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Isoenzyme variation in the genera *Phaseolus* and *Vigna* (*Fabaceae*) in relation to their systematics: aspartate aminotransferase and superoxide dismutase

VILVE JAASKA and VELLO JAASKA

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Abstract: Evolutionary variation of aspartate aminotransferase and superoxide dismutase isoenzymes in 14 wild and cultivated species of *Phaseolus* and *Vigna* has been studied by electrophoresis and isoelectric focusing in polyacrylamide gel. The American cultivated beans of the genus *Phaseolus* s. str., *P. vulgaris, P. coccineus, P. lunatus* and *P. acutifolius,* form a homogeneous group with only minor isoenzyme variation. The genus *Vigna,* on the contrary, proves to be heterogeneous in isozyme characters. Several clusters of taxa can be distinguished in close correspondence with modern treatments of the genus. The isoenzyme data support the inclusion of the Asian Azuki beans of subg. *Ceratotropis* in *Vigna,* but argue against the transfer of the S. American species *P. adenantha.* The cowpea complex *V. unguiculata* s. lato of sect. *Catiang* forms an uniform and isolated group, distinct from other sections of subg. *Vigna,* and shows affinity to *Phaseolus* s. str. by some isoenzymes. It is suggested to remove *V. unguiculata* s. lato from subg. *Vigna* and to recognize it as a separate subg. *Catiang* (DC.) JAASKA & JAASKA, stat. nov.

Generic delimitation in the *Phaseolus* L.-*Vigna* SAVI complex of subtribe *Phaseolinae* (*Fabaceae*) has been for a long time and still continues to be a taxonomic "Gordian knot" (review: VERDCOURT 1970 a). Botanical treatments of the group are based mainly on morphological, cytological and phytogeographical data and on hybridization studies (PIPER 1926, VERDCOURT 1970 a, MARÉCHAL & al. 1978, 1981).

There are also numerous attempts to employ serological (KLOZ & al. 1966, KONAREV & al. 1970, KLOZ & KLOZOVA 1974; etc.) and electrophoretical (BOULTER & al. 1967, DERBYSHIRE & al. 1976, a. o.) characteristics. It was found (KONAREV & al. 1970; etc.) that serologically the Asian beans are relatively closer to *Vigna* than to the American *Phaseolus.* On the basis of electrophoretic patterns of seed proteins the American and Asian bean species are clearly separated (DERBYSHIRE & al. 1976).

Concomittantly, it was tried to use electrophoretic isoenzyme characters for legume and bean systematics. Thus, THURMAN & al. (1967) compared electrophoretic mobility values of formic and glutamic acid dehydrogenases in legume species from different tribes, including beans. WEST & GARBER (1967 a) found that the 15

bean species they studied could be distinguished from each other by their seed esterase and leucine aminopeptidase isoenzyme spectra. The isoenzyme characters were also used in studies of interspecific bean hybrids (WEST & GARBER 1967 b, WALL 1968). It was shown that isoenzymes could be used for genetic studies of bean hybrids and their progeny. BASSIRI & ADAMS (1978 a) compared esterase, phosphatase and peroxidase isoenzyme electrophoretic patterns in 13 bean species and found that most of them were characterized by unique isoenzyme spectra. However, no taxonomic inferences were made. Other recent isoenzyme studies dealt with the intraspecific variation and the cultivar identification of the common bean, *Phaseolus vulgaris* (BASSIRI & ADAMS 1978 b, WEEDEN 1984).

In this paper we present the results of a comparative study of isoenzymes of aspartate aminotransferase (AAT, L-aspartate: 2-oxoglutarate aminotransferase, EC 2.6.1.1) and superoxide dismutase (SOD, superoxide oxidoreductase, EC 1.15, 1.1) of the *Phaseolus-Vigna* group.

Material and methods

Plant material. The seeds of cultivated species were received from the N.I. Vavilov World Collection of the All-Union Institute of Plant Industry in Leningrad (U.S.S.R.). The accessions of wild species are the gift from Dr R. MARÉCHAL, Faculty of Agronomic Sciences of the State at Gembloux (Belgium).

***Phaseolus* L., s. str.**

Ph. vulgaris L., the cultivated common bean or kidney bean – 14 accessions, originating from U.S.A. (1), Mexico (5), Guatemala (2), Columbia (1), Venezuela (1), Federal Republic of Germany (1), the Netherlands (1), and U.S.S.R. (2).

Ph. coccineus L. (= *P. multiflorus* LAM.), the Scarlet runner bean – 3 accessions received from Sweden (1) and from a local seed-shop in Estonia (2).

Ph. acutifolius A. GRAY, the cultivated tepary bean – 2 accessions from the Ukrainian S.S.R.

Ph. lunatus L., the cultivated Lima bean – 6 accessions, originating from Peru (1), Chile (1), Panama (1), U.S.A. (1), and U.S.S.R. (2).

***Vigna* SAVI**

Subg. *Vigna*, Sect. *Vigna*

V. luteola (JACQ.) BENTH. [basionym *Dolichos luteolus* JACQ., = *Dolichos repens* L., = *Phaseolus luteolus* (JACQ.) GAGNEPAIN], the type species of the genus – 4 accessions, originating from Rwanda (1), Chad (1), Brazil (1), and Antigua (1).

V. oblongifolia A. RICHARD (= *V. lancifolia* RICHARD) – 6 accessions:
a) var. *oblongifolia* (3), originating from S. Africa (1), Katanga (1), and Kenya (1);
b) var. *parviflora* (BAKER) VERD. (= *V. parviflora* BAKER) (3) – from Rwanda (1), Tanzania (1) and Nigeria (1).

V. ambacensis BAKER (= *V. abyssinica* TAUB.) (4):
a) var. *ambacensis* (2 from Zaire),
b) var. *pubigera* (BAKER) MARÉCHAL (= *V. pubigera* BAKER) (2 from Zaire).

Sect. *Catiang* (DC.) VERDC.

V. unguiculata (L.) WALP., s. lato, including:
a) subsp. *unguiculata* (basionym *Dolichos unguiculatus* L., = *Phaseolus unguiculatus* (L.) PIPER, = *V. sinensis* (L.) SAVI ex HASSK.), the cultivated cowpea – 4 accessions, originating from India (2), Nigeria (1), and Hungary (1).

b) subsp. *cylindrica* (L.) VERDC. (basionym *P. cylindricus* L., = *Dolichos catjang* BURMAN

f., = *V. cylindrica* (L.) SKEELS), the cultivated catjang, Sowpea – 5 accessions from Cuba (17), Africa (1), Mali (1), Guyana (1), and Uzbek S.S.R. (1).

c) subsp. *sesquipedalis* (L.) VERDC. (basionym *Dolichos sesquipedalis* L., = *V. sesquipedalis* (L.) FRUHW.), the cultivated yard-long bean; asparagus bean – 7 accessions, originating from China (4), India (1), Spain (1) and Uzbek S.S.R. (1).

d) subsp. *dekindtiana* (HARMS.) VERDC. (basionym *V. dekindtiana* HARMS), a wild African cowpea – 5 accessions, originating from Nigeria (1), Burundi (1), Zimbabwe (1), Zaire (1), and Chad (1).

Subgen. *Plectotropis* (SCHUM.) BAKER, sect. *Plectotropis*

V. vexillata (L.) RICHARD – 8 accessions:

a) var. *vexillata,* (basionym *Phaseolus vexillatus* L., = *Plectotropis hirsuta* K. SCHUM. & THONN., = *V. vexillata* var. *hirta* (HOOK) BAK.), originating from Brazil (1), Equador (1), Zaire (2), and Rwanda (1);

b) var. *macrosperma* MARÉCHAL, MASCHERPA & STAINER – an accession from Costa Rica (1);

c) var. *angustifolia* (K. SCHUM. & THONN.) BAKER, (basionym *Plectotropis angustifolia* K. SCHUM. & THONN., = *V. angustifolia* (R. SCHUM. & THONN.) HOOKER) – 2 accessions, originating from Australia (1), and Senegal (1.)

Subgen. *Ceratotropis* (PIPER) VERDC.

V. mungo (L.) HEPPER (= *Phaseolus mungo* L., = *Azukia mungo* (L.) MESAMUNE), urd, blackgram – 5 accessions, originating from Pakistan (3), India (1), and Egypt (1).

V. radiata (L.) R. WILCZEK. (= *Phaseolus radiatus* L., = *P. aureus* ROXB., = *Azukia radiata* (L.) OHWI, = *V. aureus* (ROXB.) HEPPER), green gram, golden gram, mungbean – 6 accessions from India (1), U.S.A. (1), China (2), Korea (1) and Japan (1).

V. angularis (WILLD.) OHWI & OHASHI (basionym *Dolichos angularis* WILLD., = *Phaseolus angularis* (WILLD.) W. F. WIGHT, = *Azukia angularis* (WILLD.) OHWI), azuki bean – 7 accessions from Japan (2) and Far East (5).

V. umbellata (THUNB.) OHWI & OHASHI (= *Dolichos umbellatus* THUNB., = *Phaseolus calcaratus* ROXB., = *V. calcarata* (ROXB.) KURZ., = *Azukia umbellata* (THUNB.) OHWI) – 3 accessions received from Cuba (1), Czechoslovakia (1), and India (1).

Subg. *Sigmoidotropis* (PIPER) VERDC., sect. *Leptospron* (BENTHAM) MARÉCHAL, MASCHERPA, & STAINER

V. adenantha (G. F. MEYER) MARÉCHAL, MASCHERPA & STAINER, (basionym *Phaseolus adenanthus* G. F. MEYER) – 3 accessions, originating from Brazil (2) and Argentina (1).

Lablab ADANSON

L. purpureus (L.) SWEET (basionym *Dolichos lablab* L., = *D. purpureus* L.), the cultivated hyacinth bean, Egyptian bean – 5 accessions, originating from India (2), Portugal (1), France (1), and Nachitzevan (1).

The botanical classification used in this paper is based on the works of VERDCOURT (1970 a) and MARÉCHAL & al. (1978).

Biochemical methods. Seeds were scarified, imbibed under water for about 20 h and germinated on moist filter paper in a thermostate at 20 °C in the dark for two or three days. The seedlings were then grown in soil under laboratory day-light conditions supplemented by fluorescent lamps for one to three weeks, mostly for two weeks. The age of the seedlings proved to be not important as it does not influence electrophoretic mobility or isoelectric point values of isoenzymes. Only shifts in the relative band intensity occur with the seedling age, as described below.

Enzyme extracts were prepared by crushing small pieces cut from first green leaves of individual seedlings in 0.2 ml of a cold buffer, containing 0.05 M tris-hydroxymethyl ami-

nomethane (tris), 0.01 M EDTA and 5 mM cysteine hydrochloride. After mechanical removal of cell debris, 20 – 50 mg aliquots of sucrose – Sephadex G-200 mixture (4 : 1) were added to every enzyme extract to increase its viscosity.

The polymerization mixture for electrophoresis contained 10% acrylamide, 0.12% N,N-methylene-bisacrylamide, 0.25 M tris-0.05 M HCl for SOD or 0.125 M tris-0.1 M HCl for AAT, 0.2% triethanolamine (or N,N,N′,N′-tetramethylene ethylene-diamine, TEMED) and 0.5 mg% riboflavine. The upper cathode buffer for electrophoresis contained 0.08 M glycine and 0.01 M tris, whereas the lower anode buffer was 0.1 M tris-acetate with an initial pH of about 8.9. Electrophoresis in the anodal direction was carried out in a refrigerated Plexiglass apparatus by applying the current at about 10 – 12 mA per slab for about 2.5 h until the marker dye, bromophenol blue, has reached the lower end of the gel.

The polymerization mixture for isoelectric focusing (IEF) contained 7.5% acrylamide, 0.15% N,N′-methylene-bisacrylamide, 1 mM EDTA-Na_2 1 mg% riboflavine, 15 mg% ammonium persulfate and 2% ampholine (LKB, Sweden) with pH ranges 4 – 6 for SOD. Photopolymerization between two fluorescent lamps was completed in 1 – 1.5 h. The upper cathode buffer was 0.02 M ethylenediamine and the anodal one was 0.02 M H_2SO_4. IEF was carried out by applying 50 V for the first 30 min and then 220 V for 2.5 h.

The reaction mixture for AAT contained 10 mM L-aspartic acid, 5 mM α-ketoglutaric acid and 10 mM calcium nitrate in 0.12 M tris-HCl at a final pH 7.8 – 8.0. After electrophoresis or IEF the gels were incubated in this reaction mixture for 30 min at 30 °C. To visualize the AAT isoenzyme bands, the gels were transferred into 1 mM tetraazotized o-dianisidine (or some other proper diazo salt) in the same buffer but without substrates (JAASKA 1980).

The reaction mixture for SOD contained 25 ml of 0.1 M tris-HCl buffer (pH 8.8), 2.0 ml of nitroblue tetrazolium (2 mg/ml), 0.1 ml riboflavine suspension in water (2 mg/ml) and 0.1 ml 10% TEMED. The gels were incubated in the mixture in the dark for 30 min at 30 °C. To visualize the SOD isoenzyme bands, the gels were rinsed with water and illuminated in water until the full development of acromatic (colourless) bands on the blue background (JAASKA & JAASKA 1982).

The stained electrophoretic and isoelectric enzymograms were fixed in 30% ethanol, containing 5% acetic acid, and photographed on the milky Plexiglas plate illuminated below. The isoenzymes encoded by separate gene loci are designated by capital letters (AAT-A, AAT-B, SOD-A etc.) followed by unified numerical values of their electrophoretic mobility or isoelectric points.

Aspartate aminotransferase (AAT)

All accessions of common bean *Phaseolus vulgaris* of different geographical origin studied revealed an identical four-banded electrophoretic phenotype of AAT (Fig. 1, lanes 1 – 3). Since common bean is known as a predominantly self-pollinating species, the four major isoenzymes are considered to be heterozymes with independent genetic control by separate loci, and will be labelled A 65, B 60, D 52 and C 44. The relative intensity of the four bands varies depending on the tissue, plant age and the accession studied. In the leaf tissue, as a rule, the fastest heterozyme dominates.

The three other American cultivated beans, *P. coccineus, P. acutifolius* and *P. lunatus,* all share the same four-banded AAT-phenotype recorded for *P. vulgaris* (Fig. 1, lanes 5 and 6), except the variation of AAT-C in *P. coccineus*. This indicates significant evolutionary conservation of AAT among the four American cultivated beans and implies close phylogenetic links between them.

Wild pantropical *Vigna luteola,* revealed a unique four-banded AAT phenotype

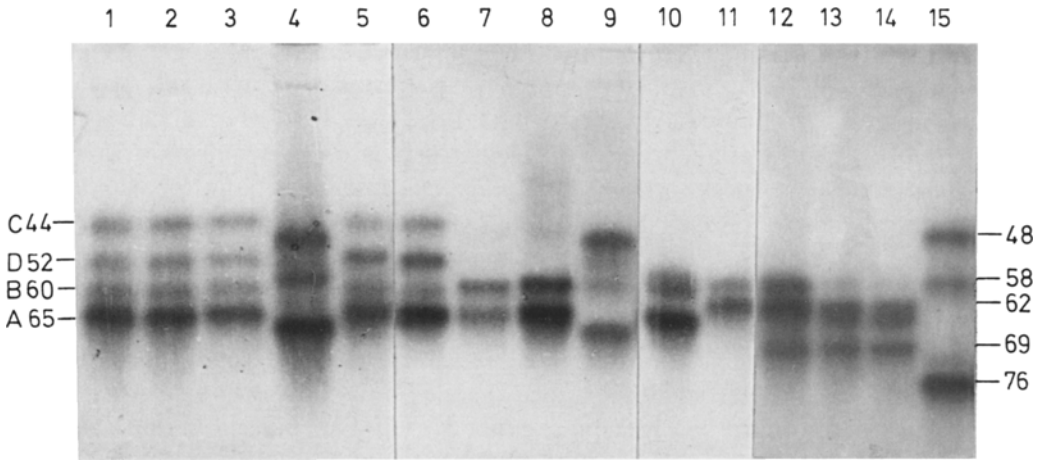

Fig. 1. Electrophoretic enzymograms of aspartate aminotransferase: *1 – 3 Phaseolus vulgaris,* accessions from the Netherlands (*1*), U.S.S.R. (*2*) and Mexico (*3*); *4 V. radiata* (= *P. aureus*); *5 P. lunatus; 6 P. acutifolius; 7* and *8 V. angularis,* two Japanese accessions; *9 V. mungo; 10 V. umbellata* (= *P. calcaratus*); *11 – 14 V. vexillata,* accessions from Brazil (*11*), Zaire (*12*), Australia (*13*), and Rwanda (*14*); *15 V. luteola* from Chad.

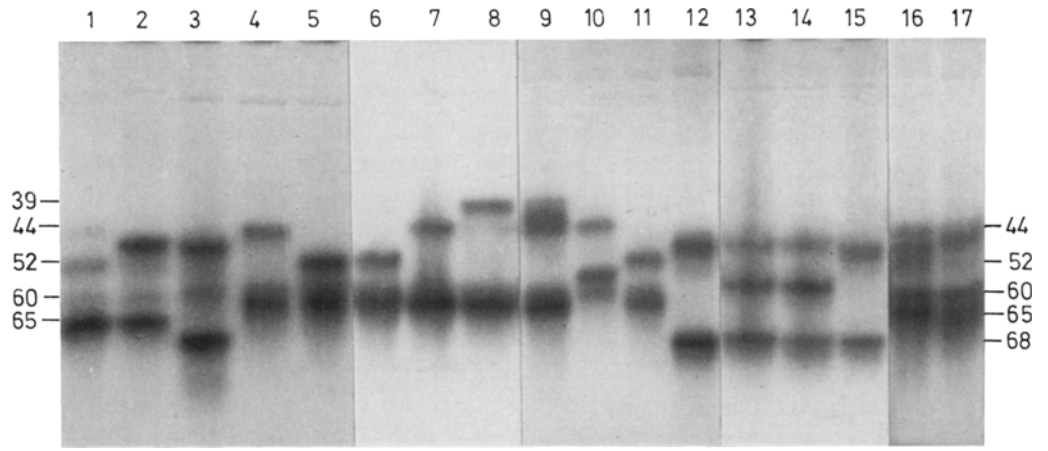

Fig. 2. Electrophoretic enzymograms of aspartate aminotransferase: *1 Phaseolus vulgaris* from Mexico; *2 V. adenantha* from Brazil; *3 V. radiata* from India; *4 V. unguiculata* subsp. *sesquipedalis* from Spain;— *5 V. unguiculata* subsp. *unguiculata* from India; *6* and *7 V. unguiculata* subsp. *cylindrica* from Cuba (*6*) and Africa (*7*); *8 – 11 V. unguiculata* subsp. *dekindtiana* from Nigeria (*8* and *9*), Burundi (*10*) and Zimbabwe (*11*); *12* and *15 V. oblongifolia* var. *parviflora* from Nigeria (*12*) and Rwanda (*15*); *13* and *14 V. oblongifolia* var. *oblongifolia* from Kenya (*13*) and Zaire (*14*); *16 V. ambacensis* from Zaire

with electromorps A 76, B 72, C 58, and D 48 for the two South American accessions and one African (Fig. 1, lane 15). The isoenzyme A 76 dominates in activity and is followed by D 48, while bands of B 72 and C 58 are weaker. One accession of *V. luteola* from Africa (Rwanda) differed only in AAT-C, displaying a faster electromorph C 60 instead of C 58.

The pantropical cultivated cowpea complex, presently treated as three subspecies or cultivar-groups of *V. unguiculata* s. lato of sect. *Catiang* (VERDCOURT 1970 a, MARÉCHAL & al. 1978), displayed limited variation of AAT with only two electromorphs for the major slow heterozyme D 52 and D 44 (Fig. 2, lanes 4–7). Electromorph D 52 was recorded for two accessions of subsp. *cylindrica* (Fig. 2, lane 6) and for one accession of subsp. *unguiculata* (Fig. 2, lane 5), whereas all accessions of subsp. *sesquipedalis* shared D 44, the usual electromorph for the species.

The major faster heterozyme A 62 and the minor one B 58 proved invariable in all accessions of cultivated cowpea (Fig. 2, lanes 4–7).

Wild cowpea *V. unguiculata* subsp. *dekindtiana* exhibited electrophoretic variation in both major heterozymes A and D, leaving only the minor heterozyme B 58 invariant throughout the species (Fig. 2, lanes 8–11). Except A 54 in one accession from Burundi, most accessions of wild cowpea shared A 62 with cultivated forms (Fig. 2, lane 10). In addition to electromorphs D 44 and D 52 shared with cultivated subspecies, a slower electromorph D 39 was frequent in one accession of wild cowpea from Nigeria (Fig. 2, lane 8), displaying heterozygous triplet phenotypes with the common electromorph D 44 in some plants (Fig. 2, lane 9). This suggests that the electromorphs of AAT-D observed in cowpea most probably represent allozymes of a dimeric enzyme. In leaves of older plants of cowpea (1.5–2 months old) an additional AAT band of slower mobility at about 34 appears and intensifies.

The seed samples of wild cowpea available for our analyses were very limited – only three to five plants of each accession. Our results, however, indicate that wild cowpea exhibits additional electrophoretic variation of two major AAT heterozymes, not scored among the cultivars. Wild and all cultivated taxa of *V. unguiculata* s. lato, however, have an unique AAT phenotype with several electromorphs in common. The results demonstrate their relationships and substantiate the hybridization evidence that all forms of cultivated cowpea, presently from different pantropical origin, have been derived from the wild subspecies *dekindtiana,* indigenous to Africa (FARRIS 1965, STEELE 1979).

The African species *V. oblongifolia* (sect. *Vigna*) revealed species-specific three-banded AAT enzymograms (Fig. 2, lanes 12–15), distinctly different from those of *V. luteola* (Fig. 1, lane 15). The fastest and slowest major heterozymes AAT-A and AAT-D proved to be essentially invariable in *V. oblongifolia*, with the electromorphs A 68 and D 48 characteristic of both varieties studied (var. *oblongifolia* and var. *parviflora*), except for one accession of var. *oblongifolia* from South Africa with a faster electromorph D 54 instead of the usual D 48.

Intraspecific variation of AAT-C correlates with the varietal differentiation of *V. oblongifolia:* accessions of the type variety show the faster electromorph C 56 (Fig. 2, lanes 13 and 14), while accessions of var. *parviflora* share the slower electromorph C 50. The latter is so close to D 48 that both are seen as a broad band (Fig. 2, lanes 12 and 15).

Vigna ambacensis, an African wild species from the same section, exhibits intraspecific variation of AAT-D with three electromorphs D 44, D 46, and D 52 among four accessions from Zaire. The appearance of a symmetrical three-banded AAT-D phenotype, combining electromorphs D 44 and D 52 as outer bands, in individual plants of one accession (Fig. 2, lane 16) suggests that the electromorphs observed presumably represent allozymes of a dimeric enzyme.

Vigna vexillata (subgen. *Plectotropis*), originating from different continents (Africa, S. America and Australia) and comprising three botanical varieties (*vexillata, macrosperma* and *angustifolia*) share the major phenotypes with isoenzymes 69, 62 and 58 (Fig. 1, lanes 11 – 14). Band 62 was supplemented by a slightly faster band 64 in three accessions. The fastest band 69 is comparatively weaker or even lacking in one accession (Fig. 1, lane 11). The intensity of the slowest band also varies in the different accessions. Despite of noticeable quantitative variation in isoenzyme activity, depending on accession and plant age, the AAT isoenzyme phenotype of *V. vexillata* is of an astonishing evolutionary stability throughout the huge intercointinental distribution area and unique among the species studied. This makes the appropriate assignment of the AAT isoenzyme bands on *V. vexillata* enzymograms difficult. The homologization made in Table 1 should be considered as tentative and may have to be revised by a more precise study.

The group of Asian cultivated beans now transferred to the genus *Vigna* subg. *Ceratotropis*, i.e. *V. mungo* (= *P. mungo*), *V. radiata* (= *P. aureus*). *V. angularis* (= *P. angularis*) and *V. umbellata* (= *P. calcaratus*), proved to be more variable in their AAT isoenzyme spectra, revealing interspecific differences and divergence from the American group.

The Asian pulses, green gram *V. radiata* and black gram *V. mungo* share an identical AAT-phenotype, with A 69 and D 48 as two major isoenzymes in common (Fig. 1, lanes 4 and 9), invariable in all accessions studied. The heterozymes of intermediate mobility, B and especially C, displayed much slighter bands, scarcely distinguishable on weaker enzymograms.

Two other cultivated Asian beans, *V. umbellata* (= *P. calcarata*) and *V. angularis*, still share the major fast heterozyme A 65 close to that of American beans, but differ in slower minor bands (Fig. 1, lanes 7, 8 and 10). All accessions of *V. umbellata* displayed an invariable three banded phenotype with A 65, D 59 and C 56. The seven accessions of *V. angularis* invariably share the two major heterozymes A 65 and D 59, and reveal intrapopulational individual variation of the minor heterozyme AAT-C, scarcely distinguishable on the enzymogram (Fig. 1, lane 8). However, on the original enzymograms the electrophoretical variants of C 36, C 46 or C 48 could be recognized, the first being the most frequent one. On some enzymograms symmetrical triplets with C 36 and C 46 as outer bands were recorded, suggesting heterozygosity for allozymes of a dimeric enzyme.

Vigna adenantha (subgen. *Sigmoidotropis*, sect. *Leptospron*), the only S. American species available, invariably has a four-banded phenotype (Fig. 2, lane 2), with AAT-A 65 and AAT-D 48 dominating, B 60 and C 52 weak. The S. American bean species *V. adenantha*, newly transferred to *Vigna*, thus shares isoenzymes A 65 and B 60 with the American species of *Phaseolus* s. str., differing in the allozymic electromorphs of AAT-D and AAT-C.

The AAT phenotype of *Lablab purpureus* reveals three bands, A 65, D 52 and C 44; thus, it closely resembles the phenotype of *Phaseolus* s. str., except the lack of B 60.

The data concerning the evolutionary differentiation of the AAT electrophoretic mobility values in the species of *Phaseolus* and *Vigna* studied are compiled in Table 1. This shows that the major fastest heterozyme AAT-A is presented by six electromorphs. Electromorph A 65 has the widest occurrence i.e. the American beans of *Phaseolus* s. str., the two Asian beans, *Vigna angularis* and *V. umbellata*, the S.

Table 1. Electrophoretic mobility [(*M*)] and isoelectric point [(*pI*)] values for aspartate aminotransferase (*AAT*) and superoxide dismutase (*SOD*) isoenzymes in the *Phaseolus, Vigna,* and *Lablab* species (*r* rare, *w* weak, *0* absence of a band, *N* number of accessions analyzed)

Species	N	AAT (M)				SOD (M)				SOD (pI)			
		A	B	C	D	A	B	C	D	A	B	C	D
P. vulgaris	14	65	60	44	52	74	55	38 30	0	4.7	5.1	5.65 5.9	0
P. coccineus	3	65	60	44 46	52	74	54	38	0 48	4.7	5.1	5.65	0 5.2
P. lunatus	6	65	60	44	52	74	55	38	0 48	4.65	5.1	5.65	0 5.2
P. acutifolius	2	65	60	44	52	74	54	38	0	4.65	5.1	5.65	0
V. adenantha (= *P. adenanthus*)	3	65	60 w	52 w	48	74	54	38	0	4.67	5.05	5.85	0 5.4
V. radiata (= *P. aureus*)	6	69	58 w	52 w	48	65	54	38	0	4.8	5.05	5.68	0
V. mungo (= *P. mungo*)	5	69	58 w	52 w 48 w	48	65	54	38	0	4.8	5.05	5.68	0
V. angularis (= *P. angularis*)	7	65	0	36 w 46 w	59 w	67	54	38	0	4.85	5.1	5.68	0
V. umbellata (= *P. calcaratus*)	3	65	0	56	59	65	54	38	0	4.8	5.1	5.68	0
V. luteola	4	76	72 w	58 60 r	48 52	65	54	38 37 r	57 0	4.8	5.05	5.68	0
V. ambacensis	4	65	62	0	44 46	65	54	38	0 48 r	–	–	–	–
V. oblongifolia	6	68	0	56 50	48 54	65	54	38	58 0	4.85	5.05	5.65	4.95 0
V. vexillata	8	69	62 0	58	64 0	65	54	38 32	59 0	4.8	5.1	5.65 5.9	4.95 0
V. unguiculata subsp. *unguiculata*	4	62	58 w	0	44 52	74	55	38	0	4.65	5.1	5.68	0
V. unguiculata subsp. *cylindrica*	5	62	58 w	0	44 52	74	55	38	0	4.65	5.1	5.68	0
V. unguiculata subsp. *sesquipedalis*	7	62	58 w	0	44	74	55	38	0	4.65	5.1	5.68	0
V. unguiculata subsp. *dekindtiana*	5	62 54 r	58 w	0	44 52 39	74 63 r	55	38 37	0	4.65	5.1	5.68	0
L. purpureus (= *D. lablab*)	4	65	0	44	52	65	54	38	0	4.65	5.1	5.68	5.0

American *V. adenantha,* the African *V. ambacensis* and the cultivated hyacinth-bean, *Lablab purpureus.* The two other Asian beans, *V. radiata* and *V. mungo,* share electromorph A 69, whereas the cowpea complex *V. unguiculata* s. l. is characterized by electromorph A 62.

The major slower heterozyme AAT-D reveales at least seven electromorphs, with D 52 and D 48 most widely occurring in the group. Electromorph D 52 is characteristic of *Phaseolus* s. str., of *Vigna unguiculata* s. l. and of the cultivated hyacinth-bean *Lablab purpureus.* Electromorph D 48 is limited to the gram beans of the *Vigna mungo – V. radiata* complex, *V. luteola, V. adenantha* and *V. oblongifolia.* Intrapopulational polymorphism of AAT-D in several species with symmetrical heterozygous triplet phenotypes, combining codominant allozymes with a hybrid isoenzyme of intermediate mobility, suggests that AAT-D is a dimeric enzyme.

Superoxide dismutase (SOD)

Electrophoretic enzymograms of SOD (Fig. 3, lanes 1 – 7) in the leaf tissues of *Phaseolus vulgaris* and *P. coccineus* reveal three major heterozymes labelled SOD-A, SOD-B and SOD-C in the order of their decreasing mobility, following the nomenclature applied in our previous paper (JAASKA 1984, where SOD-A and SOD-B were suggested to represent the chloroplastic and the cytoplasmic Cu/Zn-isoenzymes. Both are inactivated in the presence of diethyldithiocarbamate (DIECA). SOD-C is a DIECA-resistant isoenzyme.

The same three major SOD heterozymes were observed on the enzymograms of other *Phaseolus-Vigna* species studied (Figs. 3 and 4). The chloroplastic heterozyme SOD-A appeared in two most frequent electromorphs, A 74 and A 65. The faster electromorph A 74 is characteristic for all four American cultivated species, comprising *Phaseolus* s. str., and of the cowpea complex, *V. unguiculata* s. lato. An unique electromorph A 63, instead of the common A 74, turned up in *V. unguiculata* subsp. *dekindtiana* from Chad.

Wild African and pantropical beans of the genus *Vigna*, except *V. unguiculata* subsp. *dekindtiana* and *V. adenantha,* share the slower electromorph A 65 (Fig. 4, lanes 9 – 17). Three of the four Asian beans, *V. mungo, V. radiata* and *V. umbellata* had a similar SOD-A 65 pattern (Fig. 3, lanes 8, 9, and 13), whereas all six accessions of *V. angularis*, a Far-East cultivated bean, exhibit SOD-A 67, with a slight but reproducible shift towards higher mobility in comparison with the common electromorph A 65 (Fig. 4, lane 8).

The major band of SOD-A is supplemented on the enzymograms by one or two minor bands of faster mobility which reflect changes in the mobility of the basic band. This suggests that the minor bands represent modificational isoforms of the basic isoenzyme. The relative intensity of these minor bands depends on seedling age (intensity increasing with age) and the conditions of enzyme extraction.

The cytosolic heterozyme SOD-B electrophoretically is even more stable than the chloroplastic SOD-A. Only a very small and barely distinguishable shift in the mobility of SOD-B could be recorded as electromorphs B 55 and B 54. In Fig. 3 enzymograms 3, 9, 13, and 17 exemplify B 54 in comparison with B 55 on the remaining enzymograms. The small mobility shift may reflect some secondary modification of the enzyme molecule, but from Table 1 one can see that this apparently has no diagnostic value for systematics.

The slow DIECA-resistant isoenzyme SOD-C appeared in two frequent electromorphs, C 38 and C 30, and sometimes in the rare electromorphs C 32 and C 37 (Figs. 3 and 4), Table 1. Electromorph SOD-C 38 has the widest occurrence in the *Phaseolus-Vigna* group. Electromorph C 30 turned up in 7 cultivars of the common bean *P. vulgaris* (out of 14 studied), mostly from Middle America. One accession of *V. vexillata* from Rwanda also had the slow electromorph SOD-C 32, in contrast to all others with C 38. Sometimes there is a slow and weak zone above SOD-C in *V. adenantha.*

An additional DIECA-resistant isoenzyme labelled SOD-D (JAASKA 1984) was sporadically present (Fig. 4, lanes 7 and 11) in four electromorphs: D 59, D 58, D 57 and D 48 (Table 1). Electromorph D 59 is frequent in *V. vexillata* (present in six out of eight accessions. On the leaf enzymograms of SOD from older plants (two months) of this species only bands of the DIECA-resistant SOD-C 38 and SOD-D 59 are observed, whereas bands of the DIECA-sensitive Cu/Zn-isoenzymes SOD-A and SOD-B are either lacking (Fig. 4, lane 10) or weak. On the leaf enzymograms of young plants the full spectrum of all four SOD heterozymes is present (Fig. 4, lane 11). No inhibition of Cu/Zn-isoenzymes of SOD was observed in older plants of other species studied. This result suggests that the leaf tissues of *V. vexillata* with age accumulate some compounds which irreversibly inactivate the Cu/Zn-isoenzymes of SOD. The other electromorphs of SOD-D were only rarely found in occasional accessions of some species and sometimes only in some plants of such accessions, as D 48 in *P. lunatus* and *V. ambacensis.* SOD-D encountered only in a few species, thus apparently has little taxonomic value for the *Phaseolus-Vigna* group.

Fig. 3. Electrophoretic enzymograms of superoxide dismutase: *1* and *2 P. vulgaris* from Venezuela (*1*) and Mexico (*2*); *3 P. coccineus* from Sweden; *4–7 P. vulgaris* accessions from U.S.S.R. (*4*), Columbia (*5*), U.S.A. (*6*), and the Netherlands (*7*); *8 V. umbellata* (= *P. calcaratus*) from Cuba; *9 V. radiata* (= *P. aureus*) from India; *10–11 P. lunatus* from Peru (*10*) and Panama (*11*); *12 V. unguiculata* subsp. *sesquipedalis* from India; *13 V. mungo* from Pakistan; *14–16 V. unguiculata* subsp. *sesquipedalis* from Spain (*14*), subsp. *unguiculata* from Nigeria (*15*), and subsp. *dekindtiana* from Nigeria (*16*); *17 Lablab purpureus* from India

Fig. 4. Electrophoretic enzymograms of superoxide dismutase: *1 V. angularis* from Japan; *2 V. umbellata* from India; *3 V. oblongifolia* var. *parviflora* from Rwanda; *4* and *5 V. unguiculata* var. *cylindrica* from Africa (Mali) and U.S.S.R. (the Uzbek S.S.R.), respectively; *6 V. radiata* from U.S.A.; *7 P. lunatus* from U.S.S.R.; *8 V. angularis* from Japan; *9 V. vexillata* var. *vexillata* from Zaire; *10* and *11 V. vexillata* var. *vexillata* from Brazil, older plant (*10*) and young seedling (*11*); *12 V. oblongifolia* var. *parviflora* from Tanzania; *13 V. vexillata* var. *vexillata* from Rwanda; *14 V. luteola* from Brazil; *15 V. radiata* from India; *16* and *17 V. ambacensis* from Zaire

Fig. 5. Isoelectric enzymograms of superoxide dismutase: *1 P. vulgaris* from Mexico; *2 V. adenantha* from Brazil; *3 V. radiata* from India; *4–6 V. unguiculata* var. *sesquipetalis* from Spain (*4*), var. *cylindrica* from Mali (*5*), and var. *unguiculata* from India (*6*); *7 P. acutifolius* from Ukraine; *8 V. mungo* from Pakistan; *9 V. angularis* from the Soviet Far-East; *10 V. adenantha* from Brazil; *11 V. unguiculata* var. *cylindrica* from Cuba; *12 V. oblongifolia* var. *oblongifolia* from South Africa; *13 V. radiata* from India; *14 V. adenantha* from Brazil; *15 V. umbellata* from India; *16 V. oblongifolia* var. *parviflora* from Tanzania; *17* and *18 V. vexillata* var. *vexillata* from Rwanda (*17*) and from Brazil (*18*)

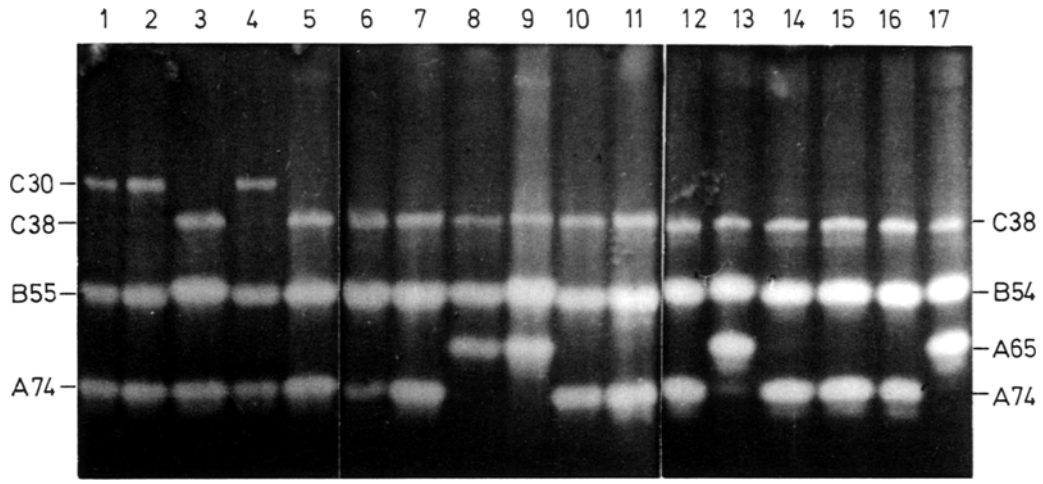

Fig. 3

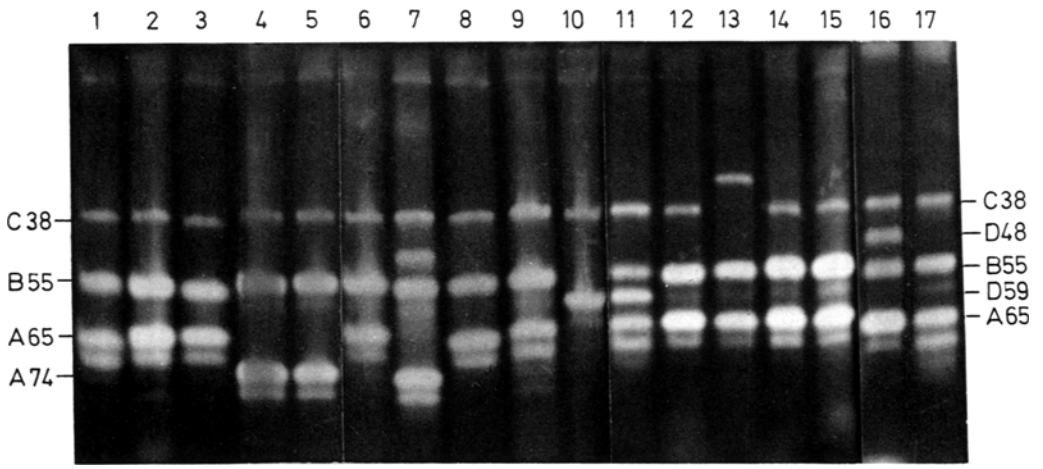

Fig. 4

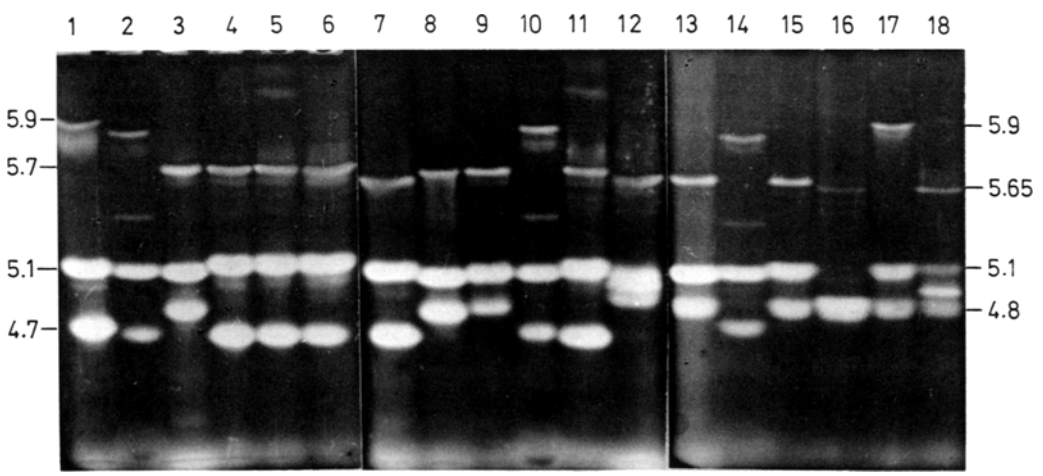

Fig. 5

To summarize the electrophoretic data on the SOD isoenzymes (Table 1), it may be concluded that this enzyme shows restricted electrophoretic variation in the *Phaseolus-Vigna* group. The American beans of *Phaseolus* s. str., the S. American wild species *Vigna adenantha,* and the cowpea complex of *V. unguiculata* s. lato are all characterized by the major electrophoretic phenotypes A 74, B 54, or B 55 and C 38 or C 30. The Asian cultivated species of the Azuki group, the wild African and pantropical species of *Vigna,* as well as the cultivated hyacinth bean *Lablab purpureus* share the phenotype A 65-B 54-C 38. Exceptionally, *P. angularis* of the Asian group has a unique phenotype A 67-B 54-C 38 with divergence in SOD-A.

Isoelectric enzymograms of SOD (Fig. 5) reveal the same three major heterozymes spaced according to their isoelectric points (pI). The isoelectric focusing (IEF) technique makes it possible to discover some additional genetic variation which is not detectable by electrophoretic analysis. Thus we were able to separate the two electromorphs A 4.7 and A 4.65, among the bean species sharing the electromorph A 74 (Table 1). Within the American cultivated beans, *P. vulgaris* and *P. coccineus* share 4.7, while *P. lunatus* and *P. acutifolius* have A 4.65 together with the cowpea complex *V. unguiculata* s. lato. The Asian beans, the African and the pantropical wild beans (except *V. unguiculata* subsp. *dekindtiana*) all have SOD-A of a higher pI, i.e. 4.8 – 4.9 (Fig. 5, Table 1).

SOD-B reveals two pI-variants, B 5.1 and B 5.05, with a small but detectable shift in pI (Fig. 5) which is not correlated with the two electromorphs B 54 and B 55 (Table 1). Thus, the American species of *Phaseolus* s. str. which exhibit the two electromorphs of SOD-B have a uniform pI-value, whereas the Asian beans which share the same electromorph B 54 diverge in their pI-morphs. The significance of this small pI-shift in SOD-B, however, is negligible for a broader *Phaseolinae* taxonomy.

Species with electromorph SOD-C 38 diverge in their pI-morphs and exhibit a small shift in pI (Fig. 5, lanes 7 – 8 and 11 – 12), which correlates with phytogeographical groups. Thus, the American beans mostly share SOD-C 5.65, while the cowpea complex *V. unguiculata* s. lato and the Asian Azuki group share C 5.68. The slower electromorphs C 30, C 32 and C 37 can be distinguished by their higher pI values (Fig. 5, lanes 1, 2, 10, 14, and 17).

SOD-D on the pI-enzymograms appears as a band between SOD-A and SOD-B (Fig. 5, lanes 12 and 18) or between SOD-B and SOD-C (Fig. 5, lanes 2 and 10) in accordance with the electrophoretic data and with no additional information of taxonomic value.

Systematic implications

The AAT and SOD isoenzyme data compiled in Table 1 for the *Phaseolus-Vigna* complex reveal several groups of taxa in close correspondence with their taxonomic treatment on the basis of morphology. The American cultivated beans of *Phaseolus* s. str. form a homogeneous group with only minor divergence and variation. However, the species now treated under *Vigna* s. lato (VERDCOURT 1970 a, MARÉCHAL & al. 1978) proved to be heterogeneous. Several natural alliances may be distinguished within *Vigna* on the basis of isoenzyme characters.

The type species of the two genera, *Phaseolus vulgaris* and *Vigna luteola* differ in electromorphs of four heterozymes of AAT, in electromorphs of SOD-A and

in pI-morphs of SOD-A, SOD-B and SOD-C. Contrary to the situation of the genus *Phaseolus* s. str., the AAT electrophoretic phenotype of *V. luteola* is unique in *Vigna* and gives no indication of genetic affinities to other species within the genus.

On the basis of the electrophoretic SOD phenotypes, the Asian Azuki beans of the subg. *Ceratotropis* form a homogeneous group and suggest a clear genetic affinity to the three species of subg. *Vigna*, i.e. *V. ambacensis, V. oblongifolia* and the type species *V. luteola*, and also to *V. vexillata* of subg. *Plectotropis*. This evidence provides new support to the taxonomic treatment of the Asian Azuki group under the genus *Vigna*.

The S. American species *V. adenantha* (= *P. adenanthus*), however, differs from other *Vigna* spp. in SOD-A, AAT-A, and AAT-B and has electromorphs of these heterozymes in common with species of *Phaseolus* s. str. Whereas MARÉCHAL & al. (1978) suggested to transfer it from *Phaseolus* s. str. to *Vigna,* the isoenzyme data argue against this. The electromorph AAT-D 48 of *V. adenantha* diverges from *Phaseolus* s. str. and signals an affinity with *Vigna*. For a sound taxonomic treatment of subg. *Sigmoidotropis,* besides *V. adenantha* of sect. *Leptospron,* more isoenzyme data are needed.

The cowpea complex *V. unguiculata* s. lato, comprising morphologically divergent cultivated and wild forms, appears to be a uniform group with common electromorphs of AAT and SOD. On the basis of SOD-A *V. unguiculata* diverges from *Vigna* and shows affinity of *Phaseolus* s. str. The AAT phenotype of *V. unguiculata*, however, is unique and shares D 52 with *Phaseolus* s. str. The isoenzyme data, thus indicate an isolated position of *V. unguiculata* s. lato in the genus *Vigna*.

The cowpea complex was recently treated (VERDCOURT 1970 a, MARÉCHAL & al. 1978) as a monotypical sect. *Catiang* (DC.) VERDC. of subg. *Vigna*. The isoenzyme data, however, suggest removal of sect. *Catiang* from subg. *Vigna*, and its recognition at a higher taxonomic level, preliminarily as a separate **subg. *Catiang* (DC.) JAASKA & JAASKA, stat. nov.,** basionym *Dolichos* L. sect. *Catiang* DC., Prodr. **2**: 398 (1825).

LINNAEUS (1753, 1763) originally has recognized the taxa of the cowpea complex under the genus *Dolichos* L. as three species, *D. unguiculatus* L., *D. biflorus* and *D. sesquipedalis* L. However, hybridization studies have proved the conspecific nature of all cultivated and wild forms of this complex, substantiating their treatment at the subspecies level (FARRIS 1965). Restoring the cowpea complex under the genus *Dolichos* is questionable, considering the retypification and revision of the genus by VERDCOURT (1970 b). Our data show that *V. unguiculata* s. lato and *V. adenantha* (= *P. adenanthus*) are intermediate between typical *Phaseolus* s. str. and *Vigna* and share isoenzyme electromorphs with both.

The isoenzyme data obtained in the present paper are clearly insufficient for broader taxonomic and phylogenetic conclusions and the "Gordian knot" of the *Phaseolus-Vigna* group still remains to be resolved. Further search for additional isoenzymes of diagnostic value in a more representative sample of wild species is needed. The present study, however, demonstrates the potential value of selected isoenzyme characters as a supplement to traditional morphological observations for elucidating phylogenetic relationships in this taxonomically complicated group.

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References

BASSIRI, A., ADAMS, M. W., 1978 a: An electrophoretic survey of seedling isozymes in several *Phaseolus* species. – Euphytica **27**: 447–449.

– – 1978 b: Evaluation of common bean cultivar relationships by means of isozyme electrophoretic patterns. – Euphytica **27**: 707–720.

BOULTER, D., THURMAN, D. A., DERBYSHIRE, E., 1967: A disc electrophoretic study of globulin proteins of legume seeds with reference to their systmatics. – New Phytol. **66**: 27–36.

DERBYSHIRE, E., YARWOOD, N. J., NEAT, E., BOULTER, D., 1976: Seeds proteins of *Phaseolus* and *Vigna*. – New Phytol. **76**: 283–288.

FARRIS, D. G., 1965: The origin and evolution of the cultivated forms of *Vigna sinensis*. – Canad. J. Genet. Cytol. **7**: 433–452.

JAASKA, V., 1980: Aspartate aminotransferase and alcohol dehycrogenase isoenzymes: Intraspecific differentiation in *Aegilops tauschii* and the origin of the D genome polyploids in the wheat group. – Pl. Syst. Evol. **137**: 259–273.

– 1984: Isoenzymes of superoxide dismutase in beans. – Proc. Acad. Sci. Estonian S.S.R., Biology **33**: 42–49. (In Russian.)

– JAASKA, V., 1982: Isoenzymes of superoxide dismutase in barley. – Biochem. Physiol. Pflanzen **177**: 375–386.

KLOZ, J., KLOZOVA, E., 1974: The Protein Euphaseolin in *Phaseolinae* – a chemotaxonomical study. – Biol. Plant. **16**: 280–296.

– – TURKOVA, V., 1966: Chemotaxonomy and genesis of protein characters with special reference to the genus *Phaseolus*. – Preslia **38**: 229–236.

KONAREV, V. G., SATBALDINA, S. T., GAVRILJUK, I. P., IVANOV, N. R., 1970: On the species specifity of bean seed proteins. – Proc. Acad. Sci. U.S.S.R. **170**: 975–977. (In Russian.)

LINNAEUS, K. VON, 1753, 1763: Species Plantarum.

MARÊCHAL, R., MASCHERPA, J. M., STAINER, F., 1978: Étude taxonomique d'un groupe complexe d'espèces des generes *Phaseolus* et *Vigna* (*Papilionaceae*) sur la base de données morphologiques et polliniques, traitées par l'analyse informatique. – Boissiera **28**: 1–273.

– – – 1981: Taxonometric study of the *Phaseolus-Vigna* complex and related genera. – In POLHILL, R. M., RAVEN, P. H., (Eds.): Advances in legume systematics, pp. 329–335. – Kew: Royal Botanic Garden.

PIPER, C. V., 1926: Studies in American *Phaseolinae*. – Contr. U.S. Natl. Herb. **22**: 663–701.

STEELE, W. M., 1979: Cowpeas *Vigna unguiculata* (*Leguminosae-Papilionatae*). – In SIMMONDS, N. W., (Ed.): Evolution of crop plants, pp. 183–185. – London, New York: Longman.

THURMAN, D. A., BOULTER, D., DERBYSHIRE, E., TURNER, B. L., 1967: Electrophoretic mobilities of formic and glutamic dehydrogenases in the *Fabaceae:* a systematic survey. – New Phytol. **66**: 37–45.

VERDCOURT, B., 1970 a: Studies in the *Leguminosae – Papilionoideae* for the Flora of Tropical East Africa. IV. – Kew Bull. **24**: 507–569.

– 1970 b: Proposal for the retypification of 3910 *Dolichos* L. by *Dolichos trilobus* L. pro parte. – Taxon **19**: 297–298.

WALL, J. R., 1968: Leucine aminopeptidase polymorphism in *Phaseolus* and differential elimination of the donor parent genotype in interspecific backcrosses. – Biochem. Genetics **2**: 109–118.

WEEDEN, N. P., 1984: Distinguishing among white seeded bean cultivars by means of allozyme genotypes. – Euphytica **33**: 199–208.

WEST, N. B., GARBER, E. D., 1967 a: Genetic studies of variant enzymes. I. An electrophoretic survey of esterases and leucine aminopeptidases in the genus *Phaseolus*. – Canad. J. Genet. Cytol. **9**: 640 – 645.

– – 1967 b: Genetic studies of variant enzymes. II. The inheritance of esterase and leucine aminopeptidases in *Phaseolus vulgaris* × *P. coccineus*. – Canad. J. Genet. Cytol. **9**: 646 – 655.

Address of the authors: VILVE JAASKA and VELLO JAASKA, Laboratory of Biochemistry, Department of Botany, Institute of Zoology and Botany, 21 Vanemuise Street, 202400 Tartu, Estonian S.S.R., U.S.S.R.