Genetic structure at the isocitrate dehydrogenase and malate dehydrogenase enzyme gene loci in cowpea (*Vigna unguiculata* (L) Walp) accessions

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ABSTRACT

Genetic structure at the isocitrate dehydrogenase and malate dehydrogenase enzyme gene loci was studied in nine cowpea accessions by applying starch gel electrophoresis. Fifty-five individual plants were sampled from each accession and used for the study. Six loci were scored for the two enzyme systems. Mean observed and expected heterozygosities were 0.231±0.03 and 0.653±0.02, respectively. F_{IS} estimate within accessions ranged from −1.00 to 1.00 (mean of 0.488±0.04). F_{IS} and F_{IT} estimates among accessions ranged from 0.046 to 0.886 (mean of 0.592±0.07) and from 0.05 to 0.844 (mean of 0.580±0.08). F_{ST} estimates ranged from 0.028 to 0.190 (mean of 0.075±0.02). Mean geneflow and outcrossing rates ranged from 8.37 to 55.75 (mean of 24.14±5.51) and from 11.20 to 80.30 (mean of 37.84±7.51), respectively. The most diverse alleles were *Idh2-4* (F_{ST} =0.530) and *Idh2-5* (F_{ST}=0.32). The accessions differed in their relative proportions of alleles and genotypes. The cowpea accessions studied showed evidence of evolutionary changes at the enzyme gene loci. Overall geneflow among the accessions indicates sufficient geneflow to prevent local differentiation. A mixed mating system was observed.

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RÉSUMÉ

ASANTE, I. K., LAING, E., DANQUAH, E. Y. & OFFEI, S. K.: *Structure génétique aux loci génétiques des enzymes d'isocitrate déshydrogénase et de malate déshydrogénase en les accessions de dolique (*Vigna unguiculata *(L) Walp).* Structure génétique aux loci de gênes d'isocitrate déshydrogénase et de malate déshydrogénase était étudiée en neuf accessions de dolique par l'application d'électrophorèse de gelée de fécule. Cinquante-cinq plantes individuelles étaient échantillonnées de chaque accessions et utilisées pour l'étude. Six loci étaient marqués pour les deux systèmes d'enzyme. Les hétérozygotes moyens observés et espérés respectivement étaient 0.231 ± 0.03 et 0.653 ± 0.02. Estimation F_{IS} en les accessions variaient entre -1.00 et 1.00 (moyenne de 0.488 ± 0.04). Les estimations de F_{IS} et F_{IT} parmi les accessions variaient de 0.046 à 0.886 (moyenne de 0.592 ± 0.07) et de 0.05 à 0.844 (moyenne de 0.580 ± 0.08). Les estimations F_{ST} variaient entre 0.028 et 0.190 (moyenne de 0.075 ± 0.02) écoulement génétique et les proportions de croisement moyen variaient respectivement entre 8.37 et 55.75 (moyenne de 24.14 ± 5.51) et entre 11.20 et 80.30 (moyenne de 37.84 ± 7.51). Les allèles les plus divers étaient *Idh 2-4* (F_{ST} = 0.530) et *Idh 2-5* (F_{ST} = 0.32). Les accessions différaient en leurs proportions relatives des allèles et des génotypes. Il y a l'évidence de changements évolutionnistes aux loci génétiques d'enzyme en les accessions de dolique étudiées. L'ensemble de découlement génétique parmi les accessions indiquent le découlement génétique suffisant pour éviter la différenciation locale. Un système d'accouplement mixte était observé.

Introduction

Cowpea (*Vigna unguiculata* (L) Walp) is an important crop of tropical Africa, Asia, and South America (Vaillancourt & Weeden, 1993). It is considered the second most important food grain legume (Onwueme & Sinha, 1991). The term

isozyme was coined by Markert & Moller (1959) to describe different molecular forms of enzymes with the same substrate specificity. The first major contribution to the evidence that multiple forms of enzymes did exist was the development of starch gel electrophoresis by Smithies (1955). The second major contribution was the demonstration that enzymes could be visualized directly on starch gel when stained with a specific histochemical stain (Hunter & Markert, 1957). Isozyme markers are codominant in inheritance and therefore make it relatively easier to distinguish between homozygotes. They are also free from epistatic/ pleiotropic effects which characterize morphological markers.

The isozyme technique has been applied in the study of genetic diversity of cowpea and its wild relatives. The technique has been used in the study of evolutionary variation of aspartate aminotransferase and superoxide dismutase in wild and cultivated species of *Phaseolus* and *Vigna* (Jaaska & Jaaska, 1988). They also used the technique to show that the Asian Azuki beans subgenus *Ceratropis* belongs to the genus *Vigna.* The isozyme technique has been applied to assess genetic distance among species of subgenus *Vigna* (Vaillancourt & Weeden, 1993).

Vaillancourt & Weeden (1993) calculated Nei's genetic distance from allelic frequencies at 26 isozyme loci and found that the range of genetic distance among species of subgenus *Vigna* was 0.41-2.69. Three clusters were observed as follows: *Vigna* (*V. luteola, V. oblongiflora* and *V. subterranea*), the second cluster grouped together section Liebrechtsia and Macrodonta, and the third cluster included *V. unguiculata, V. reticulata* and *V. vexillata. Vigna unguiculata* is relatively closer to *V. vexillata,* subgenus *Plectrotropis*, than to species belonging to section *Vigna* (Sonnante *et al.*, 1997).

Allozyme studies have shown that *V. benghelensis* has two highly divergent genepools (Sonnante *et al.*, 1997). Pasquet (1993) made an electrophoretic comparison of variation at 37 presumptive isozyme gene loci for 55 wild *Vigna unguiculata* accessions. His study confirmed a previous infraspecific classification based on morphological data. Isozyme diversity in the cowpea species complex showed that six wild accessions of cowpea showed high identity with the cultivated cowpea, and that wild cowpea may therefore be the progenitor of the cultivated types (Vallaincourt, Weeden & Barnard, 1993). Panella & Gepts (1992) also used isozyme to study genetic diversity in *V. unguiculata* to determine genetic relationships and level of genetic diversity between wild and cultivated cowpea. Isozyme-based studies on cowpea have so far dwelt on relationships and variations. Compared with other leguminous crops, little is known about the population structure of the cowpea and the partitioning of genetic diversity between cultivated and wild cowpea (Vallaincourt *et al.*, 1993).

This study aims at the genetic structure of nine cowpea accessions from three agroecological zones of Ghana at six enzyme gene loci. The study is important because the study of cowpea population structure will be useful to cowpea breeders. Aspects of Wright's F-statistics, geneflow and outcrossing rates are treated. These are areas which have not been given much attention by previous cowpea breeders and geneticists with the use of the isozyme technique.

Materials and methods

Nine cowpea landraces from the collections of the Plant Genetic Resources Centre (PGRC) of the Council for Scientific and Industrial Research (CSIR) at Bunso were used for the study. They were from three agroecological zones of Ghana: the semi-deciduous forest zone (Accession No. 87/139, 87/142 and 87/157), the Guinea savanna zone (Accession No. 87/30, 87/37 and 87/55), and the Sudan savanna zone (Accession No. 87/77, 87/81 and 87/83). Fifty-five seeds sampled from each accession were raised in the greenhouse and used for the study. The germplasm was

regenerated by the PGRC in open field in the presence of pollinating agents before the study.

Protein extraction

The source of proteins was the leaf tissue of the cowpea plants. Leaflets from 14- to 21-day-old plants from each accession were crushed in a small mortar with a pestle into a 'paste'. The crude squeezate in the 'paste' was absorbed into strips of Whatman No. 1 filter paper (about 8 mm × 4 mm), which were blotted carefully and inserted into a cut about 3.5 cm from the intended cathodal end of the starch gel.

Preparation of starch gel

For each gel, 12.5 per cent mixture of hydrolyzed starch (from SIGMA) was prepared with Tris Continuous Citrate (CTC) buffer *p*H 8.0 in a flask. The flask was constantly whirled over a Bunsen Burner till the mixture was almost a translucent gelly, usually at the point where the first large bubble formed from below. The gel was degassed with a vacuum water pump and poured quickly but carefully into a gel former. It was covered gently but quickly with a glass plate, and left to stand to form. About 2 to 3 h later, it was transferred into the refrigerator for use the next day.

Running of gel

The loaded gel was placed on an electrophoretic bath assembly "filled" with CTC buffer. Gauze wicks were soaked in the buffer and connected to the gel at both ends to allow even flow of current through the gel. The gel was covered with polythene sheet on which a glass plate was placed. Ice packs were then placed on the glass plate. The covers and ice packs were used to minimize heating of gel when current was switched on. The unit (i.e. bath with electrode buffer connected to both ends of gel, gel covered with polythene, glass plate and ice packs) was placed in a refrigerator. The cathode electrode was connected to the end where samples were loaded. Gels were subjected to between 148 and 171 V and a current of between 51 and 57 mA for 3 to 4 h.

Slicing and staining of gel

After running a gel, it was carefully trimmed at the sides with a scalpel and carefully transferred onto a glass plate for slicing. A gel (about 10 mm thick) was sliced horizontally into five 2-mm thick slices and put into staining trays. The inner cut surfaces of gel slices were stained for specific enzyme activity with the following staining recipes: isocitrate dehydrogenase (1.1.1.42): 45 mg sodium isocitric acid, 10 mg $MgCl_2$, 10 mg NADP, 30 ml Tris-HCl (0.2 M) *p*H 8.0, 7 mg MTT, trace PMS; malate dehydrogenase (1.1.1.37): 150 mg L-malic acid, 10 mg NAD, 600 mg Tris, 30 ml distilled water, 6 mg MTT, trace PMS.

Statistical analysis

Allele frequencies. Allele frequencies were calculated by the formula: (2Ho+He)/2N, where Ho = number of homozygotes, He = number of heterozygotes, and N = random sample size.

Heterozygosity. Heterozygosity expected under Hardy-Weinberg equilibrium was calculated by the formula: $H_L = 1 - \sum x^2$, where x = mean allele frequency (Ferguson, 1980).

Wright's fixation and F-statistics. Wright's fixation index was calculated for individual accession at each allele as follows (Nei, 1977): $(P-p^2)/p(1-p)$, where P = observed homozygotes and p^2 = expected homozygotes. F-statistics among accessions were calculated as follows (Nei, 1977): $F_{IS} = (P-p^2)/(p-p^2)$, $F_{IT} = (P-p^2)/(p-p^2)$, and $F_{ST} = (p^2-p^2)/(p-p^2)$; where F_{IS} is the inbreeding coefficient of an individual relative to its own sub-population, F_{IT} is the inbreeding coefficient of an individual relative to the whole population, and F_{ST} is the average inbreeding of the sub-population relative to the whole population or the genetic coefficient of differentiation among the populations.

Geneflow rate (Nm). Geneflow was estimated by the formula: $Nm = (1-F_{ST})/4F_{ST}$ (Slatkin, 1987).

Outcrossing rate (C). Outcrossing rate was estimated by the formula: $C = (1-F_{IS})/(1+F_{IS})$ (Li, 1954).

Results

Protein

Two soluble proteins involving six loci were assayed from the leaf extracts. The proteins were isocitrate dehydrogenase (1.1.1.42) and malate dehydrogenase (1.1.1.37). Two loci were scored in all accessions for the isocitrate dehydrogenase enzyme. The most cathodal locus was designated *Idh1* (with six alleles), while the anodal locus was designated *Idh2* (with five alleles). For the malate dehydrogenase (Mdh) enzyme, four loci were scored in all accessions and designated *Mdh1* (with six alleles), *Mdh2* (with nine alleles), *Mdh3* (with seven alleles), and *Mdh4* (with seven alleles).

Genotype frequencies

Table 1 shows the genotype frequencies at the six loci. Eleven different genotype classes were observed at the *Idh1* locus; five were homozygotes and six were heterozygotes. The genotype class *Idh-3/3* registered the highest frequency of 0.682 in accession 87/139. At the *Idh2* locus, five different homozygotic and four heterozygotic classes were observed. Genotype frequency ranged between 0.000 and 0.600 (accession 87/81). The six different genotype classes observed

TABLE 1

Genotype Frequencies for Nine Cowpea Accessions at Six Enzyme Loci

Genotype		*Semi-deciduous forest accessions*			*Guinea savanna accessions*			*Sudan savanna accessions*		
		87/139	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
IdhI-	1/5	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
	2/4	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.000
	2/5	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	3/5	0.023	0.000	0.025	0.000	0.000	0.000	0.028	0.121	0.191
	3/6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.064
	4/6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.021
	2/2	0.045	0.021	0.000	0.056	0.000	0.026	0.028	0.091	0.000
	3/3	0.682	0.500	0.500	0.453	0.314	0.237	0.544	0.425	0.575
	4/4	0.159	0.270	0.076	0.283	0.372	0.211	0.143	0.030	0.064
	5/5	0.091	0.188	0.325	0.208	0.314	0.526	0.257	0.333	0.085
	6/6	0.000	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000
Idh2-	1/3	0.000	0.000	0.100	0.000	0.000	0.000	0.047	0.000	0.000
	1/4	0.000	0.000	0.000	0.000	0.000	0.100	0.000	0.000	0.000
	2/4	0.333	0.000	0.300	0.500	0.050	0.100	0.143	0.100	0.193
	2/5	0.000	0.048	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	1/1	0.048	0.238	0.150	0.050	0.150	0.100	0.048	0.000	0.000
	2/2	0.333	0.619	0.150	0.550	0.550	0.350	0.476	0.600	0.346
	3/3	0.000	0.000	0.000	0.100	0.150	0.150	0.005	0.100	0.346
	4/4	0.191	0.095	0.300	0.250	0.100	0.300	0.143	0.150	0.077
	5/5	0.095	0.000	0.000	0.000	0.000	0.000	0.048	0.050	0.036
MdhI-	1/1	0.100	0.238	0.238	0.086	0.190	0.304	0.114	0.250	0.330
	2/2	0.033	0.190	0.000	0.086	0.048	0.000	0.028	0.036	0.133
	3/3	0.801	0.429	0.526	0.743	0.668	0.435	0.630	0.500	0.601
	4/4	0.033	0.048	0.047	0.000	0.000	0.000	0.000	0.000	0.000
	5/5	0.033	0.000	0.047	0.028	0.047	0.130	0.028	0.071	0.033

TABLE 1 (cont'd)

Genotype Frequencies for Nine Cowpea Accessions at Six Enzyme Loci

Genotype		*Semi-deciduous forest accessions*			*Guinea savanna accessions*			*Sudan savanna accessions*		
		87/139	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
Mdh2-	1/3	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.240	0.235
	1/4	0.000	0.091	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2/4	0.129	0.183	0.000	0.077	0.000	0.046	0.189	0.200	0.325
	2/6	0.064	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	2/8	0.000	0.000	0.000	0.038	0.000	0.000	0.000	0.000	0.000
	3/4	0.000	0.000	0.154	0.000	0.000	0.000	0.000	0.000	0.000
	3/5	0.000	0.000	0.000	0.115	0.150	0.091	0.000	0.000	0.000
	3/6	0.000	0.000	0.000	0.000	0.000	0.000	0.054	0.000	0.029
	4/7	0.000	0.136	0.077	0.038	0.150	0.000	0.054	0.000	0.059
	5/7	0.000	0.000	0.038	0.077	0.000	0.000	0.054	0.000	0.000
	6/8	0.000	0.000	0.000	0.077	0.050	0.136	0.027	0.160	0.000
	7/9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000
	8/9	0.000	0.000	0.000	0.023	0.000	0.000	0.000	0.000	0.000
	1/1	0.129	0.273	0.193	0.000	0.000	0.000	0.081	0.000	0.000
	2/2	0.064	0.091	0.077	0.077	0.000	0.136	0.027	0.040	0.088
	3/3	0.000	0.000	0.077	0.115	0.350	0.182	0.000	0.000	0.000
	4/4	0.161	0.000	0.077	0.077	0.050	0.091	0.163	0.080	0.059
	5/5	0.129	0.136	0.192	0.077	0.100	0.000	0.054	0.000	0.029
	6/6	0.097	0.045	0.115	0.155	0.100	0.091	0.108	0.000	0.029
	7/7	0.097	0.045	0.000	0.038	0.050	0.045	0.000	0.080	0.000
	8/8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.080	0.000
Mdh3-	1/2	0.000	0.087	0.040	0.000	0.000	0.000	0.057	0.000	0.000
	1/3	0.000	0.087	0.080	0.000	0.000	0.000	0.000	0.000	0.000
	1/4	0.000	0.000	0.000	0.000	0.000	0.000	0.029	0.000	0.000
	2/5	0.000	0.000	0.000	0.083	0.000	0.000	0.029	0.000	0.094
	3/6	0.034	0.043	0.000	0.043	0.000	0.000	0.200	0.000	0.000
	6/7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.048
	1/1	0.138	0.435	0.200	0.000	0.000	0.238	0.086	0.095	0.381
	2/2	0.448	0.175	0.200	0.208	0.174	0.190	0.285	0.286	0.381
	3/3	0.172	0.130	0.280	0.250	0.522	0.286	0.257	0.333	0.238
	4/4	0.138	0.000	0.080	0.083	0.087	0.143	0.000	0.095	0.095
	5/5	0.068	0.043	0.120	0.333	0.130	0.095	0.057	0.048	0.095
	6/6	0.000	0.000	0.000	0.000	0.087	0.048	0.000	0.048	0.000
	7/7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.048
Mdh4-	1/2	0.176	0.176	0.125	0.133	0.133	0.166	0.059	0.294	0.125
	1/3	0.000	0.176	0.125	0.067	0.333	0.000	0.353	0.000	0.000
	1/4	0.000	0.000	0.000	0.000	0.000	0.000	0.176	0.000	0.000
	1/5	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.000	0.000
	2/4	0.000	0.059	0.062	0.200	0.000	0.056	0.000	0.118	0.313
	2/6	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.056	0.000
	3/5	0.000	0.176	0.188	0.066	0.000	0.333	0.000	0.176	0.000
	4/5	0.050	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	4/6	0.000	0.000	0.000	0.267	0.000	0.000	0.000	0.118	0.000
	4/7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.062
	1/1	0.471	0.354	0.438	0.000	0.000	0.056	0.059	0.000	0.000
	2/2	0.235	0.059	0.062	0.200	0.537	0.278	0.294	0.176	0.125
	3/3	0.050	0.000	0.000	0.067	0.000	0.111	0.000	0.059	0.062
	4/4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.062

at the *Mdh1* locus were all homozygotes. Genotype frequency ranged from 0.000 to 0.801 (accession 87/139). At the *Mdh2* locus, eight homozygotic and 15 heterozygotic classes were observed. Genotype frequency ranged from 0.000 to 0.273 (in accession 87/142). The *Mdh3* locus had seven different homozygotic and six different heterozygotic classes with genotype frequency ranging from 0.000 to 0.448 (accession 87/139). Four different homozygotic and 10 heterozygotic classes were observed at the *Mdh4* locus. Genotype frequency ranged from 0.000 to 0.471 (accession 87/139).

Allele frequency

Table 2 shows the allele frequencies for the two enzyme systems. Forty different alleles were distributed among the six loci. At the *Idh1* locus, allele *Idh1-3* had the highest frequency with a range between 0.237 and 0.702 (mean of 0.495 $\pm$ 0.05). At the *Idh2* locus, allele *Idh2-2* had the highest frequency ranging from 0.300 to 0.650 (mean of 0.527$\pm$0.04). At the *Mdh1* locus, allele *Mdh1-3* had the highest frequency ranging from 0.435 to 0.800 (mean of 0.592$\pm$0.04). At the *Mdh3* locus, allele *Mdh3-3* had the highest frequency ranging from 0.190 to 0.522 (mean of 0.307$\pm$0.03).

Heterozygosity and Wright's fixation index (F_{IS})

Table 3 shows the observed and expected heterozygosities for the nine accessions at the six loci. Mean expected heterozygosity ranged from 0.060 to 0.860 (mean of 0.653 $\pm$ 0.02). Mean observed heterozygosity ranged from 0.000 to 0.765 (mean of 0.231 $\pm$ 0.03). The observed heterozygosity in accession 87/81 was greater than the expected at the *Idh1* and *Mdh4* loci. Table 4 shows estimates for Wright's fixation index within the nine accessions. F_{IT} values ranged from -1.000 to 1.000 (mean of 0.488 $\pm$ 0.04).

Estimates for F-statistics, gene flow rate and outcrossing rate among accessions

Table 5 shows the estimates for F-statistics, geneflow rates, and outcrossing rates. F_{IS} values ranged from 0.046 to 0.889 (mean of 0.592$\pm$0.07). F_{IT} values ranged from 0.05 to 0.854 (mean of 0.58$\pm$0.08). F_{ST} values ranged from 0.028 to 0.190 (mean of 0.075$\pm$0.02). Overall geneflow and outcrossing rates ranged from 8.37 to 55.75 (mean of 24.14$\pm$5.51) and 11.2 to 80.30 (mean of 37.84$\pm$7.51), respectively.

Discussion

The cowpea accessions differed in their relative proportions of alleles and genotypes. Allele *Idh1-1* was present in only accession 87/157. *Mdh1-4* was present in the three accessions of the semi-deciduous forest zone. *Mdh2-9* was present in accessions 87/30 and 87/142. Genotypes *Idh1-1/Idh1-5* and *Idh1-2/Idh1-4* were present in accession 87/157. The homozygote *Idh2-1/Idh2-4* was present in 87/55. Genotype *Idh2-2/Idh2-5* was present in accession 87/142. Genotype *Mdh1-4/Mdh1-4* was present in the three accessions from the semi-deciduous forest zone. The heterozygote *Mdh3-6/Mdh3-7* was present in only accessions 87/81 and 87/83. The homozygote class *Mdh4-4/Mdh4-4* was present in accession 87/83.

Observations in the genotype variation reflect polymorphism in cowpeas in Ghana. Morphological characterization of cowpea in Ghana has shown polymorphism in grain colour in Ghanaian markets (Dovlo, 1976). Polymorphism has also been established in the following traits: flower colour, growth and developmental habits like twining tendency, days to flowering and maturity, eye colour pattern, 100-seed weight, number of seeds per pod, and nodule weight (Doku, 1970; Bennett-Lartey, 1991). The foregoing evidence shows that genetic variations occur in the composition of the cowpea accessions studied; hence, evolutionary changes occur in the cowpea populations at the six enzyme gene loci.

The results showed that observed heterozygosities at the loci were less than expected in all accessions at the four loci, except

TABLE 2

Allele Frequencies at Six Enzyme Loci in Nine Accessions

	Semi-deciduous forest accessions			*Guinea savanna accessions*			*Sudan savanna accessions*		
Allele	*87/139*	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
Idh1-1	0.000	0.000	0.012	0.000	0.000	0.000	0.000	0.000	0.000
2	0.045	0.031	0.012	0.056	0.000	0.026	0.029	0.091	0.064
3	0.693	0.500	0.513	0.453	0.314	0.237	0.557	0.485	0.702
4	0.159	0.271	0.088	0.283	0.372	0.211	0.143	0.030	0.010
5	0.102	0.198	0.350	0.208	0.314	0.526	0.271	0.394	0.181
6	0.000	0.000	0.025	0.000	0.000	0.000	0.000	0.000	0.043
Idh2-1	0.048	0.238	0.200	0.050	0.150	0.125	0.071	0.000	0.000
2	0.500	0.643	0.300	0.575	0.575	0.375	0.548	0.650	0.575
3	0.000	0.000	0.050	0.100	0.150	0.150	0.119	0.100	0.100
4	0.351	0.095	0.450	0.275	0.125	0.350	0.214	0.200	0.275
5	0.095	0.024	0.000	0.000	0.000	0.080	0.048	0.050	0.050
Mdh1-1	0.100	0.233	0.238	0.086	0.190	0.305	0.114	0.250	0.030
2	0.034	0.190	0.000	0.086	0.048	0.000	0.029	0.036	0.130
3	0.800	0.429	0.524	0.743	0.667	0.435	0.629	0.500	0.600
4	0.033	0.048	0.047	0.000	0.000	0.000	0.000	0.000	0.000
5	0.000	0.095	0.143	0.057	0.048	0.130	0.200	0.143	0.200
6	0.033	0.000	0.048	0.028	0.047	0.130	0.028	0.071	0.031
Mdh2-1	0.129	0.318	0.172	0.000	0.000	0.000	0.081	0.000	0.117
2	0.161	0.182	0.141	0.180	0.425	0.228	0.027	0.120	0.132
3	0.065	0.000	0.141	0.180	0.425	0.228	0.027	0.120	0.132
4	0.226	0.205	0.234	0.100	0.125	0.114	0.378	0.200	0.294
5	0.129	0.136	0.172	0.180	0.175	0.045	0.081	0.000	0.088
6	0.129	0.045	0.094	0.200	0.125	0.159	0.243	0.100	0.088
7	0.161	0.114	0.046	0.100	0.125	0.045	0.054	0.120	0.031
8	0.000	0.000	0.000	0.080	0.025	0.250	0.014	0.160	0.000
9	0.000	0.000	0.000	0.020	0.000	0.000	0.000	0.000	0.000
Mdh3-1	0.138	0.522	0.260	0.000	0.000	0.238	0.129	0.095	0.048
2	0.448	0.217	0.220	0.250	0.174	0.190	0.329	0.286	0.380
3	0.190	0.196	0.320	0.271	0.522	0.286	0.357	0.333	0.286
4	0.138	0.000	0.080	0.083	0.087	0.143	0.014	0.095	0.095
5	0.069	0.043	0.120	0.375	0.130	0.095	0.710	0.048	0.095
6	0.017	0.022	0.000	0.021	0.087	0.048	0.100	0.048	0.048
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.048
Mdh4-1	0.559	0.529	0.563	0.100	0.233	0.139	0.382	0.147	0.067
2	0.324	0.177	0.156	0.367	0.600	0.389	0.324	0.412	0.344
3	0.059	0.177	0.156	0.133	0.177	0.278	0.177	0.147	0.313
4	0.029	0.029	0.031	0.234	0.000	0.027	0.088	0.118	0.250
5	0.029	0.088	0.094	0.033	0.000	0.167	0.029	0.088	0.000
6	0.000	0.000	0.000	0.133	0.000	0.000	0.000	0.088	0.000
7	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.031

TABLE 3

Mean Expected and Observed Heterozygosities for Nine Cowpea Accessions at the Six Enzyme Loci

	Semi-deciduous forest accessions			*Guinea savanna accessions*			*Sudan savanna accessions*		
Allele	*87/139*	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
Idh1	0.482	0.636	0.606	0.668	0.664	0.622	0.595	0.060	0.468
	0.021	**0.042**	**0.100**	**0.057**	**0.000**	**0.000**	**0.028**	**0.121**	**0.277**
Idh2	0.611	0.520	0.665	0.581	0.609	0.699	0.632	0.525	0.581
	0.333	**0.048**	**0.400**	**0.050**	**0.050**	**0.100**	**0.190**	**0.100**	**0.250**
Mdh1	0.347	0.712	0.644	0.429	0.512	0.684	0.550	0.661	0.581
	0.000	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**
Mdh2	0.843	0.790	0.835	0.849	0.741	0.818	0.766	0.860	0.804
	0.323	**0.409**	**0.406**	**0.480**	**0.350**	**0.273**	**0.568**	**0.720**	**0.735**
Mdh3	0.720	0.640	0.761	0.716	0.665	0.794	0.732	0.776	0.749
	0.034	**0.217**	**0.240**	**0.125**	**0.000**	**0.000**	**0.314**	**0.000**	**0.095**
Mdh4	0.577	0.649	0.625	0.764	0.554	0.723	0.709	0.758	0.716
	0.235	**0.588**	**0.500**	**0.733**	**0.467**	**0.556**	**0.647**	**0.765**	**0.500**

Bold figures are observed heterozygosities

TABLE 4

Mean Estimates of Wright's Fixation Index (F_{IS}) for Nine Accessions at the Six Enzyme Loci

	Semi-deciduous forest accessions			*Guinea savanna accessions*			*Sudan savanna accessions*		
Locus	*87/139*	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
Idh1	0.637	0.586	0.611	0.667	0.500	0.667	0.640	0.584	0.390
Idh2	0.438	0.478	0.219	0.629	0.611	0.560	0.452	0.578	0.272
Mdh1	0.833	0.833	0.833	0.833	0.830	0.499	0.827	0.833	0.833
Mdh2	0.453	0.360	0.447	0.334	0.283	0.491	0.235	0.128	0.135
Mdh3	0.675	0.423	0.638	0.510	0.715	0.715	0.378	1.000	1.000
Mdh4	0.292	0.021	0.065	0.024	0.034	0.111	0.038	-0.010	0.162

at the *Idh1* and *Mdh1* loci where the observed heterozygosities were higher than expected in accession 87/81 (Table 3). Heterozygosities from the work reported here were higher than those observed by Vaillancourt *et al.* (1993). They observed expected and observed values of 0.168 and 0.029, respectively.

Panella & Gepts (1992) also observed a total genetic diversity of 0.085 in their work on 34 cultivated accessions and 56 wild accessions of *V. unguiculata.* Balagtas & Ramirez (1991), in a study of Philippine cowpea collections, observed an expected heterozygosity of 0.360. Results of the work reported here show a trend in high genetic diversity in Ghanaian cowpeas, despite the limited number of accessions that were analyzed. The results also presuppose that Ghanaian cowpea farmers have been able to maintain high genetic diversity in Ghanaian cowpeas possibly through human selection,

TABLE 5

F-Statistics, Geneflow and Outcrossing Rates at the Six Enzyme Gene Loci in the Nine Cowpea Accessions

Allele	F_{IS}	F_{IT}	F_{ST}	*Nm*	*C*
Idh1-1	-0.004	-0.002	0.003	11.03	100.00
2	0.912	0.916	0.018	15.36	5.07
3	0.874	0.897	0.033	7.39	6.09
4	0.975	0.977	0.043	18.70	1.49
5	0.869	0.875	0.590	4.42	7.46
6	0.333	0.333	0.010	2.66	66.67
Mean	0.660	0.666	0.028	9.93	31.13
Idh2-1	0.594	0.819	0.039	7.64	10.90
2	0.880	0.519	0.040	14.40	27.00
3	0.557	0.633	0.025	97.62	34.80
4	0.733	0.622	0.530	16.93	26.10
5	0.232	0.254	0.032	142.17	70.60
Mean	0.599	0.569	0.190	55.75	33.89
Mdh1-1	0.999	0.936	0.147	5.21	0.47
2	1.000	0.250	0.054	5.98	0.00
3	1.000	0.940	0.063	8.28	0.00
4	0.333	0.333	0.001	34.64	66.67
5	0.999	0.976	0.024	23.52	0.07
6	1.000	0.990	0.015	8.37	0.00
Mean	0.889	0.854	0.051	14.33	11.20
Mdh2-1	0.427	0.413	0.026	4.07	50.58
2	0.133	0.228	0.188	39.26	29.13
3	0.418	0.453	0.077	3.54	26.31
4	0.275	0.275	0.009	205.99	61.75
5	0.778	0.763	0.022	31.03	16.63
6	0.609	0.600	0.021	18.78	30.52
7	0.309	0.329	0.168	7.17	56.04
8	0.254	0.231	0.060	1.68	82.12
9	0.010	-0.020	0.011	11.91	100.00
Mean	0.357	0.364	0.065	35.94	50.34
Mdh3-1	0.854	0.763	0.102	7.19	8.26
2	0.914	0.893	0.023	25.74	4.52
3	0.861	0.900	0.027	25.70	7.72
4	0.953	0.799	0.177	12.79	2.53
5	0.946	0.930	0.018	21.97	2.79
6	0.427	0.422	0.011	24.81	48.60
7	0.333	0.322	0.011	2.44	66.67
Mean	0.755	0.718	0.053	17.23	20.16
Mdh4-1	0.047	0.057	0.047	38.65	64.42
2	0.421	0.329	0.182	14.55	41.43
3	0.180	0.175	0.026	9.62	72.45
4	-0.119	0.049	0.087	2.59	96.39
5	-0.113	-0.107	0.045	9.87	95.05
6	-0.083	-0.076	0.049	2.22	94.31
7	-0.010	-0.010	0.007	4.17	98.06
Mean	0.046	0.050	0.063	11.67	80.30
Grandmean	0.592 ± 0.07	0.580 ± 0.08	0.075 ± 0.02	24.14 ± 5.15	37.84 ± 7.51

natural selection, and seed exchange among local cowpea farmers. The high genetic diversity of the analyzed population makes them suitable materials for breeding programmes.

Mean fixation index (F_{IS}) values at all the loci were all positive. Positive F_{IS} values suggest heterozygote deficiencies and therefore, deviation from Hardy-Weinberg equilibrium law as a result of non-random mating, selection, genetic drift, mutation, or limited geneflow (Gehring & Lindhart, 1992). Cowpea is a cultivated crop and therefore the observed Wright's fixation index values could be due to human selection for desirable agronomic traits. Mean genetic differentiation was 0.075 ± 0.02, thus, giving an overall 4.8 per cent genetic differentiation among the cowpea accessions over the six loci. This suggests that the greatest average percentage of genetic diversity of the cowpea accessions studied resided within accessions on the basis that 92.5 per cent genetic differentiation was observed within the accessions.

There was therefore an important level of geneflow linking the accessions. The most diverse alleles were *Idh2-4* (F_{ST} =0.530) and *Idh2-5* (F_{ST}=0.32). Overall geneflow estimate among accessions was 24.14, indicating sufficient geneflow to prevent local differentiation. This rules out the possibility of genetic drift as the cause of differences in allele frequencies of the cowpea accessions studied. Outcrossing produced overall 37.84 per cent individuals. This indicates that there was mixed mating in the cowpea accessions used for the study. The way the germplasm was multiplied before this study in the presence of pollinating agents might have contributed to the geneflow estimate. The resulting diversity observed in the study shows that the accessions are predominantly outcrossed, given the presence of extrafloral nectaries in cowpea.

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