion of polymorphic loci (L), and number of alleles at polymorphic loci (A). for each group, the number of accessions studied is given in brackets

		Total (21)	*V. frutescens* (7)	*V. friesiorum* (1)	*V. membranacea* subsp. *caesia* (4)	*V. membranacea* subsp. *hapalantha* (4)	*V. membranacea* subsp. *membranacea* (4)	NI 1526
Adh1	103	0.143	0	0	0	0	0.75	0
	100	0.809	1	1	1	1	0	1
	97	0.048	0	0	0	0	0.25	0
Adh2	100	1	1	1	1	1	1	1
Amp2	103	0.333	0.715	1	0	0	0	1
	100	0.667	0.285	0	1	1	1	0
Amp4	104	0.047	0	1	0	0	0	0
	102	0.047	0	0	0	0	0.250	0
	100	0.667	0.857	0	1	1	0	0
	98	0.167	0.143	0	0	0	0.375	1
	96	0.071	0	0	0	0	0.375	0
Enp	103	0.119	0.357	0	0	0	0	
	101	0.286	0	1	0.25	0	1	0
	100	0.500	0.357	0	0.75	1	0	1
	98	0.047	0.143	0	0	0	0	0
	97	0.047	0.143	0	0	0	0	0
Est3	111	0.143	0	0	0.25	0.50	0	0
	109	0.143	0	0	0.75	0	0	0
	107	0.238	0	0	0	0.50	0.75	0
	105	0.071	0.214	0	0	0	0	0
	103	0.048	0	1	0	0	0	0
	100	0.309	0.786	0	0	0	0	1
	96	0.048	0	0	0	0	0.25	0
Fdh	100	0.762	0.857	0	1	0.75	0.50	1
	97	0.119	0.071	1	0	0.25	0	0
	94	0.119	0.071	0	0	0	0.50	0
Fle1	108	0.095	0	0	0	0	0.50	0
	100	0.619	0.857	1	1	0.25	0	1
	96	0.238	0	0	0	0.75	0.50	0
	92	0.048	0.143	0	0	0	0	0
Fle3	100	0.857	1	1	1	0.75	0.50	1
	92	0.143	0	0	0	0.25	0.50	0

βGal	104	0.143	0.428	0	0	0	0	0
	102	0.143	0.428	0	0	0	0	0
	100	0.476	0.143	1	1	1	0	0
	99	0.190	0	0	0	0	0.75	1
	97	0.048	0	0	0	0	0.25	0
Gdh	102	0.238	0	0	1	0	0.25	0
	100	0.667	1	0	0	1	0.75	0
	96	0.095	0	1	0	0	0	1
Gpd	106	0.143	0	0	0	0	0.75	0
	100	0.429	1	0	0	0	0.25	1
	97	0.238	0	1	1	0	0	0
	94	0.190	0	0	0	1	0	0
Idh2	100	0.548	1	1	0	0	0.625	1
	96	0.238	0	0	0	1	0.250	0
	92	0.214	0	0	1	0	0.125	0
Mdh1	100	1	1	1	1	1	1	1
Mdh2	100	1	1	1	1	1	1	1
Mdh3	100	1	1	1	1	1	1	1
Mdh4	100	0.952	0.857	1	1	1	1	1
	93	0.048	0.143	0	0	0	0	0
Me	108	0.167	0	0.5	0.750	0	0	0
	106	0.238	0	0	0	1	0	1
	102	0.167	0	0	0.125	0	0.75	0
	100	0.381	1	0.5	0.125	0	0	0
	96	0.047	0	0	0	0	0.25	0
Mnr1	102	0.0470	0.143	0	0	0	0	0
	100	0.905	0.857	1	1	1	1	0
	98	0.047	0	0	0	0	0	1
Mpi	103	0.071	0	0	0	0	0.375	0
	100	0.500	0	1	0.5	1	0.625	1
	98	0.119	0.071	0	0.5	0	0	0
	96	0.095	0.286	0	0	0	0	0
	94	0.214	0.642	0	0	0	0	0
Pgd1	100	0.810	1	1	1	1	0	1
	96	0.190	0	0	0	0	1	0
Pgd2	100	0.762	1	1	1	0	1	0
	98	0.048	0	0	0	0	0	1
	96	0.190	0	0	0	1	0	0

—continued overleaf

Table 3—*continued*

		Total	*V. frutescens*	*V. friesiorum*	*V. membranacea* subsp. *caesia*	*V. membranacea* subsp. *hapalantha*	*V. membranacea* subsp. *membranacea*	NI 1526
		(21)	(7)	(1)	(4)	(4)	(4)	
Pgi1	100	0.952	1	1	1	1	1	0
	96	0.048	0	0	0	0	0	1
Pgi2	104	0.047	0.071	0	0.125	0	0	0
	100	0.738	0.857	1	0.875	0	1	1
	96	0.190	0	0	1	0	0	0
	94	0.024	0.071	0	0	0	0	0
Pgi3	104	0.190	0.428	0	0	0.25	0	0
	102	0.190	0.143	0	0	0.75	0	0
	100	0.333	0	1	1	0	0.25	1
	96	0.286	0.428	0	0	0	0.75	0
Pgm1	100	0.976	0.928	1	1	1	1	1
	97	0.024	0.071	0	0	0	0	0
Pm2	105	0.190	0.143	0	0	0	0.75	0
	103	0.024	0.071	0	0	0	0	0
	100	0.643	0.643	1	1	0.50	0.25	1
	99	0.048	0.143	0	0	0	0	0
	97	0.095	0	0	0	0.50	0	0
Sdh	103	0.190	0	0	0	0.75	0.25	0
	101	0.095	0	1	0	0	0	1
	100	0.524	1	0	1	0	0	0
	99	0.143	0	0	0	0	0.75	0
	97	0.048	0	0	0	0.25	0	0
Sod	100	1	1	1	1	1	1	1
H		0.388	0.184		0.065	0.099	0.225	
L		0.83	0.48		0.17	0.24	0.52	
A		3.67	2.78		2.20	2.00	2.13	

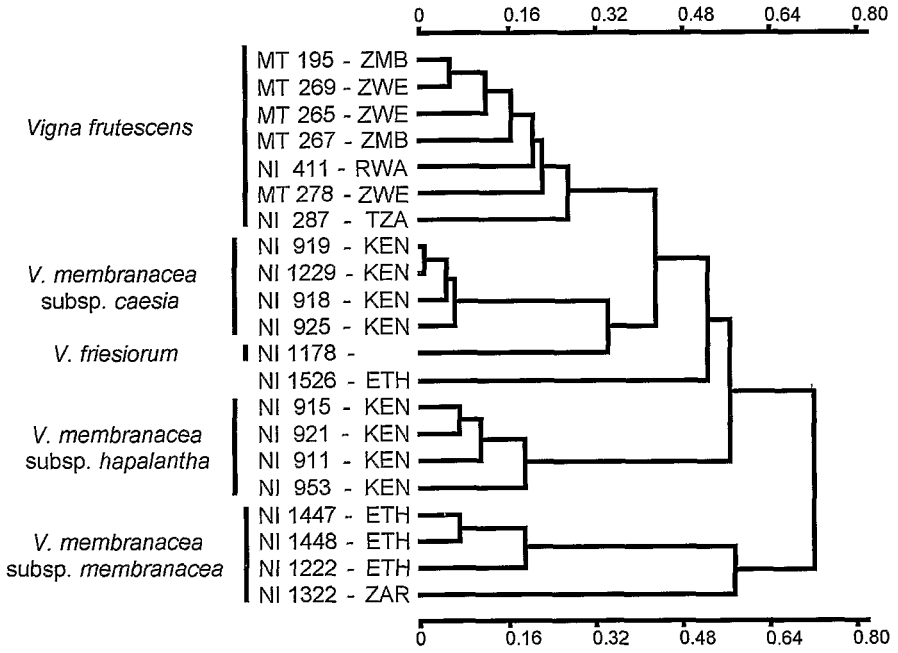

Fig. 1. Nei's distances UPGMA.

Vaillancourt and Weeden obtained their seeds from Florida. This may explain why they considered *V. frutescens* close to *Macroptilium purpureum* in their cpDNA study (Vaillancourt et al., 1993a) and why their results showed so much diversity within *V. frutescens* in their isozyme study (Vaillancourt and Weeden, 1993). Two of their accessions were true *V. frutescens* and one was *Macroptilium atropurpureum* (they reported that MT 267 showed more similarity with NI 411 than with MT 265).

However, despite this MT 265 misidentification, *V. frutescens* (mainly because of accessions NI 411 and MT 267) appeared close to *V. membranacea* and *V. friesiorum* in their isoenzyme study (Vaillancourt and Weeden, 1993).

Vigna frutescens, *V. friesiorum*, and *V. membranacea* subsp. *caesia* seem to be very closely related. This could be a criterion for merging these three taxa within one species; distances between these are lower than those encountered within *V. vexillata* (Garba and Pasquet, 1998b) or *V. unguiculata* (Pasquet, in press). The UPGMA (Fig. 1) showed *V. frutescens*, *V. friesiorum* and subsp. *caesia* intermixed within the same cluster, and the Principal Coordinate Analysis (Fig. 2) showed these three taxa grouped together as opposed to subsp. *membranacea* and subsp. *hapalantha*. This agrees with previous studies. Separation between *V. membranacea* and *V. friesiorum* was based only upon habital features (Verdcourt, 1970). While studying the same accessions we did not observe the difference in standard appendages reported by Maréchal et al. (1978). Intermediate herbarium specimens between *V. frutescens* and

Table 4
Nei's distances between accessions (minimum, average, and maximum) from same or different taxa. for each group, the number of accessions studied is given in brackets

	V. frutescens (7)	*V. friesiorum* (1)	*V. membranacea* subsb. *caesia* (4)	*V. membranacea* subsb. *hapalantha* (4)	*V. membranacea* subsb. *membranacea* (4)
V. frutescens (7)	0.055 0.209 0.305				
V. friesiorum (1)	0.423 0.484 0.642	-			
V. membranacea subsb. *caesia* (4)	0.321 0.421 0.511	0.297 0.348 0.371	0.010 0.050 0.084		
V. membranacea subsp. *hapalantha* (4)	0.525 0.624 0.729	0.586 0.618 0.651	0.329 0.447 0.609	0.071 0.144 0.276	
V. membranacea subsp. *membranacea* (4)	0.517 0.706 0.998	0.547 0.700 0.794	0.437 0.688 0.865	0.618 0.744 0.916	0.073 0.362 0.684
NI 1526 (1)	0.363 0.523 0.618	0.414	0.546 0.575 0.609	0.595 0.663 0.802	0.684 0.872 0.969

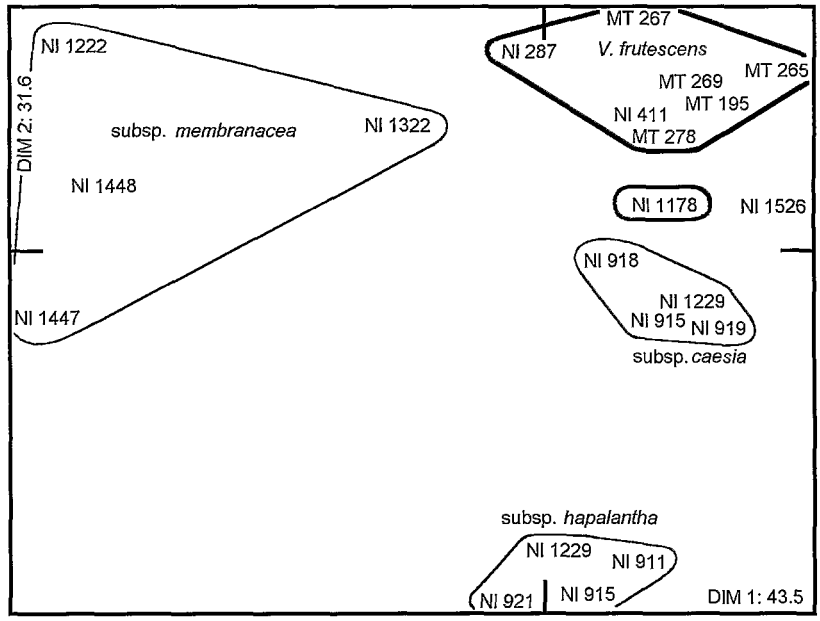

Fig. 2. Principal coordinate analysis.

V. membranacea were reported. For example, specimen Bally 9473 (EA) looks like a subsp. *caesia* specimen (including high ovule number) but shows long-beaked style and larger seeds of *V. frutescens* (Verdcourt, 1971). Similarly, the intermediate features displayed by accession NI 1526, obtained from seeds of specimen Gilbert, Enselmu and Vollesen 7994 (K), might be an argument for merging these species.

However, there are some reasons for considering the possible segregation of several species from within the *V. frutescens–V. friesiorum–V. membranacea* complex. First, Nei distances (Table 4) for subsp. *membranacea* and subsp. *hapalantha* are higher than those encountered within *V. unguiculata* (Pasquet, 1999) and within *V. vexillata* (Garba and Pasquet, 1998b). Moreover, since the grand mean Nei (1972) distance for populations of congeneric species is 0.4 (Crawford, 1989), it seems difficult to accept that two accessions with a distance of 0.916 belong to the same species. Next, all the accessions of subsp. *hapalantha* and subsp. *caesia* originated from the area where both taxa overlap alone. Although we studied only eight accessions, no indication of introgression was noted. Both groups of accessions showed no alleles in common for eight out of the 29 loci studied (Table 3). Additionally, no overlap was detected for flower size and ovule number data obtained from herbarium sheets. Instead, these data showed a marked gap for these characters (Table 1), and Verdcourt (1970), while not considering ovule number, reported that both taxa were clearly separated. Therefore, in our opinion, subsp. *membranacea* and subsp. *hapalantha* should be considered as different species. If this view is accepted, one might also consider the

remaining subspecies as specifically distinct: subsp. *membranacea*, subsp. *hapalantha* as well as subsp. *caesia*. However, this would be inconsistent with the morphological characters.

However, some material still needs investigation: subsp. *membranacea* and subsp. *macrodon* from Kenya and Uganda, subsp. *caesia* from Ethiopia and inland Kenya, an unnamed subspecies from the Red Sea hills characterised by its low ovule number (according to manuscript notes on Kew herbarium sheets), *V. friesiorum* from northern Kenya (as NI 1178 is morphologically closer to herbarium specimens from South Kenya and Tanzania), *V. frutescens* from Ethiopia, and especially populations from northern central Africa, i.e. Nigeria, Cameroon and Sudan.

Finally, without hybridisation data, these results are insufficient to define species limits within the *V. frutescens–V. friesiorum–V. membranacea* complex.

Nevertheless, the results presented here do show that the three species concerned are close enough to suggest that the section Liebrechtsia and Macrodontae should be merged with section Macrodontae. The following synonymy is proposed:

Section Macrodontae Harms in Engl., Pflanzenw. Afr. 3(1): 688 (1915)
genus Liebrechtsia De Wild., Ann. Mus. Congo, sér. 4, 1: 70 (1902) syn. nov.
Section Liebrechtsia (De Wild.) Bak. f., Leg. Trop. Afr.: 397 (1929) syn. nov.

This section is characterised by the following morphological characters: stipules bilobed at the base, tertiary nerves of the leaflets not parallel, flowers purple with symmetric standard and keel without prominent beak or marked pocket on left side.

Acknowledgements

We thank Jean Hanson (ILRI, Addis Ababa, Ethiopia) and Helen Moss (IBPGR, Harare, Zimbabwe) for providing some of the seed samples used in this study.

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