Theor Appl Genet (2000) 101:211–219

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Allozyme diversity of cultivated cowpea *Vigna unguiculata* (L.) Walp.

Received: 18 February 1999 / Accepted: 4 November 1999

Abstract A survey of allozyme variation in cultivar-groups of cowpea [*Vigna unguiculata* (L.) Walp.] was undertaken by examining 21 enzyme systems encoded by 36 loci in 271 accessions representing the five cultivar-groups. Very low levels of variation were found within accessions, which is typical of self-pollinating species. Little variation was also found among accessions. Compared with other legume crops, *V. unguiculata* is depauperate in allozyme variation. We found an average of 1.61 alleles per locus with 42% of the loci polymorphic and a total heterozygosity of 0.061. Of the variation present, 90% was found within cultivar-groups, while 10% was among cultivar-groups. Data analyses revealed continuous variation among cultivar-groups and geographic regions with the accessions failing to segregate into discrete morphophysiological or geographic clusters. However, evolved cultivar-groups (cv.-gr. Melanophthalmus and cv.-gr. Sesquipedalis) appear to be less diverse than their putative primitive cultivar-group progenitors. Due to the lack of availability of critical material, no clear center of origin can be established. However, the data presented suggest that Northeast Africa could be a possible center of domestication.

Key words Cowpea · *Vigna unguiculata* · Allozymes · Crop evolution · Genetic diversity

Introduction

Although a wide array of biochemical and molecular procedures allow genetic diversity to be estimated at various levels of biological organization, allozyme studies still have numerous advantages for investigations of crops and their wild progenitors. These include a wide applicability, low cost and speed. Additionally, allozymes are codominant markers, encoded by loci scattered over the genome, and they have never been under direct human selection. Therefore, allozyme studies can provide insight into the evolution of the crop and give answers to classical questions concerning crop evolution: for example – What wild taxon is ancestral to the cultigen? Has there been a loss of genetic variation as a result of domestication? Where are the centers of origin and centers of domestication? What is the relationship between morphological diversity and allozyme polymorphism? (Doebley 1989; Hamrick and Godt 1997).

Cowpea, *Vigna unguiculata* (L) Walp, is currently cultivated in all tropical areas and in some temperate areas like the Mediterranean Basin and the southern states of the USA. World cowpea acreage is estimated to exceed 12.5 million hectares, and annual production is over 3 million tons world-wide (Singh et al. 1997). Therefore, it is one of the main grain legume crops in the world and is the principal grain legume throughout much of Africa. In addition to use as a pulse crop, it is cultivated as a fodder and has also been used as a fiber crop, although the latter use is currently disappearing.

Despite the fact that cowpea was known in India during the first millenium B.C. (Steele and Mehra 1980) and despite its wide distribution in Asia, all evidence points to its origin in Africa, although where the crop was first domesticated is uncertain. Ethiopia (Vavilov 1926; Steele 1972), West Africa (Murdock 1959; Faris 1963; Rawal 1975; Maréchal et al. 1978; Vaillancourt and Weeden 1992; Ng 1995) and eastern and southern Africa (Baudoin and Maréchal 1985) have all been considered probable centers of domestication, while a "diffuse" domestication in the savanna after the dispersal of cereals has also been hypothesized (Chevalier 1944; Steele 1976). The latter hypothesis was supported by Harlan (1971) who considered that cowpea was domesticated in his African Non-Center.

Communicated by I. Dvorak

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The species includes cultivated forms, i.e. *V. unguiculata* ssp. *unguiculata* var. *unguiculata*, wild annual forms, i.e. ssp *unguiculata* var. *spontanea* (Schweinf.) Pasquet, and ten wild perennial subspecies (Pasquet 1993a, 1993c, 1997). This classification is based on results from morphological (Pasquet 1993a; Padulosi 1993), allozyme (Panella and Gepts 1992; Vaillancourt et al. 1993; Pasquet 1993b, 1999) and cpDNA studies (Vaillancourt and Weeden 1992; Pasquet unpublished data). *V. unguiculata* ssp. *unguiculata* var. *spontanea* is the likely progenitor of the cultivated cowpea (Pasquet 1999).

Although cultivated cowpea classification was based on three groups for a long time (Piper 1912; Westphal 1974), it is now based on five cultivar-groups (cv.-gr.) (Pasquet 1998):

- cv.-gr. Textilis (long inflorescence peduncle, usually 40°cm–1°m) in West Africa;
- cv.-gr. Sesquipedalis (fleshy pod, wrinkled when ripe, longer than 30 cm, kidney-shaped seeds spaced within the pod, more than 17 ovules), chiefly in East Asia;
- cv.-gr. Melanophthalmus (seed testa thin and often wrinkled, flower and seed partly white, fewer than 17 ovules, plant able to flower quickly from the first nodes under inductive conditions), originally in West Africa;
- cv.-gr. Biflora (seed testa thick and shiny, flower and seed most often coloured, fewer than 17 ovules, plant able to flower quickly from the first nodes under inductive conditions);
- cv.-gr. Unguiculata (seed testa thick and shiny, flower and seed most often coloured, more than 16 ovules, plant flowering late, even under inductive conditions).

Two main groups can be separated on the basis of their physiology and their number of ovules: cv.-gr. Biflora and cv.-gr. Melanophthalmus display a low number of ovules and can flower quickly under inductive conditions, while cv.-gr. Unguiculata and cv.-gr. Sesquipedalis display a high number of ovules and cannot flower quickly under inductive conditions. Each main group is subdivided into a primitive subgroup and an evolved subgroup. The primitive cultivars (from cv.-gr. Unguiculata and especially cv.-gr. Biflora) are characterized by pods more or less dehiscent and small seeds with thick testa and wild colours (tan, gray, mottled and speckled), and their morphological diversity is low. In contrast, cv.-gr. Melanophthalmus and cv.-gr. Sesquipedalis look like the outcome of two divergent lineages and are characterized by the expression of several recessive genes (Pasquet 1998).

However, these hypotheses inferred from morphological data, have never been examined using molecular data. While previous studies of allozyme variability included cultivated accessions, they still used Piper's classification (Panella and Gepts 1992; Vaillancourt et al. 1993).

Table 1 Country of origin, cultivar group and identification number of the cowpea accessions included in this study[a]

Country[b]	Identification number
Cultivar-group Textilis	
CMR	NO 27, NO 40, NO 198, NO 262, NO 577, NO 654, NO 668, NO 1898, NO 2300, NO 2613, NO 2798, NO 3076.
TGO	TO 7.
Cultivar-group Biflora	
BRA	EX 79.
CHN	EX 15, EX 16.
CMR	NO 2, NO 89, NO 106, NO 117, NO 165, NO 175, NO 177, NO 179, NO 183, NO 251, NO 252, NO 253, NO 309, NO 311, NO 324, NO 325, NO 326, NO 1425, NO 1669, NO 1795, NO 1878, NO 1880, NO 2063, NO 2174, NO 2295, NO 2606, NO 2633, NO 3113, NO 3336.
DZA	AG 2, AG 4, AG 5, AG 6, AG 7.
EGY	EG 1.
ERI	ET 25.
ETH	ET 1, ET 2, ET 4, ET 5, ET 6, ET 7, ET 8, ET 9, ET 10, ET 11, ET 12, ET 13, ET 14, ET 15, ET 16, ET 23, ET 26, ET 27, ET 28, ET 30, ET 31, ET 40, ET 41.
HVO	HV 1, HV 2, HV 5, HV 7, HV 9.
IND	EX 14, EX 35, EX 53, EX 56, EX 63, EX 74, EX 78.
IRQ	EX 18, EX 58.
LAO	EX 37.
MDG	MG 5.
PAK	EX13, EX 39, EX 49, EX 50, EX 51, EX 52, EX 54.
PHL	EX 41, EX 42.
SUN	EX 17.
TGO	TO 1, TO 3, TO 4, TO 5.
THA	EX 40.
UGA	UG 4, UG 10.
USA	EX 29, EX 30, EX 31.
YEM	ET 33, ET 34, ET 39.
ZAR	ZR 3, ZR 7.
Unknown	EX 34 [c].

Table 1 (continued)

Country[b]	Identification number
Cultivar group Melanophthalmus	
CMR	NO 7, NO 14, NO 17, NO 28, NO 95, NO 110, NO 122, NO 129, NO 133, NO 137, NO 144, NO 173, NO 189, NO 193, NO 260, NO 574, NO 643, NO 649, NO 661, NO 670, NO 760, NO 927 A, NO 927B, NO 1223, NO 1319, NO 1467, NO 1559, NO 1732, NO 2208, NO 2253, NO 2296, NO 2308, NO 2348, NO 2529, NO 2547, NO 2616, NO 2629, NO 2711, NO 2720, NO 2721, NO 2751, NO 2806, NO 2836, NO 2837, NO 2844, NO 3098, NO 3148, NO 3157, NO 3160, NO 3256.
DZA	AG 1, AG 3.
HVO	HV 10.
ITA	EX 20, EX 21, EX 23, EX 24.
SUN	EX 25.
USA	EX 28.
Cultivar-group Unguiculata	
AGO	AN 1.
BRA	EX 73, EX 75.
COL	EX 19.
CMR	CS 5, CS 7, CS 14, CS 15, CS 20, CS 23, CS 45, CS 52, CS 53B, CS 54, CS 56B, CS 56 C, CS 56D, CS 56 E, CS 67, CS 85, CS 151, CS 152, CS 154, NO 74, NO 90, NO 125, NO 195, NO 576, NO 2206, NO 2220, NO 2304, NO 2466, NO 2973, NO 3123, OU 23, OU 24B, OU 28, OU 31, OU 59 A, OU 65, OU 100, OU 130, OU 134D, OU 150B, OU 150F, OU 152B, OU 152 C, OU 158, OU 159, OU 174, OU 176 C.
ETH	ET 3, ET 20, ET 21, ET 35.
IND	EX 55, EX 57, EX 59.
MDG	MG 10.
SOM	ET 32.
TGO	TO 6.
UGA	UG 3, UG 5, UG 8, UG 11, UG 12.
USA	EX 27, EX 82.
YEM	ET 38.
ZAF	AS 1 A, AS 2B, AS 2 C, AS 3D, AS 3 E, AS 8, AS 10 A, AS 10 C, AS 10F.
ZAR	ZR 1, ZR 2, ZR 4, ZR 8.
Cultivar-group Sesquipedalis	
CHN	EX 12, EX 33, EX 36.
CMR	NO 1036.
GUY	EX 32.
IDN	EX 48, EX 81.
IND	EX 11.
NCL	EX 38.
PHL	EX 43, EX 83.
THA	EX 71, EX 72.

[a] Additional information regarding accessions can be obtained directly from the author.
[b] AGO, Angola; BRA, Brazil; CMR, Cameroon; CHN, China; COL, Colombia; DZA, Algeria; EGY, Egypt; ERI, Eritrea; ETH, Ethiopia; GUY, Guyana; HVO, Burkina Faso; IDN, Indonesia; IND, India; IRQ, Iraq; ITA, Italia; LAO, Laos; MDG, Madagascar; NCL, New-Caledonia; PAK, Pakistan; PHL, Philipins; SOM, Somalia; SUN, Georgia; THA, Thailand; TGO, Togo; UGA, Uganda; YEM, Yemen; ZAF, South-Africa; ZAR, Zaire.
[c] Although of unknown origin, EX 34 morphology suggests an Asian origin

In this article, we report on allozyme analyses of 271 cultivated accessions, representing a geographical distribution extending from America to South-East Asia, with a special emphasis on areas (West, Central and Northeast Africa) where cowpea could have originated. The aim of the investigation was to use allozymes to examine the genetic variation within and among *V. unguiculata* cultivar-groups and to try to develop hypotheses on cowpea domestication.

Materials and methods

Plant materials

Allozyme diversity was assayed in 271 cultivated accessions (Table 1). Of these, 132 accessions were provided by various institutes, including Agricultural University (Wageningen, The Netherlands), Botanical Research Institute (Pretoria, South Africa), EMBRAPA (Golânia, Brazil), Faculté des Sciences Agronomiques (Gembloux, Belgium), FAL Völkenrode (Braunschweig, Germany), Jardin Botanique National de Belgique (Meise, Belgium), Kasetsart University (Thailand), USDA, ARS (Charleston and Griffin, USA) and Zentralinstitut für Genetik und Kultupflanzenforschung (Gatersleben, Germany). An additional 139 accessions came from the ORSTOM cultivated cowpea collection maintained in Montpellier, now duplicated in IITA (Ibadan, Nigeria) and USDA, ARS (Griffin, Ga, USA). These 139 accessions were collected in Cameroon during an ethnobotanical survey (Pasquet and Fotso 1994). They belong to 67 of the 89 cultivars identified (3 cultivars from cv.-gr. Textilis, 12 cultivars from cv.-gr. Biflora, 31 from cv.-gr. Melanophthalmus, 20 from cv.-gr. Unguiculata and a cultivar from cv.-gr. Sesquipedalis). Additional information about the accessions can be obtained directly from the author.

Each accession was made of two self-pollinated lines and maintained as such, with each of these lines coming from one seed

of the original stock. Therefore, two plants were assayed per accession.

Isozyme electrophoresis

The methods for sample preparation, horizontal starch gel electrophoresis (histidine/citrate system at pH 6.0 was used for all enzymes, and the gel mixture contained 14% starch) and enzyme staining are described in Pasquet (1999). The 21 enzyme systems studied were alcohol dehydrogenase (ADH), aminopeptidase (AMP), NADH diaphorase (DIA), endopeptidase (ENP), esterase (EST), fluorescent esterase (FLE), formate dehydrogenase (FDH), glucose-6-phosphate dehydrogenase (G6PD), β-glucosidase (βGLU), glutamate dehydrogenase (GDH), glutamate oxaloacetate transaminase (GOT), glutathione reductase (GR), isocitrate dehydrogenase (IDH), mannose phosphate isomerase (MPI), malate dehydrogenase (MDH), malic enzyme (ME), phosphoglucomutase (PGM), phosphogluconate dehydrogenase (PGD), phosphoglucose isomerase (PGI), shikimate dehydrogenase (SDH), and superoxyde dismutase (SOD). Isozyme and allozyme nomenclature follow the outline given by Pasquet (1999).

Data analysis

Electrophoretic data were analyzed using BIOSYS-1 version 1.7 (Swofford and Selander 1981) and NTSYS version 1.80 (Rohlf 1993). Data for accessions were entered as allele frequencies. In

Table 2 Mean allele frequencies for major groups of cultivated cowpea examined in this study. Locus/allele nomenclature is described in the Materials and methods. The 21 monomorphic loci, i.e. *Adh1*, *Adh2*, *Dia1*, *Dia2*, *Est1*, *Est3*, *Fle1*, *Gdh*, *βGlu1*, *βGlu3*, *Got1*, *Gr*, *Idh1*, *Idh2*, *Mdh1*, *Mdh2*, *Mdh3*, *Mpi*, *Pgd1*, *Pgm2*, and *Sod2* are not included in the Table

		Total (271)[b]	S[a] (13)	U (82)	M (59)	T (13)	B (104)	B-WA (42)	B-AS (21)	B-NEA (27)	B-O (14)
Amp2	102	0.965	1	0.988	1	0.692	0.957	0.976	0.976	0.889	1
	100	0.035	0	0.012	0	0.308	0.043	0.024	0.024	0.111	0
Amp3	103	0.093	0	0	0.034	0	0.221	0.381	0.214	0.056	0.071
	100	0.907	1	1	0.966	1	0.779	0.619	0.786	0.944	0.929
Amp3b	100	0.966	1	0.945	0.949	1	0.985	0.964	1	1	1
	95	0.030	0	0.055	0.051	0	0.005	0.012	0	0	0
	90	0.004	0	0	0	0	0.010	0.024	0	0	0
Amp4	100	0.991	1	1	1	1	0.976	0.988	0.905	1	1
	96	0.002	0	0	0	0	0.005	0.012	0	0	0
	93	0.007	0	0	0	0	0.019	0	0.095	0	0
Enp	105	0.089	0.308	0.061	0	0	0.144	0	0.095	0.407	0.142
	100	0.706	0.615	0.646	0.780	0.923	0.697	0.821	0.810	0.556	0.429
	98	0.205	0.077	0.293	0.220	0.077	0.159	0.179	0.095	0.037	0.429
Fdh	102	0.242	0	0.171	0.254	0.538	0.284	0.571	0.095	0.074	0.107
	100	0.758	1	0.829	0.746	0.462	0.716	0.429	0.905	0.926	0.893
Fle3	100	0.153	0.038	0.244	0	0.154	0.183	0.060	0	0.593	0.036
	96	0.847	0.962	0.756	1	0.846	0.817	0.940	1	0.407	0.964
G6pd	105	0.081	0	0.012	0	0.308	0.163	0	0	0.630	0
	100	0.919	1	0.988	1	0.692	0.837	1	1	0.370	1
Got2	110	0.179	0.038	0.512	0	0	0.058	0	0.190	0.074	0
	100	0.821	0.962	0.488	1	1	0.942	1	0.810	0.926	1
Me	100	0.843	0.769	0.902	0.958	0.885	0.735	0.952	0.762	0.315	0.857
	97	0.153	0.231	0.098	0.042	0.115	0.255	0.048	0.190	0.685	0.143
	94	0.004	0	0	0	0	0.010	0	0.048	0	0
Pgd2	100	0.991	1	1	1	1	0.976	1	1	0.907	1
	96	0.009	0	0	0	0	0.024	0	0	0.093	0
Pgi2	115	0.007	0	0	0	0.077	0.010	0.024	0	0	0
	107	0.004	0	0	0	0	0.010	0	0	0.037	0
	100	0.989	1	1	1	0.923	0.980	0.976	1	0.963	1
Pgi3	100	0.993	1	0.988	1	1	0.990	0.976	1	1	1
	92	0.007	0	0.012	0	0	0.010	0.024	0	0	0
Pgm1	105	0.004	0	0	0	0.077	0	0	0	0	0
	100	0.996	1	1	1	0.923	1	1	1	1	1
Sdh	105	0.004	0	0	0	0	0.010	0	0	0.037	0
	100	0.979	0.846	1	1	0.923	0.975	1	0.952	0.963	0.964
	95	0.015	0.154	0	0	0.077	0.010	0	0.048	0	0
	90	0.002	0	0	0	0	0.005	0	0	0	0.036

[a] S, cv.-gr. Sesquipedalis; U, cv.-gr. Unguiculata; M, cv.-gr. Melanophthalmus; T, cv.-gr. Textilis; B, cv.-gr. Biflora; B-WA, cv.-gr. Biflora from West Africa; B-AS, cv.-gr. Biflora from Asia; B-NEA, cv.-gr. Biflora from Northeast Africa; B-O, cv.-gr. Biflora from other origins

[b] Numbers of accessions are in parenthesis

addition, a hierarchical arrangement of the accessions was established at the cultivar-group level so that the data could be analyzed within and among cultivar-groups and summarized per cultivar-group. Allelic compositions of each cultivar-group were determined at 36 presumed loci.

Genetic variability was assessed using the proportion of polymorphic loci (*P*), the mean number of alleles among all loci (*A*) and among polymorphic loci (A_p), and the total diversity (H_t) (Nei 1973). Total diversity was partitioned into the weighted average diversity within cultivar-groups (H_s) and the between-cultivar-group gene diversity (D_{st}) (Nei 1973). These quantities are related by the expression $H_t=H_s+D_{st}$. The proportion of total allelic diversity found among cultivar-groups (G_{st}) was calculated as the ratio D_{st}/H_t (Nei 1973). The genetic distances of Nei (1972) were calculated for all possible pairwise comparisons among the 271 accessions. The unweighted pair group method with arithmetic averaging (UPGMA) was then performed on the distance matrix (Sneath and Sokal 1973). Multivariate relationships among accessions were revealed through principal coordinate (PCO) analysis of distances.

Results

Genetic variability in *V. unguiculata* var. *unguiculata*

The 21 enzyme systems screened revealed 36 scorable loci, which were presumed to be the products of individual coding loci. In the present study we were able to score *Amp3b*. The *Amp3b* (only stained with alanine-β-naphtylamide) band was weak and usually hidden below *Amp2* in the wild accessions. However since only the fastest *Amp2* alleles ($Amp2^{102}$ and $Amp2^{100}$) were present in cultivated cowpea, the faint Amp3b band was always scorable.

Fifteen loci were polymorphic (approx. 0.71 loci per enzyme system), and 58 alleles (approx. 1.61 alleles per locus and 2.46 per polymorphic locus) were resolved. Two-thirds of the loci were weakly polymorphic, as 10 out of 15 loci showed allele frequencies ≥0.9 for the most frequent allele (Table 2). The estimated heterozygosity was low (H_t=0.061). The mean estimated heterozygosity for cultivar-groups ranged from 0.027 for cv.-gr. Melanophthalmus to 0.072 for cv.-gr. Biflora and 0.078 for cv.-gr. Biflora from Northeast Africa (Table 3).

Inter-cultivar-groups relationships in *V. unguiculata* var. *unguiculata*

Within-accession diversity was only 0.002, therefore between-accession diversity was responsible for most of the genetic diversity, and the coefficient of gene differentiation was 0.970 between the total sample and accessions. Within cultivar-group diversity (H_s) was 0.055, between-cultivar-group diversity (D_{st}) was 0.006 (considering five cultivar-groups), and the coefficient of gene differentiation (G_{st}) was 0.100 (between total sample and cultivar-group). Considering six groups instead of the five cultivar-groups by splitting the northeastern and

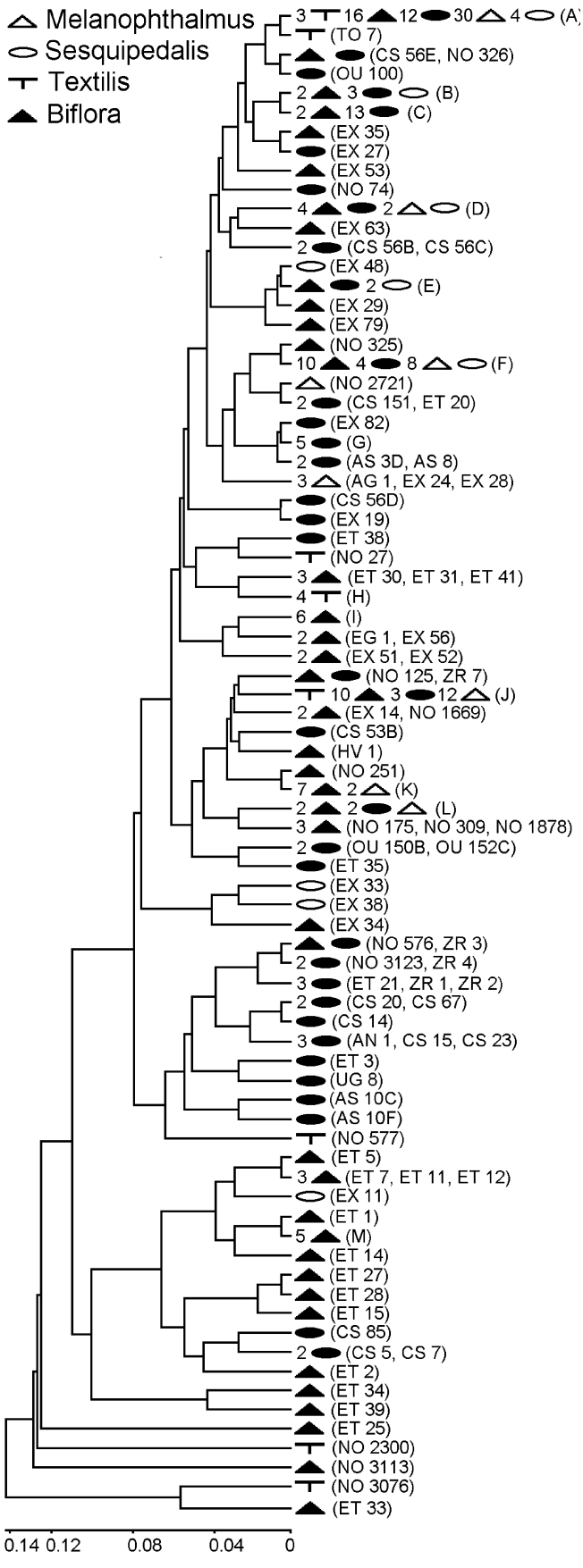
Table 3 Genetic diversity statistics for cultivated accessions of *V. unguiculata*

Group	N[a]	A	Ap	P	H_s	H_t
Cv.-gr. Sesquipedalis	13	1.17	2.20	0.14	0.002	0.036
Cv.-gr. Unguiculata	82	1.22	2.11	0.25	0.002	0.055
Cv.-gr. Melanophthalmus	59	1.14	2.00	0.14	0	0.027
Cv.-gr. Biflora	104	1.58	2.50	0.39	0.003	0.072
Cv.-gr. Textilis	13	1.25	2.00	0.25	0.001	0.066
Cv.-gr. Biflora (Asia)	21	1.25	2.12	0.22	0.004	0.051
Cv.-gr. Biflora (Northeast Africa)	27	1.33	2.09	0.31	0.004	0.078
Cv.-gr. Biflora (West Africa)	42	1.31	2.10	0.28	0.001	0.047
Cv.-gr. Biflora (other)	14	1.19	2.17	0.17	0.003	0.037
Total	271	1.61	2.47	0.42	0.002	0.061

[a] *N*, Number of accessions; *A*, average number of alleles per locus; *Ap*, average number of alleles per polymorphic locus; *P*, proportion of loci polymorphic; *Hs*, mean diversity within accessions; *Ht*, total diversity

Table 4 Partition of total diversity (H_t) for each polymorphic locus and averaged for the 36 loci: diversity within cultivar-groups (H_s), between-cultivar-group gene diversity (D_{st}) and proportion of total allelic diversity found among cultivar groups (G_{st}), considering five cultigroups or six groups (cv.-gr. Biflora split between northeastern and non-northeastern accessions)

Loci	H_t	H_s	D_{st}	G_{st}	H_s	D_{st}	G_{st}
		(5 groups)			(6 groups)		
Amp2	0.068	0.060	0.008	0.121	0.058	0.009	0.139
Amp3	0.167	0.146	0.021	0.125	0.139	0.028	0.170
Amp3b	0.064	0.063	0.001	0.017	0.063	0.001	0.018
Amp4	0.018	0.019	0.000	0.013	0.018	0.000	0.020
Enp	0.451	0.440	0.016	0.034	0.421	0.030	0.066
Fdh	0.367	0.346	0.019	0.051	0.336	0.030	0.083
Fle3	0.259	0.242	0.017	0.066	0.197	0.062	0.240
G6pd	0.149	0.133	0.017	0.111	0.074	0.075	0.503
Got2	0.294	0.196	0.097	0.331	0.196	0.097	0.332
Me	0.266	0.249	0.017	0.063	0.200	0.065	0.246
Pgd2	0.018	0.018	0.000	0.015	0.017	0.002	0.084
Pgi2	0.022	0.021	0.001	0.027	0.021	0.001	0.034
Pgi3	0.015	0.015	0.000	0.004	0.015	0.000	0.005
Pgm1	0.007	0.007	0.001	0.074	0.007	0.001	0.074
Sdh	0.040	0.037	0.002	0.062	0.037	0.003	0.066
36 loci	0.061	0.055	0.006	0.100	0.050	0.011	0.187



non-northeastern cv.-gr. Biflora led to values of 0.050 for H_s, 0.011 for D_{st} and 0.187 for G_{st}. The contribution of different loci to this variability partition showed that *Got2* was the main contributor, along with *Amp2*, *Amp3* and *G6pd*. However, when six groups were considered (instead of the five previous cultivar-groups), *Fle3*, *G6pd*, *Got2* and *Me* were highlighted (Table 4).

One of the trees resulting from the UPGMA analysis of pairwise genetic distances of Nei (1972) is shown in Fig. 1. For the clarity of the tree, genetically identical accessions were pooled, and the tree shows the 78 different profiles observed. The low genetic distances between *V. unguiculata* var. *unguiculata* accessions are emphasized. The trees separate a group of primitive accessions from Eritrea (ET 33), Yemen (ET 25) and Cameroon (NO 2300, NO 3113, NO 3076) from the rest of the accessions. Another group was formed by accessions mostly from northeastern Africa (from ET 39 to ET 5 in Fig. 1). All cv.-gr. Melanophthalmus accessions and most cv.-gr. Sesquipedalis accessions are encountered in the upper part of the trees (from NO 577 upward in Fig. 1). The top of the tree shows that accessions from every cultivar-group are very closely intermixed. The first line (A) includes 65 genetically identical accessions from the five different cultivar-groups.

Principal coordinate analysis gave a very similar result (Fig. 2). Most northeastern cv.-gr. Biflora accessions and most cv.-gr. Unguiculata accessions are grouped in the upper right-hand quadrat, while all accessions from non northeastern cv.-gr. Biflora, cv.-gr. Melanophthalmus and cv.-gr. Sesquipedalis are concentrated in the middle of the figure. Within northeastern cv.-gr. Biflora, the 4 accessions at the top of the figures are those from Yemen and Eritrea. They are among the few northeastern

Fig. 1 UPGMA phenogram of 271 accessions of *Vigna unguiculata* var. *unguiculata* using Nei (1972) genetic distances values. Accessions with identical profiles are clustered. *A* AG 6, CS 45, CS 54, CS 154, ET 4, ET 40, EX 13, EX 18, EX 36, EX 39, EX 49, EX 50, EX 54, EX 57, EX 59, EX 71, EX 74, EX 81, HV 2, HV 5, MG 5, NO 2, NO 17, NO 40, NO 95, NO 110, NO 129, NO 133, NO 137, NO 144, NO 260, NO 574, NO 649, NO 661, NO 927 A, NO 1036, NO 1319, NO 1467, NO 1559, NO 1732, NO 1795, NO 1898, NO 2296, NO 2308, NO 2466, NO 2547, NO 2616, NO 2711, NO 2720, NO 2798, NO 2806, NO 2836, NO 2837, NO 2844, NO 2973, NO 3098, NO 3148, NO 3160, NO 3256, OU 31, OU 130, OU 134D, TO 3, TO 6, UG 12, *B* ET 6, EX 12, EX 41, OU 24B, OU 158, ZR 8, *C* AS 1 A, CS 52, ET 32, EX 37, EX 73, EX 75, EX 78, MG 10, NO 2220, OU 23, OU 59 A, OU 150F, OU 152B, OU 176 C, UG 11, *D* AG 2, CS 152, ET 23, EX 32, EX 40, NO 14, NO 28, TO 5, *E* EX 42, EX 43, EX 55, EX 72, *F* AG 3, AG 4, AG 5, AG 7, ET 9, EX 15, EX 20, EX 21, EX 23, EX 25, EX 30, EX 31, EX 58, EX 83, NO 122, NO 927B, NO 2529, OU 28, OU 65, OU 159, UG 4, UG 5, UG 10, *G* AS 2B, AS 2 C, AS 3 E, NO 2206, UG 3, *H* NO 198, NO 262, NO 668, NO 2613, *I* NO 117, NO 177, NO 253, NO 311, NO 324, NO 1880, *J* AS 10 A, EX 17, HV 7, HV 10, NO 7, NO 106, NO 173, NO 643, NO 654, NO 670, NO 760, NO 1223, NO 2063, NO 2253, NO 2295, NO 2304, NO 2348, NO 2606, NO 2629, NO 2633, NO 2751, NO 3157, NO 3336, OU 174, TO 1, TO 4, *K* EX 16, NO 89, NO 165, NO 179, NO 193, NO 252, NO 1425, NO 2174, NO 2208, *L* HV 9, NO 90, NO 183, NO 189, NO 195, *M* ET 8, ET 10, ET 13, ET 16, ET 26, *F* EX 16, NO 89, NO 165, NO 179, NO 193, NO 252, NO 1425, NO 2174, NO 208

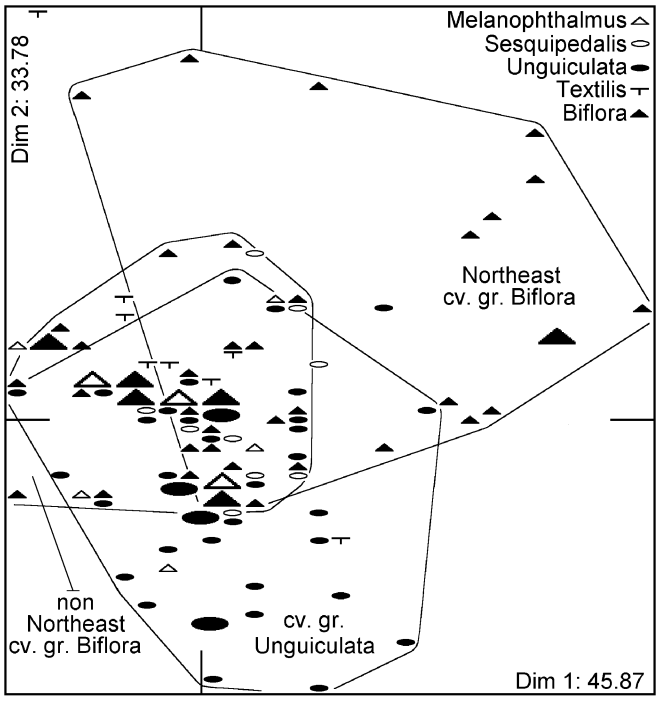


Fig. 2 Principal coordinate analysis of 271 accessions of *Vigna unguiculata* var. *unguiculata* based on the Nei (1972) distance matrix. The *first two axes* account for 45.9% and 33.8% of the total variance, respectively. *Large symbols* represent more than 5 accessions. *Lines encircle* northeastern cv.-gr. Biflora, non-northeastern cv.-gr. Biflora and cv.-gr. Unguiculata. Cv.-gr. Textilis, cv.-gr. Melanophthalmus and cv.-gr. Sesquipedalis are *not encircled* by any *lines*

cv.-gr. Biflora accessions that display alleles $Amp2^{100}$, Fdh^{102}, $Got2^{110}$, $Pgd2^{96}$ and Sdh^{105}. Cv.-gr. Textilis appears heterogeneous with NO 3076 at the top of the figure and NO 577 among the cv.-gr. Unguiculata accessions (due to alleles Enp^{98} and $Fle3^{100}$).

Mean allele frequencies for *V. unguiculata* var. *unguiculata* across the 15 polymorphic loci are presented in Table 2 for each cultivar-group and for each geographical origin of cv.-gr. Biflora accessions. With the exception of *Got2* and *Fdh*, all loci showed the same most common allele between cultivar-groups. *Got2* was the only locus where the G_{st} was higher than 0.2 (Table 4). $Got2^{110}$ characterized more than half of the cv.-gr. Unguiculata accessions, 1 cv.-gr. Sesquipedalis accession, 5 cv.-gr. Biflora accessions from Asia and 3 cv.-gr. Biflora accessions from Ethiopia and Yemen, while it was not encountered in the West African cultivar-groups. In addition to *Got2* and *Fdh*, several loci showed marked differences in allele frequencies between the cv.-gr. Biflora group of accessions: *Enp*, *Fdh*, *Fle3*, *G6pd* and *Me*. In most cases, the geographical group involved is the northeastern group (Table 2).

Discussion

Relationships between wild and cultivated cowpea

Since *V. unguiculata* ssp. *unguiculata* var. *spontanea* is the progenitor of the cultivated cowpea (Pasquet 1999), a comparison of Table 2 with similar var. *spontanea* data shows inversions in frequencies between both ssp. *unguiculata* varieties with respect to loci *Amp2*, *Enp*, *Fle3* and *Me*. The profile $Amp2^{102}$–$Fle3^{96}$–Me^{100} has been observed in 200 cultivated accessions belonging to all cultivar-groups, but this profile has been found in only 1 weedy accession from Niger out of 95 var. spontanea accessions (Pasquet 1999). Such genetic differentiation is fairly rare in cultivated plants (Doebley 1989) but not without precedent: a somewhat similar situation can be found in annual sunflower (Cronn et al. 1997) and Tepary bean (Garvin and Weeden 1994).

Since several markers show high frequencies in cultivated cowpeas and low frequencies in wild ones, these markers could be useful in localizing the center of origin of cowpea. However, $Amp2^{102}$, Enp^{98}, $Fle3^{96}$ and Me^{100} are encountered in wild accessions from West Africa, East Africa and Southern Africa, which suggests a widely distributed crop-weed complex. In addition, the lack of availability of wild accessions from Northeast Africa limits further attempts to identify the center of origin of cultivated cowpea (Pasquet 1999).

Genetic variability in *V. unguiculata* var. *unguiculata*

Total genetic diversity in cultivated cowpea (H_t=0.061 within 271 accessions) was found to be higher than previously reported, i.e. H_t=0.018 within 34 accessions (Panella and Gepts 1992) and H_t=0.029 within 112 accessions (Vaillancourt et al. 1993). The percentage of polymorphic loci (P=0.42) was also higher than those previously reported, i.e. P=0.04 within 34 accessions (Panella and Gepts 1992) and P=0.23 within 112 accessions (Vaillancourt et al. 1993). This can be explained by the greater diversity of material sampled since Vaillancourt et al. (1993) studied only 7 accessions from Ethiopia and Somalia and no cv.-gr. Textilis accessions, while Panella and Gepts (1992) examined only 1 accession from Ethiopia and only 1 cv.-gr. Textilis accession (NI 816=TO 7). The higher variability observed here may also reflect the greater number of loci studied. Of the 7 loci with H_t>0.1 (Table 4), Vaillancourt et al. (1993) studied only 3 (*Fdh*, *Got2*, and *Me*), while Panella and Gepts (1992) examined only 4 (*Fle3*, *G6pd*, *Got2*, and *Me*).

Nevertheless, these cultivated cowpea diversity values are lower than those reported in many other crops (Doebley 1989), particularly in tropical legume crops (Pasquet 1999), although the values are slightly higher than those reported in yam bean (H_t=0.055 and P=0.42) by Potter and Doyle (1992) and in Bambara groundnut (H_t=0.052 and P=0.17) by Pasquet et al. (1999). The

low diversity of cultivated cowpea could be explained by a single domestication event (Pasquet 1999) and self-pollinating breeding system (Steele 1972; Kumar et al. 1976; Ladeinde and Bliss 1977; Williams and Chambliss 1980). Self-pollinating species have slightly lower diversity than outbreeding species (Hamrick and Godt 1997).

The low diversity may be also explained by a double bottleneck effect – from wild to primitive cultivar-groups and from primitive to evolved cultivar-groups. Cultivar-groups Biflora and Textilis (Table 3) show the highest cultivar-group diversity, while that of cultivar-groups Sesquipedalis, and especially Melanophthalmus, have less than half the diversity of cv.-gr. Biflora (Table 3). Compared to the West African cv.-gr. Biflora, cv.-gr. Melanophthalmus shows a reduction of 13% for *A*, 50% for *P*, and 43% for H_t.

Inter-cultivar-group relationships in *V. unguiculata* var. *unguiculata*

Cultivar-group Sesquipedalis diversity appears as a subset of cv.-gr. Unguiculata and/or Asian cv.-gr. Biflora, while cv.-gr. Melanophthalmus diversity is a subset of West-African cv.-gr. Biflora diversity (Table 2). Therefore, the emergence of these cultivar-groups from their primitive progenitors appears to be the most recent events within cultivated cowpea evolution.

Separation between the two main morphophysiological groups is only partly supported by allele *Got2*^{110} and *Amp3*^{103}. Such a *Got2* polymorphism has already been reported by Panella and Gepts (1992) and Vaillancourt et al. (1993), but the classification they used did not allow them to determine that this polymorphism is mainly concentrated in cv.-gr. Unguiculata and Asian cv.-gr. Biflora. Unlike *Phaseolus vulgaris* (Singh et al. 1991), the two groups do not show contrasting allozyme polymorphism, which supports a single domestication hypothesis. The separation between the two main morphophysiological groups looks like a recent event.

Northeastern accessions from cv.-gr. Biflora show the highest diversity and a unique allozyme polymorphism. With the exception of few rare alleles (*Amp3b*^{95}, *Amp3b*^{90}, *Amp4*^{96}, *Pgi2*^{115} and *Pgi3*^{92}), all of the alleles encountered in West African accessions from cv.-gr. Biflora and cv.-gr. Melanophthalmus are encountered in northeastern cv.-gr. Biflora, while two alleles common within northeastern cv.-gr. Biflora (*Enp*^{105} and *G6pd*^{105}) are not encountered in West Africa. The same can be said about Asian cv.-gr. Biflora and cv.-gr. Biflora from other areas. The same situation occurs with cv.-gr. Unguiculata and cv.-gr. Sesquipedalis. Here again, with the exception of few rare alleles (*Amp3b*^{95}, *Pgi3*^{92} and *Sdh*^{95}), all alleles encountered in cv.-gr. Unguiculata and cv.-gr. Sesquipedalis are encountered in northeastern cv.-gr. Biflora. *Got2*^{110}, which characterizes more than half of the cv.-gr. Unguiculata accessions, is encountered in 3 northeastern cv.-gr. Biflora accessions (ET 2, 6, 34), and *Fle3*^{100}, which characterizes 1 out of 4 cv.-gr. Unguiculata accessions, is encountered in more than half of the northeastern cv.-gr. Biflora accessions. Although the number of cv.-gr. Textilis accessions studied was low, one can notice also a link between northeastern cv.-gr. Biflora and cv.-gr. Textilis. They both show the highest *Amp2*^{100} frequencies, and *G6pd*^{105} is encountered in both cultivar-groups. These lines of evidence suggest that the northeastern cv.-gr. Biflora appears to be a pivot group that may be considered to be the progenitor of all cultivar-groups and that Northeast Africa may be a possible center of domestication.

Although some indirect arguments could favor the hypothesis of a secondary domestication of cowpea from weedy forms in West Africa (Garba and Pasquet 1998), the high genetic diversity of the northeastern cv.-gr. Biflora accessions and their pivot position in the middle of the other cultivar-groups makes Northeast Africa a good candidate for a center of domestication. The parallel between the structure of the gene pools of cowpea and of pearl millet (Tostain and Marchais 1989; Tostain 1992) and sorghum (Ollitrault et al. 1989; Morden et al. 1989; Aldrich et al. 1992) supports this hypothesis.

Acknowledgements The author thanks T.H. Arnold (Botanical Research Institute, Pretoria, South Africa), N. Echikh (Faculté des Sciences Agronomiques, Gembloux, Belgium), R.L. Fery (USDA, ARS, Charleston, USA), M. Freire (EMBRAPA, Golânia, Brazil), C.O. Lehmann (Zentralinstitut für Genetik und Kultupflanzenforschung, Gatersleben, Germany), G. Lovell and A. Graves Gillaspie (USDA, ARS, Griffin, USA), L. Seidewitz (FAL Völkenrode, Braunschweig, Germany), Peerasak Srinives (Kasetsart Universty, Thailand), T. Vanderborght (Jardin Botanique National de Belgique, Meise, Belgium) and L.J.G. Van der Maesen (Agricultural University, Wageningen, The Netherlands) for seed samples and passport data.

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