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Molecular heterogeneity of Cowpea (*Vigna unguiculata Fabaceae*) seed storage proteins

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Abstract: 81 wild forms and 110 cultivated cowpea, *Vigna unguiculata*, accessions from 21 countries of Africa were screened for variability in seed storage proteins. Total seed proteins, albumin and globulin fractions were investigated by means of sodium·dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing (IEF) of non-reduced and/or reduced samples in one- and two-dimensional procedures. The globulin fraction is heterogeneous in molecular weight and contains both legumin-like components and three to six nondisulfide-linked subunits. Three globulin subunits, with molecular weights 110, 76, and 41 kD were found to be composed of disulfide-linked polypeptides. In the nondisulfide-linked fraction, both cultivated and wild forms exhibited patterns of four types (A–D). This fraction contains polypeptide subunits of molecular weights 62, 56, and 52 kD for A type, 62, 56, 54, and 52 kD for B type, 62, 56, 52, and 50 kD for C type, and at least 62, 56, 54, 52, 50, and 49 kD for D type. These subunits present similar multiple charge forms but C and D types possess more basic specific 50 and 49 kD nondisulfide linked components. Major albumin fraction contains subunits of 94, 86, 32, and 24 kD. No infraspecific variation was observed in albumin or legumin-like fractions. The discussion is focussed on the relations between genetic variability assessed by storage protein coding genes and phenotypic variability.

The cowpea, *Vigna unguiculata* (L.) WALP., is cultivated in many tropical and subtropical regions, Africa being the only continent where wild and cultivated forms are found. For the populations of these countries and especially of W Africa, cowpeas are mainly utilized as dry cooked beans and constitute a primary source of protein (~ 25%) and carbohydrate (~ 63%) (BRESSANI 1985). Like other legume seeds (DERBYSHIRE & al. 1976), cowpeas contain large amounts of salt soluble proteins, mainly vicilin 7S globulin and to lesser amount legumin-like 11S globulins. Both classes of proteins consist of several polypeptides which have been described in detail (CARASCO & al. 1978, KHAN & al. 1980, MURRAY & al. 1983). As seed proteins are deficient overall in sulphur amino acids (EVANS & BOULTER 1974, OLOGHOHO & FETUGA 1982), the selection of cultivars crossed with related wild taxons would permit improvement of their nutritional quality. In this aim the knowledge of a global view on the genetic pool of *V. unguiculata* is prerequired

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with special reference to the still underemployed variability of the spontaneous species. The majority of the reports presented (CARASCO & al. 1978, SEFAH-DEBEH & STANLEY 1979, KHAN & al. 1980, BHATTY 1982, HERNANDEZ & al. 1983, MURRAY & al. 1983, VIDOVIC & MURRAY 1984) and more recently PEDALINO & al. (1990) resulted in investigations on cultivated material supplied by I.I.T.A. (International Institute of Tropical Agriculture, Ibadan, Nigeria). The studies of PAINO D'URZO & al. (1990) are the only ones dealing with spontaneous material of *V. unguiculata*. The present paper characterizes the major cowpea seed proteins using materials from all regions of its cultivation. The result of this investigation is compared with taxonomic data obtained from phenotypic studies (PASQUET 1992).

Material and methods

Plant material and taxonomic data. Eighty-one wild and 110 cultivated accessions of cowpea were studied. Their origin and location are listed in Tables 1 and 2. Among the cultivated samples, CS, NO, and OU are landraces from Cameroon (ORSTOM collection). The TO and ZR accessions come from the IBPGR *Phaseolinae* collection maintained in Meise Jardin Botanique National, Belgium, the ET accessions from the National Botanical Institute, Pretoria, South Africa and the WESTPHAL collection maintained in Wageningen, The Netherlands. EG 1 comes from Zentralinstitut für Genetik und Kulturpflanzenforschung, Gatersleben, Germany. The source of the wild material originates from CIAT, Centro International de Agricultura Tropical, Cali, Colombie. (FLG accession), IBPGR *Phaseolinae* collection (NI accessions), IBPGR/University of Zimbabwe collection (MT accessions), IITA, International Institute of Tropical Agriculture, Ibadan, Nigeria. (TVNU accessions), and ORSTOM (SP accessions). Among the annual accessions, four of them (SP 33, 34, 37, 38) can be considered as "weedy types" morphologically intermediate between wild and cultivated accessions.

According to the classification of PASQUET (1992) based on morphological traits, the studied accessions of spontaneous material contain especially the annual subsp. *unguiculata* var. *spontanea* (SCHWEINF.) PASQUET, and some accessions of the eight groups of perennials: subspp. *baoulensis* (CHEV.) PASQUET, *letouzeyi* PASQUET, *burudiensis* PASQUET, *pubescens* (WILCZEK) PASQUET, *tenuis* (MEY.) MARÉCHAL, MASCHERPA & STAINER, *stenophylla* (HARV.) MARECHAL, MASCHERPA & STAINER. Also two perennial non-described groups from Congo (littoral forms) and Malawi (higher altitude forms) were studied.

For the cultivated Africa accessions the morphological studies of PASQUET (results not shown) have demonstrated an accordance with the classification of CHEVALIER (1944). So cv. '*unguiculata*' (L.) WESTPHAL may be divided into three groups. The cv. '*campestris*' (smooth seeds) and cv. '*melanophtalmus*' (wrinkled seeds) may be considered as the Northern geographical group (subsaharian savanahs from Senegal to Ethiopia) and the cv. '*oleraceus*' such as the Southern geographical group (austral Africa and forest areas from Sierra Leone to Zaire and Kenya).

The accessions of these three groups have been studied as well as accessions of cv. '*texilis*' (CHEV.) WESTPHAL and cv. '*sesquipedalis*' (L.) WESTPHAL. This latter originates from Asia. Subsp. *protacta* is now becoming subsp. *stenophylla* (PASQUET 1992) according to the nomenclatural priority rules. Plants were cultivated in Abidjan (Centre d'Adiopodoumé, Ivory Coast) from September 1987 to June 1989 and later in Niamey (Cameroon).

Protein extraction and fractionation. Total saline soluble proteins (crude extract), albumin and globulin fractions were performed as described by RAYMOND & al. (1991). Meal of mature dehulled dry seeds was extracted (100 mg meal/ml buffer) for 30 min at room temperature with 0.1 M potassium phosphate buffer, pH 8.0, containing 0.4 M NaCl. For the preparation of globulin and albumin fractions, the crude extract was dialysed against

Table I. Characteristics and banding patterns of the cultivated *Vigna unguiculata*. ZAF South Africa, CMR Cameroon, EGY Egypt, ETH Ethiopia, TGO Togo, ZAR Zaire; further abbreviations see Material and methods

Banding pattern	Subsp.	Accession number	Country	Locality		Other code n°
A	*oler.*	AS 2B	ZAF		Mdlondlweni, Kwa Zulu, Natal	
A	*oler.*	AS 2C	ZAF		Mdlondlwani, Kwa Zulu, Natal	
C	*oler.*	AS 3E	ZAF		Nhlamfunda, Kwa Zulu, Natal	
A	*oler.*	AS 7A	ZAF		Masamane, Bophuthatswana, Transvaal	
C	*oler.*	AS 8	ZAF		Setlagole, Bophuthatswana, Transvaal	
C	*oler.*	AS 10F	ZAF		Phameni, Ka Ngwane, Transvaal	
A	*oler.*	CS 5	CMR	3° 57′ N 10° 41′ E	Minomindjock	
B	*oler.*	CS 6	CMR	3° 49′ N 10° 15′ E	Song Ndong	
B	*oler.*	CS 7A	CMR	3° 56′ N 10° 32′ E	Song Simouth	
A	*oler.*	CS 7B	CMR	3° 56′ N 10° 32′ E	Song Simouth	
B	*oler.*	CS 14	CMR	4° 42′ N 12° 48′ E	Vela	
B	*oler.*	CS 23B	CMR	4° 15′ N 10° 55′ E	Likound	
B	*oler.*	CS 45	CMR	4° 52′ N 11° 15′ E	Zock	
B	*oler.*	CS 52	CMR	4° 39′ N 09° 53′ E	Lamba	
B	*oler.*	CS 53A	CMR	4° 39′ N 09° 53′ E	Lamba	
B	*oler.*	CS 53D	CMR	4° 39′ N 09° 53′ E	Lamba	
A	*oler.*	CS 54	CMR	4° 39′ N 09° 53′ E	Lamba	
B	*oler.*	CS 56C	CMR	4° 47′ N 13° 03′ E	Wall	
B	*oler.*	CS 56D	CMR	4° 47′ N 13° 03′ E	Wall	
B	*oler.*	CS 56E	CMR	4° 47′ N 13° 03′ E	Wall	
A	*oler.*	CS 67	CMR	4° 09′ N 11° 10′ E	Nkolassa	
B	*oler.*	CS 85	CMR	3° 28′ N 10° 37′ E	Makot	
A	*oler.*	CS 151	CMR	4° 02′ N 14° 59′ E	Bouno II	
A	*oler.*	CS 154	CMR	4° 37′ N 14° 18′ E	Garoua Sembe	
A	*camp.*	EG 1	EGY			Wig 79/82
B	*camp.*	ET 1	ETH			WP 2556
A	*camp.*	ET 2	ETH			WP 2556
A	*oler.*	ET 3	ETH			WP 2556
A	*camp.*	ET 4	ETH			VIG 8674
B	*camp.*	ET 5	ETH			WP 3186
B	*camp.*	ET 8	ETH			WP 3492
B	*camp.*	ET 9	ETH			WP 3186
B	*camp.*	ET 10	ETH			WP 2556
B	*camp.*	ET 12	ETH			WP 3186
B	*camp.*	ET 13	ETH			WP 3186
B	*camp.*	ET 14	ETH			WP 2556
B	*camp.*	ET 15	ETH			
B	*mela.*	NO 7	CMR	10° 27′ N 13° 34′ E	Amsa Jiri	
B	*mela.*	NO 17	CMR	08° 38′ N 12° 38′ E	Bimlerou	
A	*mela.*	NO 28	CMR	10° 23′ N 13° 33′ E	Choua	
D	*text.*	NO 40	CMR	10° 06′ N 15° 16′ E	Djougoumta	
B	*oler.*	NO 74	CMR	10° 01′ N 15° 26′ E	Gobo	
B	*mela.*	NO 95	CMR	10° 37′ N 14° 46′ E	Goudou Goudoum	
B	*mela.*	NO 110	CMR	11° 02′ N 14° 09′ E	Mora	
A	*mela.*	NO 122	CMR	10° 04′ N 14° 08′ E	Lam	
A	*mela.*	NO 129	CMR	10° 11′ N 14° 31′ E	Lara	
A	*mela.*	NO 133	CMR	11° 47′ N 15° 06′ E	Logone Birni	
A	*mela.*	NO 137	CMR	11° 11′ N 14° 14′ E	Magdeme	
A	*mela.*	NO 142A	CMR	10° 07′ N 13° 27′ E	Maboudji	
A	*mela.*	NO 144	CMR	10° 07′ N 13° 27′ E	Magoudji	
A	*mela.*	NO 173	CMR	10° 47′ N 13° 52′ E	Midere	
A	*camp.*	NO 183	CMR	10° 36′ N 13° 34′ E	Mogode	
A	*mela.*	NO 189	CMR	10° 12′ N 14° 11′ E	Moutouroua	
A	*mela.*	NO 193	CMR	10° 07′ N 14° 19′ E	Moumour	
D	*camp.*	NO 251A	CMR	10° 57′ N 14° 04′ E	Tala Mokolo	

Table 1 (continued)

Banding pattern	Subsp.	Accession number	Country	Locality		Other code n°
B	*mela.*	NO 574	CMR	08° 09′ N 15° 02′ E	Mbakla	
B	*oler.*	NO 576	CMR	06° 45′ N 14° 32′ E	Zaoroudua	
D	*text.*	NO 577	CMR	08° 00′ N 15° 11′ E	Laran Nda	
B	*mela.*	NO 643	CMR	10° 02′ N 15° 15′ E	Guibi	
A	*mela.*	NO 649	CMR	10° 04′ N 15° 16′ E	Bongor	
B	*text.*	NO 654A	CMR	08° 00′ N 15° 11′ E	Danike	
B	*mela.*	NO 661	CMR	10° 02′ N 15° 15′ E	Harkouna	
B	*mela.*	NO 670	CMR	10° 12′ N 15° 16′ E	Guinane	
B	*mela.*	NO 760	CMR	09° 58′ N 15° 29′ E	Tehantako	
B	*mela.*	NO 927A	CMR	10° 04′ N 13° 51′ E	Gorom	
B	*mela.*	NO 927B	CMR	10° 04′ N 13° 51′ E	Gorom	
B	*sesq.*	NO 1036	CMR	11° 47′ N 15° 06′ E	Logone Birni	
B	*mela.*	NO 1223	CMR	10° 31′ N 14° 26′ E	Mogom	
B	*mela.*	NO 1292	CMR	10° 07′ N 14° 24′ E	Poukebi	
A	*mela.*	NO 1387	CMR	10° 37′ N 13° 53′ E	Pomla	
B	*mela.*	NO 1467	CMR	10° 05′ N 15° 03′ E	Walia	
B	*mela.*	NO 1559	CMR	09° 58′ N 14° 46′ E	Danhou	
B	*mela.*	NO 1732	CMR	10° 12′ N 13° 52′ E	Ourlang	
B	*camp.*	NO 1878	CMR	10° 41′ N 13° 47′ E	Sirak	
B	*camp.*	NO 1880	CMR	10° 41′ N 13° 47′ E	Sirak	
B	*text.*	NO 1898	CMR	10° 47′ N 13° 59′ E	Roua	
A	*camp.*	NO 2174	CMR	10° 43′ N 14° 07′ E	Douroum	
A	*oler.*	NO 2206	CMR	10° 54′ N 14° 22′ E	Mazangai	
A	*mela.*	NO 2253	CMR	10° 17′ N 14° 56′ E	Kalfou Centre	
B	*mela.*	NO 2296	CMR	07° 49′ N 12° 29′ E	Alme	
B	*mela.*	NO 2308	CMR	07° 55′ N 12° 27′ E	Maalti	
B	*mela.*	NO 2348	CMR	07° 57′ N 12° 15′ E	Kontcha	
B	*mela.*	NO 2399	CMR	07° 21′ N 13° 22′ E	Laokobong	
A	*mela.*	NO 2404	CMR	07° 37′ N 14° 10′ E	Baka	
B	*mela.*	NO 2408	CMR	07° 37′ N 14° 10′ E	Baka	
B	*mela.*	NO 2425	CMR	07° 53′ N 14° 51′ E	Laggoy	
B	*oler.?*	NO 2460	CMR	08° 38′ N 12° 38′ E	Bimlerou haut	
A	*mela.*	NO 2529A	CMR	07° 26′ N 13° 54′ E	Ngang Ha	
A	*mela.*	NO 2715A	CMR	11° 03′ N 14° 26′ E	Djaoude	
B	*text.*	NO 2798	CMR	08° 33′ N 13° 16′ E	Sangbe	
B	*mela.?*	NO 2956	CMR	07° 11′ N 14° 51′ E	Yandia	
B	*oler.*	OU 31	CMR		Sang Guneku	
A	*oler.*	OU 59A	CMR	04° 59′ N 09° 26′ E	Kombone Bafaw	
A	*oler.*	OU 100B	CMR	05° 23′ N 10° 25′ E	Bandjoun	
A	*oler.*	OU 130	CMR	06° 31′ N 10° 44′ E	Tabenken	
A	*oler.*	OU 134D	CMR	06° 25′ N 09° 55′ E	Bajine Esimbi	
A	*oler.*	OU 150B	CMR	05° 19′ N 10° 05′ E	Fontsa Toula	
A	*oler.*	OU 150F	CMR	05° 19′ N 10° 05′ E	Fontsa Toula	
A	*oler.*	OU 152B	CMR	04° 37′ N 10° 04′ E	Benga	
A	*oler.*	OU 152C	CMR	04° 37′ N 10° 04′ E	Benga	
A	*oler.*	OU 158	CMR		Beba (Wum)	
A	*oler.*	OU 159	CMR		Beba (Wum)	
B	*camp.*	TO 5	TGO			GP 562
D	*camp.*	TO 6	TGO			GP 562
D	*text.*	TO 7	TGO			NI 816
B	*oler.*	ZR 1	ZAR	02° 19′ S 28° 45′ E	Mulungu, Kivu	NI 22
B	*oler.*	ZR 2	ZAR	02° 19′ S 28° 45′ E	Mulungu, Kivu	NI 23
B	*camp.?*	ZR 3	ZAR	06° 45′ S 23° 57′ E	Gandajika, Kasai	NI 202
B	*oler.*	ZR 4	ZAR	06° 45′ S 23° 57′ E	Gandajika, Kasai	NI 203
B	*oler.*	ZR 8	ZAR			NI 479

Table 2. Characteristics and banding patterns of the wild *Vigna unguiculata*. KEN Kenia, ZWE Zimbabwe, BWA Botswana, ZAR Zaire, BDI Burundi, NGA Nigeria, TZA Tanzania, NER Niger, SEN Senegal, ZAF South Africa, MR Cameroon, TCD Chad, CIV Ivory Coast, AGO Angola, MDG Madagascar, MWI Malawi, ZMB Zambia, COG Congo

Banding pattern	Subsp.	Accession number	Country	Locality	
A	*spon.*	FLG 4901A	KEN	03° 37′ S 39° 5′ E	Kilifi
A	*spon.*	FLG 4903B	KEN	02° 17′ S 40° 54′ E	Lamu
A	*spon.*	FLG 4904B	KEN	02° 17′ S 40° 54′ E	Lamu
C	*ten.*	MT 4	ZWE	17° 45′ S 31° 05′ E	
C	*paw.*	MT 53			
D	*spon.?*	MT 55B	ZWE	19° 03′ S 32° 44′ E	Vumba, 1220 m
A	*spon.*	MT 76B	ZWE	19° 57′ S 32° 05′ E	
B	*prot.*	MT 509A	BWA	24° 19′ S 25° 20′ E	19 km W Molepolole
D	*prot.*	MT 564B	BWA	23° 10′ S 20° 50′ E	21 km S Charles Hill
C	*spon.*	MT 621B	BWA	22° 22′ S 26° 52′ E	15 km S Serowe
A	*spon.*	NI 198A	ZAR	04° 01′ N 19° 19′ E	Kutubongo near Libenge (Ubangi)
A	*spon.*	NI 198B	ZAR	04° 01′ N 19° 19′ E	Kutubongo near Libenge (Ubangi)
A	*sb/pub*	NI 301	TZA	10° 20′ S 40° 28′ E	Msimbati
A	*spon.*	NI 319	ZAR	06° 45′ S 23° 57′ E	Gandajika (Kasai), 800 m
C	*spon.*	NI 320	ZAR		Muja Riv. (Kasai), 570 m
A	*spon.*	NI 423	ZWE	13° 08′ S 28° 25′ E	Luanshya
D	*bur.*	NI 456	BDI		
C	*baou.*	NI 794	NGA	07° 30′ N 03° 54′ E	IITA Campus Ibadan
C	*spon.*	NI 817	ZWE	18° 12′ S 31° 34′ E	Marandelas
A	*pub.*	NI 856	TZA	06° 43′ S 38° 23′ E	86 km E Morogoro
C	*spon.*	NI 874	ZWE	18° 12′ S 31° 34′ E	Marandelas
A	*pub.*	NI 910	TZA	06° 43′ S 38° 23′ E	86 km E Morogoro
A	*spon.*	NI 945	NER		Bara (Bara)
B	*spon.*	NI 951	NGA	12° 00′ N 08° 30′ E	Dalaram (Kano)
A	*pub.*	NI 957	TZA	05° 14′ S 38° 47′ E	40 km W Tanga
B	*spon.*	NI 963	SEN	12° 32′ N 16° 45′ E	Shirring cape, sea side
A	*pub.*	NI 989	KEN	03° 55′ S 39° 46′ E	Whispering Palms Hotel (Kilifi)
B	*spon.*	NI 991	NER	13° 29′ N 11° 57′ E	Campus Niamey University
A?	*spon.?*	NI 1167	ZAF		
A?	*spon.?*	NI 1171	ZWE	11° 23′ S 29° 31′ E	Bangwelu lake
A	*spon.*	NI 1228A			
A	*spon.*	NI 1228B			
A	*spon.*	NI 1232	BDI	04° 07′ S 29° 30′ E	Kigwena lake, 25 km S Rumonge
A	*spon.*	SP 3	CMR	11° 08′ N 14° 18′ E	Wumdare
B	*spon.*	SP 5	CMR	11° 08′ N 14° 18′ E	Wumdare
B	*spon.*	SP 33	CMR	07° 53′ N 14° 41′ E	Ndok – Bem
B	*spon.*	SP 34	CMR	07° 53′ N 14° 41′ E	Ndok – Bem
A	*baou.*	SP 36	CMR	06° 24′ N 11° 35′ E	Mayo Djinga
B	*spon.*	SP 37	CMR	08° 14′ N 14° 56′ E	Sorombeo
B	*spon.*	SP 38	CMR	07° 57′ N 14° 42′ E	Ndok
C	*baou.*	SP 39	CMR	03° 52′ N 11° 27′ E	Akokndoue
C	*baou.*	SP 45	CMR	04° 14′ N 11° 02′ E	km 1 Binoum – Keleng
A	*spon.*	SP 46	CMR	11° 24′ N 14° 34′ E	Waza
C	*let.*	SP 47	CMR	03° 49′ N 12° 01′ E	km 6 Nyodo – SODECAQ station
A	*let.*	SP 48	CMR	03° 49′ N 12° 01′ E	km 3 Nyodo – SODECAQ station
A	*spon.*	SP 52	CMR	05° 04′ N 14° 02′ E	km 2 Bambouti – Bertoua
C	*baou.*	SP 55	CMR	04° 18′ N 12° 15′ E	km 8 Nginda II – Nkobiba
B	*spon.*	SP 57B	CMR	10° 28′ N 13° 41′ E	Liri – Gova
A	*baou.*	SP 63	CMR	06° 41′ N 10° 43′ E	Nikambe – Berare
A	*spon.*	SP 64	TCD		Kournari

Table 2 (continued)

Banding pattern	Subsp.	Accession number	Country	Locality	
B	*spon.*	SP 65	NER		
A	*spon.*	SP 66	NER		Kambra road
C	*baou.*	SP 69	CIV	06° 43′ N 05° 50′ W	NE Proziblonfa
C	*baou.*	SP 72	CIV	06° 14′ N 05° 05′ W	Lamto
C	*spon.*	SP 74	AGO	09° 50′ S 13° 15′ E	Luanda
A	*spon.*	SP 75	TZA		Igawa, 155 MI S Iringa, 4000 ft
A	*spon.*	SP 78A	NER		Tenda (C. Sabongari)
A	*spon.*	SP 78B	NER		Tanda (C. Sabongari)
A	*spon.*	SP 81A	MDG	12° 40′ S 49° 18′ E	Antsakoafe, prov. Diego Suarez
A	*spon.*	SP 81B	MDG	12° 40′ S 49° 18′ E	Antsakoafe, prov. Diego Suarez
A	*spon.*	SP 83A	TZA	05° 05′ S 38° 28′ E	Korogwe
A	*spon.*	SP 83B	TZA	05° 09′ S 38° 28′ E	Korogwe
A	*spon.*	SP 85A	AGO		between Maria Teresa and Colomboluca
C	*spon.*	SP 85B	AGO		between Maria Teresa and Culomboluca
A	*spon.*	SP 87A	KEN		Weiwei (Katuw)
A	*spon.*	SP 88A	MWI	16° 30′ S 34° 50′ E	Lengwe Game Res.
A	*spon.*	SP 90	BWA	20° 03′ S 23° 19′ E	Road Xanakuna-Moshu
D	*let.*	SP 95A	CMR	05° 39′ N 14° 09′ E	Mari falls
C	*baou.*	SP 135B	CIV	06° 17′ N 05° 05′ W	Kokoti-Kouamekro
C	*spon.*	TVNU 265A	BWA		
C	*spon.*	TVNU 267	TZA		
A	*spon.*	TVNU 269B	BWA		
B	*spon.*	TVNU 272A	BWA		
D	*spon.*	TVNU 284A			
C	*spon.*	TVNU 290A	BWA		
C	*spon.*	TVNU 294B	TZA		
A	*spon.?*	TVNU 299A	TZA		
A	*spon.*	TVNU 300A	TZA		
A	*spon.*	TVNU 352A	ZMB		
D	*cong.*	TVNU 645A	COG		
A	*spon.?*	TVNU 660B	COG		
C	*baou.*	TVNU 784A	NGA	07° 30′ N 03° 54′ E	IITA Campus Ibadan

33 mM sodium acetate buffer, pH 4.8 at 4 °C overnight and centrifuged (20,000 g, 20 min, 4 °C). The precipitate of globulin proteins was resuspended in distilled water and the albumins in the supernatant were dialyzed against distilled water and lyophilized.

Immunoassays. Antibodies against pea legumin and pea vicilin were supplied by the Institut National de la Recherche Agronomique (INRA, Nantes). The electrophoretic transfer of proteins from acrylamide gels to nitrocellulose filters (LKB) was carried out according to TOWBIN & al. (1979) using an LKB 2005 Transphor apparatus. Blots were incubated with horseradish peroxydase-conjugated goat anti-rabbit IgG. The developing solution contained 0.6 mg/ml 4-chloro-l naphtol, dissolved in methanol, and 0.1% H_2O_2.

Electrophoresis procedures. The saline soluble extracts were analysed by polyacrylamide gel electrophoresis in the presence of SDS (SDS-PAGE) using 10%, 12.5%, or a 10–20% polyacrylamide (PAA) gradient. The gel containing 10% of PAA gave the most efficient separation of polypeptide components (results not shown) and were used in all subsequent SDS-PAGE analyses. One-dimensional SDS-PAGE was carried out (L.K.B. 2001 vertical electrophoresis unit) in 0.75 mm slabs (LAEMMLI 1970). The running gel was overlaid with

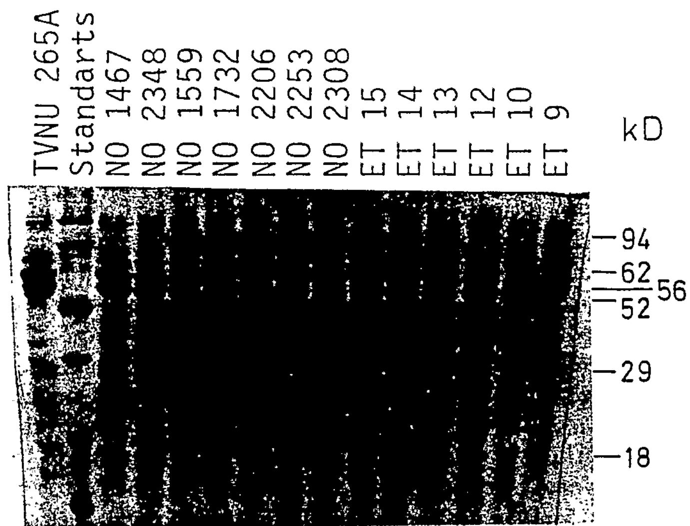
4% PAA as a stacking gel. Samples were solubilized by heating at 100 °C for 5 min in 50 mM Tris-HCl buffer pH 6.8 containing 5% (w/v) SDS, 30% (v/v) glycerol (sample buffer) and, in reducing conditions, 3% (v/v) 2-mercaptoethanol (2-ME). Gels were stained with 0.25% (w/v) coomassie brilliant blue R in methanol/acetic acid/water (50:10:40) and destained with the same solvent. Isoelectric focusing (IEF) was made on a L.K.B. multiphor 2117 apparatus. Slab gel (1 mm) contained 5% (w/v) PAA, 7.8 M urea, and 5% ampholines (Serva) of pH range 3.5 to 10 with omission of nonidet P40. Analyses of protein samples solubilized in 7 M urea were performed as previously described (DALGALARRONDO & al. 1984). Molecular weights were estimated using standard proteins (Pharmacia) run in the same gel and pHs on isoelectric focussed gel by surface electrode. Known molecular weight standard polypeptides were phosphorylase B (94 kD), bovine serum albumin (67 kD), ovalbumin (43 kD), carbonic anhydrase (30 kD), soybean trypsin inhibitor (20.1 kD), and lactalbumin (14 kD). For the two-dimensional electrophoresis, stained bands of the first run (ID) were excised and incubated (2 × 15 min) with the buffer suitable for the next electrophoresis (DALGALARRONDO & al. 1984, 1985). In order to improve the visualization of low abundance proteins the 2 D gels were miniaturized by the procedure described by MOHAMED & al. (1989).

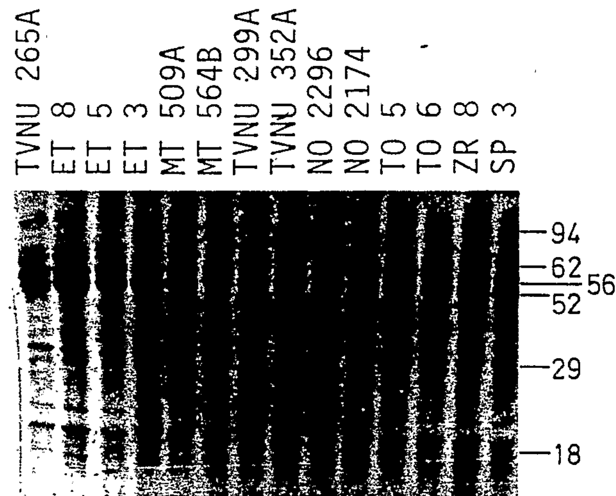
Results

Total protein extracts. The total protein extracts of 110 cultivated and 81 wild *Vigna unguiculata* accessions have been analyzed in non-reducing conditions as shown in Fig. 1. SDS-PAGE patterns were found to be composed of numerous bands. Major components of 94, 62, 56, 52, 29, and 18 kD could be identified in all accessions together with minor components. Additional subunits of 54, 50, and 49 kD were also detected. Based on this variability, four types of seed protein patterns (A–D) of wild and cultivated forms of cowpea can be distinguished. The different types contain polypeptide subunits of 62, 56, and 52 kD for A; 62, 56, 54, and 52 kD for B; 62, 56, 52, and 50 kD for C and 62, 56, 54, 52, 50, 49 kD for D (Fig. 2 A). The assignment of all accessions to the types described is reported in Tables 1 and 2. In the attempt to identify which particular proteins are responsible for such polymorphism, the albumin and globulin fractions were analyzed separately.

Seed albumins. As shown, for example, in the pattern of the A type (Fig. 2 B, line 2), the albumin fraction of all the samples analyzed exhibits in both non-reducing and reducing conditions the same major components of 94, 86, and 32 kD. Only the 24 kD albumin disappears after reduction. This phenomenon may be related to the presence of disulfide-linked low molecular weight albumins of other families (DECHERF-HAMEY & al. 1990). The results are reported in Table 3.

Seed globulins. The isoelectric precipitated globulin fraction was analyzed in monodimensional electrophoresis with or without 2 ME. In non-reducing conditions the globulin fraction of the A type shows protein bands of 110, 76, 62, 56, 52, and 41 kD molecular weights (Fig. 2 B, line 1). The three other types present additional components (54, 50, and 49 kD according to the accessions). SDS-PAGE (1 D) enabled the separation of seed globulins into two fractions: (i) the 110, 76, and 41 kD proteins which disappear in reducing conditions while bands of 58, 29, 18, and 15 kD appear. Thus these proteins are disulfide-linked and may be related to 11 S legumin-like globulins (KHAN & al. 1980). (ii) The 62, 56, and 54 kD subunits which are unaltered after reduction and constitute the nondisulfide-linked globulin fraction (NDL).





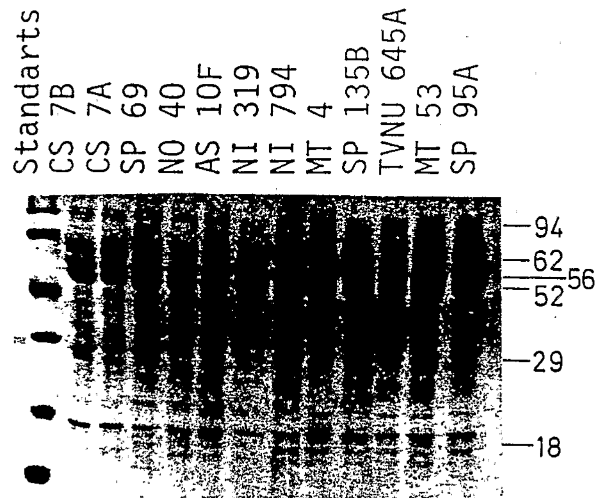


Fig. 1. Monodimensional electrophoresis (SDS-PAGE) of *Vigna unguiculata* seed storage proteins in non-reducing conditions

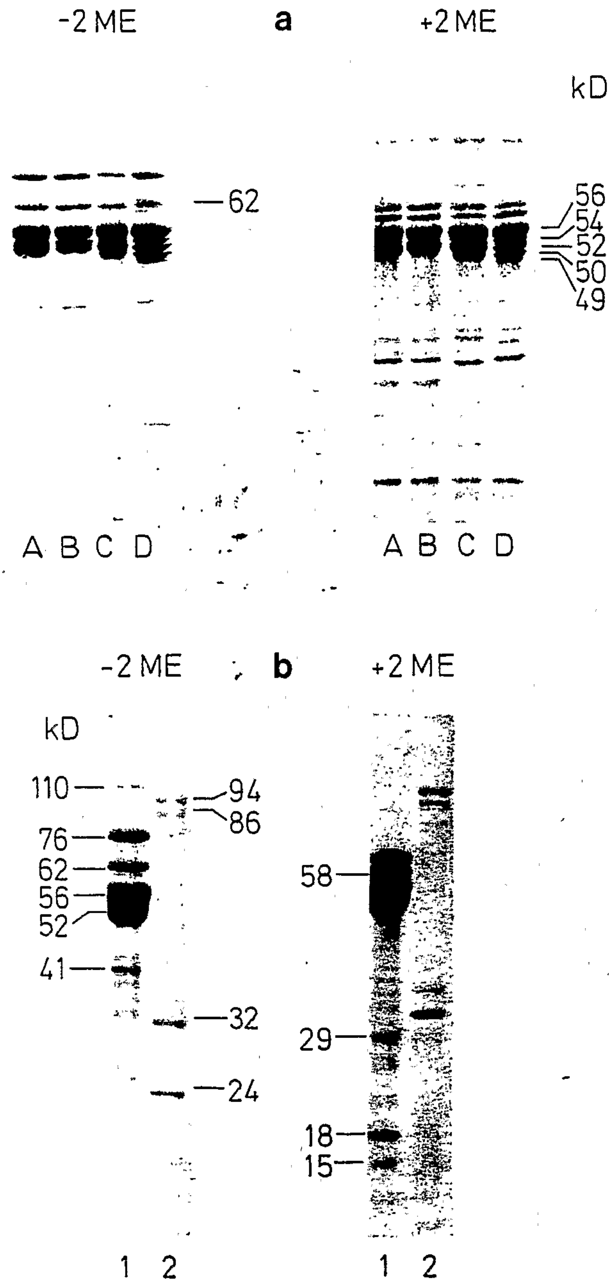


Fig. 2. Monodimensional electrophoresis (SDS-PAGE) of *Vigna unguiculata* seed storage proteins in reducing and non-reducing conditions. *a* Crude extracts of the A, B, C, and D types. *b* Separated globulins (1) and albumins (2) of the A type

The four types of seed protein patterns were also analyzed by two-dimensional SDS-PAGE (Fig. 3). Concerning the legumin-like globulins, the 2 D analysis enables a determination of the polypeptide composition of these proteins. For A and B patterns, it was evident that 58 and 18 kD proteins as well as polypeptides of 29 and 15 kD were disulfide-linked to form a prominent 76 and 41 kD subunit. The

Table 3. Apparent molecular weight (MW × 10^{-3}) and isoelectric point (IP) of nondisulfide-linked, legumin-like, and albumin cowpea seed fractions (unreduced and reduced)

MW (kD)	Nondisulfide-linked fraction							
	A	B	C	D	IP			
62	+	+	+	+	6.2			
56	+	+	+	+	5.4	5.5	5.8	6.1
54	−	+	−	+	5.4	5.45[a]	5.75[a]	6.1
52	+	+	+	+	5.4[b]	5.45[c]	5.75[b, c]	6.1
50	−	−	+	+	5.4[d]	6.1[d]	6.7[e]	6.8[e]
49	−	−	+	+	7.0	7.1		

a absent in D type, *b* absent in D type, *c* absent in C type, *d* absent in C type, *e* absent in D type

MW legumin-like fraction (kD)			MW albumin fraction
(αβ) pairs	α	β	
110	non-determined		94
76	58	18	86
41	29	15	32
			24 (αβ)

110 kD globulin, a minor species, is also supposed to be a legumin-like component and is considered to be composed of two 56 kD subunits polypeptides, as described by KAHN & al. (1980). These polypeptides are only faintly visible in the 2 D (Fig. 3 A, black arrow). The 2 D analysis permit to confirm that the 62, 56, 54 kD subunits were unaltered after reduction (NDL fraction). This is also the case for the 52, 50, and 49 kD subunits of the B, C, and D types. The composition of the whole globulin fraction is reported in Table 3.

Legumes in general contain globulin type storage proteins which can be assigned to two main types: legumin and vicilin. Although considerable variations in the relative amounts of these two types of storage proteins exist, it appears that *V. unguiculata* contains a substantial excess of nondisulfide-linked proteins over legumin-like proteins. Although *V. unguiculata* globulins resembles the other legume 11 S and 7 S fractions (molecular weights 300–400 kD and 170 kD, respectively, on G 200 gel filtration, results not shown), the 7 S fraction will be referred to as nondisulfide-linked (NDL) fraction in the text and may corresponds to vicilins (subunits of 45–60 kD in SDS, heterogeneous and nondisulfide-linked) despite the serological cross-reaction with pea vicilin failed to give a positive precipitation.

Among the 81 samples of wild forms of cowpea analyzed, the NDL fraction of A type represents 56.6%, B: 10%, C: 26.6%, and D: 7.6% while among the 110 cultivated forms it represents 30%, 57.6%, 2.7%, and 3.6%, respectively.

The IEF/SDS-PAGE (+ 2 ME) two-dimensional profiles of the four types revealed a high heterogeneity in the polypeptidic composition (Fig. 4). The major

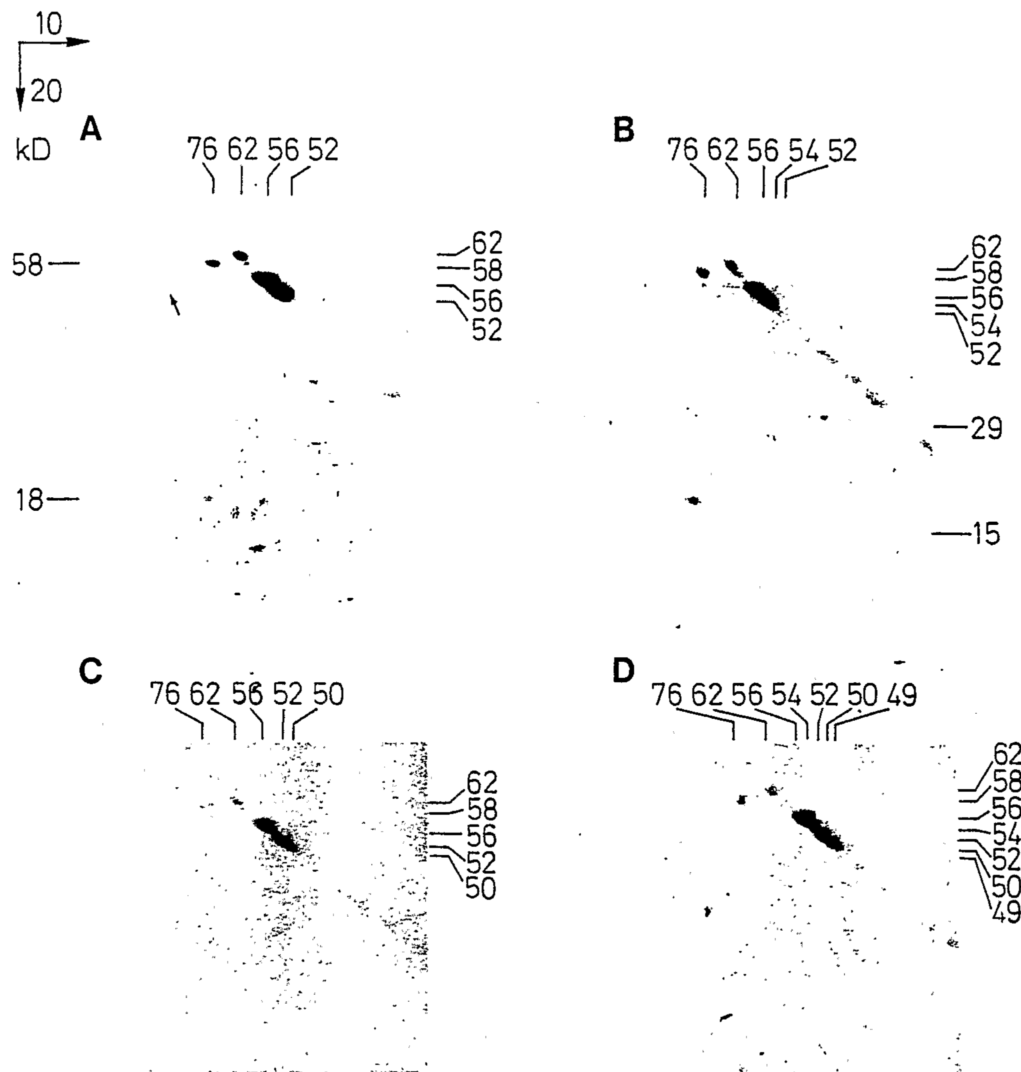


Fig. 3. Two-dimensional electrophoresis of *Vigna unguiculata* seed storage proteins (1 D: SDS-PAGE; 2 D: SDS-PAGE + 2-ME). A–D patterns correspond to A–D types, respectively. Arrow indicates faintly visible 56 kD polypeptides

components, concerning only NDL proteins, exhibited pI's in the range 5.4–6.2. Except for the 62 kD molecular weight all the others showed charge polymorphism. The most important differences concern the presence of more basic components (pI 7.0) of 50 and 49 kD molecular weights only represented in the C and D types (arrows on C and D patterns in Fig. 4). Additional differences can be observed in the pH range 5.4–6.2 where some spots present in A and B types are obviously absent in the C and D types (arrows on A and B patterns in Fig. 4). Concerning the legumin-like fraction, the 76 kD subunit is composed of a 58 kD polypeptide (pI 6.2) bound to a 18 kD polypeptide (pI 7.0) by disulfide(s). The 41 kD subunit is constituted of a 29 kD polypeptide (pI 7.0) plus a 15 kD polypeptide (not detected in the 2 D). By analogy with other legumin-like 11 S globulins (Derbyshire & al.

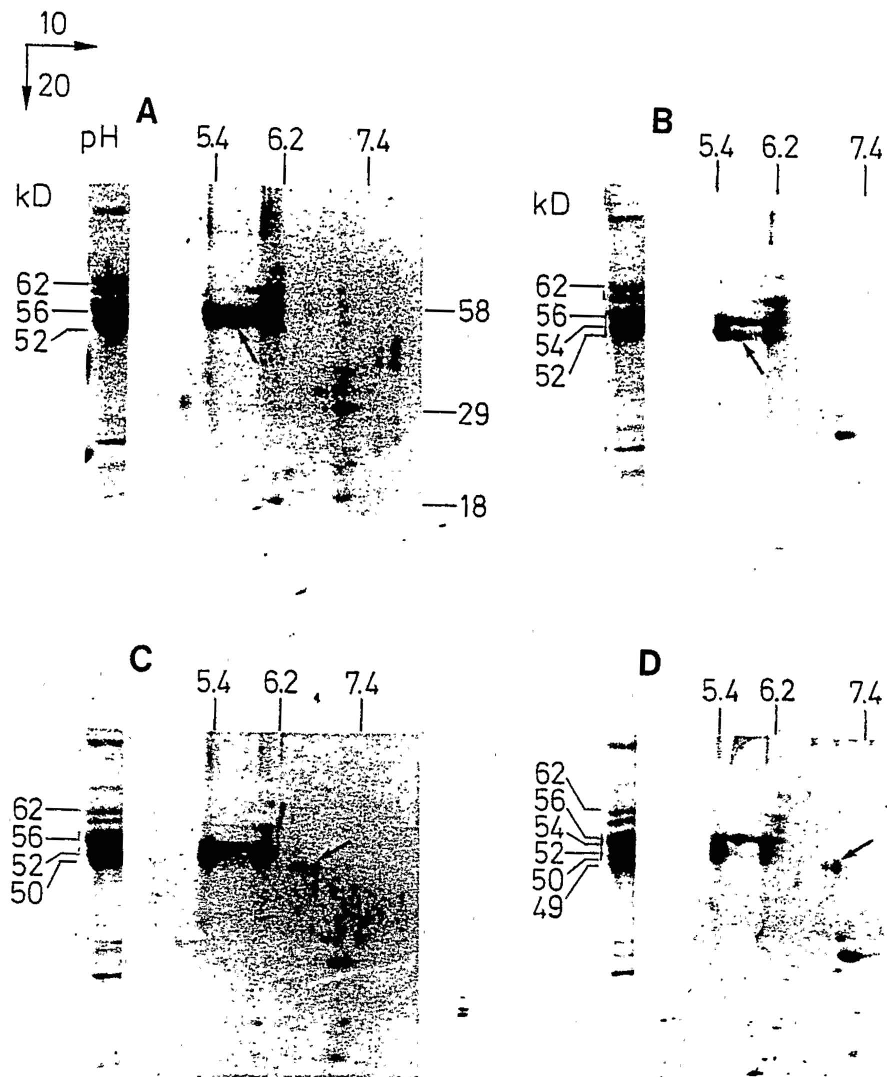


Fig. 4. Two-dimensional analysis of *Vigna unguiculata* seed storage proteins (1 D: IEF; 2 D: SDS-PAGE + 2-ME). A–D patterns correspond to the entire A–D types. Arrows indicate the main charge polymorphism differences observed between the types

1976) the higher molecular weights polypeptides might well be acidic and those of lower molecular weights, basic. The results are summarized in Table 3.

Discussion

The infraspecific analysis deals with 191 accessions. Eight of the nine perennial taxons (with 23 accessions) have been considered although 23 is not important enough. The 9th taxon, subsp. *dekindtiana* sensu stricto from the south of Angola does not exist in living collections. The sampling of the annual forms, with accessions coming from 17 different countries is enough representative. Among the cultivated

forms, there is only one accession of cv. '*sesquipedalis*' but we have six accessions of cv. '*textilis*' (among which five were collected by R. PASQUET).

The usefulness of seed protein variability for discriminating among cultivars and wild accessions as well as for studying the genetic relationships among lines has been widely reported for legumes (LADIZINSKY & HYMOWITZ 1979, KRISHNA & MITRA 1988, GEPTS & al. 1988, SCHINKEL & GEPTS 1988, KROCHKO & al. 1990, PASQUALINI & al. 1991, TUCCI & al. 1991). The knowledge of the infraspecific variation is necessary if protein patterns are used additionally for the characterization of species. Working on cultivated cowpea, KHAN & al. (1980), PEDALINO & al. (1990) have described two different vicilin banding patterns. Our results collate more data concerning the infraspecific polymorphism of wild and cultivated forms and we present evidence for four types based on the variability of the NDL components which constitute the major protein fraction. The four protein types contain the three major subunits of the A type (62, 56, and 52 kD molecular weights). Besides, type B contains a particular subunit of 54 kD, type C of 50 kD, while type D exhibits both subunits of 54 and 50 kD plus an other subunit of 49 kD molecular weight. The given molecular weights are in good agreement with those precedently described by CARASCO & al. (1978) (52000, 54000, and 56000), KHAN & al. (1980) (52000, 58000, and 63000) and MURRAY & al. (1983) with 52500, 56000, and 63000 daltons.

The cultivated accessions are mainly represented by A or B types (90%) (Table 4 A). A well balanced proportion of A and B types is found in the *oleraceus* and *melanophtalmus* groups. This is not the case in the *campestris* group. In this group the Ethiopian accessions (2 A type and 9 B type) are responsible for the apparent disequilibrium as the remaining *campestris* group possess an equilibrated proportion of A, B, and D profiles. The C type appears in the three accessions (*oleraceus*) from South Africa (the three others are of A type). So a bipolarity between the Ethiopian group and the South African group can be observed. Cv. '*oleraceus*' belongs to the south morphological group while the two cv. '*campestris*' and '*melanophtalmus*' are representative of the north morphological group. As shown by their morphological traits (PASQUET 1992) traces of introgression between these two geographic groups of cultivated accessions have been found especially in the south Cameroun where a major part of the accessions are derived. These introgressions account for the absence of C type and the abundance of B type in the Cameroun subspecies. The D profile appears in two accessions of cv. '*textilis*' and two accessions of the '*campestris*' group. Curiously enough, in cv. '*textilis*' the D type appears in NO 40 and in NO 577 but neither in NO 654A nor in NO 2798 which are respectively very close (Table 1).

Concerning the wild accessions, the annual accessions (subsp. *unguiculata* var. *spontanea*) which are morphologically and isoenzymically close to the cultivated ones must be separated from the perennial forms which are more distant (PASQUET 1992). The perennial forms present a majority of C (11) and D (4) profiles. Subsp. *pubescens* presents only the A type (Table 4 B). This taxon is the nearest of the annual forms as shown by isoenzymes (PASQUET 1993). As plant breeding systems influence the degree of genetic diversity among populations (LOVELESS & HAMRICK 1984) the internal variability of perennial forms is reinforced by the fact that most of these plants are rather outcrossed as compared to the annual or the few perennial

Table 4. Distribution of the protein patterns among the *Vigna unguiculata* subspecies

Subspecies	A	B	C	D
A. Cultivated accessions				
Campestris	5	13	–	2
Melanophtalmus	17	22	–	–
Oleraceus	21	20	3	–
Sesquipedalis	–	1	–	–
Textilis	–	4	–	2
Total cultivated forms	43	59	3	4
B. Wild perennial accessions				
Pubescens	4	–	–	–
F. Congo	–	–	–	1
Stenophylla	–	1	–	1
Baoulensis	2	–	8	–
Letouzeyi	1	–	1	1
Burundiensis	–	–	–	1
Tenuis	–	–	1	–
F. Malawi	–	–	1	–
Total perennial forms	7	1	11	4
C. Wild annual accessions				
Spontanea North	10	11	–	–
Spontanea South	26	1	10	2
Total annual forms	36	12	10	2

forms (such as subsp. *stenophylla* and subsp. *pubescens*) which are rather self-crossed. Among the A type of subsp. *baoulensis*, one accession comes from Cameroun, the other from Ivory Coast. In contrast, subsp. *letouzeyi* whose accessions come from a relatively small area (60 km in diameter) presents three different profiles. It is likely that the finer study of a greater number of perennial forms would increase the degree of variability observed and would also strengthen the arguments provided by morphological and isoenzymic data showing the different perennial subspecies to be far from each other and far from the complex annual forms/cultivated forms.

The annual forms present mainly A, B, and C types (Table 4 C). However, a different protein type spectrum is observed between the accessions on both sides of the equatorial forest. The northern group shows only A (10) and B (10) profiles while the southern group present mainly A (26) and C (10) types and only one B type (TVNU 272 from Botswana) and two D type (MT 55B from Zimbabwe and TVNU 284A from Botswana). Thus the wild and cultivated forms of the northern group appear to be identical. In contrast those of the annual southern group are different by the almost total lack of B type and the relative abundance of the C type. It may be noticed that the A and C types found in the annual accessions of the south are also present in the cultivated South African forms (3 A type and 3 C type). Thus, whatever the geographical zone, a quasi identity between wild annual forms and cultivated forms may be observed. The difference between the cluster

cultivated-wild annual of a similar geographical zone is lower than the difference between the northern and the southern groups due to C pattern. This correlation between wild annual and cultivated forms is in large accordance with the taxonomic data used which cluster in subsp. *unguiculata* the spontaneous annual forms (var. *spontanea*) and the cultivated forms (var. *unguiculata*) since the difference between annual and cultivated forms is not any greater than those between cultivated-annual north and cultivated-annual south forms.

Studies of *Glycine max.* concerned, firstly, cultivated forms and dealt with enzymes (Hymowitz & Kaizuma 1979, 1981) and to a lesser degree the 7 S fraction of seed proteins (Kitamura & al. 1984, Davies & al. 1985, Tsukada & al. 1986). For *Cajanus cajan* (Ladizinski & Hamel 1980), *Psophocarpus tetragonolobus* (Kortt 1983, Blagrove & Gillespie 1978), *Phaseolus polyanthus* (Schmit & Debouck 1991) and *Vigna radiata* (Tomooka & al. 1992), little or no variability is observed within the cultivated forms. The observed variability in the cultivated forms of cowpea is low but not negligible. This variability is very poorly correlated to morphological and biogeographical groups as it is in *Phaseolus vulgaris* (Brown & al. 1981, Gepts & Bliss 1985, Romero-Andreas & Bliss 1985, Gepts & al. 1986, Gepts & Bliss 1986) and *Phaseolus lunatus* (Debouck & al. 1989, Maquet & al. 1990). This would be an argument favouring the unique domestication of *Vigna unguiculata* contrary to these two other species. Among the few studies dealing with wild accessions of neighbouring species, those of Romero-Andreas & Bliss (1985) on *Phaseolus vulgaris* and Schinkel & Gepts (1988) on *Phaseolus acutifolius* display quite a lot of different patterns (8 for *P. vulgaris* and 15 for *P. acutifolius*). Such an increase in variability has not been observed with *V. unguiculata* but the number of cowpea perennial accessions is too low to draw definite conclusions, except that they are well separated from the annual forms and present some specific nondisulfide linked components.

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