Isozyme Variation and Genetic Diversity at 3 Phosphoglucose-Isomerase (PGI) [Glucose-1-phosphate] Gene Loci in Nine Cowpea Accessions (*Vigna unguiculata* (L.) Walp) from Three Agroecological Zones

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Abstract

Isozyme variation and genetic diversity in three loci of the phosphoglucose-isomerase [glucose-1-phosphate] gene loci were studied within nine accessions of cowpea [*Vigna unguiculata* (L.) Walp] landraces by means of starch gel electrophoresis. These nine accessions, namely 87/139, 89/142, 87/157 (Deciduous forest accessions), 87/30, 87/37, 87/55 (Guinea savanna accessions) and 87/77, 87/81, 87/83 (Sudan savanna accessions) were sampled from three agroecological zones. The three PGI loci *PGI2**, *PGI3** and *PGI4** are polymorphic. Results show that there was clinal trend in all the three enzyme loci, and the cowpea landraces studied showed ecogeographical racial differences in the incidence of genes for the phosphoglucose-isomerase enzyme. Genetic distances within Deciduous forest, Guinea savanna and Sudan savanna agroecological zones were 0.068, 0.048 and 0.128, respectively. Between Deciduous forest and Sudan savanna zones accessions genetic distance ranged from 0.030 to 0.234 with a mean genetic distance of 0.128 ± 0.020, between Deciduous forest and Guinea savanna zones accession genetic distance ranged from 0.007 to 0.127 with a mean value of 0.053 ± 0.010, while between Guinea savanna and Sudan savanna zones accessions genetic distance ranged from 0.052 to 0.139 with a mean value of 0.087 ± 0.011. The observed pattern of allozyme distribution is explained by Neo-Darwinian evolutionary models, in which natural selection plays a predominant role.

Introduction

Isozymes are direct gene products and are the most widely used molecular markers by plant breeders. They have been used by several workers to identify biochemical species, as well as population and cultivar markers (Kephart, 1990). Among other advantages isozyme analysis is simple and low cost; isozymes have simple molecular basis of polymorphism and a reasonable genome coverage of 10 to 50 loci per species. The technique has been useful in the breeding of cultivated crops. By using isozyme analysis Gutierrez *et al.*(1998) determined genetic relationships among 24 collections of common bean (*Phaseolus vulgaris* L). Twenty-four collections, 21 of Andean origin and three from Central America were characterized. Eight out of 22 isozyme systems showed good resolution. Six of the enzymes malate dehydrogenase (MDH), shikimate dehydrogenase (SKDM), ribulose-bisphosphate (Rubisco; RBCS), isocitrate dehydrogenase (IDH) and glutamate dehydrogenase (GDH) were polymorphic. The two others, glutamate oxalacetate transaminase (GOT) [aspartate aminotransferase] and endopeptidase (EP), were monomorphic.

Isozymes have also been useful in the study of *Vigna unguiculata* and the wild forms of the species (Panella & Gepts, 1992; Pasquet, 1993; Vaillancourt & Weeden, (1993). Danquah *et al.* (2000) applied isozyme markers to study genetic polymorphism in four accessions of sor-

ghum. Natural population of organisms existing in different localities may be described in terms of the relative proportion in them of the different allelic types with regards to agroecological adaptive changes. Races of species have different chromosomes or alleles since they occur in different habitats within the geographic-distribution area of the species. Generally, in response to the environmental differences in the different geographic-distribution areas, certain genotypes are selected for and thus result in racial differences. Clinal trends are apparent in relation to climatic variation and sharp discontinuities in the local environments due to edaphic or biotic influences can also lead to allelic differentiation (Breese, 1989).

Total fitness or survival of an allele, therefore, depends on its response and adaptation to a complex of interacting environmental forces such as climatic, edaphic and biotic (Breese, 1989). In some situations the same pair of alleles are found in uniform frequencies over a wide distribution range of a species, or frequencies of alleles are uniform (Kimura, 1982). Such observations indicate the active participation of natural selection in maintaining genetic polymorphism. Population of organisms which inhabit different territories and which differ in relative frequencies of the chromosomal types may be described as races. Races are, therefore, populations that differ in the relative frequencies of gene alleles or of chromosome structures. The study of allelic frequencies of an organism would, therefore, give an insight into evolutionary trends of the organism. The aim of the study was to examine the distribution pattern, allelic frequency differences and genetic distance at three phosphoglucoseisomerase gene loci in the cowpea.

Materials and methods

Collection of cowpea accessions

Table 1 shows collection sites, agroecological zones and geographical origins of the cowpea accessions studied. The cowpea accessions were 87/139, 87/142, 87/157; (from Deciduous forest agroecological zone), 87/30, 87/37, 87/55;(from Guinea savanna agroecological zone) and 87/77, 87/81, 87/83 (from the Sudan savanna agroecological zone). These were samples of the 1987 collections from Plant Genetic Resources Centre of the Council for Scientific and

TABLE 1

List of nine cowpea accessions studied

Accession	*Collection site*	*Longitude*	*Latitude*	*Agroecological zone*
87/139	Akora Darko	00°24'W	06°22'N	Deciduous forest
87/142	Akora Darko	00°24'W	06°22'N	Deciduous forest
87/157	Abene	00°34'W	06°38'N	Deciduous forest
87/30	Boterly	00°29'W	09°25'W	Guinea savanna
87/37	Zan	00°16'W	09°25'N	Guinea savanna
87/55	Limoh	01°13'W	09°29'N	Guinea savanna
87/77	Buoti	02°07'W	10°53'N	Sudan savanna
87/81	Buoti	02°07'W	10°53'N	Sudan savanna
87/83	Nandom	03°15'W	10°50'N	Sudan savanna

Industrial Research, Bunso, Ghana.

Enzyme extraction

The protein source was the leaf tissue of cultivated cowpea plants. Leaflets were crushed in a small mortar with a pestle into a 'paste'. Crude squeezate in the 'paste' was absorbed into strips of Whatman No.1 filter paper (about 8×4 mm), which were blotted carefully and inserted into a cut about 3.5 cm from the intended cathodal end of the gel.

Starch gel electrophoresis

Electrophoretic procedure adopted was similar to what was described by Hunter & Market (1957). Electrophoresis was performed in 12.5 % hydrolysed starch resolving gels using Tris Continuous Citrate buffer (*p*H 8.0). Gels were subjected to between 148 V to 171 V and a current of between 51 mA to 57 mA for 3-4 h

Starch gel staining

After electrophoresis, gels were trimmed and cut into slices. Sliced gels were stained in a solution of 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 and PMS (trace) and incubated for 30 min at 25 °C. The stain was poured away and the gels were rinsed in distilled water for a few minutes.

Isozyme phenotyping

After staining, enzyme activities in gels showed as bands. Plants phenotypically having only one band are homozygous and those having three bands (including heterodimer with two homodimers) are heterozygous. Loci and alleles were numbered by using Arabic numbers, counting from cathodal end of gel.

Data analysis

Allele frequencies were scored for each accession and used to compute Nei's coefficient of genetic identity (I) as follows:

$$I = \frac{\sum x_i y_i}{\sqrt{(\sum x_i^2 \sum y_i^2)}} \qquad \text{(Furgeson, 1980)}$$

where x_i and y_i are the frequencies of the i-th allele in populations X and Y respectively. Genetic distance (D) was calculated by

$$D = -\log_e I \qquad \text{(Ferguson, 1980)}$$

The genetic distance matrix was used to generate dendrograms using the Unweighted Paired-Group Arithmetic Average (UPGM) clustering method (Sneath & Sokal, 1973) as illustrated by Ferguson (1980).

Results

Allozymes and their frequencies

The number of allozymes detected per locus in the analysed accessions is shown in Table 2. All the three loci were polymorphic and the largest number of alleles was detected at locus *PGI 2** with five alleles, while loci *PGI 3** and *PGI 4** exhibited four alleles each. Accession 87/139, from the Deciduous forest zone, had five alleles at locus *PGI 2**, while all other accessions had four alleles each.

The accessions differed in the frequency of the detected alleles as shown in Table 2. Some alleles were common to all accessions and some were rare or very rare. Alleles that were present in all or the majority of the accessions were referred to as common, while those present in several accessions in low frequencies were referred to as rare. Alleles found in a few individual accessions in low frequencies were referred to as very rare. Allele

TABLE 2

Allele frequencies of cowpea accessions (sample sizes are given in brackets)

Cowpea accessions	*87/139*	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
Locality	*Deciduous forest*			*Guinea savanna*			*Sudan savanna*		
PGI2 1*	.076	.000	.000	.000	.000	.000	.000	.000	.000
2	.788	.533	.814	.641	.783	.638	.893	.893	.928
3	.030	.300	.043	.269	.133	.207	.071	.071	.048
4	.091	.150	.100	.026	.067	.138	.019	.019	.012
5	.015	.017	.043	.064	.017	.017	.020	.017	.011
	(33)	(30)	(35)	(39)	(30)	(29)	(28)	(28)	(42)
PGI3 1*	.379	.233	.200	.222	.172	.074	.115	.175	.119
2	.121	.267	.300	.278	.328	.426	.385	.327	.381
3	.394	.467	.429	.361	.379	.315	.404	.288	.357
4	.106	.033	.071	.139	.121	.185	.096	.212	.143
	(33)	(30)	(35)	(36)	(28)	(27)	(26)	(26)	(42)
PGI4 1*	.190	.095	.138	.300	.173	.227	.259	.043	.019
2	.548	.432	.550	.400	.579	.409	.222	.196	.789
3	.143	.182	.267	.240	.250	.273	.482	.587	.173
4	.119	.091	.050	.060	.058	.091	.037	.174	.119
	(21)	(22)	(30)	(25)	(26)	(22)	(27)	(23)	(26)

PGI 2-2* was common with frequency ranging from 0.533 to 0.928. Allele *PGI2*-3* was rare except that accession 87/142 had frequency of 0.300. Alleles *PGI 2*-4* and *PGI 2*-5* were also rare. Allele *PGI 2*-1* was very rare and was found in only one accession (87/139). Allele *PGI 3*-3* was common except in accession (87/81) where its frequency was 0.288. Allele *PGI 3*-2* was also common except in three accessions (87/139, 87/142 and 87/30) where frequencies were low. Alleles *PGI 3*-1* and *PGI 3*-4* were rare. Allele *PGI 4*-2* was common, however frequencies were low in accessions 87/77 and 87/81. Alleles *PGI 4*-1* and *PGI 4*-4* were rare.

Some specific characteristics of the individual accessions could be deduced on the basis of average frequencies of characteristic alleles at the loci (Fig. 1-3). The highest average frequencies of alleles *PGI 2*-2, PGI 3*-2, PGI 3*-4, PGI 4*-3* and *PGI 4*-4* were registered in the accessions from the

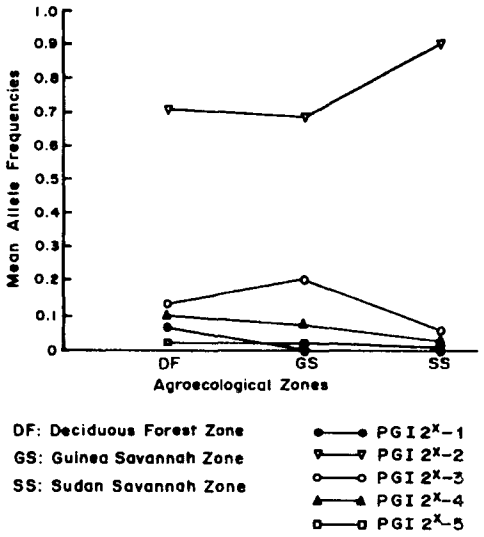

Fig. 1. Mean allele frequencies distribution at the PGI 2* locus in the three agroecological zones

Sudan savanna agroecological zone. The highest average frequencies of alleles *PGI 2*-4, PGI 3*-1, PGI 3*-3* and *PGI 4*-2* were registered in the Deciduous forest agroecological zone accessions, while the highest average frequencies of alleles *PGI*

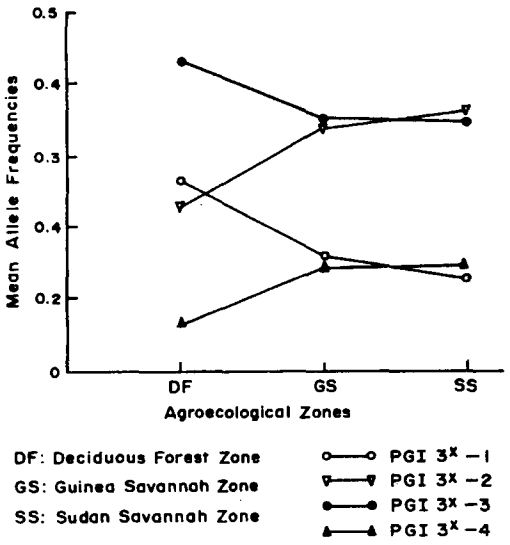

Fig. 2. Mean allele frequencies distribution at the PGI 3* locus in the three agroecological zones

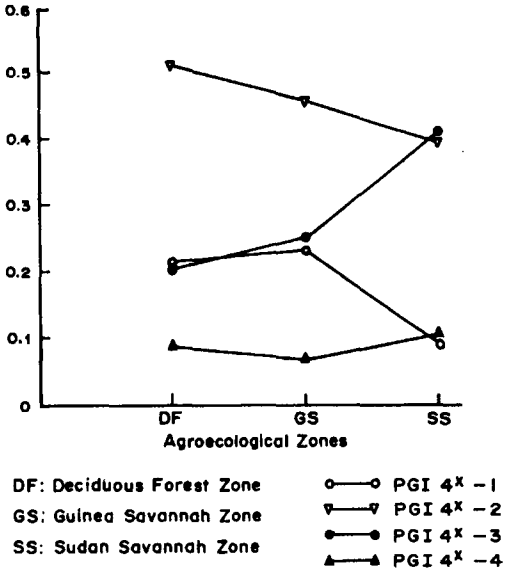

Fig. 3. Mean allele frequencies distribution at the PGI 4* locus in the three agroecological zones

2-3* and *PGI 4*-1* were registered in the Guinea savanna agroecological zone accessions. It could be deduced that allele *PGI 2*-2* is approaching fixation and allele *PGI 2*-1* is approaching extinction (Fig. 1). Alleles *PGI 3*-2* and *PGI 3*-4* increase in average frequencies from the Deciduous forest agroecology to the Sudan savanna agroecology, while alleles *PGI 3*-1* and *PGI 3*-3* increase in average frequencies from the Sudan savanna agroecological zone to the Deciduous forest agroecological zone (Fig. 2). Alleles *PGI 4*-3* and *PGI 4*-4* increase in average frequencies from the Deciduous forest agroecological zone to the Sudan savanna agroecological zone, while allele *PGI 4*-2* shows the reverse (Fig. 3).

Genetic diversity. Genetic diversity among accessions was assessed based on genetic distance and cluster analysis.

Genetic distance. Genetic distances between accessions are presented in a matrix form in Table 3. Genetic distance among accessions ranged from 0.007 (between accessions 87/157 and 87/37) to 0.234 (between accessions 87/142 and 87/81) D = 0.087. Average genetic distance (D) within agroecological zone was calculated by summing the genetic distances among the three accessions in an agroecological zones and finding the average. Within the Deciduous forest agroecological zone genetic distance among the accessions ranged from 0.036 to 0.093 (D=0.068±0.014), within the Guinea savanna agroecological zone genetic distance ranged from 0.028 to 0.034 (D = 0.048±0.009) and within Sudan savanna agroecological zone genetic distance among the accessions ranged from 0.038 to 0.234 (D = 0.128±0.020).

Average genetic distance (D) among two agroecological zones accessions were calculated by pooling genetic distances among all accessions between the two agroecological zones together and the average estimated. Genetic distance between Deciduous forest agroecological zone accessions and Sudan savanna agroecological

TABLE 3

Matrix of genetic identity (above diagonal) and genetic distance (below diagonal) for cowpea accessions

	87/139	*87/142*	*87/157*	*87/30*	*87/37*	*87/55*	*87/77*	*87/81*	*87/83*
87/139		.911	.965	.967	.955	.881	.861	.826	.931
87/142	.093		.927	.973	.942	.940	.860	.791	.862
87/157	.036	.076		.948	.993	.948	.932	.893	.969
87/30	.034	.027	.053		.971	.966	.925	.870	.896
87/37	.046	.060	.007	.029		.972	.949	.900	.969
87/55	.127	.062	.060	.035	.028		.937	.892	.917
87/77	.150	.151	.062	.078	.052	.065		.963	.858
87/81	.191	.234	.113	.139	.105	.114	.038		.841
87/83	.071	.149	.031	.110	.031	.087	.153	.173	

D: Mean = 0.087±0.009. Range = 0.007-0.234

zone accessions ranged from 0.030 to 0.234, with a mean of 0.128±0.020; genetic distance between Deciduous forest agroecological zone accessions and Guinea savanna agroecological zone accessions ranged from 0.007 to 0.127, about a mean of 0.053±0.010, while the genetic distance between Guinea savanna agroecological zone accessions and Sudan savanna agroecological zone accessions ranged from 0.052 to 0.139, with a mean of 0.087±0.011.

Fig. 4 presents a dendrogram resulting from cluster analysis of the accessions. Three distinct population clusters were defined. Group 1 is a cluster of four accessions from all three agroecological zones, namely; accessions 87/139, 87/157 (Deciduous forest types) 87/37 (Guinea savanna type) and 87/83 (Sudan savanna type). Group 2 is a cluster of two Guinea savanna type accessions (87/30 and 87/55) and one Deciduous forest type (87/142). Group 3 is a cluster of two Sudan savanna types (87/77 and 87/81). The two Sudan savanna accessions 87/

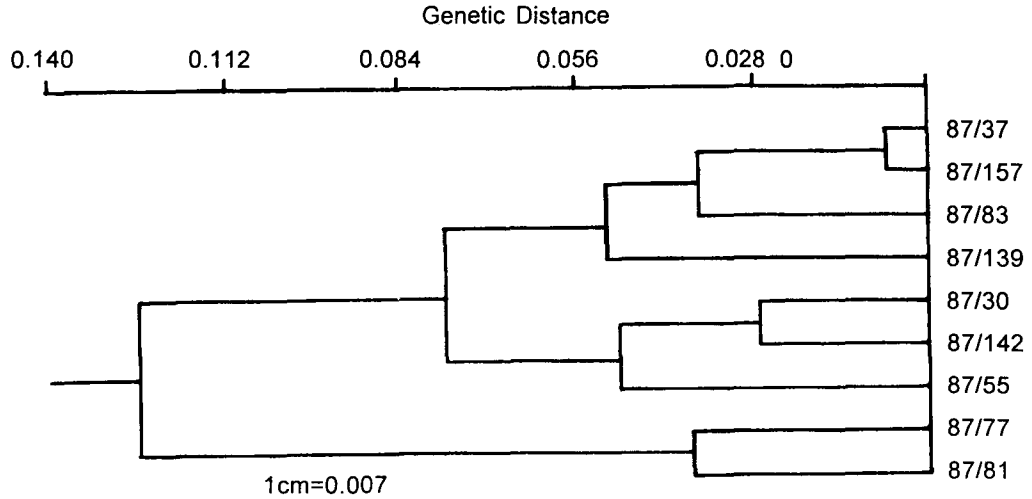

Fig. 4. Cluster analysis for allozyme data of a cowpea accessions (UPGMA)

77 and 87/81 forming one cluster is not surprising since they were collected from the same site (Buoti), however, the two Deciduous forest accessions 87/139 and 87/142 were collected from the same site (Akora Darko) they did not fall into the same cluster. The two Sudan savanna accessions are highly diverse.

Discussion

Each type of gene has a definite frequency in the gene pool of a population generally, and these frequencies are characteristic for each population. If, however, the same population is sampled repeatedly in different locations the relative frequencies of genes of different types may be observed to change from location to location, hence genetic changes in the composition of population is observed giving an evidence of evolutionary changes in nature (Sinnot *et al*, 1958). The experimental results showed differences in the allelic frequencies for instance, allele *PGI 2*-2* is approaching fixation, while allele *PGI 2*-3* approaches loss.

Cowpea plants that carried alleles *PGI 2*-2, PGI 3*-2, PGI 3*-4, PGI 4*-3* and *PGI 4*-4* were for some reason more successful in survival and reproduction in the Sudan savanna zone, whereas cowpea plants that carried alleles *PGI 3*-1, PGI 3*-3* and *PGI 4*-2* were more successful in survival and reproduction in the Deciduous forest zone, and cowpea plants that carried the alleles *PGI 2*-4* and *PGI 4*-1* were more successful in the Guinea savanna zone. This is an indication that the accessions in general were functionally specific from a biochemical point of view (Geric, Zlokolica & Geric, 1989). Natural selection is believed to be actively maintaining genetic polymorphism if there is a cline among other factors (Kimura, 1982). In all the three PGI enzyme gene loci there were clinal effects between the longest distances from where cowpea accessions were collected. Clinal effect in this situation indicates that the frequencies of the different alleles change with ecogeographical factor. The cowpea accessions studied showed racial differences in the incidence of genes for the PGI enzyme and that the genes that determine PGI enzyme production vary in frequency in the cowpea.

Genetic distance can be considered as a measure of the average number of electrophoretically detectable allelic substitutions per locus that have accumulated since the separation of two populations from a common ancestor (Simpson & Withers, 1986). From the results of the present study, the average genetic distance of 0.087 among the accessions implies that at the PGI enzyme gene loci there was about 9% allelic substitution among the accessions. Average genetic distance values of 0.068, 0.048 and 0.128 within Deciduous forest, Guinea savanna and Sudan Savanna zones, respectively, also implied that within these agroecological zones there were about 7%, 5% and 13% allelic substitutions, respectively at the PGI enzyme loci. It is evident that there was relatively high genetic diversity within the Sudan savanna agroecological zone accessions.

Generally average genetic distance between Deciduous forest and Sudan savanna accessions was 0.128. This indicates that these two groups of accessions have accumulated about 13% allelic substitution, since they separated from their common ancestor; average genetic distance between Deciduous forest and Guinea savanna accessions was 0.053, implying that they have

accumulated about 5% allelic substitutions since they diverged from their common ancestor. Similarly, the average genetic distance of 0.087 between Guinea savanna and Sudan savanna zones accessions implies that they have accumulated about 9% allelic substitutions from the period they separated from their common ancestor. It may be deduced that the different levels of allelic substitutions at the PGI gene loci among accessions of the different agroecological zones indicate that the PGI enzyme gene loci could be functionally specific from a biochemical point of view.

Populations with higher genetic variability, other conditions being the same, are superior source of materials (Geric *et al.*, 1986). Considering the extent of genetic diversity, accessions 87/77 and 87/81 should become important genetic sources, with reference to the PGI enzyme gene loci, for the improvement of the sum of genetic variability in the materials used in cowpea breeding.

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