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Genetic relationships within *Vigna unguiculata* (L.) Walp. based on isozyme analyses

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Summary

Isozyme analyses of genetic diversity in *Vigna unguiculata* were performed to determine genetic relationships and level of genetic diversity between wild and cultivated cowpea. Thirty-four cultivated accessions of *V. unguiculata*, 56 wild accessions of *V. unguiculata*, and six accessions representing five related wild *Vigna* species were analyzed. Ten enzyme systems were polymorphic within *Vigna unguiculata*: AAT, ACO, G6PDH, DIAP, LAP, MUE, ME MDH, PRX, and SOD. Fourteen of 24 putative loci (58%) were polymorphic within wild *V. unguiculata*, but only one locus (4%) was polymorphic within cultivated cowpea; when five related *Vigna* species were examined, 21 of the 24 bands of activity showed polymorphisms (88%) adding 33 alleles to the 48 identified within *V. unguiculata*. In one F_2 population of 68 plants (UCDVg 36 × UCDVg 21) a loose linkage was indicated between *Diap-2* and *G6pd-1* ($\chi^2 = 15.39$; $p = 0.004$) with an estimated distance of 36.0 cM ± 5.02 (recombination (r) = 0.31). Also in another F_2 population of 38 plants (CB 88 × UCDVg 21) a loose linkage was indicated between *Lap-1* and *Prx* ($\chi^2 = 9.62$; $p = 0.047$) with an estimated distance of 39.8 cM ± 7.0 ($r = 0.33$). Total genetic diversity (H_T) was 0.085 over all of the accessions including the one classified as *V. nervosa*. Within accession diversity (H_S) approached zero and between accession diversity (D_{ST}) was responsible for all of the genetic diversity present. Therefore the coefficient of gene differentiation ($G_{ST} = D_{ST}/H_T$) approached 1. Absolute gene differentiation (D_m) was 0.087. Two of the nine segregations in this study were skewed. In general, results of this study concurred with the taxonomic classification within *V. unguiculata* and provided a strong indication that a severe genetic bottleneck occurred during the domestication process of cowpea.

Abbreviations: AAT – aspartate amino-transferase, ACO – aconitase, ALD – aldolase, AUS – Australia, BDI – Burundi, BWA – Botswana, CHN – China, CMR – Cameroon, DIAP – diaphorase, DZA – Algeria, ETH – Ethiopia, G6PDH – glucose-6-phosphate dehydrogenase, GDH – glutamate dehydrogenase, GHA – Ghana, GUY – Guyana, IDH – isocitrate dehydrogenase, IND – India, KEN – Kenya, LAO – Laos, LAP – leucine aminopeptidase, MDH – malate dehydrogenase, ME – malic enzyme, MEX – Mexico, MOZ – Mozambique, MUE – methylumelliferyl-esterase, MWI – Malawi, MYS – Malaysia, NER – Niger, NGA – Nigeria, PRX – peroxidase, RBSC – ribulose-bisphosphate carboxylase, SEN – Senegal, SLE – Sierra Leone, SOD – superoxide dismutase, TGO – Togo, TZA – Tanzania, USA – United States of America, XDH – xanthine dehydrogenase, ZAF – South Africa, ZAR – Zaire, ZIM – Zimbabwe, ZMB – Zambia.

Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.), also known as blackeyed bean, blackeyed pea, field pea, southern pea, or crowder pea is grown on some 80,000 hectares in the US (Fery, 1990), which represents a small percentage of the total world production estimated in 1981 at 2.27 million tons harvested from 7.7 million hectares (Rachie, 1985). Cowpea is grown in Africa and

Table 1. Identification, source, taxonomy and origin of the materials used in this study

Number	Donor's number[a]	*Vigna unguiculata* ssp[b]	Cultigroup or botanical variety	Origin[c]
UCDVg 182	RM 53	*dekindtiana*		—
UCDVg 827	TVNu 3661	*dekindtiana*		ZAR
UCDVg 828	TVNu 3662	*dekindtiana*		ZAR
UCDVg 16	NI 794	*dekindtiana*	*coerulea*	NGA
UCDVg 36	NI 1171	*dekindtiana*	*coerulea*	—
UCDVg 38	NI 1228	*dekindtiana*	*coerulea*	KEN
UCDVg 4	NI 198	*dekindtiana*	*dekindtiana*	ZAR
UCDVg 10	NI 301	*dekindtiana*	*dekindtiana*	TZA
UCDVg 11	NI 320	*dekindtiana*	*dekindtiana*	ZAR
UCDVg 12	NI 423	*dekindtiana*	*dekindtiana*	ZMB
UCDVg 19	NI 874	*dekindtiana*	*dekindtiana*	ZIM
UCDVg 21	NI 933	*dekindtiana*	*dekindtiana*	GHA
UCDVg 22	NI 945	*dekindtiana*	*dekindtiana*	NER
UCDVg 24	NI 951	*dekindtiana*	*dekindtiana*	NGA
UCDVg 26	NI 963	*dekindtiana*	*dekindtiana*	SEN
UCDVg 27	NI 964	*dekindtiana*	*dekindtiana*	NGA
UCDVg 31	NI 991	*dekindtiana*	*dekindtiana*	NER
UCDVg 32	NI 993	*dekindtiana*	*dekindtiana*	NGA
UCDVg 33	NI 1026	*dekindtiana*	*dekindtiana*	NGA
UCDVg 35	NI 1167	*dekindtiana*	*dekindtiana*	—
UCDVg 40	NI 1232	*dekindtiana*	*dekindtiana*	BDI
UCDVg 46	RM 76	*dekindtiana*	*dekindtiana*	ZIM
UCDVg 48	RM 105	*dekindtiana*	*dekindtiana*	ZIM
UCDVg 49	RM 131	*dekindtiana*	*dekindtiana*	TZA
UCDVg 53	RM 340	*dekindtiana*	*dekindtiana*	MWI
UCDVg 66	RM 621	*dekindtiana*	*dekindtiana*	BWA
UCDVg 67	RM 623	*dekindtiana*	*dekindtiana*	BWA
UCDVg 45	RM 62	*dekindtiana*	*dekind/sten*	ZIM
UCDVg 47	RM 102	*dekindtiana*	*dekind/sten*	ZIM
UCDVg 50	RM 218	*dekindtiana*	*huillensis*	ZMB
UCDVg 58	RM 498	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 59	RM 499	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 60	RM 502	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 62	RM 546	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 63	RM 574	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 64	RM 593	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 65	RM 612	*dekindtiana*	*kgalagadiensis*	BWA
UCDVg 13	NI 456	*dekindtiana*	*mensensis*	BDI
UCDVg 52	RM 320	*dekindtiana*	*mensensis*	ZIM
UCDVg 56	RM 449	*dekindtiana*	*mensensis*	ZMB
UCDVg 57	RM 461	*dekindtiana*	*mensensis*	MWI
UCDVg 18	NI 856	*dekindtiana*	*protracta*	TZA
UCDVg 20	NI 910	*dekindtiana*	*protracta*	TZA
UCDVg 23	NI 947	*dekindtiana*	*protracta*	GHA
UCDVg 25	NI 957	*dekindtiana*	*protracta*	TZA
UCDVg 30	NI 979	*dekindtiana*	*protracta*	KEN
UCDVg 34	NI 1029	*dekindtiana*	*protracta*	TZA
UCDVg 39	NI 989	*dekindtiana*	*protracta*	KEN
UCDVg 68	TVNu 112	*dekindtiana*	*protracta*	—
UCDVg 131	TVNu 110	*dekindtiana*	*protracta*	—
UCDVg 44	RM 55	*dekindtiana*	*stenophylla*	ZIM
UCDVg 51	RM 319	*dekindtiana*	*stenophylla*	ZIM
UCDVg 41	RM 5	*dekindtiana*	*tenuis*	ZIM

Continued opposite

Table 1. – continued

Number	Donor's number[a]	*Vigna unguiculata* ssp[b]	Cultigroup or botanical variety	Origin[c]
UCDVg 42	RM 26	*dekindtiana*	*tenuis*	ZIM
UCDVg 43	RM 38	*dekindtiana*	*tenuis*	ZIM
UCDVg 55	RM 365	*dekindtiana*	*tenuis*	MWI
UCDVg 3	NI 147	*unguiculata*	*biflora*	—
UCDVg 14	NI 478	*unguiculata*	*biflora*	LAO
UCDVg 15	NI 778	*unguiculata*	*biflora*	IND
UCDVg 28	NI 972	*unguiculata*	*biflora*	ZMB
UCDVg 29	NI 973	*unguiculata*	*biflora*	ZMB
UCDVg 1	NI 126	*unguiculata*	*sesquipedalis*	GUY
UCDVg 2	NI 146	*unguiculata*	*sesquipedalis*	AUS
UCDVg 7	NI 262	*unguiculata*	*sesquipedalis*	CHN
UCDVg 8	NI 268	*unguiculata*	*sesquipedalis*	CHN
UCDVg 9	NI 269	*unguiculata*	*sesquipedalis*	CHN
UCDVg 37	NI 1184	*unguiculata*	*sesquipedalis*	LAO
UCDVg 829	CM	*unguiculata*	*sesquipedalis*	MYS
UCDVg 831	CM	*unguiculata*	*sesquipedalis*	MYS
UCDVg 833	CM	*unguiculata*	*sesquipedalis*	MYS
UCDVg 17	NI 816	*unguiculata*	*textilis*	TGO
UCDVg 5	NI 230	*unguiculata*	*unguiculata*	ZAR
UCDVg 6	NI 227	*unguiculata*	*unguiculata*	ZAR
UCDVg 136	TVu 9944	*unguiculata*	*unguiculata*	—
UCDVg 147	TVu 4742	*unguiculata*	*unguiculata*	NER
UCDVg 171	TVu 13536	*unguiculata*	*unguiculata*	BWA
UCDVg 173	TVu 7144	*unguiculata*	*unguiculata*	ETH
UCDVg 177	TVu 9063	*unguiculata*	*unguiculata*	IND
UCDVg 179	TVu 9069	*unguiculata*	*unguiculata*	IND
UCDVg 183	RM 481	*unguiculata*	*unguiculata*	—
UCDVg 185	LK 87RSP1	*unguiculata*	*unguiculata*	CMR
UCDVg 445	TVu 12347	*unguiculata*	*unguiculata*	MOZ
UCDVg 556	TVu 1643	*unguiculata*	*unguiculata*	ZAF
UCDVg 755	TVu 1476	*unguiculata*	*unguiculata*	DZA
UCDVg 771	TVu 10750	*unguiculata*	*unguiculata*	SLE
UCDVg 844	NSS V9	*unguiculata*	*unguiculata*	MEX
CB 46	IWB	*unguiculata*	*unguiculata*	USA
CB 88	IWB	*unguiculata*	*unguiculata*	USA
US 566	RF	*unguiculata*	*unguiculata*	USA
US 568	RF	*unguiculata*	*unguiculata*	USA

[a]Donor's identification: RM – Dr. R. Mithen; TVNu or TVu – Dr. N.Q. Ng; NI – Dr. R. Maréchal; CM – Dr. C. Mak; NSS – Native Seed Search; LK – Dr. L. Kitch; IWB – Dr. I.W. Buddenhagen; RF – Dr. R. Fery.
[b]Taxonomic classification was provided by the donor.
[c]Country abbreviations from FAO/IBRGR Plant Genetic Resources Newsletter, 1973, 49:45–48.

Asia for the most part as a sustenance crop – i.e. most of what has been grown does not reach commerce.

Cowpea, which belongs to the pan-tropical genus *Vigna* (also containing mung bean (*Vigna radiata* (L.) Wilczek), rice bean (*Vigna umbellata* (Thunb.) Ohwi & Ohashi), and adzuki bean (*Vigna angularis* (Willd.) Ohwi & Ohashi) – all of Asian origin), is now believed to have been domesticated in Africa. Different taxonomists have separated wild cowpeas at species, subspecies, and varietal levels (Baudoin & Maréchal, 1985). There have also been conflicting reports as to the fertility of hybrids among these wild taxa and between wild and cultivated cowpeas (Sakupwanya et al., 1989). Maréchal and his co-workers revised the taxonomy of the genus *Vigna* (Maréchal et al., 1978) based on plant morphology and pollen structure. They divided the species into four subspecies – three wild subspecies and one cultivated subspecies

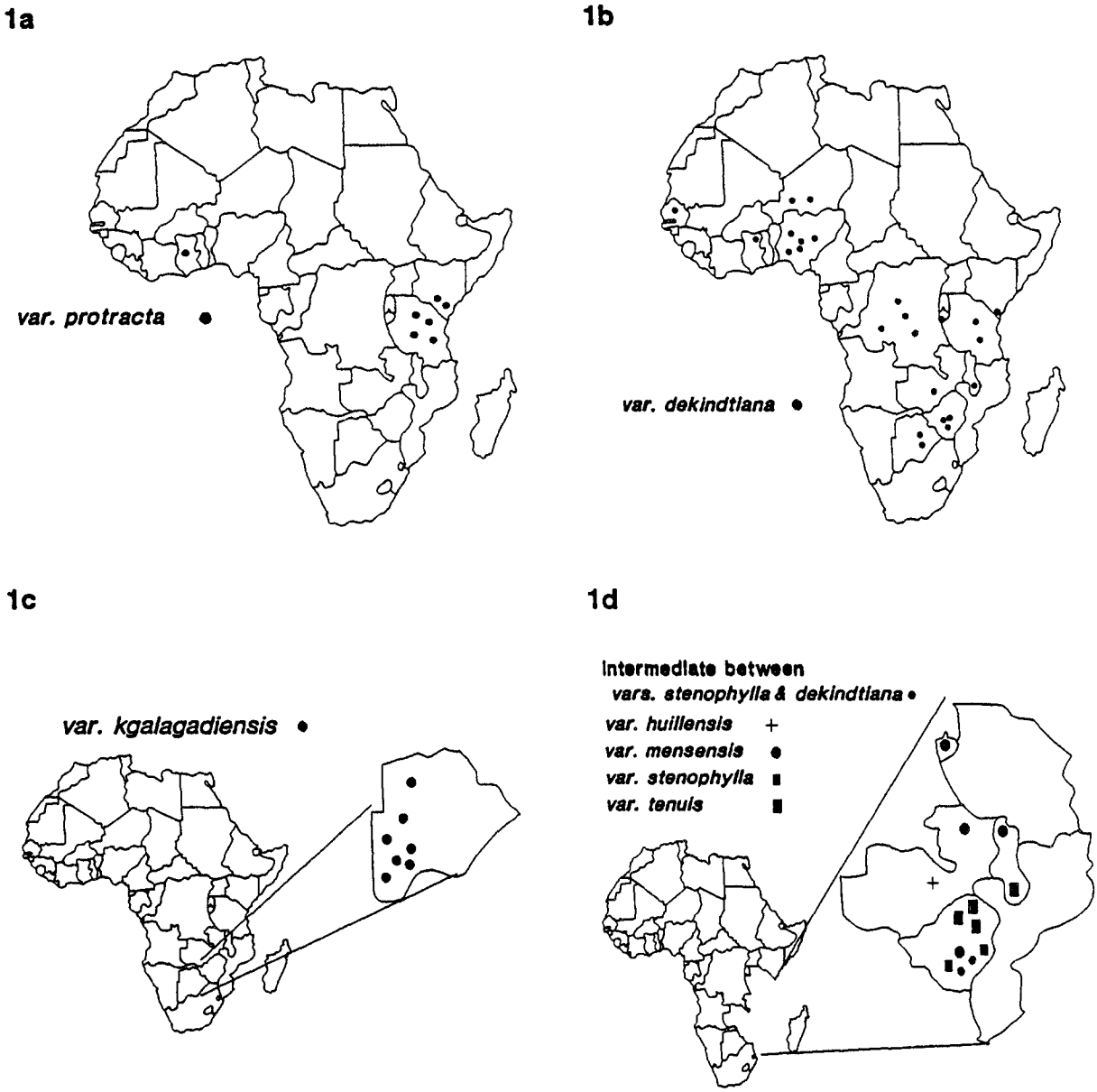

Fig 1. Approximate origins of *Vigna unguiculata* ssp. *dekindtiana* accessions.

containing four "cultivar groups" (cultigroups or cv-gr). Nonetheless, Mithen (1987) felt that this present intraspecies taxonomy did not reflect the variation found within the wild taxa of *Vigna unguiculata*. He recently divided the species into a wild and cultivated subspecies (Mithen & Kibblewhite, n.d.) following the gene pool model of Harlan & de Wet (1971). Among cultivated cowpeas, two cultigroups were distinguished by pod length and among wild subspecies, seven varieties were distinguished by morphological and physiological characteristics.

Starch gel electrophoresis of isoxymes has been used to explore the variation within many crop species as well as their evolutionary relationships (e.g., maize (*Zea mays* L.) – Cardy & Kannenberg, 1982; Doebley et al., 1984; rice (*Oryza* ssp.) – Second, 1982; *Triticeae* – McIntyre, 1988;

sorghum (*Sorghum bicolor* (L.) Moench) – Morden et al., 1989; common bean (*Phaseolus vulgaris* L.) – Koenig & Gepts, 1989b; and tepary bean (*Phaseolus acutifolius* A. Gray) – Garvin et al., 1989; Schinkel & Gepts, 1989). Isozyme and analyses have provided evidence to support the existence of two major centers of genetic diversity of common bean (Koenig & Gepts, 1989a; Singh et al., 1991b; Sprecher, 1988). Very few results of isozyme analyses within the species *Vigna unguiculata* have been published. Jaaska & Jaaska (1988) used two enzyme systems, amino aspartate transferase (AAT) and superoxide dismutase (SOD) to examine evolutionary variation between and within the genera *Phaseolus* and *Vigna*. Based on this study, they suggested placing cowpea in a separate subgenus, *Catiang*, within the genus *Vigna*. A preliminary report of a study using isozymes to measure diversity among *V. unguiculata* and related species was presented by Vaillancourt & Weeden (1990). Fifteen of 28 loci examined within *V. unguiculata* were found to be polymorphic. Forty-two cultivated accessions and 19 wild accessions of *V. unguiculata*, and 11 species of the subgenus *Vigna* were examined.

The objectives of the present study were to: (1) compare the levels of diversity among wild and cultivated cowpeas, (2) determine genetic distances among wild and cultivated cowpeas, and (3) provide additional information for the taxonomic classification within the species.

Materials and methods

Plant materials

Thirty-four cultivated accessions of *V. unguiculata*, 56 wild accessions of *V. unguiculata*, and six accessions representing five related wild *Vigna* species were analyzed. The cultivated accessions were chosen to represent a wide geographical and taxonomic range (Table 1) and all four of the cultivar groups described by Maréchal et al. (1978) were represented. Analysis of the seed protein banding patterns was also used to choose a diverse collection within cv-gr *unguiculata* (Panella & Gepts, 1990). All seven of the wild varieties described by Mithen & Kibblewhite (n.d.) were represented. The place of origin of each accession is provided whenever known (Fig. 1). Accession number, taxonomic classification as determined by the donor, and country where originally collected are listed in Table 1.

The two F_2 populations analyzed – CB 88 × UCDVg 21 and UCDVg 21 × UCDVg 36 – were the result of hand pollinations made in Davis among wild and cultivated materials grown in the greenhouse in the winter of 1988/89. The F_1 plants were grown in the greenhouse at Davis to prevent insect vectored cross pollination and the F_2 plants were grown in the greenhouse in 1990 and 1991. CB 88 is a large-seeded commercial blackeye variety grown in California and classified as *V. unguiculata* ssp. *unguiculata* cv-gr *unguiculata*. UCDVg 21 is a wild cowpea with large purple flowers and an open pollinated breeding system. UCDVg 36 is a wild cowpea with small purple flowers and is autogamous. Both of these wild cowpeas are classified as *V. unguiculata* ssp. *dekindtiana* with UCDVg 21 in var. *dekindtiana* and UCDVg 36 in var. *coerulea* (Table 1). Eighty-nine and sixty-nine individuals were analyzed from crosses CB 88 × UCDVg 21 and UCDVg 21 × UCDVg 36, respectively; however, it was not possible to score every individual for each segregating allozyme locus.

Isozyme analysis

Seeds used in the isozyme analyses had a portion of the raphe end of the seed removed for protein analysis; further scarification was unnecessary. For the small-seeded, wild accessions this represented approximately one-half of the seed; it was much smaller in proportion for the large-seeded, cultivated accessions. The small seeds were germinated in petri dishes in the laboratory and transplanted into six-packs filled with coarse vermiculite, four to six days after germination. The large, cultivated seeds were planted directly into the vermiculite-filled six-packs. The six-packs were placed on flats of sterilized sand in the greenhouse and watered daily with liquid fertilizer added to the water.

At three to five weeks of age, leaf tissue was harvested from newly expanding leaves and from the roots had grown into the sand and were washed clean before being harvested. After sampling, the seedlings were transplanted into six-inch pots and grown to maturity in the greenhouse. Whenever

Table 2. Enzyme systems assayed, loci, alleles, and stain recipes

Isozyme	Buffer[a]	Loci[b]	Alleles	Tissue[c]	Stain recipes
AAT Aspartate amino-transferase E.C. 2.6.1.1	L	Aat-1* Aat-2	100 100, 104, 96	L	25.0 ml 0.1 M Tris.HCl pH 8.0; 50 mg L-aspartic acid; 25 mg α-keto-glutaric acid; 2 mg Pyridoxal 5′-phosphate; 25.0 mg Fast Blue BB salt.[1]
ACO Aconitase E.C.4.2.1.3	H	Aco-1* Aco-2	100, 104, 0 100, 94, 0	L	25.0 ml 0.1 M Tris.HCl pH 8.0; 2.5 ml 0.1 M $MgCl_2$; trace of Meldola Blue; 4.0 mg MTT; 0.25 ml isocitrate dehydrogenase; 5.0 mg NADP; 25.0 mg cis-aconitic acid.[2]
ALD Aldolase E.C. 4.1.2.13	L	Ald*	100	L	10.0 ml 0.2 M Tris.HCl pH 8.0; 25.0 mg sodium arsenate (arsenic acid); 10.0 mg MTT; 80.0 U glyceraldehyde-3-phosphate dehydrogenase; 1.0 mg PMS; 10.0 mg NAD; 35.0 mg fructose-1,6-diphosphate.[1]
DIAP Diaphorase E.C. 1.6.4.3	L	Diap-1* Diap-2	100, 90 100, 107, 90	R	25.0 ml 0.1 M Tris.HCl pH 8.0; trace 2,6-dichlorophenol-indophenol (DCPIP); 7.0 mg NADH; 10.0 mg MTT.[1]
G6PDH Glucose-6-phosphate dehydrogenase E.C. 1.1.1.49	H	G6pd-1 G6pd-2*	100, 103, 0 100, 97, 0	R	25.0 ml 0.1 M Tris malate pH 7.2; trace of Meldola blue; 4.0 mg MTT; 4.0 mg NADP; 6.0 mg 6-phosphogluconate.[3]
GDH Glutamate dehydrogenase E.C. 1.4.1.2	L	Gdh*	100	L	25.0 ml 0.1 M Tris.HCl pH 7.0; trace of meldola Blue; 1.0 g glutamate (monosodium salt–glutamic acid); 4.0 mg NAD; 10.0 mg MTT.[2]
IDH Isocitrate dehydrogenase E.C. 1.1.1.41/42 [NAP/NADP forms]	H	Idh*	100	R	25.0 ml 0.1 M Tris.HCl pH 7.1; 0.25 ml 0.1 M $MnCl_2$; 30.0 mg sodium isocitrate (isocitric acid); trace of Meldola Blue; 4.0 mg MTT; 15.0 mg NADP.[1]
LAP Leucine aminopeptidase E.C. 3.4.11.1	L	Lap-1 Lap-3*	100, 98 100, 103, 95	L	25.0 ml 0.1 M potassium phosphate pH 6.0; 2.5 ml 0.1 M $MgCl_2$; 10.0 mg Fast Black K salt; 10.0 mg L-leucine β-napthylacride (in 1.0 ml NN-dimethyl-formamide).[3]
MDH Malate dehydrogenase E.C. 1.1.1.37	H	Mdh-1* Mdh-2*	100 100, 102	R	25.0 ml 0.1 M Tris-HCl pH 8.0; 20.0 mg L(−)malic acid; 4.0 mg MTT; trace of PMS; 10.0 mg NAD.[1]
ME Malic enzyme E.C. 1.1.1.40	L	Me*	100, 102, 98	R	25.0 ml 0.1 M Tris malate pH 7.2; trace of Meldola Blue; 10.0 mg L(−)malic acid; 3.0 mg MTT; 3.0 mg NADP.[4]
MUE Methylumbelliferyl-esterase E.C. 3.1.1.-	L	Mue-1* Mue-2*	100, 97, 93 100	L	10.0 ml 0.1 M potassium phosphate pH 6.0; 3.0 mg 4-methylumbelliferylacetate (in 1.0 ml acetate); bands are visible under UV light and develop within five minutes.[2]
PRX Peroxidase E.C. 1.11.1.7	L	Prx	100, 104	L	25.0 ml 0.1 M sodium acetate pH 5.0; 20.0 mg 3-amino-9-ethylcarbazole (in 3.0 ml NN-dimethyl-formamide); 6 drops of 30% H_2O_2.[1]

continued opposite

Table 2. – continued

Isozyme	Buffer[a]	Loci[b]	Alleles	Tissue[c]	Stain recipes
Rubisco Ribulose-bisphosphate carboxylase E.C. 4.1.1.39	L	Rbsc*	100	L	This appears as a red band on the LAP stained gel (unpublished data).
SOD Superoxide dismutase E.C. 1.15.1.1	H	Sod-1* Sod-2* Sod-3*	100, 95, 0 100 100	L	*SOLUTION A*: 25.0 ml 0.1 M sodium phosphate pH 7.1; 5.0 mg MTT *SOLUTION B*: 25.0 ml 0.1 M sodium phosphate pH 7.1; 1.0 mg Riboflavin; 0.1 ml TEMED. *To stain*: Mix just before use and pour solution A over gel slice. Incubate on shaker for 20 minutes in the dark. Drain off solution A and pour in solution B. Incubate under illumination until achromatic bands appear.[1]
XDH Xanthine dehydrogenase E.C. 1.1.1.204	H	Xdh*	100	R	25.0 ml 0.05 M Tris.HCl pH 8.0; 100.0 mg hypoxanthine; 5.0 mg MTT; trace of PMS; 15.0 mg NAD.[1]

[a]Gepts et al., 1991 adapted from Selander et al., 1971 (L: lithium hydroxide TRIS borate) and Cardy et al., 1980 (H: histidine citrate).
[b]Loci designations marked with an asterisk are tentative, pending identification through segregation analysis.
[c]Either leaf (L) or root (R) tissue was used.

[1]Adapted from Vallejos, E. 1983.
[2]Adapted from Weeden, N.F. and J.F. Wendel. 1989.
[3]Adapted from Shaw, C.R. and R. Prasad. 1970.
[4]Adapted from Brewer, G.J. 1970.

possible, seeds (resulting from self-pollination) and herbarium samples were preserved.

The tissue samples were macerated in a grinding buffer consisting of: 3.75% reduced glutathione adjusted to pH 7.6 with 1 M TRIS (unacidified). The resulting liquid was absorbed onto filter paper wicks which were subjected to electrophoresis in 10% starch gels (Gepts et al., 1992 – adapted from Weeden, 1984a). After examining 25 enzyme systems, 15 were judged to give the most consistent results. The systems with the tissue assayed, buffer system used, and stain recipes are listed in Table 2.

The loci were sequentially named with the most anodal band of activity being assigned the number 1 (e.g., *Lap-1*). The cowpea cultivar "California Blackeye 46" (CB 46) was run as a control and its alleles were designated as 10 (e.g., $Lap\text{-}1^{100}$). Other alleles were measured in millimeters migrated compared to the control (Koenig & Gepts, 1989b).

Genetic diversity analysis

Genetic diversity parameters of Nei (1973) such as genetic distance, total gene diversity, and inter- and intra-population diversity were calculated using computer programs obtained from Dr. K. Ritland (Department of Botany, University of Toronto, Canada). The program also generated a dendrogram using the unweighted pair group method with arithmetic means (Sneath & Sokal, 1973) based on Nei's distance. The LINKAGE-1 software from Suiter et al. (1983) was used to calculate linkage distances and chi-square values in segregating F_2 populations.

Results and discussion

Isozyme loci: detection, polymorphism, genetic control, and segregation

Ten enzyme systems were polymorphic within *Vigna unguiculata*: AAT, ACO, G6PDH, DIAP, LAO, MUE, ME, MDH, PRX, and SOD (Table 2). There were 24 putative loci evaluated and 14 showed polymorphism (58%) (Table 3) which is consistent with the observation of Hamrick and Godt (1990) that on average about 50% of a plant species' loci are polymorphic. Segregation of two F_2 populations was used to examine the genetic

Table 3. Isozyme constitution of the *Vigna unguiculata* accessions

UCD Vg #	*Aat-2*	*Aco-1*	*Aco-2*	*G6pd-1*	*G6pd-2*	*Diap-1*	*Diap-2*	*Lap-1*	*Lap-3*	*Mue-1*	*Me*	*Mdh-2*	*Prx*	*Sod-1*
182	100	0	100	0	100	100	100	98	100	100	—	100	100	100
827	104	100	100	100	100	100	100	100	100	100	100	100	100	100
828	100	100	100	—	—	100	100	100	100	100	—	100	100	100
16	96, 100	100	94	100	100	100	107	98	100	100	—	100	104	100
36	104	100	100	100	100	100	100	100	100	100	100	100	100	100
38	104	100	100	100	100	100	100	100	100	100	—	100	100	0
4	100	104	100	100	100	100	100	98	100	100	—	100	100	100
10	100	100	100	100	100	100	100	100	95	100	—	100	100	100
11	100	100	100	—	—	100	100	100	100	100	—	100	100	100
12	100	104	100	—	—	100	100	100	100	100	—	100	100	95
19	104	100	100	0	100	100	100	98	100	100	—	100	100	100
21	100	100	94	103	100	100	107	98	100	100	100	100	104	100
22	100, 104	100	100	100	100	100	100	100	100	100	100	100	100	100
24	100	0	100	100	100	100	100	100	100	—	—	100	100	100
26	100	100	100	100	100	100	100	100	100	—	—	100	100	100
27	100	100	100	100	100	100	100	—	—	100	100	100	100	100
31	100	0	100	100	100	100	100	—	—	100	—	100	100	100
32	100	100	94	100	100	100	107	98	100	100	—	100	104	100
33	100	100	100, 94	100	100	100	107	98	100	100	—	100	104	100
35	100	100	100	100	100	100	100	100	100	100	100	100	100	100
40	100	100	100	100	100	100	100	100	100	100	100	100	100	100
46	100	100	100	100, 0	100	100	100	100	100	100	100	100	100	100
48	100	100	100	100, 0	100	100	100	100	100	100	100	100	100	100
49	100	100	94	100	100	100	100	100	100	100	100	100	100	100
53	100	100	100	100	100	100	100	100	100	100	—	102	100	100
66	100	100	100	0	100	100	100	100	100	100	100	100	100	100
67	100, 104	100	100	0	100	100	100	100	100	100	100	100	100	100
45	100	100	100	100	100	90	90	98	100	100	—	100	100	100
47	100	100	100	—	—	100, 90	100, 90	100	100	100	—	100	100	100
50	100	100	100	100, 0	100, 0	100	100	100	100	100	102	100	100	100
58	100, 104	100	100	0	100	100	100	98	100	100	100	100	100	100
59	100, 104	100	94, 100	100	97	100	100	100	100	—	—	100	104	100
60	100	100	100	100	100	100	100	100	100	—	—	100	100	100
62	104	100	100, 94	100	97	100	100	100	100	100	—	100	100	100
63	104	100	100	100	97	100	100	100	100	100	—	100	100	100
64	104	100	100, 0	100	97	100	100	100	100	100	100	100	100	100
65	100, 104	100	100	0	100	100	100	100	100	93	100	100	100	100
13	100	100	100	100	100	100	100	98	103	100	—	100	100	100
52	100	100	100	—	—	100	100	100, 98	100	100	—	100	100	100
56	100	100	100	100	100	100	100	98	95	—	—	100	100	100
57	100	100	100	100	100	100	100	98	100	—	—	100	100	100
18	100	100	100	100	100	100	100	100	100	100	—	100	100	100
20	100	100	100	100	100	100	100	100	100	100	100	100	100	100
23	100	100	100	100	100	100	100	98	100	—	—	100	100	100
25	100	100	100	100	100	100	100	100	100	—	—	100	100	100
30	100	100	100	100	100	100	100	98	100	100	100	100	100	100
34	100	100	100	100	100	100	100	100	100	100	100	100	100	100
39	100	100	100	100	100	100	100	100	100	100	98	100	100	100
68	100	—	—	100	100	100	100	100	100	100	100	100	100	0
131	100	100	100	100	100	100	100	100	95	—	100	100	100	100
44	100	100	100	100	100	90	90	98	100	97	100	100	100	100
51	100	100	100	0	100	90	90	100	100	100	100	100	100	0
41	100	100	100	100	100	100	100	—	—	100	—	100	100	100
42	100	100	100	100	100	100	100	—	—	100	—	100	100	100

continued overleaf

Table 3. – continued

UCD Vg #	*Aat-2*	*Aco-1*	*Aco-2*	*G6pd-1*	*G6pd-2*	*Diap-1*	*Diap-2*	*Lap-1*	*Lap-3*	*Mue-1*	*Me*	*Mdh-2*	*Prx*	*Sod-1*
43	100	100	100	100	100	100	100	100	100	100	100	100	100	100
55	—	100	94	100	100	100	100	100	100	100	100	100	100	100
3	100	100	100	100	100	100	100	100	100	100	100	100	100	100
14	104	100	100	100	100	100	100	100	100	—	100	100	100	100
15	104	100	100	100	100	100	100	100	100	—	100	100	100	100
28	100	100	100	100	100	100	100	100	100	100	100	100	100	100
29	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	100	100	100	—	—	100	100	100	100	100	—	100	100	100
2	100	100	100	100	100	100	100	100	100	100	100	100	100	100
7	100	100	100	100	100	100	100	100	100	100	100	100	100	100
8	100	100	100	100	100	100	100	100	100	100	100	100	100	100
9	100	100	100	100	100	100	100	100	100	100	100	100	100	100
37	100	100	100	100	100	100	100	100	100	100	100	100	100	100
829	100	100	100	100	100	100	100	100	100	—	100	100	100	100
831	100	100	100	100	100	100	100	100	100	—	100	100	100	100
833	100	100	100	100	100	100	100	100	100	—	—	100	100	100
17	100	100	100	100	100	100	100	100	100	100	100	100	100	100
5	104	100	100	100	100	100	100	100	100	100	100	100	100	100
6	100	100	100	100	100	100	100	100	100	100	—	100	100	100
136	104	100	100	100	100	100	100	100	100	100	100	100	100	100
147	100	100	100	100	100	100	100	100	100	100	100	100	100	100
171	104	100	100	100	100	100	100	100	100	100	100	100	100	100
173	100	100	100	100	100	100	100	100	100	100	100	100	100	100
177	100	100	100	100	100	100	100	100	100	—	100	100	100	100
179	100	100	100	100	100	100	100	100	100	—	100	100	100	100
183	104	100	100	100	100	100	100	100	100	100	100	100	100	100
185	100	100	100	100	100	100	100	100	100	100	100	100	100	100
445	104	100	100	100	100	100	100	100	100	100	100	100	100	100
556	104	100	100	100	100	100	100	100	100	100	100	100	100	100
755	104	100	100	100	100	100	100	100	100	100	100	100	100	100
771	100, 104	100	100	100	100	100	100	100	100	100	100	100	100	100
844	104	100	100	100	100	100	100	100	100	—	100	100	100	100
CB 46	100	100	100	100	100	100	100	100	100	100	100	100	100	100
CB 88	100	100	100	100	100	100	100	100	100	100	100	100	100	100
US 566	100	100	100	100	100	100	100	100	100	100	100	100	100	100
US 568	100	100	100	100	100	100	100	100	100	100	100	100	100	100

control of loci *Aat-2*, *Diap-2*, *G6pd-1*, *Prx*, *Lap-1*, and *Aco-2* (Table 4). All other loci assignments are tentative pending confirmation by genetic analysis. When five related *Vigna* species were examined, 21 of the 24 bands of activity showed polymorphisms adding 33 alleles to the 48 identified within *V. unguiculata*. However, since these species do not hybridize, genetic confirmation is currently not possible. Three enzyme systems, GDH, XDH, and ALD, failed to show polymorphisms among the individuals tested. In the following paragraphs were summarized our observations for each enzyme system.

Aldolase (ALD). This enzyme was visualized as a single blurred band of activity (tentatively *Ald*) in Vigna (Fig. 2a). Weeden & Wendel (1989) reported that this enzyme is frequently represented by two tetrameric isozymes. Although reported to be polymorphic in pea (*Pisum sativum* L.) (Weeden & Gottlieb, 1980), this locus was monomorphic throughout *Vigna unguiculata* and the related *Vigna* species examined.

Glutamate dehydrogenase (GDH). This enzyme was visualized as a faint single band of activity (tentatively *Gdh*) in *Vigna* (Fig. 2a). Goodman & Stuber

Table 4. Single locus segregation data[a]

F_2 family	Segregation classes				Goodness of fit	
(P1P1 × P2P2)	P1P1	P1P2	P2P2	Total	χ^2	p
UCDVg 21 × UCDVg 36						
Aat-2	31	32	4	69	21.90	<0.001
Diap-2	10	36	23	69	5.03	0.081
G6pd-1	13	37	18	68	1.26	0.531
Prx	11	21	12	44	0.20	0.905
CB 88 × UCDVg 21						
Lap-1	20	40	25	85	0.88	0.643
Diap-2	21	42	22	85	0.03	0.983
G6pd-1	23	50	10	83	7.55	0.023
Aco-2	4	15	2	21	4.24	0.120
Prx	11	14	13	38	2.84	0.241

[a]All loci were tested against a 1:2:1 ratio.

(1983) reported two unlinked loci in maize and Quiros (1983) reported one band of activity present in the leaves of alfalfa (*Medicago sativa* L.). This locus was monomorphic throughout *V. unguiculata* and the related *Vigna* species examined.

Isocitrate dehydrogenase (IDH). This enzyme was visualized as a faint single band of activity (tentatively *Idh*) in *Vigna* (Fig. 2a). Kephart (1990) reported this as a dimeric enzyme coded by one locus in most plant species; however, Kiang & Gorman (1983) reported two bands in soybean (*Glycine max* L. Merill). This locus was monomorphic throughout *V. unguiculata*, but showed polymorphism in the related *Vigna* species examined.

Malic enzyme (ME). This enzyme was visualized as a strong band of activity (tentatively *Me*) in *Vigna* (Fig. 2a). Goodman & Stuber (1983) reported that it is a tetramer in maize. It was visualized as a single, strong band of activity in common bean (Weeden, 1984b). This locus was polymorphic and three alleles were identified within *V. unguiculata.*

Ribulose-bisphosphate carboxylase, small subunit (Rubisco). This enzyme was visualized as a single, red band of activity (tentatively *Rbsc*) in *Vigna* (Fig. 2a), cathodal to the *Lap-3* band, using the stain for LAP. This locus was monomorphic throughout *V. unguiculata*, but showed polymorphism in the related *Vigna* species examined.

Xanthine dehydrogenase (XDH). This enzyme was visualized as a faint single band of activity (tentatively *Xdh*) in *Vigna* (Fig. 2a). This enzyme is not often used in plant research; however, McLeod et al. (1983) reported a single locus with three alleles in pepper and its relatives (*Capsicum* sp.). This locus was monomorphic throughout *V. unguiculata* and the related *Vigna* species examined.

Aconitase (ACO). This enzyme was visualized as two bands of activity (*Aco-1, Aco-2*) close together (Fig. 2b) in *Vigna*. This agreed with what was reported in barley (*Hordeum vulgare* L.) by Brown (1983). Kephart (1990) reported that it is usually monomeric in plants. Segregation of *Aco-2* in one of the crosses (Table 4) could be explained by a monomeric enzyme (two bands of activity – $\frac{1}{2}\alpha$ and $\frac{1}{2}\beta$) segregating in typical Mendelian fashion. The other band of activity was tentatively assigned the locus *Aco-1* pending genetic confirmation. Three alleles were seen at each locus. One of these was scored as a null allele. However, until genetic data are available, there is no way to visually discriminate between a null allele and the product of *Aco-1* and *Aco-2* migrating to the same place in the gel.

Glucose-6-phosphate dehydrogenase (G6PDH). This enzyme was visualized as two bands of activity (*G6pd-1, G6pd-2*) close together (Fig. 2b) in *Vigna*. In common bean it has been described as a single band of activity with no reported polymorphisms (Koenig & Gepts, 1989a). Kephart (1990) has reported G6PDH as usually dimeric with two loci in plants. In both segregating F_2 populations analyzed here, the *G6pd-1* heterozygote showed a banding pattern expected of a dimer (three bands of activity – $\frac{1}{4}\alpha\alpha$, $\frac{1}{2}\alpha\beta$, $\frac{1}{4}\beta\beta$). This is similar to what has been seen in chickpea (*Cicer* sp.) (Gaur & Slinkard, 1989). In one of the populations, the segregation was skewed from the expected in favor of the maternal parent (Table 4); however, in the other population it did not deviate significantly from expectations. Distorted segregation is not uncommon, especially in crosses between taxa which are not closely related (Koenig & Gepts, 1989b; Weeden & Wendel, 1989) and, therefore, *G6pd-1* is proposed as an allozyme locus. The other band of activity was tentatively assigned the locus *G6pd-2* pending genetic confirmation.

Malate dehydrogenase (MDH). This enzyme was visualized as two bands of activity (tentatively

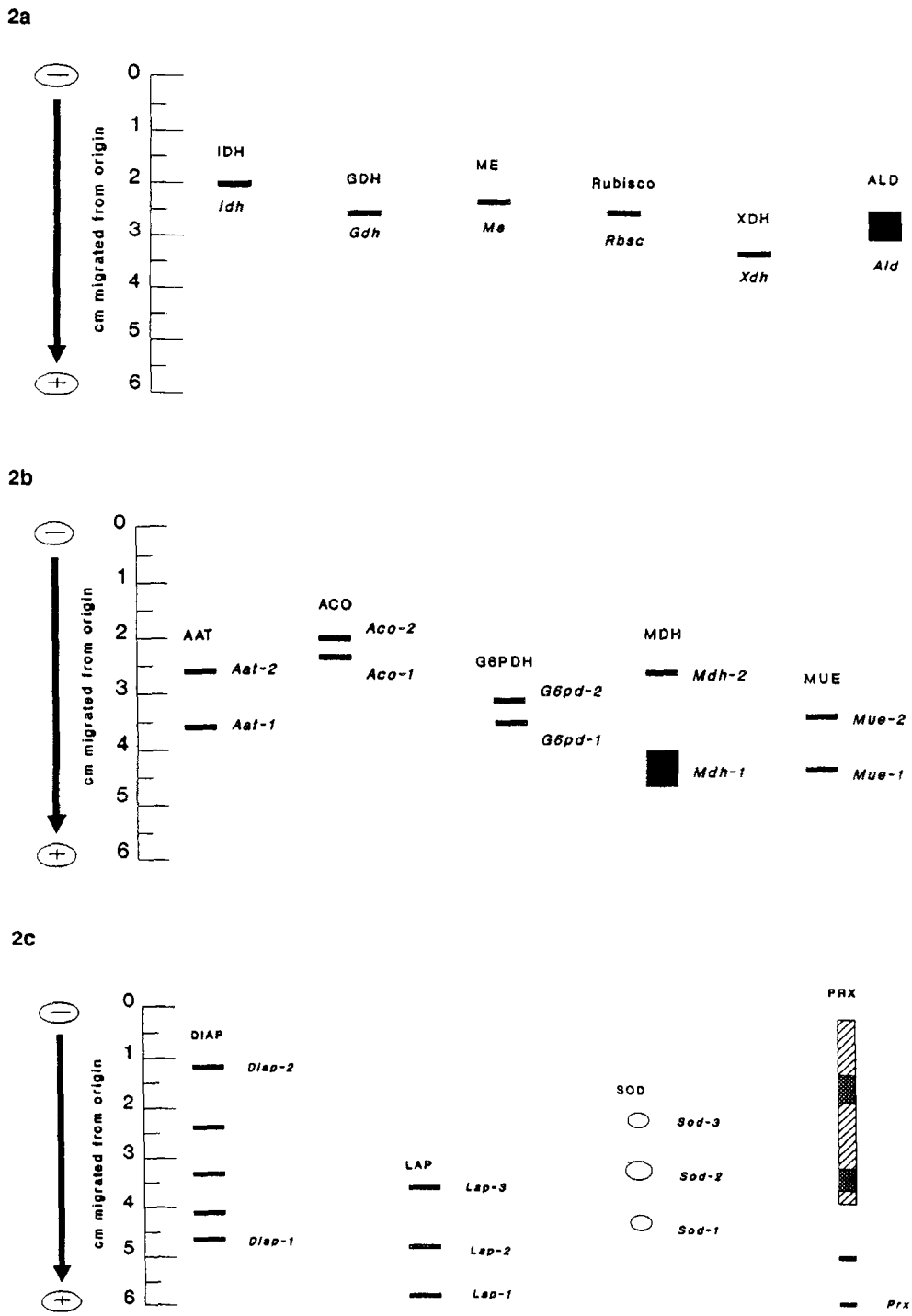

Fig 2. (2a) Zymograms of the enzyme systems visualized as a single band of activity. (2b) Zymograms of the enzyme systems visualized as two bands of activity. (2c) Zymograms of the enzyme systems visualized with complex banding patterns of activity.

Mdh-1 and *Mdh-2*) (Fig. 2b) in *Vigna*. Kephart (1990) has reported MDH as usually dimeric with three loci in plants; however, it has been characterized as two monomeric loci in common bean (Koenig & Gepts, 1989b; Singh et al., 1991b). *Mdh-2* was polymorphic and two alleles were identified within *V. unguiculata*. *Mdh-1* was monomorphic throughout *V. unguiculata*, but showed polymorphism in the related *Vigna* species examined.

Methylumbelliferyl-esterase (MUE). There are whole families of esterases which can sometimes make it difficult to describe them genetically, but they can often be separated by tissue and substrate specificity (Weeden & Wendel, 1989). MUE was assayed only under the conditions given (Table 2) and was visualized as two bands of activity (tentatively *Mue-1* and *Mue-2*) in *Vigna* (Fig. 2b). The bands of activity appeared quickly (five to ten minutes) and could be seen only under UV illumination. *Mue-1* was polymorphic and three alleles were identified within *V. unguiculata*. *Mue-2* was monomorphic throughout *V. unguiculata*, but showed polymorphism in the related *Vigna* species examined.

Aspartate amino-transferase (AAT). This enzyme was visualized as two bands of activity (*Aat-1*, *Aat-2*) in these analyses of *Vigna* (Fig. 2b). Jaaska & Jaaska (1988) reported four bands of activity in common bean and three in cowpea. The two most anodal bands (*Aat-A* and *Aat-B*) were very close together and monomorphic within cultivated cowpea. Polymorphism was observed only at the most cathodal band (*Aat-D*) among cultivated cowpea although one wild cowpea accession showed polymorphism for the *Aat-A* band. In faba beans (*Vicia faba* L.) three bands of activity were also seen; *Aat-1* was invariable, *Aat-2* and *Aat-3* were dimeric with multiple alleles (Mancini et al., 1989). Weeden & Gottlieb (1980) found three bands of activity in pea. *Aat-2* and *Aat-3* were invariable, while *Aat-1* was a polymorphic dimer. *Aat-1* was located in the chloroplast, *Aat-3* in the cytoplast, and *Aat-2* could not be localized. There are two bands of activity in common bean, but in 83 wild accessions Koenig & Gepts (1989a) observed no polymorphisms. Both Jaaska & Jaaska (1988) and Mancini et al. (1989) used polyacrylamide gels in their work. The photographs of the zymograms published by Jaaska & Jaaska (1988) appear to be very similar to what was seen in the study of *Vigna* if *Aat-B* and *Aat-A* were to comigrate. Observed differences might be due to different gel matrices (polyacrylamide versus starch). In the segregating F_2 population of cowpea, the *Aat-2* heterozygote showed a banding pattern expected of a dimer (three bands of activity – $\frac{1}{4}\alpha\alpha$, $\frac{1}{2}\alpha\beta$, $\frac{1}{4}\beta\beta$), although the segregation was skewed from the expected in favor of the maternal parent (Table 4). Skewed segregation of *Aat* has been reported also in lentil crosses (*Lens culinaris* Medak) (Harvey & Muehlbauer, 1989).

Diaphorase (DIAP). This enzyme was visualized as five co-segregating bands of activity (*Diap-1*, *Diap-2*) in *Vigna* (Fig. 2c). In common bean this system has been well characterized as a tetrameric enzyme controlled by two interacting, tightly linked loci (Sprecher, 1988; Weeden, 1984b; Weeden & Liang, 1985) giving the expected five band pattern when both loci are homozygous ($\alpha\alpha\alpha\alpha$, $\alpha\alpha\alpha\beta$, $\alpha\alpha\beta\beta$, $\alpha\beta\beta\beta$, $\beta\beta\beta\beta$). In both segregating F_2 populations, the *Diap-2* heterozygote was what would be expected of a tetramer (15 bands of activity if one locus were heterozygous – $\alpha\alpha\alpha\alpha$, $\alpha'\alpha\alpha\alpha$, $\alpha'\alpha'\alpha\alpha$, $\alpha'\alpha'\alpha'\alpha'$, $\alpha'\alpha'\alpha'\alpha'$, $\alpha\alpha\alpha\beta$, etc. – see Sprecher (1988) for more detailed discussion) and the segregation ratio did not deviate significantly from expectations ($p = 0.05$) (Table 4).

Leucine aminopeptidase (LAP). This enzyme was visualized as three bands of activity (*Lap-1*, *Lap-2*, *Lap-3*) (Fig. 2c) in *Vigna*. Activity at the *Lap-2* locus was low and the band did not visualize consistently, thus no data from this locus were included in the analyses. Kephart (1990) reported LAP as either two or three monomeric loci in plants. LAP has shown three bands of activity in common bean and polymorphism at the *Lap-3* locus has been reported (Koenig & Gepts, 1989b). In the segregating F_2 population of cowpea, it was not possible to distinguish if the *Lap-1* heterozygote was a monomer or dimer; however, the segregation ratio did not deviate significantly from the expectations (Table 4).

Superoxide dismutase (SOD). This enzyme was visualized as three achromatic bands of activity

(*Sod-1*, *Sod-2*, *Sod-3*) in *Vigna* (Fig. 2c), with a possible fourth band (or perhaps a "ghost" band) anodal to *Sod-1*. Jaaska & Jaaska (1988) have reported similar findings (three bands of activity with polymorphisms in one) in their studies of *Phaseolus* and *Vigna* and the published photographs match closely the bands seen in this study. Mancini et al. (1989) showed three loci in faba bean with polymorphisms at all three. *Sod-1* and *Sod-2* were dimers, while *Sod-3* was a tetramer. Both Mancini et al. (1989) and Jaaska & Jaaska (1988) used a polyacrylamide gel matrix which may have given a resolution different than the starch matrix used in this study. In this study *Vigna* showed a polymorphism at the *Sod-1* locus while the *Sod-2* and *Sod-3* loci remained unvaried.

Peroxidase (PRX). There are a number of peroxidases active in the cell and often the banding pattern does not represent the activity of a single enzyme (Weeden & Wendel, 1989), although Brewbaker et al. (1985) used tissue and substrate specificity and to distinguish among 13 peroxidases in maize. This enzyme was visualized as multiple bands and streaks of activity. The single most anodal band of activity (*Prx*) was evaluated (Fig. 2c). A similar banding pattern to that seen in this study was reported in common bean (Bassiri & Adams, 1978) and a polymorphism in a cathodal band of activity (Weeden, 1984b) has been used in studies of common bean evolution (Koenig & Gepts, 1989a; Singh et al., 1991a, 1991b). Segregation of the banding pattern in an F_2 population (Table 4) was not different from the expected segregation. The banding pattern of the heterozygote could not be used to distinguish between monomeric or dimeric enzyme constitution and Kephart (1990) reported both as being present in plant peroxidases.

In crosses between taxa which are not closely related, segregation ratios skewed from expected ratios (3:1 or 1:2:1) are not unusual (Weeden & Wendel, 1989). Zamir & Tadmor (1986) showed that 54% of the loci in interspecific crosses and 13% of the loci in intraspecific crosses of the genera *Lens*, *Capsicum*, and *Lycopersicon* deviated from the expected segregation ratios. Koenig & Gepts (1989b) studied populations from crosses among common beans from the Mesoamerican and Andean gene pools and found that 36% of the total segregation ratios were skewed. Two of the nine segregation ratios in this study showed skewed distributions. The two crosses evaluated represented the most distantly related groups of taxa *V. unguiculata* present in this study (D = 0.288).

Linkage

In the F_2 population of cross UCDVg 36 × UCDVg 21, a loose linkage between *Diap-2* and *G6pd-1* was indicated ($\chi^2 = 15.39$; $p = 0.004$) with an estimated distance of 36.0 cM ± 5.02 (recombination (r) = 0.31). Linkage between these loci in the other F_2 population (CB 88 × UCDVg 21) was not indicated although 82 plants, scored for both enzymes, were analyzed. In this population, however, the segregation of *G6pd-1* was skewed which could have accounted for the lack of observed linkage. Also in the CB 88 × UCDVg 21 F_2 population, an analysis of 38 plants scored for both *Lap-1* and *Prx* revealed a loose linkage ($\chi^2 = 9.62$; $p = 0.047$) with an estimated distance of 39.8 cM ± 7.0 (r = 0.33).

Isozyme linkage maps have been developed for other related legume species. One of the most complete is in pea (Weeden, 1985). It shows *Aldo-p* (*Ald*), *Aat-m* (*Aat-3*), and *Skdh* on chromosome 2 which shares some colinearity with lentil which has *Aat-mb* (*Aat-1*), *Skdh* and *Aat-m* (*Aat-3*) in one linkage group (Muehlbauer et al., 1989) and with chickpea which has *Ald-p* (*Ald*) and *Aat-m* (*Aat-2*) in linkage group II (Gaur & Slinkard, 1990).

In common bean, the degree of linkage between *Me* and *Rbsc* varies among crosses (*Rbsc*/*Me* – Weeden, 1984b, 1986) and until an intermediate marker was found (*Rbsc*/*Lec*/*Me* – Koenig & Gepts, 1989b) it was difficult to validate the linkage. With an integrated map using RFLPs, isozymes and morphological markers in common bean (Nodari et al., 1992), it has become clear that these markers are linked. In the common bean map (Nodari et al., 1992), there is a linkage of 15.9 cM between the *Diap* locus and the *Aco-2* locus. This linkage was not judged significant by a chi square test in the F_2 population segregating at these loci (CB 88 × UCDVg 21). However, there were only 20 plants which could be scored for both of these allozymes and further data from this and other populations will provide more information on these loci. Therefore, until more data are

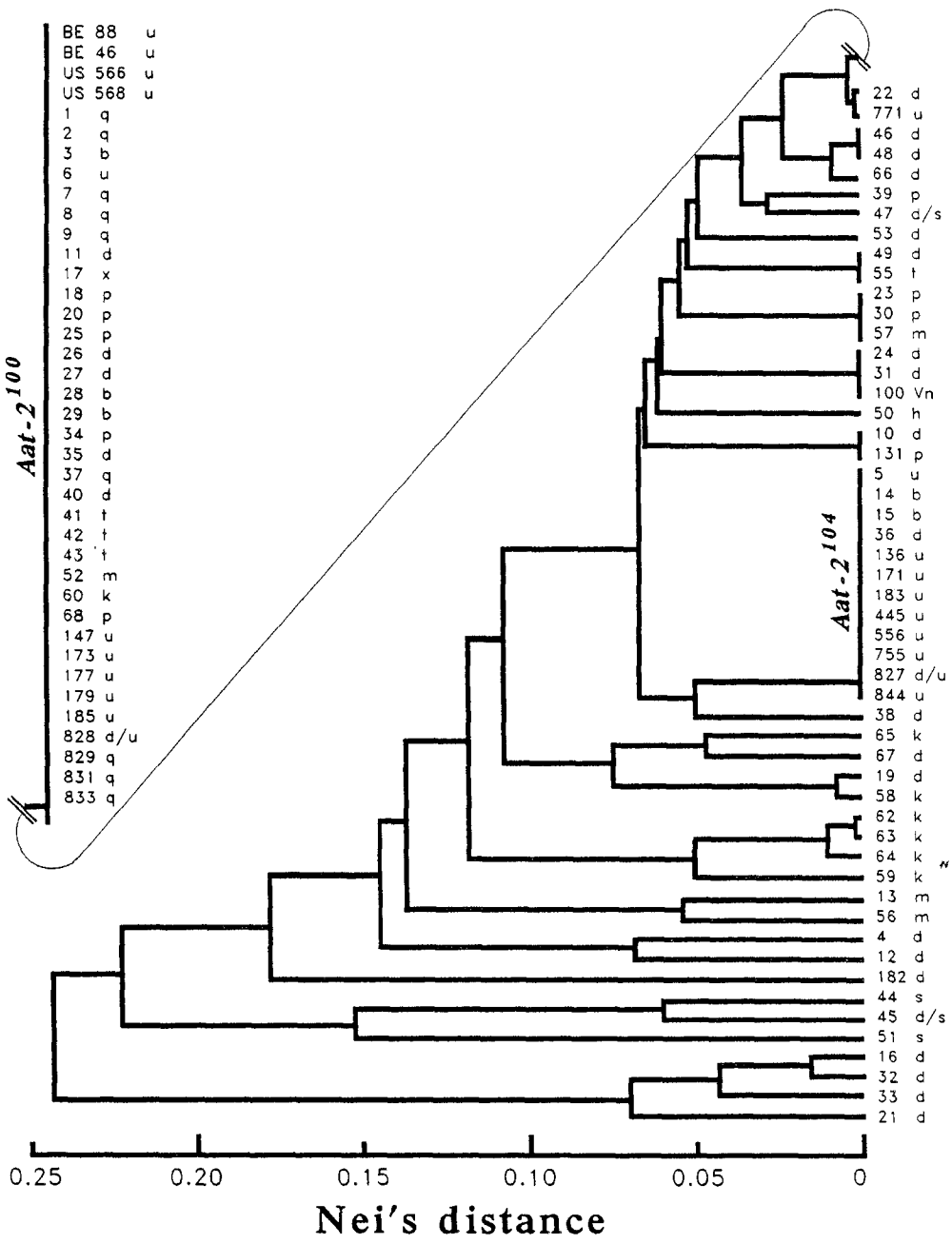

Fig 3. Dendrogram is constructed using unweighted pair group method on arithmetic means of Nei's distance between accessions. Number code is the UCDVg number identifying accessions and the letter code give the taxonomic classification: u = *Vigna unguiculata* ssp. *unguiculata* (*V.u.u.*) cv-gr *unguiculata*; q = *V.u.u.* cv-gr *sesquipedalis*; b = *V.u.u.* cv-gr *biflora*; x = *V.u.u.* cv-gr *textilis*; p = *Vigna unguiculata* ssp. *dekindtiana* (*V.u.d.*) var. *protracta*; d = *V.u.d.* var. *dekindtiana*; t = *V.u.d.* var. *tenuis*; m = *V.u.d.* var. *mensensis*; k = *V.u.d.* var. *kgalagadiensis*; h = *V.u.d.* var. *huillensis*; s = *V.u.d.* var. *stenophylla*; Vn = *Vigna nervosa*.

available in *V. unguiculata*, all of the linkages described above should be regarded as tentative.

Genetic diversity and relationship with taxonomic data

Nei's measurements of genetic diversity (1973) were calculated on the 90 *V. unguiculata* accessions and one *V. nervosa* accession. (*V. nervosa* is the only other member of the section, *Catiang*, to which *V. unguiculata* belongs.) Total genetic diversity (H_T) was 0.085 over all the accessions including the one classified as *V. nervosa.* Within accession diversity (H_S) approached zero, therefore between accession diversity (D_{ST}) was responsible for all of the genetic diversity present and the coefficient of gene differentiation ($G_{ST} = D_{ST}/H_T$) approached 1. Absolute gene differentiation (D_m) was 0.087. The relative values of these were similar to those of a population of 83 wild *Phaseolus vulgaris* accessions: $H_T = 0.132$, $H_S = 0.006$, $D_{ST} = 0.126$ (Koenig & Gepts, 1989a). Like *P. vulgaris*, the diversity present in *V. unguiculata* was primarily among populations. Hamrick & Godt (1990) reviewed allozyme data published between 1968 and 1988 correlating a number of species characteristics to Nei's measures of distance. Annual and short-lived perennials (such as cowpeas) in general had a low H_S. Selfing species were characterized by high D_{ST} and low H_S which is what was seen in *V. unguiculata.*

Nei's distance was used to construct a dendrogram (Fig. 3) using the unweighted pair group method with arithmetic averages (Sneath & Sokal, 1973). Based on the dendrogram, the cultivated cowpeas were divided into two groups – those with all alleles the same as the control (henceforth – allele^{100} group) and those which differed by having the *Aat-2*^{104} allele. Most of the wild accessions differed from the allele^{100} group at only one or two loci and some of the alleles were found in very few accessions. *Me*^{98}, *Mdh-2*^{102}, *Mue-1*^{93}, *G6pd-1*^{103}, *Sod-1*^{95}, and *Mue-1*^{97} were all found in only one accession each.

The grouping of the wild accessions by the allozyme analysis agreed fairly well with the taxonomic classification. Three of the four var. *tenuis* accessions (41, 42, 43) were grouped with the allele^{100} group; all originated in Zimbabwe (Fig. 1d). The fourth accession (52), from Malawi, differed by having the *Aco-2*^{94} allele. The var. *mensensis* accessions were divided into two groups – one accession (52-from Zimbabwe) belonged to the allele^{100} group and the other three (57-Malawi, 56-Zambia, and 13-Burundi; see Fig. 1d) differed by the *Lap-1*^{98} allele; two of these also had different alleles at the *Lap-3* locus. The variety *protracta* was represented by nine accessions (Fig. 1a) – five of these (18, 20, 25, 34, 68) were in the allele^{100} group (all were from Tanzania). Two of the remaining four differed by the *Lap-1*^{98} allele (23-Kenya and 30-Ghana), one by the *Lap-3*^{95} allele (131-origin unknown), and one by the *Me*^{98} allele (39-Kenya). All seven of the var. *kgalagadiensis* accessions originated in Botswana (Fig. 1c). They showed variation at the *Aat-2* locus. One accession (60) belonged to the allele^{100} group, three had the *Aat-2*^{104} allele, and the other three contained both alleles. Four accessions (59, 62, 63, 64) containing the *Aat-2*^{104} allele also contained the *G6pd-2*^{97} allele and one or more other loci with alleles different from 100. The one *huillensis* accession (50) differed from the allele^{100} group by containing the *Me*^{102} allele and the 100 and null (0) alleles at both the *G6pd-1* and *G6pd-2* loci.

The largest group of wild accessions was from the variety *dekindtiana.* They represented a wide geographic area across Africa (Fig. 1b). Accessions from this variety were distributed throughout the dendrogram. In most cases they represented a change of alleles at one or two loci; however, a group of four accessions can be distinguished by differences at the *Diap-2*, *Prx*, *Aco-1*, and *Lap-1* loci. These four accessions differed at the *Aat-2*, *Aco-2*, and *G6pd-1* loci among themselves. All of these accessions were from Nigeria (16, 32, 33) and Ghana (21) and all exhibited a tendency to outcross. They had large, aromatic flowers and a style holding the stigma well past the anthers. In the greenhouse these accessions were self fertile but set little seed unless mechanically self-pollinated. Also distanced from the other accessions by the *Aco-1*^{0}, *Lap-1*^{98}, and *G6pd-1*^{0} alleles was accession 182. Like the other four *dekindtiana* accessions, it shared the characteristics of an outcrossing plant.

Another outlying group contained the two var. *stenophylla* accessions (44, 51) and one accession (45) thought to be intermediate between the varieties *stenophylla* and *dekindtiana* (Fig. 1d). All three of these accessions contained the slow alleles

at the *Diap* loci (*Diap-1*^{90} and *Diap-2*^{90}) and contained alleles other than 100 at the *G6pd-1*, *Lap-1*, or *Mue-1* loci. The other accession (47), classified as an intermediate between the varieties *stenophylla* and *dekindtiana*, contained both the slow and normal alleles at the two *Diap* loci.

For the most part, the taxonomic structure of Mithen & Kibblewhite (n.d.) was supported by this isozyme analysis. This was especially true of the accession within taxonomic groups from the same geographic locations. For example, the one accession (55) of *V. unguiculata* ssp. *dekindtiana* var. *tenius* which showed a different isozyme constitution from the other var. *tenuis* accessions (41, 42, 43) was from a different geographic location (Fig. 1d). The same can be seen in var. *mensensis* where the one accession from Zimbabwe (52) differed at specific alleles from the others (13, 56, 57) (Fig. 1d). The var. *stenophylla* accessions (44, 51) had different alleles at the *Diap* loci and those accessions (45, 47) classified as intermediate between var. *dekindtiana* and var. *stenophylla* showed the same loci or were polymorphic at those loci.

Variety *dekindtiana* is pan-African (Fig. 1b) and showed a wide range of allelic polymorphisms. The four accessions (16, 21, 32, 33) from this variety which exhibited an outcrossing breeding habit showed the largest number of alleles different from the allele^{100} control (six alleles). The difference in breeding system (outcrossing versus selfing) is one of the major characteristics influencing levels of diversity within populations (Hamrick & Godt, 1990).

The range of variation present in this taxon was consistent with Hamrick & Godt's (1990) observation that widespread species have more variation than endemic species. However, this variety has long been classified as the wild subspecies with *V. unguiculata* (Faris, 1965; Rawal, 1975; Lush & Evans, 1981). It has been only recently that taxonomists have discriminated more finely within the wild taxa belonging to *V. unguiculata* (Verdcourt, 1970; Maréchal et al., 1978), therefore it is possible that populations not clearly fitting into any other classification have been lumped together as var. *dekindtiana* by default.

The accession labelled as *V. nervosa* (100) differed only at the *Me* locus (D = 0.049 from the domestic control). Although this is a rare allele, this accession does not seem to merit separate species status based on this isozyme data. The plant is currently being grown to maturity to study the classification. It will be selfed to provide seed to allow an attempt at hybridization with other *V. unguiculata* accessions and re-evaluation of morphological and physiological characteristics. Two of the accessions classified as *V. unguiculata* ssp. *dekindtiana* (827, 828) may have been better classified as weedy *V. unguiculata* ssp. *unguiculata* judged by morphological (especially seed sizes) and isozyme data.

Accessions representing all four of Maréchal's cultigroups were included in the allele^{100} group. All of the cv-gr *sesquipedalis* (nine) and cv-gr *textilis* (one) accessions were in this group. Three of the five cv-gr *biflora* accessions were in the allele^{100} group as were one-half of the cv-gr *unguiculata* accessions. The rest of the cv-gr *unguiculata* accessions differed by having the *Aat-2*^{104} allele present, except one accession (771) which had both alleles (*Aat-2*^{100} and *Aat-2*^{104}) present. With the exception of accession 755, all of the cv-gr *unguiculata* accessions containing the *Aat-2*^{104} allele came from southwestern Africa (Table 1). The two cv-gr *biflora* accessions containing the *Aat-2*^{104} allele were of Asian origin.

Effect of domestication on allozyme diversity

In general this study concurred with the taxonomic classification within *V. unguiculata* although the variety *dekindtiana* should be studied further and more accessions from the other wild taxa should be examined as they become available. There was significant genetic diversity within this species, but almost all of it was to be found among rather than within the populations. Genetic variation within the four *V. unguiculata* ssp. *unguiculata* culti-groups (34 accessions) consisted of variation between two alleles of the *Aat-2* locus (Fig. 2). Therefore, only 4.2% of the loci were polymorphic within the domesticated subspecies. Within the wild taxa, *V. unguiculata* ssp. *dekindtiana*, 58% of the loci were polymorphic. This is a strong indication that a severe genetic bottleneck has occurred during the domestication process, in spite of substantial variation in seed color, seed coat patterns, plant type pod type, and seed size among the cultivated cowpeas. A similar situation has been found in common bean (Singh et al., 1991a, 1991b) where morphological markers, often controlled by

single or few genes, indicated much diversity, and biochemical and molecular markers indicated very little diversity. The selectively neutral nature of the biochemical and molecular markers (Kimura, 1983) may provide a more accurate way to assess the total genetic diversity present in a species.

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