Genetic Resources and Crop Evolution **43:** 157–165, 1996.

Relationships of *Vigna unguiculata* (L.) Walp., *V. vexillata* (L.) A. Rich. and species of section *Vigna* based on isozyme variation

Gabriella Sonnante[1], Angela R. Piergiovanni[1], Quat N. Ng[2] & Pietro Perrino[1]
[1]*Germplasm Institute, CNR, Via Amendola, 165/A, 70126 Bari, Italy;* [2]*International Institute of Tropical Agriculture, Oyo Road, PMB 5320, Ibadan, Nigeria*

Received 5 April 1995; accepted in revised form 19 April 1995

Key words: cowpea, isozyme, Nei's genetic distance, relationship, variation, *Vigna* species

Summary

Isozyme variation in 25 accessions of wild and cultivated *Vigna unguiculata*, 49 accessions of seven wild species belonging to section *Vigna*, and 11 accessions of *V. vexillata* (subgenus *Plectrotropis*) was scored at 17 putative loci to assess genetic relationships within and among species. The wild species selected for this study are among those which carry important agronomical traits useful in cowpea (*V. unguiculata*) breeding programs. Allelic frequencies were calculated and Nei's genetic distances were obtained. Low levels of intraspecific variation were observed for *V. heterophylla, V. luteola* and *V. racemosa*, whereas the other species showed a higher polymorphism. *Vigna unguiculata* possessed intraspecific genetic distances comparable to those previously found by other authors. Most of the isozyme variation was apportioned among species. Although *V. luteola* and *V. marina* had an interspecific genetic distance resembling the range observed at intraspecific level, all the other species showed very high interspecific distances. *Vigna unguiculata* was relatively closer genetically to *V. vexillata* than to the species belonging to section *Vigna*.

Abbreviations: AUS – Australia; BDI – Burundi; BRA – Brazil; BWA – Botswana; CAF – Central African Republic; GHA – Ghana; CMR – Cameroon; COG – Congo; RI – Costa Rica; EGY – Egypt; ETE – Ethiopia; GAB – Gabon; GRC – Greece; ITA – Italy; KEN – Kenya; MOZ – Mozambique; NER – Niger; NGA – Nigeria; PAN – Panama; RWA – Rwanda; TCD – Chad; TZA – Tanzania; ZAF – South Africa; ZAR – Zaire; ZMB – Zambia.

Introduction

Vigna is a large diverse genus of more than 100 species, grouped in 7 subgenera, 4 of which (*Vigna, Haydonia, Plectrotropis* and *Macrorhyncha*) are distributed in Africa: the first two are endemic to that continent (Maréchal et al., 1978; Ng, 1990). Subgenus *Vigna* contains 9 sections; cowpea (*V. unguiculata*), the domesticated species widely cultivated in tropical Africa, belongs to the section *Catiang* and is believed to have originated in Africa (Ng & Maréchal, 1985).

The taxonomy of this genus is not completely satisfactory. Recent studies, in fact, report new taxa (Pienaar, 1992) or revise both the *V. unguiculata* species complex (Ng, 1995; Padulosi, 1993; Pasquet, 1993b) and the section *Catiang* (Jaaska & Jaaska, 1988). Furthermore, the secondary genepool of *V. unguiculata* has not been defined yet (Ng & Padulosi, 1991). Further knowledge of the genetic relationships between cowpea and other related taxa would be useful, because several wild species carry traits for resistance to biotic and abiotic stresses and thus represent a source of important genes for cowpea breeding. In particular, section *Vigna*, belonging to subgenus *Vigna*, contains 20 African species, at least two of which (*V. luteola* and *V. oblongifolia*) are highly resistant to the cowpea storage weevil (Ng, 1990), whereas *V. marina* exhibits resistance to salty environments (Padulosi & Ng, 1993). Moreover, several accessions of *V. vexillata* (subgenus *Plectrotropis*) show high levels of resistance to some major pests of cowpea, due to their epidermal

vestiture and secondary plant metabolites (Chiang & Singh, 1988; Fatokun & Singh, 1987; IITA, 1988).

The placement of individuals into different subgenera, sections, species and subspecies is primarily based on morphological attributes that do not necessarily reflect real genetic relationships. To better define these relationships in the genus *Vigna*, cytogenetic (Galasso et al., 1993), biochemical (Paino D'Urzo et al., 1990) and RFLP (Fatokun et al., 1993) studies have been reported recently. Isozyme analysis may also clarify taxonomic and phylogenetic relationships in plants (Mowrey & Werner, 1990; Lu & Pickersgill, 1993; Jaaska, 1994). Two taxonomic studies have been carried out on *Vigna* species using isozyme data: Jaaska and Jaaska (1988) tested the enzyme systems GOT and SOD to assess variation in species of *Vigna* and *Phaseolus*, while Vaillancourt and Weeden (1993) evaluated the degree of similarity among eight species belonging to all the six sections of subgenus *Vigna*.

The objective of this investigation was to study systematic relationships, based on the analysis of seventeen isozyme loci, within and among *V. unguiculata*, *V. vexillata*, and seven species belonging to section *Vigna*.

Materials and methods

Plant material

The accessions analysed were provided by IITA (Ibadan, Nigeria) and University of Gembloux (Belgium). Some of the *V. unguiculata* were obtained from the Germplasm Institute (Bari, Italy). Table 1 lists accessions analysed. For the subgenus *Vigna* section *Catiang*, both cultivated (*V. unguiculata* subsp. *unguiculata*) and wild cowpea (subsp. *dekindtiana*) were considered. *Vigna racemosa*, *V. oblongifolia*, *V. ambacensis* Bak., *V. luteola*, *V. marina*, *V. gracilis*, and *V. heterophylla* are species in the subgenus *Vigna* section *Vigna* which were analysed. Finally, for subgenus *Plectrotropis*, *V. vexillata* was considered. The accessions tested were chosen so that different geographical regions were represented. From 4 to 8 plants per accession were analysed.

Isozyme analysis

To analyze the enzyme systems listed in Table 2, seeds were germinated in Petri dishes at 24 °C, then transplanted to, and grown in, a greenhouse. After 4–6 weeks, active growing leaves were collected and extracted according to Bringhurst et al. (1981). Leaf extracts were aliquoted and stored at −80 °C. Paper wicks were soaked in the extracts and loaded on a 11% starch, 2% sucrose gel. Gel and electrode buffers were prepared as in Gepts et al. (1992), adapted from Selander et al. (1971) and Cardy et al. (1980). A lithium hydroxide-tris citrate buffer pH 8.3 was used for the enzyme systems AAT and ME, while the other enzymes were analysed by a histitine-citrate buffer pH 6.5. The electrophoretic run was carried out under the conditions reported by Panella & Gepts (1992). Enzymes AAT, G6PD, IDH, MDH, ME and PRX were stained according to Panella & Gepts (1992); DIA, SKD and SOD following Wendel and Weeden (1989).

Data analysis

After staining, bands were scored and alleles were assigned to the putative loci which were designated sequentially starting from the anodal end.

Allelic frequencies were determined for each accession and intraspecific genetic distances were calculated according to Nei (1972). Homologous isozymes between species were detected using band position and intensity. To evaluate interspecific distances, frequencies of each allozyme were averaged over accessions belonging to the same species.

The UPGMA clustering method (Sneath & Sokal, 1973; Sokal & Sneath, 1963) grouped accessions and species on the basis of Nei's genetic distance.

Results and discussion

One locus was scored for IDH, ME and SKD, two zones of activity were observed for the enzymes AAT, DIA, G6PD and PRX; three isozymes were scored for MDH and SOD. Representative electrophoretic profiles showing isozyme variation for the systems ME, IDH and G6PD are shown in Figure 1.

In total, 158 allozymes were observed for all polymorphic loci. The distribution of alleles per locus and species is shown in Table 3. For each locus, alleles are labelled by the first letter of the species name in which they were first observed (e.g. u, u′ are two allozymes for a polymorphic locus in *V. unguiculata*); when the same allele is shared by two or more species, the same letter is used. The absence of bands for a locus was considered as a null allele. Of the 17 putative loci

Table 1. List of the material analysed

Number	*Vigna* sp.	Subsp.	Botanical variety	Origin
1 MG 103236	*unguiculata* (L.) Walp	*unguiculata*		ITA
2 MG 103264	"	"		"
3 MG 103442	"	"		"
4 MG 103464	"	"		ETE
5 MG 106811	"	"		GRC
6 MG 106819	"	"		"
7 MG 110844	"	"		EGY
8 MG 110845	"	"		"
9 MG 110846	"	"		"
10 IT 82D716	"	"		NGA
11 IT 81D994	"	"		"
12 IT 81D1137	"	"		"
13 IT 81D1151	"	"		"
14 TVu 2027	"	"		"
15 MG 112920	"	"		ETE
16 MG 113016	"	"		"
17 MG 113017	"	"		"
18 MG 113018	"	"		"
19 MG 113107	"	"		NGA
20 MG 112989	"	*dekindtiana* (Harms) Verdc.	*pubescens* (Wilcz.) Marech et al.	TZA
21 MG 116102	"	"	"	
22 MG 116103	"	"	"	
23 MG 116105	"	"	*protracta* (Mey.) Verdc.)	
24 MG 116106	"	"		
25 MG 116108	"	"	*mensensis* (Schweinf.) Marech. et al.	
26 NI 339	*vexillata* (L.) A. Rich.		*macrosperma* Marech. et al.	CRI
27 NI 336	"		*vexillata*	CRI
28 NI 557	"		*vexillata*	ZAF
29 NI 620	"		*angustifolia* (Schum. et Thonn.) Bak.	AUS
30 NI 827	"		*vexillata*	BRA
31 NI 932	"		*angustifolia*	PAN
32 TVNu 240	"			CAF
33 TVNu 292	"			TZA
34 TVNu 593	"			NE R
35 TVNu 635	"			COG
36 TVNu 719	"			BWA
37 NI 200	*luteola* (Jacq.) Benth.			TCD
38 NI 326	"			BRA
39 TVNu 172	"			"
40 TVNu 254	"			CAF
41 TVNu 500	"			BWA
42 TVNu 1174	*marina* (Burm.) Merrill			GAB
43 TVNu 1179	"			GAB
44 TVNu 1386	"			GAB
45 TVNu 1441	"			MOZ
46 NI 440	*ambacensis* Bak.		*ambacensis*	ZAR
47 NI 464	"		"	ZAR

Table 1. Continued

Number	*Vigna* sp.	Subsp.	Botanical variety	Origin
48 NI 997	"		*pubigera* (Bak.) Marech. et al.	NGA
49 NI 1371	"		*ambacensis*	CMR
50 TVNu 11	"			ZAR
51 TVNu 147	"			GHA
52 NI 282	*oblongifolia* A. Rich.		*parviflora* (Bak.) Verdc.	TZA
53 NI 123	"		*oblongifolia*	KEN
54 NI 335	"		"	CRI
55 NI 387	"		*parviflora*	RWA
56 NI 389	"		"	RWA
57 NI 461	"		*oblongifolia*	ZAR
58 NI 777	"		"	NGA
59 NI 954	"		"	ZMB
60 NI 1173	*gracilis* (Guill. et Perr.) Hook. f.			CMR
61 TVNu 173	"			RWA
62 TVNu 1120	"			COG
63 TVNu 1180	"			GAB
64 NI 239	*racemosa* (G. Don) Hutch. et Dalz.		*racemosa*	ZAR
65 NI 447	"		"	ZAR
66 NI 815	"		"	NGA
67 NI 995	"		"	NGA
68 NI 996	"		"	NGA
69 NI 1245	"		"	BDI
70 NI 1250	"		"	BDI
71 NI 1254	"		"	BDI
72 NI 1446	"		"	CMR
73 NI 122	*heterophylla* A. Rich.			KEN
74 TVNu 19	"			

Country abbreviations from FAO/IBPGR (1973).
Note. Codes of accessions are those used by the donor genebank. MG accessions are from the Germplasm Institute (Italy); IT, TVu and TVNu accessions are from IITA (Nigeria); NI accessions are from the University of Gembloux (Belgium).

Table 2. Enzyme systems tested and their international code

AAT	Aspartate amino-transferase	E.C. 2.6.1.1
DIA	Diaphorase	E.C. 1.6.4.3
G6PD	Glucose-6-phosphate dehydrogenase	E.C. 1.1.1.49
IDH	Isocitrate dehydrogenase	E.C. 1.1.1.41
MDH	Malate dehydrogenase	E.C. 1.1.1.37
ME	Malic enzyme	E.C. 1.1.1.40
PRX	Peroxidase	E.C. 1.11.1.7
SKD	Shikimate dehydrogenase	E.C. 1.1.1.25
SOD	Superoxide dismutase	E.C. 1.15.1.1

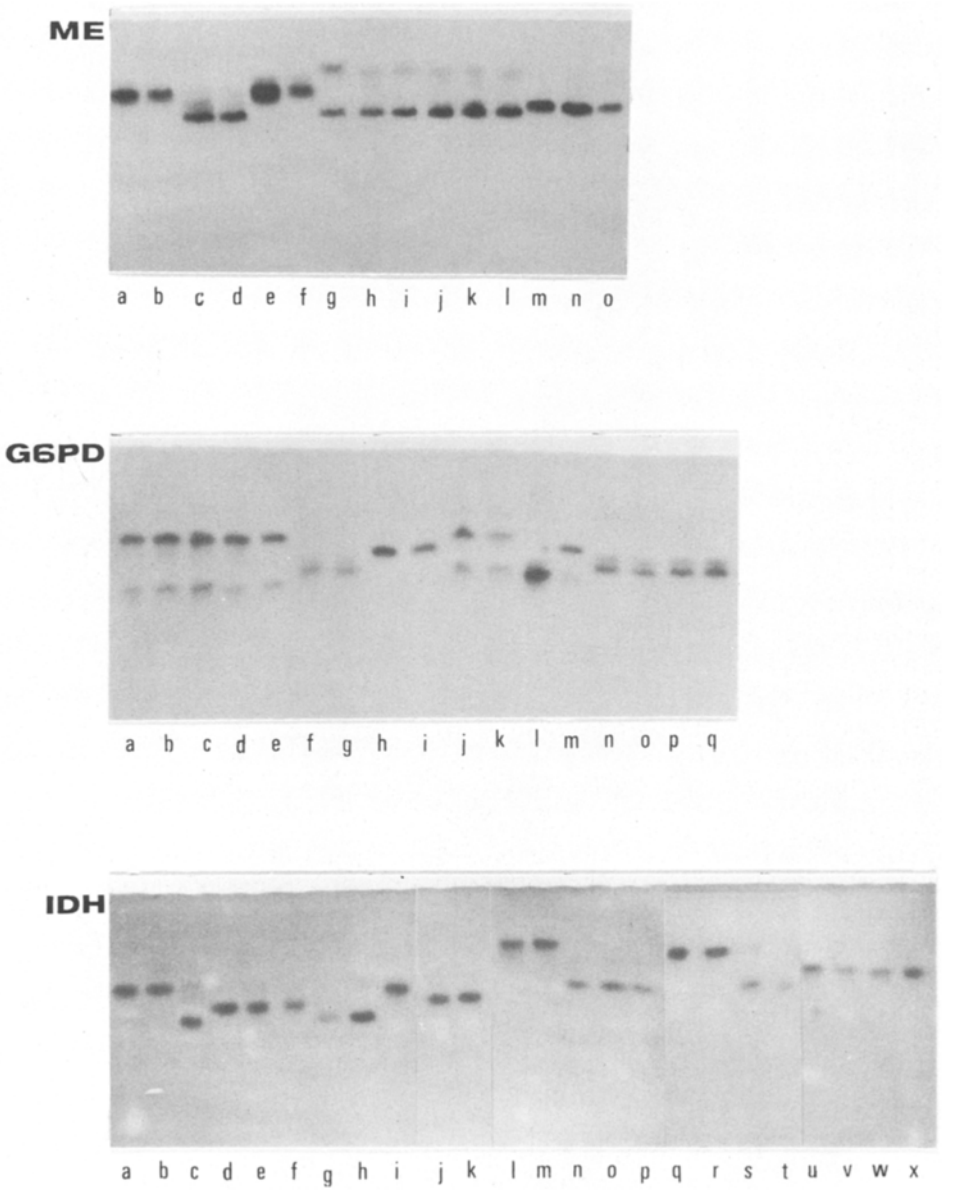

Fig. 1. Representative gel stainings for the enzymes: ME, G6PD, IDH. Samples are as follows. ME, lanes a–f: *V. gracilis*; g–l: *V. heterophylla*; m–o: *V. racemosa*. G6PD, lanes a–e: *V. racemosa*; f–g: *V. unguiculata*; h–m: *V. gracilis*; n–q: *V. heterophylla*. IDH, lanes a–b: *V. racemosa*; c–d: *V. marina*; e–f: *V. luteola*; g–i: *V. ambacensis*; j–k: *V. oblongifolia*; l–m: *V. unguiculata*; n–p: *V. vexillata*; q–t: *V. gracilis*; u–v: *V. heterophylla*; w–x: *V. racemosa*.

Table 3. Allele distribution per locus and species

	ungu	ungd	vex	lut	mar	amb	obl	rac	gra	het
Aat1	u	u	v	l	l m	a	o	o	o	h
Aat2	u u′	u u′	v v′	l	l	a a′	o o′	r	o o′ g	h
Dia1	u	u	v	l	l	a	u o	u	g g′	a
Dia2	u	u	v	l	l	a	o	r	g g′ g″	a
G6pd1	u	u	v v′	l	l	l	o o′	r	g g′	h
G6pd2	u u′	u u′	v	l	l	a	o o′	r	g g′	h
Idh	u u′	u	v	l	l m	a a′ m	o	a	g g′	h
Mdh1	u	u d	u v	u	u	u	u o	r	u	u
Mdh2	u u′	u u′	v	l	l	l a	l o	r	g	g
Mdh3	u	u	u	u	u m	u	u	u	u	u
Me	u	u	v v′	l	l	a a′	o o′	u	g g′	h
Prx1	u n	u d n	v	l	l	a a′	o	r	g g′	h
Prx2	u u′	u	u v	l	l	a a′	o o′	r r′ r″	g g′	r
Skd	u u′	u	v v′	l	l	a	o	r	g	l
Sod1	u u′	u d	v v′	l l′	m m′	a a′	o o′	r	g g′	h
Sod2	u	u	u	l	m	a	o	r	g g′	h
Sod3	u	u	u	u	u	u	u	u	u	u

n: null allele

ungu: *V. unguiculata unguiculata*; ungd: *V. unguiculata dekindtiana*; vex: *V. vexillata*; lut: *V. luteola*; mar: *V. marina*; amb: *V. ambacensis*; obl: *V. oblongifolia*; rac: *V. racemosa*; gra: *V. gracilis*; het: *V. heterophylla*.

scored in this study, 16 were polymorphic between species. The most variable locus among and within species was Sod-1, for which each species included two alleles (except for *V. racemosa* and *V. heterophylla* which were monomorphic), and all the allozymes were peculiar to one species. On the other hand, Sod-3 was monomorphic and Mdh-3 was polymorphic only for *V. marina*. *Vigna luteola* and *V. marina* shared a high number of alleles.

Table 4 summarizes levels of intraspecific variation by the proportion of polymorphic loci (P), the average number of alleles per locus (A) and the range of Nei's genetic distance within species.

Vigna heterophylla was monomorphic for all the loci under consideration but, in this case, only two accessions were analysed. Conversely, *V. gracilis* was the most diverse, with 11 polymorphic loci, a high average number of alleles per locus and remarkable intraspecific genetic distances (D). Very low variation was found within *V. luteola* with only one polymorphic locus and an average of 1.06 alleles per locus. The accessions of *V. racemosa* were also very similar, the range of D being 0.00–0.06 and with only one polymorphic locus. Both *V. oblongifolia* and *V. ambacensis* were more variable, with a proportion of polymorphic loci of 0.53 and 0.47 respectively, a similar range of intraspecific genetic distance and the same average number of alleles per locus (1.53 each). *Vigna vexillata* revealed a range of intraspecific genetic distance of 0.00–0.15, and a quite high proportion of polymorphic loci (0.43) and average number of alleles per locus (1.43). *Vigna unguiculata* showed intraspecific genetic distances comparable to those obtained by oth-

Table 4. Proportion of polymorphic loci (P), average number of alleles per locus (A) and range of intraspecific Nei's genetic distances (D)

Species	P	A	D
V. heterophylla	0.00	1.00	0.00–0.00
V. luteola	0.06	1.06	0.00–0.06
V. racemosa	0.06	1.12	0.00–0.06
V. marina	0.18	1.18	0.00–0.27
V. vexillata	0.43	1.43	0.00–0.15
V. ambacensis	0.47	1.53	0.06–0.35
V. oblongifolia	0.53	1.53	0.06–0.43
V. unguiculata	0.53	1.65	0.00–0.33
V. gracilis	0.65	1.76	0.06–0.71

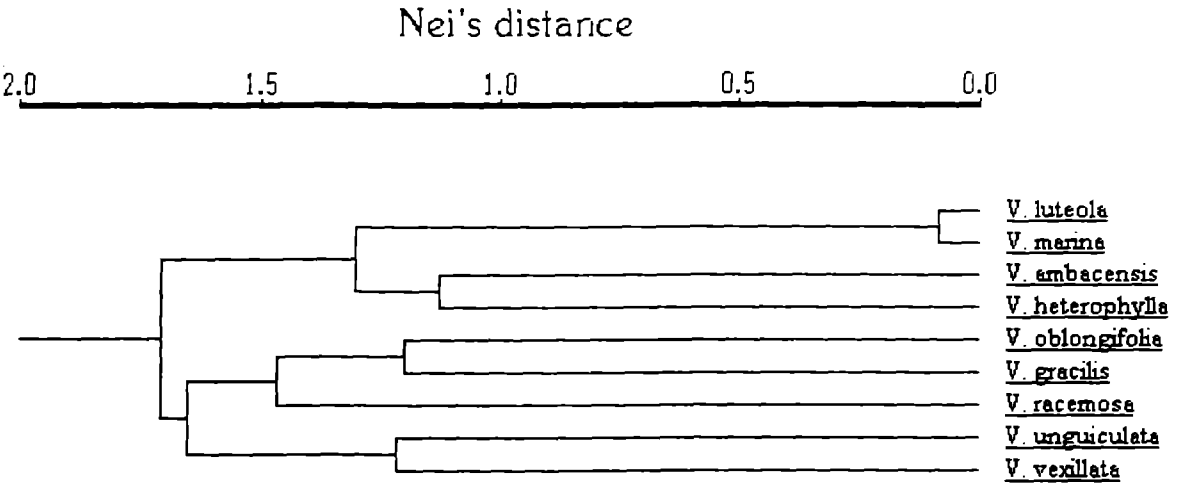

Fig. 2. Dendrogram of interspecific relationships based on Nei's genetic distances.

Table 5. Nei's genetic distances between *Vigna* species

Species	het	lut	rac	mar	vex	amb	obl	ung	gra
het	0.00								
lut	1.43	0.00							
rac	1.82	2.11	0.00						
mar	1.46	0.08	2.21	0.00					
vex	1.79	1.77	2.11	1.84	0.00				
amb	1.13	1.15	1.79	1.16	1.69	0.00			
obl	1.77	1.54	1.38	1.59	1.80	1.50	0.00		
ung	1.70	1.68	1.38	1.74	1.21	1.60	1.45	0.00	
gra	1.28	1.55	1.55	1.61	1.62	1.47	1.20	1.53	0.00

Abbreviations are as in Table 3. Ung includes both cultivated and wild cowpea.

er authors (Panella & Gepts, 1992), with a relatively high average number of alleles per locus (1.65) and proportion of polymorphic loci (0.53). Even though *V. unguiculata* is a self-fertilizing species, its proportion of polymorphic loci reveals to be much higher than the average value observed for 28 selfers (0.18) by Gottlieb (1981) and confirms that cowpea maintains more isozyme diversity than do other self-fertilizing species (Vaillancourt et al., 1993). In our study, both cultivated and wild cowpea were included in the calculation of these values. If considered separately, wild *V. unguiculata* showed a lower diversity compared to cultivated cowpea (e.g. six polymorphic loci versus eight). This can be attributed to the few accessions investigated in the present study, both for cultivated and, above all, for wild cowpea, compared to other studies which dealt with isozyme variation only in the *V. unguiculata* species complex (Panella & Gepts, 1992; Pasquet, 1993a; Vaillancourt et al., 1993).

When evaluating intraspecific variation, it was usually neither possible to establish correlations between geographical origin of accessions and their allozyme genotypes, nor to group accessions according to varieties. But, the three accessions of *V. marina* from Gabon (TVNu 1174, TVNu 1179, TVNu 1386) are allied much closer to one another than to the accession from Mozambique (TVNu 1441). The isozyme differences observed between the accessions from Western and from Eastern Africa seem to correspond to morphological differences which suggest the existence of two subspecies in *V. marina* (Padulosi & Ng, 1993).

The UPGMA dendrogram in Figure 2 groups the different species on the basis of Nei's genetic distances calculated for the 17 loci scored (Table 5).

Relatively high genetic distances between species were observed. Similar values were obtained by Vaillancourt and Weeden (1993) who analysed eight species of subgenus *Vigna*. Within the section *Vigna*, the species shared by their study and ours were *V. oblongifolia* and *V. luteola* which they found to be more closely allied than did our study. This can be explained by the different enzyme systems analysed and by the presence of different species in the clusters of the two papers. In the present study, the highest genetic distance (2.21) was found between *V. racemosa* and *V. marina*. Other plant genera belonging to the Leguminosae may behave differently. In fact, in the section *Arachis* of the genus *Arachis* Nei's genetic distance among species was low (between 0.00 and 0.45; Lu & Pickersgill, 1993). Conversely, among species of *Cicer*, distances ranging from 0.24 to 2.39 have been observed (Ahmed et al., 1992).

Vigna luteola and *V. marina* (Fig. 2) represented an interesting exception to the general pattern of genetic diversity, due to the very small distance (0.08) separating the two species. This value is comparable to those observed at intraspecific level and suggests that these taxa are genetically very close. In fact, *V. marina* and *V. luteola* are very similar morphologically (Padulosi & Ng, 1993), differing mainly by their habitat occupied: the former grows along seashores and the latter along fresh water shores.

The dendrogram (Fig. 2) also shows that *V. unguiculata* is isozymatically closer to *V. vexillata* of the subgenus *Plectrotropis*, than it is to the other species of subgenus *Vigna* section *Vigna*. However, the distance observed in the present study between *V. vexillata* and cowpea (1.21) is still high and these two species cannot be considered as closely related. A similar result was obtained by Vaillancourt & Weeden (1993) using partly different isozyme markers. Fatokun et al. (1993) constructed a dendrogram based on RFLP analysis of different species belonging to 4 subgenera of the genus *Vigna* and observed that *V. vexillata* was closer to subgenus *Vigna*, section *Catiang* (*V. unguiculata*) than it was to the Asiatic *Vigna* species. The very low genetic similarity (*D* ranging from 1.21 to 1.74) observed between cowpea and the other species considered in this study agrees with previous results. This could explain why all crosses attempted so far between cowpea and other species of the same subgenus or with *V. vexillata* have failed (Fatokun, 1991; Ng, 1990). The genetic divergence observed between cowpea and other species of subgenus *Vigna* led Jaaska and Jaaska (1988) to suggest that *V. unguiculata* be placed in a diferent subgenus, even though they used only two enzyme systems to analyse their material.

Vigna ambacensis and *V. heterophylla* formed a cluster relatively closer to that of *V. luteola* and *V. marina*, whereas *V. oblongifolia, V. gracilis* and *V. racemosa* were grouped separately, slightly closer to *V. unguiculata* and *V. vexillata*.

In general, the high distances observed for all the taxa examined may be due to the highly polymorphic loci analysed, for which the different species shared a very low number of allozymes (Table 3).

Conclusions

The high degree of isozyme divergence observed among the species of *Vigna* may suggest that these taxa diverged anciently. None of the species under consideration can be considered as closely related to the cultivated cowpea, therefore, attempts of gene transfer from these wild species into *V. unguiculata* by conventional crosses have very little probability of being successful.

Further studies at different levels and considering additional accessions are being performed to better clarify the relationships between *V. luteola* and *V. marina* and to assess whether they deserve the status of separate species.

References

Ahmad, F., P.M. Gaur & A.E. Slinkard, 1992. Isozyme polymorphism and phylogenetic interpretations in the genus *Cicer* L. Theor. Appl. Genet. 83: 620–627.

Bringhurst, R.S., S. Arulsekar, J.F. Hancock Jr. & V. Voth, 1981. Electrophoretic characterization of strawberry cultivars. J. Amer. Soc. Hort. Sci. 106: 684–687.

Cardy, B.J., C.W. Stuber & M.M. Goodman, 1980. Techniques for starch gel electrophoresis of enzymes from maize (*Zea mays* L.). Department of Statistics Mimeo Series, No. 1317, North Carolina State University, Raleigh, NC.

Chiang, H.S. & S.R. Singh, 1988. Pod hairs as a factor in *Vigna vexillata* resistance to the pod sucking bug. Entomologia Experimentalis et Applicata 47: 195–199.

FAO/IBPGR, 1973. Plant Genetic Resources Newsletter 49: 45–48.

Fatokun, C.A., 1991. Wide hybridization in cowpea; problems and prospects. Euphytica 54: 137–140.

Fatokun, C.A., D. Danesh, N.D. Young & E.L. Stewart, 1993. Molecular taxonomic relationships in the genus *Vigna* based on RFLP analysis. Theor. Appl. Genet. 86: 97–104.

Fatokun, C.A. & B.B. Singh, 1987. Interspecific hybridization between *Vigna pubescens* and *V. unguiculata* (L.) Walp. through embryo rescue. Plant Cell, Tissue and Organ Culture 9: 229–233.

Galasso, I., D. Pignone & P. Perrino, 1993. Cytotaxonomic studies in *Vigna*. II. Heterochromatin characterization in *Vigna unguiculata* and three related wild species. Caryologia 4: 275–282.

Gepts, P., V. Llaca, R.O. Nodari & L. Panella, 1992. Analysis of seed proteins, isozymes and RFLPs for genetic and evolutionary studies in *Phaseolus*. In: H.F. Linskens & J.F. Jackson (Eds.), pp. 63–93, Springer, Berlin Heidelberg, New York.

Gottlieb, L.D., 1981. Electrophoretic evidence and plant populations. In: L. Feinhold (Ed.) Progress in Phytochemistry, Vol. 7, pp. 1–46, Pergamon Press, New York.

International Institute of Tropical Agriculture, 1988. IITA Annual Report and Research Highlight 1978/1988, Ibadan, Nigeria.

Jaaska, V., 1994. Isoenzyme evidence on the systematics of *Hordeum* section *Marina* (Poaceae). Pl. Sys. Evol. 191: 213–226.

Jaaska, V., & V. Jaaska, 1988. Isoenzyme variation in the genera *Phaseolus* and *Vigna* (*Fabaceae*) in relation to their systematics: aspartate aminotransferase and superoxide dismutase. Pl. Syst. Evol. 159: 145–159.

Lu, J., & B. Pickersgill, 1993. Isozyme variation and species relationships in peanut and its wild relatives (*Arachis* L. – Leguminosae). Theor. Appl. Genet. 85: 550–560.

Maréchal, R., J.M. Mascherpa & F. Stainier, 1978. Etude taxonomique d'un groupe d'espèces des genres *Phaseolus* et *Vigna (V. unguiculata)* sur la base de données morphologiques et polliniqes, traitées pour l'analyse informatique. Boissiera 28: 1–273.

Mowrey, B.D. & D.J. Werner, 1990. Phylogenetic relationships among species of *Prunus* as inferred by isozyme markers. Theor. Appl. Genet. 80: 129–133.

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

Ng, N.Q., 1990. Recent developments in cowpea germplasm collection, conservation, evaluation, and reseearch at the Genetic Resources Unit, IITA. In: N.Q. Ng & L.M. Monti (Eds.), Cowpea Genetic Resources, pp. 13–28, IITA, Ibadan, Nigeria.

Ng, N.Q., 1995 (in press). Cowpea *Vigna unguiculata* (Leguminosae-Papilionideae). In: J. Smartt & N.W. Simmonds (Eds.), Evolution of crop plants, 2nd edition, Longman, England.

Ng, N.Q. & R. Maréchal, 1985. Cowpea taxonomy, origin and germplasm. In: S.R. Singh & R.O. Rachie (Eds.), Cowpea Research Production and Utilization, pp. 10–21, John Wiley & Sons, New York, NY.

Ng, N.Q. & S. Padulosi, 1991. Cowpea gene pool distribution and crop improvement. In: N.Q. Ng, P. Perrino, F. Attere & Z. Zedan (Eds.), Crop Genetic Resources of Africa, Vol. II, pp. 161–174, IITA, IBPGR, UNEP and CNR, The Trinity Press, UK.

Padulosi, S., 1993. Genetic diversity, taxonomy and ecogeographic survey of the wild relatives of cowpea (*Vigna unguiculata* (L.) Walp.). PhD Thesis, Faculty of Science, Université Catholique de Louvain-La Neuve, Belgique.

Padulosi, S. & N.Q. Ng, 1993. A useful and unexploited herb, *Vigna marina* (*Leguminosae-Papilionideae*) and the taxonomic revision of its genetic diversity. Bull. Jard. Bot. Nat. Belg. 62: 119–126.

Paino D'Urzo, M., M. Pedalino, S. Grillo, R. Rao & M. Tucci, 1990. Variability in major seed proteins in different *Vigna* species. In: N.Q. Ng & L.M. Monti (Eds.), Cowpea Genetic Resources, pp. 90–100, IITA, Ibadan, Nigeria.

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

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

Pasquet, R.S., 1993b. Classification infraspécifique des formes spontanées de *Vigna unguiculata* (L.) Walp. (Fabaceae) á partir des données morphologiques. Bull. Jard. Bot. Nat. Belg. 62: 127–173.

Pienaar, B.J., 1992. A taxonomic revision of the genus *Vigna* Savi (Fabaceae) in Southern Africa. Masters Thesis, Faculty of Science, University of Pretoria, South Africa.

Selander, R.K., M.H. Smith, S.Y. Yang, W.E. Johnson & J.B. Gentry, 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*: I. Variation in the old field mouse (*Peromyscus polionotus*). Univ. Tex. Pub. 7103: 49–90.

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

Sokal, R.R. & P.H.A. Sneath, 1963. Principles of numerical taxonomy. Freeman, San Francisco.

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

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

Wendel, J.F. & N.F. Weeden, 1989. Visualization and interpretation of plant isozymes. In: D.E. Soltis & P.M. Soltis (Eds.), Isozymes in Plant Biology, pp. 5–45, Dioscorides Press, Portland, Oregon.