Allozyme Variation and Ecogeographical Variation Correlation in Cowpea *(Vigna unguiculata* (L.) *Walp)* Accessions

I. K. Asante and E. Laing
Department of Botany, University of Ghana, P. O. Box 55, Legon, Accra

Abstract

Allozymic-ecogeographical correlation was studied in the cowpea to find out whether cowpea allozyme distribution pattern favours the selectionist theory of selection. The study was based on 22 loci and geographical, temperature and moisture parameters. The cowpea accessions were collections from three agroecological zones of Ghana, namely semi-deciduous forest, Guinea savanna and Sudan savanna. The enzymes were adenylate kinase, fumarate hydratase, hexokinase, isocitrate dehydrogenase, malate dehydrogenase, malic enzyme, 6-phosphogluconate dehydrogenase and phosphoglucoisomerase (glucose-1-phosphate). A total of 110 different alleles were observed; an average of 5.00 alleles per locus. The most polymorphic locus was the *Mdh2* enzyme locus with nine different alleles. The *Pgi1* locus was monomorphic in all the nine accessions. The highest allele frequency of 0.905 occurred in the Sudan savanna zone. The alleles *Ak1-3, Mdh3-6, Mdh4-3, Mdh4-3* and *Pgm1-3* showed clinal effects with allele frequencies increasing from the semi-deciduous forest zone to the Sudan savanna zone. Frequencies for the alleles *Me2-1, Pgdh1-5* and *Pgi2-5* increased from the Sudan savanna zone to the semi-deciduous forest zone. Allozyme frequencies were significantly correlated with geographical, temperature and moisture factors. Therefore, allozyme polymorphism in the nine cowpea accessions studied were partly adaptive. Allozyme frequency distribution appears to be primarily affected by environmental variables such as monthly cloudiness, minimum temperature, mean monthly rainfall and longitude. The findings of the study do not favour the neutralist theory of selection.

Introduction

Cowpea is cultivated in all the six agroecological zones of Ghana. The greatest production occurs in the savanna zones and the margins of the semi-deciduous forest zone (GGDP, 1990). Constraints in cowpea production, among other factors, include drought and heat (Singh *et al.*, 1992). Sub-characters of cowpea yield components are affected by diverse environmental conditions. For instance, reproductive development, yield potential and seed yield in cowpeas are sensitive to the weather (Marfo & Hall, 1992). It has been documented that warmer temperatures can hasten the appearance of flowers on both photoperiod-sensitive and insensitive genotype (Singh & Rachie, 1985). Percentage pod set per plant and number of days to pod maturation in cowpea have been found to decrease from the semi-deciduous forest zone to the savanna zone and might be selected for by the climatic factors mean monthly, total cloud and number of rain days per year (Asante, 1998).

Some workers believe that most molecular variation will prove to be physiologically meaningful and, hence, under selective control and important in adaptation; but others regard it as evolutionary "noise" without phenotypic effect and thus selectively neutral (Ayala, 1976). Kimura & Ohta (1971) proposed that isozyme variability polymorphisms are selectively neutral and they would all thus function equally well. However, evidence of the frequency and the patterns of the polymorphism have failed to correspond with the predictions of the neutralist theory. It now appears likely that the polymorphisms are due to some forms of balancing selection. A study of allozyme frequency distribution in the cowpea showed that allele *Pgm2-4* was found to be absent in both the semi-deciduous forest and Guinea savanna zones of Ghana (Asante, 2000). If protein and other molecular polymorphism are adaptive, as claimed by the selectionists, one should expect to find correlation between genetic variability and some environmental factors (Lewontin, 1974).

Natural selection is believed to be actively maintaining genetic polymorphism if (i) the same pair of alleles are found in uniform frequencies over a wide distribution range of a species; (ii) different alleles are fixed in different local populations; (iii) there is a cline; (iv) frequencies of alleles are uniform within each locality but different among localities (Kimura, 1982). However, the neutralist theory claims that distribution of different morphs do not correlate with any climatic, edaphic or geographic variables, and that a particular allele in a given species has been brought to that frequency by random drift but not by adaptation to the living condition of that species (Kimura, 1981).

The objective of the study was to find out whether cowpea allozyme distribution pattern in nine cowpea accessions from three agroecological zones in Ghana favours the selectionist theory of selection.

Materials and methods

Collection sites and ecogeographical data

Nine cowpea landraces sampled from 20 accessions for convenience were used for the study. These were collected from the Plant Genetic Resources Centre, Council for Scientific and Industrial Research, Bunso. They were samples from 1987 collections from three agroecological zones of Ghana and were distributed as follows: semi-deciduous forest zone (accessions 87/139, 87/142 and 87/157); Guinea savanna zone (87/30, 87/37 and 87/55) and Sudan savanna zone (87/77, 87/81 and 87/83). Ecogeographical data over a period of 10 years (1977-1987) for the collecting sites of the cowpea were collected from the Ghana Meteorological Department. Collection sites and their corresponding ecogeographical data are shown in Table 1. The ecogeographical data are as follows: longitude (L_n), latitude (L_t), mean monthly maximum temperature (T_{max}), mean monthly minimum temperature (T_{min}), mean monthly cloudiness (Cl), mean monthly sunshine (S_m), mean annual rainfall (R_a), number of rainy days per year (R_d), relative humidity per year at 1500 h (Rh1500) and 0600 h (Rh0600), mean monthly vapour pressure at 1500 h (P1500) and 0600 h (P0600).

Isozyme analysis

A modified form of Tanksley & Orton's (1983) procedure was used for enzyme extraction, starch gel preparation, electrophoresis and enzyme staining. A maximum of 50 seedlings from each of the nine accessions were raised in the greenhouse and used for the study. The source of protein was the median leaflet of 14-30-day-old plants. Crude squeezate from the leaflets was absorbed into strips of Whatman No. 1 filter paper (about 8 × 4 mm), and loaded unto a gel prepared from 12.5% mixture of hydrolyzed starch (from SIGMA). The gel was subjected to 148 V and a current of 51 mA for 4 h.

Inner cut surfaces of gel slices were stained for specific enzyme activity with the following staining recipes: Adenylate kinase (2.7.4.3): 40 mg glucose, 30 mg adenosine diphosphate (ADP), 7.5 mg nicotinamide adenine dinucleotide phosphate (NADP), 20 mg

TABLE 1

Cowpea accessions, collection sites and their corresponding ecogeographical data

Accession	Locality	Longitude	Latitude	Mean Temp. °C Maximum	Mean Temp. °C Minimum	Annual rain-fall (mm)	Raindays	Relative humidity 1500 h	Relative humidity 0600 h	Mean sunshine	Vapour pressure 1500 h	Vapour pressure 0600 h	Mean monthly cloud (knot)
87/139	Akora Darko	00 °24'W	06 ° 22'N	31.3	21.7	112.0	11	66.0	96.0	5.5	27.5	25.8	5.5
87/142	Akora Darko	00 ° 24'W	06 ° 22'N	31.3	21.7	112.0	11	66.0	96.0	5.5	27.5	25.8	5.5
87/157	Abene	00 ° 34'W	06 ° 38'N	28.1	18.4	110.0	11	67.8	92.3	-	23.2	23.1	-
87/30	Boterly	00 ° 29'W	09 ° 25'N	32.5	21.3	90.4	8	50.5	85.1	7.0	-	22.0	4.5
87/37	Zan	00 ° 16'W	09 ° 25'N	33.4	21.8	103.0	8	47.0	79.0	7.3	21.8	22.2	4.6
87/55	Limoh	01 ° 13'W	09 ° 29'N	33.8	22.3	86.1	8	45.5	76.8	7.4	21.5	22.0	4.7
87/77	Buoti	02 ° 07'W	10 ° 53'N	34.3	22.4	82.4	7	39.8	69.0	7.9	19.5	20.0	4.0
87/81	Buoti	02 ° 07'W	10 ° 53'N	34.3	22.4	82.4	7	39.8	69.0	7.9	19.5	20.0	4.0
87/83	Nandom	03 ° 15'W	10 ° 50'N	33.2	22.0	70.0	7	44.0	72.8	7.9	20.0	20.5	3.9

Source: Ghana Meteorological Survey

magnesium chloride ($MgCl_2$), hexokinase/glucose-6-phosphate dehydrogenase (HK/G6PDH), 30 ml tris-HCl (0.2 *M*) *p*H 8.0, trace MTT, trace PMS; Fumarate hydratase (4.2.1.2): 30 mg nicotinamide adenine dinucleotide (NAD), 200 mg fumic acid, 30 ml tris-HCl (0.2 *M*) *p*H 7.0, 50 ml malate dehydrogenase (MDH), trace MTT, trace PMS; Hexokinase (2.7.1.1): 60 mg glucose, 100 mg $MgCl_2$, 15 mg adenosine triphosphate

(ATP), 5 mg NADP, 30 ml tris-HCl (0.2 *M*) *p*H 7.0, 20 ml G6PDH, 7 mg MTT, trace PMS; 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; 6-Phosphogluconate dehydro-genase (1.1.1.44): 30 mg 6-phosphogluconate, 10 mg $MgCl_2$, 10 mg NADP, 30 ml tris-HCl (0.2 *M*) *p*H 8.0, 7 mg MTT, trace PMS; Phosphoglucose isomerase (5.3.1.9): 20 mg fructose-6-phosphate, 10 mg $MgCl_2$, 10 mg NADP, 30 ml tris-HCl (0.2 *M*) *p*H 8.0, 50 ml G6PDH, 7 mg MTT, trace PMS; Phospho-glucomutase (5.4.2.20): 50 mg glucose-1-phosphate, 70 mg $MgCl_2$, 3 mg NADP, 30 ml tris-HCl (0.2 *M*) *p*H 8.0, 50 ml G6PDH, 7 mg MTT, trace PMS.

Data analysis

Allele frequencies were calculated by the formula: (2Ho + He)/2N, where Ho = number of homozygotes, He = number of heterozygotes and N = sample size (Fergurson, 1980). Spearman correlation and stepwise multiple regression were computed by using SPSS/PC Statistical Software package. The test of multiple regression was conducted to find linear associations between allozyme variation and the ecogeographical variables.

Results and discussion

Mean frequencies of detected alleles in the nine accessions are presented in Table 2. A total of 110 different alleles were distributed among the 22 loci for an average of 5.00 alleles per locus. The locus for *Mdh2* was most polymorphic with nine different alleles. The locus for *Pgi1* was monomorphic in all the accessions studied. The alleles *Ak2-3, Idh1-1, Mdh1-4, Mdh2-1* and *Pgi2-1* were present in only semi-deciduous forest zone accessions, with frequencies less than 0.05, except allele *Mdh2-1* that had a frequency of 0.206. Alleles *Hk1-4, Mdh4-7, Me1-7, Pgdh1-5* and *Pgm2-1* were present in the Sudan savanna accessions but absent in accessions of semi-deciduous and Guinea savanna zones. Alleles *Idh1-6, Idh2-5 and Me1-1* were absent in only Guinea savanna zone accessions.

Generally, the highest allele frequency was 0.905 found in the Sudan savanna zone accessions in allele *Pgi2-2*. The highest allele frequencies for semi-deciduous forest and Guinea savanna zones accessions were 0.823 and 0.847, respectively, for allele *Ak2-1. Alleles Ak1-3, Mdh3-6, Mdh4-3, Mdh4-4* and *Pgm1-3* showed clinal effects with their allele frequencies increasing from the semi-deciduous forest zone to the Sudan savanna zone. Similarly, frequencies for alleles *Me2-1, Pgdh1-5* and *Pgi2-5* increased from Sudan savanna zone to the semi-deciduous forest zone.

Environmental correlates of allozyme variation are shown in Table 3. Allozyme frequencies were observed to be significantly correlated with geographical, temperature and moisture factors. Alleles *Hk1-4, Mdh1-5, 6pgd2-3, 6pgd2-1, Me1-4* and *Idh1-4* were positively and significantly correlated with longitude. The alleles *Idh2-3, Me1-6* and *Pgd3-1* were positively and significantly correlated with latitude, while allele *Mdh1-4* and latitude were negatively and significantly correlated. Allele *Pgd3-1* was positively and significantly correlated with minimum temperature. Alleles *Pgd1-1* and *Pgd2-5* were positively and significantly correlated with annual rainfall, while alleles *Hk1-4, Idh2-3, Mdh1-5* and *Pgd3-1* were negatively and significantly correlated with annual rainfall. Microgeographical variation and allozyme variation correlates have also been established in *Avena barbata,* and pattern of genetic

TABLE 2

Mean allele frequency distribution at 22 gene loci in three agroecological zones

Locus	Allele	Agroecological zone: Semi-deciduous forest	Guinea savanna	Sudan savanna
Ak1–	1	0.498	0.527	0.422
	2	0.397	0.359	0.421
	3	0.056	0.057	0.118
	4	0.049	0.057	0.037
Ak2–	1	0.823	0.847	0.813
	2	0.133	0.153	0.187
	3	0.043	0.000	0.000
Fh1–	1	0.708	0.046	0.127
	2	0.180	0.759	0.471
	3	0.112	0.195	0.402
Hk1–	1	0.343	0.397	0.384
	2	0.494	0.503	0.372
	3	0.162	0.100	0.168
	4	0.000	0.000	0.076
Hk2–	1	0.624	0.711	0.683
	2	0.207	0.115	0.190
	3	0.142	0.134	0.127
Idh1–	1	0.004	0.000	0.000
	2	0.029	0.027	0.061
	3	0.569	0.335	0.581
	4	0.173	0.289	0.061
	5	0.286	0.349	0.282
	6	0.008	0.000	0.014
Idh2–	1	0.162	0.108	0.024
	2	0.481	0.508	0.591
	3	0.017	0.133	0.106
	4	0.299	0.250	0.230
	5	0.040	0.000	0.049
Mdh1–	1	0.190	0.194	0.131
	2	0.075	0.045	0.065
	3	0.584	0.615	0.576
	4	0.043	0.000	0.000
	5	0.079	0.078	0.181
	6	0.027	0.068	0.043
Mdh2–	1	0.206	0.000	0.000
	2	0.161	0.100	0.171
	3	0.069	0.278	0.093
	4	0.222	0.113	0.291
	5	0.146	0.133	0.027
	6	0.089	0.161	0.145
	7	0.107	0.090	0.068
	8	0.000	0.118	0.058
	9	0.000	0.007	0.013
Mdh3–	1	0.307	0.079	0.091
	2	0.295	0.205	0.332
	3	0.235	0.360	0.325
	4	0.073	0.104	0.068
	5	0.077	0.200	0.071
	6	0.013	0.052	0.065
	7	0.000	0.000	0.048
Mdh4–	1	0.550	0.157	0.197
	2	0.219	0.452	0.360
	3	0.131	0.196	0.212
	4	0.030	0.087	0.152
	5	0.070	0.067	0.039
	6	0.000	0.044	0.029
	7	0.000	0.000	0.010
Me1–	1	0.059	0.000	0.049
	2	0.093	0.136	0.187
	3	0.533	0.342	0.526
	4	0.141	0.088	0.015
	5	0.166	0.377	0.150
	6	0.008	0.057	0.069
	7	0.000	0.000	0.005
Me2–	1	0.119	0.084	0.070
	2	0.290	0.302	0.429
	3	0.483	0.513	0.228
	4	0.000	0.000	0.067
	5	0.025	0.058	0.196
	6	0.095	0.043	0.050
	7	0.000	0.000	0.020
Pgdh1–	1	0.157	0.117	0.080
	2	0.675	0.534	0.615
	3	0.097	0.019	0.015
	4	0.071	0.333	0.263
	5	0.000	0.000	0.019
Pgdh2–	1	0.047	0.018	0.093
	2	0.151	0.145	0.000
	3	0.112	0.110	0.224
	4	0.216	0.307	0.239
	5	0.269	0.189	0.144
	6	0.166	0.166	0.222
	7	0.022	0.027	0.060
	8	0.016	0.038	0.019
Pgi1–	1	1.000	1.000	1.000
Pgi2–	1	0.025	0.000	0.000
	2	0.712	0.687	0.905
	3	0.124	0.203	0.063
	4	0.114	0.077	0.017
	5	0.025	0.033	0.016
Pgi3–	1	0.271	0.156	0.136
	2	0.229	0.344	0.364
	3	0.430	0.352	0.350
	4	0.070	0.148	0.150
Pgi4–	1	0.208	0.233	0.107
	2	0.510	0.443	0.402
	3	0.197	0.254	0.414
	4	0.087	0.070	0.111
Pgm1–	1	0.500	0.462	0.449
	2	0.380	0.407	0.382
	3	0.120	0.121	0.162
Pgm2–	1	0.640	0.351	0.344
	2	0.360	0.559	0.507
	3	0.000	0.097	0.097
	4	0.000	0.000	0.051

variability of the species was attributed to Neo-Darwinian evolutionary models in which selection plays a predominant role (Hamrick & Allard, 1972).

Significant coefficient of multiple regression values (R^2) for environmental predictors of allozyme variation are presented in Table 4. Linear association between allozyme variability and the variable combination of monthly cloudiness, monthly minimum temperature and longitude explained 95.9% of the variation in each of alleles *Idh1-6* and *Mdh4-7*. Linear association between allozyme variability and the four variable combination of monthly cloudiness, minimum

Table 3

Spearman correlation values between ecogeographical parameters and cowpea allozymes (only significant values were used)

	Longitude	*Latitude*	*Mean annual rainfall*	*Rain day*	*Mean sunshine*
Hk1-4	0.959*		-0.848*		
Idh1-4	-0.842*				
Idh2-3		0.876*	-0.842*	-0.867*	-0.841*
Mdh1-5	0.880*		-0.879*		
Mdh1-4		-0.935**		0.947**	-0.954**
Mdh2-5					
Mdh4-4	0.922*				
Me1-6		0.868*		-0.883*	0.874*
Me1-4	-0.859*				
Pgdh1-1	0.856*		0.853*		
Pgdh2-5			0.846*		
Pgdh2-1	0.836*				
Pgdh3-1		0.907*	-0.907*	-0.903*	0.897*
Pgm2-4	0.930*				
Pgm2-1					-0.838*

	Relative humidity		*Vapour pressure*		*Mean monthly*	*Mean temperature*	
	1500 h	*0600 h*	*1500 h*	*0600 h*	*cloud*	*Max*	*Min*
Hk1-4							
Idh1-4							
Idh2-3		0.866*					
Mdh1-5							
Mdh1-4	0.950*	0.927*	0.945**	0.916*		-0.925*	
Mdh2-5							-0.849*
Mdh4-4							
Me1-6	-0.852*		-0.866*				
Me1-4							
Pgdh1-1							
Pgdh2-5							
Pgdh2-1							
Pgdh3-1	-0.889*		-0.904*	-0.903*	-0.892*	0.841*	
Pgm2-4							
Pgm2-1	0.835*						

TABLE 4

Linear association values (R^2) between cowpea allozymes and ecogeographical parameters (only significant values were recorded) (R_a = mean annual rainfall, Cl = Mean monthly cloud, L_n = longitude and T_{min} = mean monthly minimum temperature)

Dependent variable	*Cl L_n T_{min}*	*R_a Cl L_n T_{min}*
Hk1-4		0.999**
Hk1-3		0.977*
Hk1-2		0.985*
Hk1-1		0.999**
Idh1-6	0.960*	
Idh2-3		0.996**
Mdh1-4		0.977*
Mdh2-2		0.994*
Mdh3-3		0.998**
Mdh4-7	0.960*	
Mdh4-4		0.995*
Pgdh1-4		0.995*
Pgdh1-2		0.997**
Pgdh2-1		0.989*
Pgdh3-5		0.992*
Pgi4-2		0.986*
Pgm2-3		0.999

* = $P < 0.01$; ** = $P < 0.001$

temperature, monthly annual rainfall and longitude explained 97.7% of variation in each of alleles *Hk1-3* and *Mdh1-4*; 98.5% in allele *Hk1-2*; 99.9% of variation in each of alleles *Hk1-1* and *Pgm2-3*; 99.6% of variation in *Idh2-3*; 99.4% of variation in allele *Mdh2-2*; 99.8% in allele *Mdh3-3*; 99.5% of variation in each of alleles *Mdh4-4* and *Pgdh1-4*; 99.7 %; 98.9% in allele *6pgdh2-1*; 99.2 % of variation in allele *Pgdh3-5*; 98.6 % of variation in allele *Pgi4-2;* 97.5% of variation in allele *Me1-5*; and 97.9% in allele *Me1-5*.

It could be deduced from the results that genetic diversity in the cowpea accessions used for the study is related to ecogeographic parameters and was partially attributed to geographical, temperature and moisture factors. However, since cowpea is a cultivated crop human selection might also influence genetic diversity of the cowpea accessions used for the study. The findings of this work, therefore, do not favour the neutralist theory of selection. If allozymic diversity indeed varies dynamically with the environment, then during multiplication and regeneration of Ghanaian cowpea landraces, it is important for cowpea breeders and curators to carry these out as near to the region of their natural distribution as possible (Breese, 1989).

References

Asante I. K. (1998). Environmental predictors of three cowpea (*Vigna unguiculata* (L.) Walp) yield sub-characters. *Ghana J. Sci.* **38**: 101-106.

Asante I. K. (2000). Allozyme frequency distribution patterns at two gene loci of the phosphoglucomutase and one gene locus of fumarate hydratase enzymes in the cowpea (*Vigna unguiculata* (L.) Walp) landraces in three agroecological zones. *J. Ghana Sci. Ass.* **2**(3): 136-143.

Ayala F. J. (Ed.) (1976). *Molecular evolution.* Sinauer Associates Publishers.

Breese L. (1989). Multiplication and regeneration of germplasm. In *Scientific management: Characterization, evolution and enhancement*. Ed. H. T. Stalker and C. Chapman. Pp. 41-45. IBPGR, Rome/ Department of Crop Science, North Carolina State University.

Ferguson A. (1980). *Biochemical systematics and evolution.* Blackie, Glasgow and London.

Ghana Grains Development Project (1990). *Maize and cowpea production guide.* Ghana/CIDA Grains Development Project.

Hamrick J. L and **Allard R. W.** (1972). Microgeographical variation in allozyme frequencies in *Avena barbata. Proc. Natl Acad. Sci. U.S.A.* **69**:2100-2104.

Kimura M. (1982). *The neutral theory of molecular evolution.* Cambridge University Press, London and New York.

Kimura M. and **Ohta T.** (1971). Protein polymorphism as a phase of molecular evolution. *Nature* **229**: 467-469.

Lewontin R. C. (1974). *The Genetic basis of evolutionary change.* Columbia University Press, New York.

Marfo K. O. and **Hall A. E.** (1992). Inheritance of heat tolerance during pod set in cowpea. *Crop Sci.* **32**: 912-918.

Singh S. R., Jachal L. E., Thottappilly G., Cardwell K. F. and **Myers G. O.** (1992). Status of research on constraints to cowpea production. In *Biotechnology: Enhancing research on tropical crops in Africa.* Ed. G. Thottappilly, L. M. Monti, D. R. Mohan Raj, and A. W. Moore. CTA/IITA, Ibadan, Nigeria.

Singh S. R. and **Rachie K. O.** (1985). *Cowpea research production and utilization.* John Wiley and Sons, Chichester.

Tanksley S. D. and **Orton T. J** (1983). *Isozymes in plant genetic and breeding,* Part A. Elsevier Sciences Publishers. B.V., Amsterdam.