POPULATION GENETICS AND CHARACTERIZATION OF COWPEA ACCESSIONS (*VIGNA UNGUICULATA* (L) WALP) FROM THREE AGROECOLOGICAL ZONES OF GHANA BASED ON ISOZYME.

A thesis submitted for the degree of Doctor of Philosophy of the University of Ghana, Legon

By

ISAAC KOJO ASANTE, B.Sc. Hons. (Botany); M. Phil. (Botany)
University of Ghana, Legon

DEPARTMENT OF BOTANY
UNIVERSITY OF GHANA

OCTOBER, 1996

DECLARATION

I certify that this work was carried out under the supervision of Professor E. Laing, Dr. E.K. Abban and Dr. (Mrs.) Angelina Jumah, and that this thesis does not contain any material previously published or written by another person except where due reference is made in the text; and to the best of my knowledge this work does not contain any material previously submitted for a degree or diploma in any University.

I.K. ASANTE

E. Laing
.....
PROF E. LAING
(Supervisor)

.....
Student

.....
DR. E.K. ABBAN
(Supervisor)

.....
DR.(MRS.) A. JUMAH
(Supervisor)

ABSTRACT

Allozyme variation in protein encoded at 22 loci was studied in 9 cowpea accessions from three agroecological zones of Ghana (namely Deciduous forest, Guinea savannah and Sudan savannah) by electrophoresis. In addition, variation in thirteen morphological agronomic traits of the accessions were studied. The results indicated that:

(i) 21 out of 22 loci were polymorphic (95.5%);

(ii) cowpea accessions displayed average estimates of mean alleles per locus, A = 5.00; mean expected heterozygosity, $\bar{H}_L$ = 0.561±0.037, mean observed heterozygosity, $\bar{H}_o$ = 0.264±0.007; total genetic diversity, H_t = 0.648±0.032; mean Wright's fixation index, F_{is} = 0.573±0.096; frequency of cross-pollination, 0.244±0.067

(iii) Coefficient of genetic distance (D_m) based on allozyme data among Deciduous forest zone accessions ranged from 0.130 to 0.167 with a mean of 0.143±0.010, among Guinea savannah zone accessions the range was 0.101 to 0.190 with a mean of 0.140±0.021 and in Sudan savannah zone accessions the range was 0.163 to 0.285 with a mean of 0.235±0.030 Coefficient of meristic distance (D_m) based on meristic data among Deciduous forest zone accessions ranged from 0.063 to 0.285 with a mean of 0.103±0.017, among Guinea savannah zone accessions the range was 0.047 0.190 with a mean of 0.123±0.034 and among Sudan savannah zone accessions the range was 0.010 0.057 with a mean of 0.038±0.012.

(iv) Deviations from Hardy-Weinberg equilibrium were found in several loci in some of the cowpea accessions

(v) 86.5% of total genetic variation was found to be present within accessions while 13.4% resided among accessions.

(vi) Allozymic and environmental variations were partly correlated; morphological and environmental factors were partially correlated.

(vii) Allozyme variations in several gene loci and morphological variations were significantly correlated with and predictable by environmental variables, primarily related to geographic, temperature and moisture indices.

Pattern of genetic variation within and between accessions suggested that:

(a) climatic 'selection' plays a primary role in allozymic and morphological differentiation into ecologically adaptive patterns;

(b) the environmental variation model seems to be a good predictor of genetic variation in cowpea accessions.

Results suggested that use of isozyme technique can

(a) lead to discovery of genetic markers for quantitative traits in cowpea breeding;

(b) help in collection, multiplication and regeneration of cowpea seed germplasm.

Results further suggested that cowpea accessions from Sudan savannah zone of Ghana should serve as good source of genetic material for improvement of the sum of genetic variability in

materials used in cowpea breeding.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

CHAPTER ONE

INTRODUCTION

1.1 The cowpea plant:diversity, origin and distribution

The plant commonly called cowpea and scientifically known as *Vigna unguiculata* (L) Walp belongs to the plant family Papilionaceae. Physically, the plant can be erect, semi-erect, prostrate (trailing) or climbing, and its growth habit ranges from indeterminate to fairly determinate. The leaves of the plant are trifoliate. Flowers are white or purple; they are zygomorphic and hermaphrodite. There are five sepals which are more or less fused to encircle the base of the flower after maturity There are five petals which are of three types: a posterior petal called the 'standard petal', two smaller lateral ones called the 'wings' and two others which are fused along their lower margins to form a keel. The keel is a boat-shaped structure which encloses the reproductive parts, the gynoecium and androecium.

Cowpea belongs to the genus *Vigna* and subgenus *Catiang* (Rachie, 1985). The subgenus Catiang includes only two known distinct species; *V. unguiculata* and *V. nervosa* (Rachie 1985.) *V. unguiculata* is subdivided into five subspecies of which three are cultivated and two are closely related wild species (Ng and Marechal,1985) The three cultivated subspecies are *V. unguiculata, V. cylindrica* and *V. sesquipedalis* (Rachie, 1985; Onwuene and Sinha, 1991) while the two wild species are *V. dekindtiana* and *V. mensensis* (Rachie, 1985).

Cowpea is considered to have originated from West Africa (Faris, 1965, Rachie and Rawal, 1976) with Nigeria as the centre of diversity mainly because wild and weedy species have been found there (Rawal, 1973; Steele, 1976)

Africa leads in world production of cowpea. Nigeria, Burkina Faso, Niger, Ghana, Kenya and Uganda are the major producing countries (Dovlo, 1976; Rachie, 1985) The plant is also grown extensively in Latin America and to a limited extent in the southern part of the U.S.A (Onwuene and Sinha,1991)

1.2 Economic importance of cowpea

Cowpea is considered the second most important food grain legume (Onwueme and Sinha,1991) This is because its protein content ranges from 18 to 29% (IITA, 1983) and constitutes a cheap source of plant protein for humans. The plant also provides feed, forage, hay and silage for livestock. It serves as green manure and cover crop for maintaining soil productivity (Onwueme and Sinha, 1991) As food, the fresh seeds and immature pods of cowpea are eaten as spinach. As a soil improving material, cowpea compensates for the loss of nitrogen removed by cereals when intercropped with them (Onwueme and Sinha, 1991)

1.3 Cowpea cultivation in Ghana

Ghana has six agro-ecological zones as indicated in figure 1, and cowpea is cultivated in all the zones. Greatest production occurs in the Savannah zones and the margins of the semi-deciduous

forest zone (GGDP, 1990). Cultivation is mostly by peasant farmers on small scale and by intercropping with cassava, sorghum, millet and yams (Bennet-Lartey, 1991). However, commercial cowpea farming is on the increase (Bennet-Lartey,1991) Cowpea varieties cultivated by farmers are mostly landraces which are widely diverse in character. Constraints in cowpea production are caused by factors including insect pests, diseases, plant parasite weeds, drought and heat (Singh *et al.*, 1992) Hot weather during pod set reduces productivity of cowpea by inhibition of floral bud development; while high night temperature results in low pod set, as a result of sterility associated with anther indehiscence and incomplete pollen development. Heat tolerance is controlled by a single dominant gene, but there are minor genes and environmental effects as well (Marfo and Hall, 1992)

1.4 Cowpea variability study

Variability resulting from selection under diverse environments is found in cultivated species of which cowpea is not an exception (Ng and Marechal, 1985) Variability studies of several characters of cowpea have been facilitated by characterization and growth performance evaluation of cowpea germplasm. For example cowpea grain colour polymorphism was studied in Ghanaian markets (Dovlo, 1976). Variation in growth and development habits including twining tendency, flower colour, days to flowering and maturity, eye colour and pattern, susceptibility to insect pests and diseases, 100-mean seed weight, number of seeds

per plant, number of peduncles per plant, peduncle length and yield per plant have been studied (Porter *et al.*, 1974; Bennet-Lartey,1991). de Mooy (1985) in a study of 108 accessions of Botswana cowpea germplasm observed polymorphism in terminal leaflet length and width, peduncle length, pod length, number of pods per plant and number of seeds per plant. Variability in seed size, seed colour, pod length, number of seeds per pod, 100-seed weight and grain yield has been observed in cowpea accessions from Ghana and Nigeria (Amoatey, 1987). In a study of 39 Ghanaian and exotic varieties, Doku (1970) observed variability in weight of nodules per plant. Asante (1991) in a study of inheritance and genetical linkage in cowpea reported of variability in pigmentation of unripe pod tip, flower bud tip and sepal, seed coat colour and eye pattern, flower colour and paleness in leaf chlorophyll.

1.5 Isozyme characterization and evaluation of plant genetic resources

Characterization and evaluation result in a recording of a number of traits which help in identifying accessions. Usually such studies aim at identifying accessions with desirable traits for use in crop improvement (Ramanatha Rao and Riley, 1994). Until recently, most of the characterization and evaluation of plant genetic resources had been based on comparison of meristic and morphological characters (Ramanatha Rao and Riley, 1994) However, such traits are often under complex genetic control and subject to edapho-climatic influences (Asiedu, 1992) To refine the basis for evaluation and breeding programmes, genetic characterization of

species and populations of several plants and animal groups have proved very useful (Asiedu,1992). Among genetic characterization approaches, isozyme and allozyme analysis have been used by several workers to identify biochemical species, population and cultivar markers (Asiedu *et al.*, 1990; Kephart, 1990) Allozyme analysis could also indicate the genetic relationships among related group of organisms (Ferguson,1980).

The method has also been used to identify genotypes, and study genetic diversity (Asiedu, 1992; Balagtas and Ramirez, 1991; Bhat *et al.*, 1992; Vaillancourt *et al.*, 1993). Isozyme technique has also been used in confirming hybrids, tracking alien chromosome segments, tagging genes and screening backcross populations more efficiently (Asiedu, 1992).

Generally,isozyme and allozyme analysis have several advantages over morphological markers. For instance, isozyme markers are co-dominant in inheritance and therefore make it relatively easier to distinguish homozygotes from heterozygotes. They are also free from epistatic/pleiotropic effects which characterize morphological markers (Asiedu,1992) This technique is effectively non-destructive and can also be applied to screen large numbers of plants rapidly (Moore and Collins,1983)

Although isozyme markers are sometimes subject to ontogenic variation, limited in number and only DNA regions coding for soluble proteins can be sampled, analysis of isozymes will usually have an advantage over DNA based methods such as RFLP and RAPDS in terms of cost (Asiedu, 1992).

1.6 Some basis for isozyme variation

Isozyme variation can be classified into two with reference to genetic analysis (Pollock *et al.*,1984). The first class constitutes variant forms of an enzyme with different primary structures coded at different loci within the genome. The second type of variation is often called allozymic variation which is most frequently used in genetic analysis. The former types may have different locations within the cell/organism and may be synthesized at different times during development. They are often different in their catalytic/regulatory properties allied to differences in function and show large differences in their electrophoretic mobilities. Generally, all members of a population will possess such multiple form (Pollock *et al.*,1984)

Allozymes are different forms of a polypeptide coded at a single locus and often differ in only a few amino acid residues (Pollock *et al.*,1984). Usually, they have similar catalytic and regulatory properties, and within a population there may be a large number of different forms. Expression of these forms in any individual as a multiple-banding pattern on an electrophoretogram will depend on the number of alleles and heterozygosity at the locus involved, ploidy level and number of polypeptide chains in the functionally active protein (Pollock *et al.*,1984)

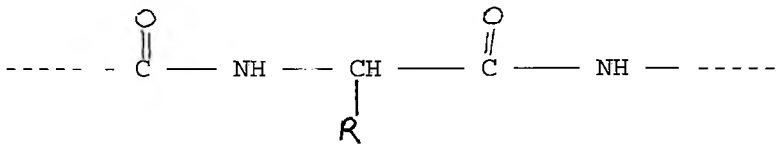
1.7 Electrophoresis

The primary structure of proteins bears a direct relationship to the sequence of nucleotides within the genome (Pollock *et*

al.,1984). Proteins are therefore useful for direct genetic study, since they are immediate products of gene action. Since many proteins are enzymes, enzyme variability has been used as an indicator of genetic diversity.

Protein separation.

When condensed into a polypeptide, amino acids can generally and basically be chemically represented as follows (Lewontin, 1974):



where R is specific to the amino acid. Twenty amino acids make up the primary structure of proteins and fall into three categories with respect to this R side chain. In one group, the R group chains are non-ionizable, they are electrostatically neutral (Lewontin, 1974). In a second group, arginine, lysine, hydroxylysine and tyrosine, the R chains are made of an ammonia (NH_2) group, which is in a dynamic equilibrium between a neutral and a positively charged form. At physiological pH (about 7-8) they are positively charged, since they are basic amino acids (Lewontin, 1974, Pollock *et al.*, 1987). A third group, made of aspartate, glutamate and histidine, has a carboxylic (-COOH) acid R group which is in a dynamic equilibrium between neutral and negatively charged forms At physiological pH they are negatively charged, since they are acidic amino acids. The net electrical

charge of a polypeptide comprising these three groups of amino acid reflects the relative proportions of acidic and basic amino acids. In most soluble enzyme proteins the molar proportions of acidic amino acids exceeds that of basic amino acids (Pollock *et al.*, 1984). As the pH is lowered, more and more of the NH_2 group will become positively charged NH_3^+ ions, while the acidic COO^- ions will be saturated and become neutral, resulting in the whole polypeptide taking on a positive charge (Lewontin, 1974) The reverse will happen as the pH is raised. Eventually, a pH is reached where the negative and positive charges just balance out to give a neutral polypeptide. This is the isoelectric point (pI), which for most plant enzymes has values in the acidic range.

At alkaline pH most enzymes are negatively charged and will move away from the negative cathode region of an electric field and towards the anode (+) The rate of migration of the protein is determined by its charge:mass ratio. In general terms, electrophoresis is an analytical procedure which exploits differences in charge:mass ratio to fractionate proteins in a mixture by means of an electric field (Pollock *et al.*, 1984)

If an allelic change at a locus results in the replacement of an amino acid in a neutral polypeptide with an amino acid from another polypeptide, the isoelectric point of the protein will be altered, as will the net charge on the protein at any given pH. Such change in net charge can be used to separate proteins and thus to distinguish allelic forms of the same gene product by the use of electrophoresis (Lewontin, 1974). However electrophoresis has its

limitations which can be summarised in two statements (Pollock *et al.*, 1984) viz: not all distinct bands on electrophoretograms are true isoenzymes, and secondly not all isoenzymes can be resolved by electrophoresis.

Enzymic protein and polymorphism.

Interpretation of banding patterns evident on stained gels is aided considerably by the quaternary structure of many enzymes (Kephart, 1990) Most enzymes assayed for electrophoretic mobility function as monomers (single polypeptide), dimers (two sub-unit enzyme) or tetramers (four sub-unit enzyme) Since most allozymes of a single gene are co-dominantly inherited, the polypeptide sub-units encoded by each allele are interpreted as phenotypes on the gel with heterozygotes showing multiple bands. If the proteins coded for by, for example, alleles A and B differ by a single amino acid, and the substitution results in a change in electric charge or in conformation of the molecule they will have different electrophoretic mobilities. Then in a diploid species three electrophoretic patterns could be observed for a monomeric enzyme (Fig.1a) In the heterozygotes, only two bands are found on the gel. In a dimeric protein, as a result of the production of two different polypeptides in the heterozygote, three different dimers are found, in this case a hybrid dimer is produced (Fig.1b). For a tetrameric protein, five different tetramers consisting of two polypeptides are possible resulting in a five-banded heterozygote pattern (Fig1.c).

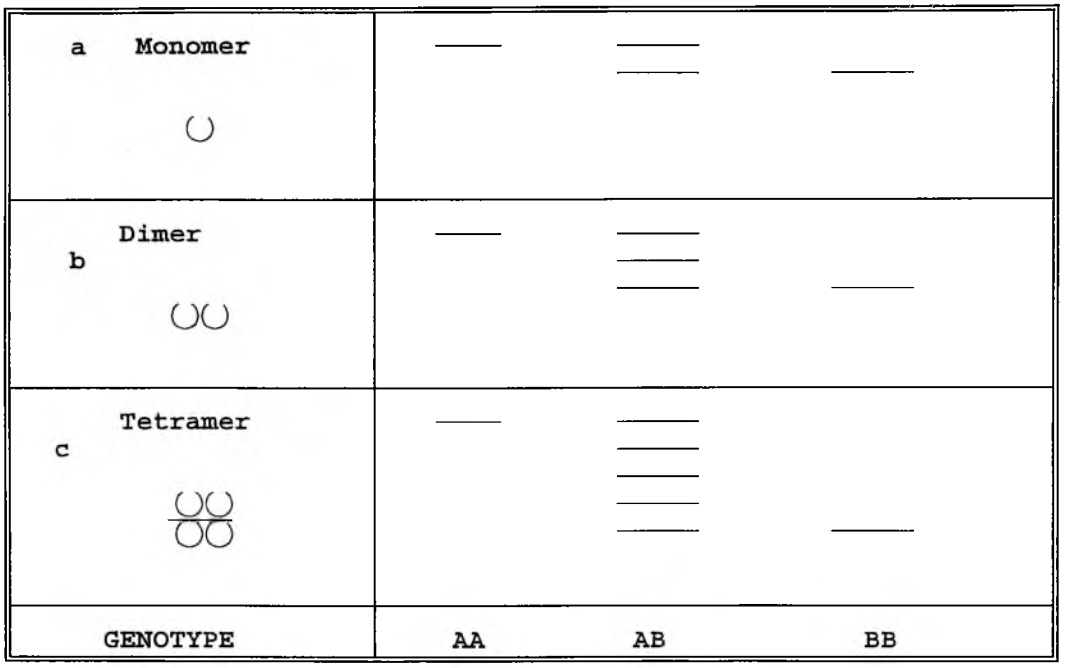


Fig 1: Hypothetical phenotype (bands) for soluble proteins formed by association of one, two, and four polypeptides.

1.8 Purpose of Study

Available evidence indicates that cowpea breeding in Ghana has been based to a very large extent on conventional morphological character states, which are usually influenced by environmental factors. As a result of this, morphological markers in characterization and plant breeding programmes are not reliable, unless they are complemented with genetic markers. Therefore, in this study allozymes at 22 loci of nine cultivars of cowpea landraces from three agroecological zones in Ghana were studied. Principal objectives of the study were to:

(i) study the genetic structure of selected Ghanaian cowpea landraces

(ii) identify allele frequency differences among their cultivars with special references to those of different agroecological zones

(iii) select biochemical markers for cowpea cultivars identification.

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection of cowpea germplasm

Nine landraces of cowpea germplasm were used for the study. These were collections from Plant Genetic Resources Centre (PGRC) of the Council for Scientific and Industrial Research at Bunso. The nine landraces were representatives from three agroecological zones of Ghana, namely, Deciduous Forest, Guinea Savannah and Sudan Savannah zones. A list of the nine collections showing their origin is presented in Table 1 and a map of Ghana showing their collection sites is presented in Fig.2.

TABLE 1

LIST OF NINE ACCESSIONS STUDIED

ACCESSION	COLLECTION SITE	LONG.	LAT.	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'N	Guinea Savannah
87/37	Zan	00°16'W	09°25'N	Guinea Savannah
87/55	Limoh	01°13'W	09°29'N	Guinea Savannah
87/77	Buoti	02°07'W	10°53'N	Sudan Savannah
87/81	Buoti	02°07'W	10°53'N	Sudan Savannah
87/83	Nandom	03°15'W	10°50'N	Sudan Savannah

Source: PGRC, Bunso

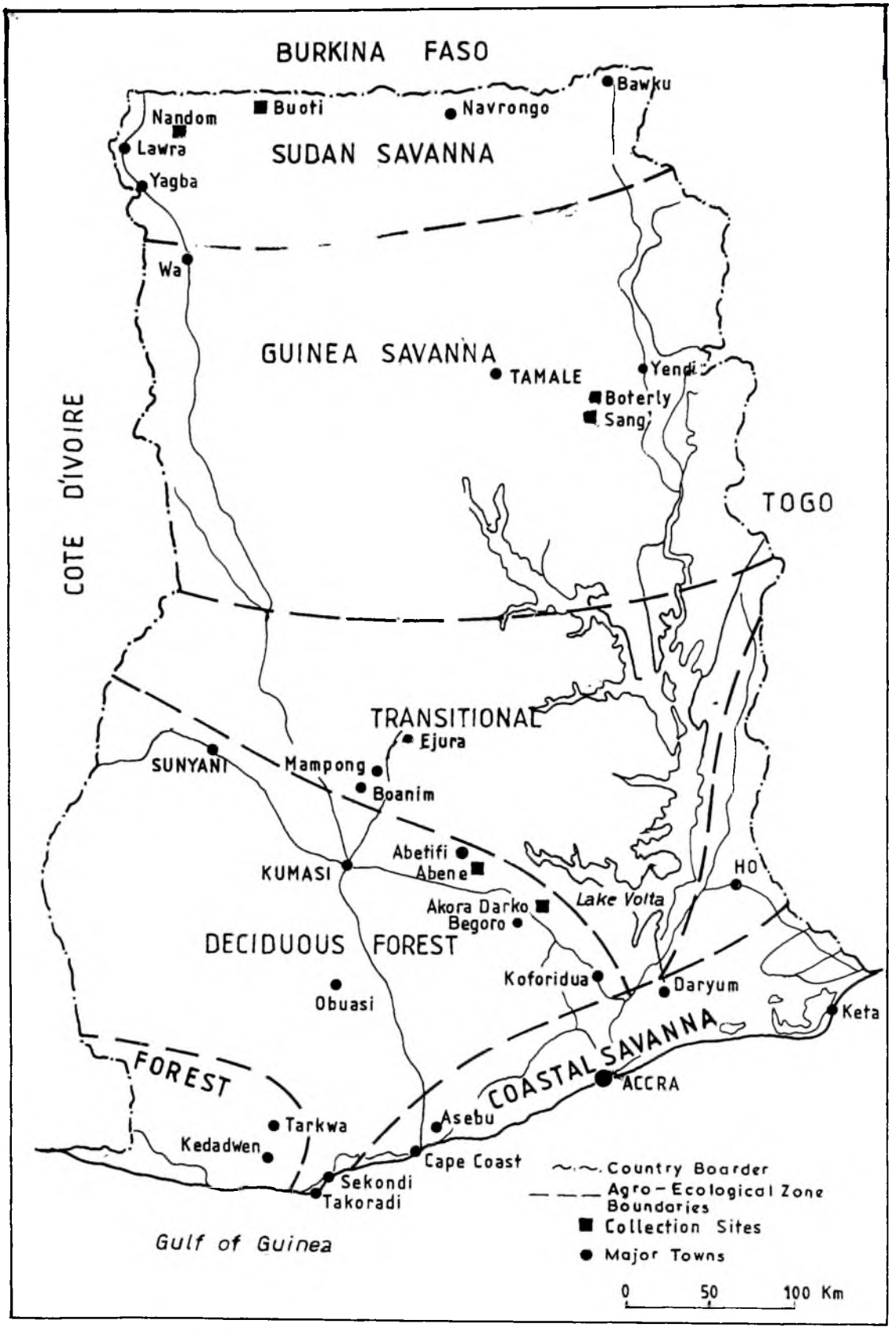


Fig. 2. Map of Ghana showing cowpea accessions collection sites.

2.2 Experimental plot

An experimental plot was developed at the Department of Botany, University of Ghana, Legon. Nine beds, each of dimensions 0.5 x 1.2m were prepared. Beds were labelled according to accession types planted on them. Crops were watered at least once a day and water loss through evaporation was limited by covering the beds with dry leaves. Weeds were regularly removed from the beds.

2.3 Seed multiplication

Twenty seeds from each of the nine accessions were sown on the experimental plot, during the third week of August 1993. Pods were harvested in late November 1993 and percentage seed germination for each accession was estimated. Seeds were bagged and stored in a refrigerator at 18°C.

2.4 Morphological characterization

Twenty-five seeds were selected from each of the nine accessions and sown on the experimental plot in the third week of February 1994. A table of random numbers was used to select twenty plants from each accession for the following morphological characterization: days to flowering, percentage pod set, days to pod maturation, pod length, percentage pod damage by legume borer, number of seeds per pod, number of ovules per pod, seed weight, percentage seed set, percentage seed damage by insect, fecundity rate of *Callosobruchus maculatus* infestation of seed and percentage leaf spots cover.

2.4.1 Percentage seed germination(PSG)

Number of seeds that germinated was expressed as a percentage of seeds sown and scored for percentage seed germination.

2.4.2 Days to flowering(DTF)

Number of days from sowing to when 75% of the plants had flowered was recorded. The means and standard errors of days to flowering were then calculated for the various accessions.

2.4.3 Percentage pod set per plant(PPS)

Number of flowers that opened on a plant was recorded daily until time of appearance of first pod. Total number of flowers that opened during the period was recorded together with total number of pods harvested per plant. Difference between these two figures was expressed as a percentage and scored as percentage pod set per plant. Means and their standard errors of this character were then calculated for the various accessions.

2.4.4 Days to pod maturation(DPM)

Number of days from flowering to when 75% of the pods had matured was recorded and the means and their standard errors were calculated.

2.4.5 Pod length(PL)

A minimum number of three dried pods chosen at random from a plant was used for scoring pod length. To measure pod length, a

thread was used to follow the course of a pod from stylar end to the point of attachment of pod to the stalk. The thread was stretched out on a metre rule and pod length was read off in centimetres. The mean value and standard error of pod length were then calculated per accession.

2.4.6 Percentage pod damage by legume pod borer(PPD)

Number of pods with holes from each plant were counted and expressed as a percentage of pods harvested from a plant. This result was used to assess the percentage pod damage by legume pod borer. The mean and its standard error of this character were calculated per accession.

2.4.7 Number of seeds per pod(NSP)

Pods used to score pod length were opened by hand. Number of seeds in each pod was counted and recorded. The mean and its standard error were calculated for each accession.

2.4.8 Number of ovules per pod(NOP)

Total number of ovules was recorded for every pod opened. A mean value and its standard error were then calculated for each accession.

2.4.9 Percentage seed set(PSS)

Total number of seeds (as recorded in 2.4.7) was expressed as a percentage of ovules (as recorded in 2.4.8) and scored for

percentage seed set for each accession.

2.4.10 50-mean seed weight(MSW)

Score for 50-mean seed weight was based on the average weight of three batches of seeds, with each batch having a total sample of 50 seeds. Seeds were dried in the sun after harvest for seven days to ensure that, seeds were of about the same dryness. Seeds were weighed carefully by using Ohaus Galaxy 1200 balance. THe mean value and its standard error for seed weight were then calculated per accession.

2.4.11 Percentage Seed damage by insect(PSI)

Number of seeds with holes in them were counted and expressed as a percentage over total number of seeds. This result was then used to assess percentage seed damage by insect for each accession.

2.4.12 Fecundity rate of *Callosobruchus maculatus* infestation(FRM) of seed

Two hundred seeds from each accession were bagged separately and sterilized in an incubator at a temperature of 35°C for 15 hours. Five replicates of thirty seeds from each accession were weighed and placed in petri dishes. A pair of young adults of *C. maculatus* of opposite sex fed on cowpea food media was placed in each petri dish. Two weeks after the set up, number of eggs laid on each seed were counted and scored for number of eggs laid per petri dish. Seeds were later placed back into the petri dishes.

Number of adults that emerged per petri dish was scored after three

weeks,and expressed as a percentage of total number of eggs laid. This was then expressed as fecundity rate for each accession.

2.4.13 Percentage leaf spots cover (LS)

Five plants were randomly sampled from each cowpea accession 30 days after germination. The first five leaves from each plant were harvested. Total leaf area and leaf area covered by leaf spots were taken by a planometer Leaf area covered by leaf spots was expressed as a percentage of total leaf area and used as score for percentage leaf spots cover for each accession.

2.5 Starch gel electrophoresis

Plants from the nine cowpea accessions were raised on an improvised experimental plot at Institute of Aquatic Biology of Council for Scientific and Industrial Research, where allozyme experiments were undertaken. A minimum of 15 individuals from each accession were studied. Soluble proteins from crude homogenates of 14 to 30-day-old leaves of the accessions were subjected to starch gel electrophoresis to study accession marker proteins, allozyme polymorphism and variability Results obtained formed the basis for the estimation of various population/accession characteristics and relationship among the materials studied.

2.5.1 Procedure

Electrophoretic procedure adopted was similar to what was described by Hunter and Market (1957) as zymogram technique and as

horizontal starch gel electrophoresis by other workers. For convenience, the procedure could be subdivided into four stages as follows:

(a) Preparation of starch gel

(b) Sample preparation and application to gel

(c) Running of gel

(d) Slicing and staining of gel

2.5.1.1 Preparation of starch gel

For each gel, 12.5% mixture of hydrolysed starch(from SIGMA) was made with Tris Continuous Citrate (CTC) buffer pH 8.0 (Appendix 14) in a flask. The flask was constantly whirled over a Bunsen Burner till the mixture came to an almost translucent gelly state, usually this was achieved at the point where the first large bubble formed from below. Gel was degassed with a vacuum water pump and poured quickly but carefully into a gel former. The gel was covered gently but quickly with a glass plate, and left to stand to form. About 2 to 3 hours later, it was transferred in the refrigerator for use the next day

2.5.1.2 Sample preparation and application to gel

The tissue used as the source of proteins for screening was the leaf of cultivated plants. Samples were obtained from the median leaflets which were collected from the field between 6.00 am and 6.30 am into labelled plastic containers. The leaflets were cleaned gently with tissue paper and 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 8x4mm),which were blotted carefully and inserted into a cut about 3.5 cm from intended cathodal end of the gel. After loading the gel with 18-27 samples, the cut part of the gel was pushed into place. Spacers were then inserted into former together with the gel to ensure a very close contact of samples to gel.

2.5.1.3 Running of gel

Loaded gel was placed on 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. Later, 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. Cathode electrode was connected to the end where samples were loaded. Gels were subjected to between 148 V to 171 V and a current of between 51 mA to 57 mA for 3 to 4 hours.

2.5.1.4 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 10mm thick) was sliced horizontally into five 2mm thick slices and put into staining trays. Inner cut surfaces of gel slices were stained for specific enzyme activity. Appropriate staining mixtures (see Appendix 14) were prepared and poured onto gels which were then incubated in an oven at 35-40°C, until sites of activity reached optimum visibility. Stained gels were washed and fixed in appropriate gel fixing solution. (see Appendix 14).

2.6 Scoring of gels

Sites of enzyme activity indicated by horizontal bands on stained gels were examined visually. Number of loci per enzyme system were determined by inspection of band pattern and knowledge of protein involved. The band patterns were then recorded. Other parameters recorded were date, gel running time, running voltage and current, name of enzyme system and name of locality of samples. Loci and alleles were numbered by using Arabic numbers, counting from cathodal end of gel. Single bands were interpreted as homozygotes while vertically close double or triple bands (depending on whether enzyme system is monomeric or dimeric) were interpreted as heterozygotes. Gels with clear bands were saved to be photographed.

2.7 Analysis of electrophoretic data

2.7.1 Allele frequency

Assuming there are only two alleles, A_1 and A_2 which are codominant in a population which is at Hardy-Weinberg equilibrium, genotypes expected would be:

genotypes	A_1A_1	A_1A_2	A_2A_2	Total	
observed numbers	a	b	c	N	(1)

From (1)

N = a + b + c individuals which is equivalent to a random sample of 2N genes

Estimate of the proportions of A_1 allele is given by

$$p(A_1) = \frac{2a+b}{2N} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2a)$$

and estimate of proportions of A_2 allele is given by

$$q(A_2) = \frac{2c+b}{2N} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (2b)$$

so that $p + q = 1$

Generally under the assumptions stated above, allelic frequencies can be calculated by the formula.

$$\frac{2H_o + He}{2N}$$

where; H_o . number of homozygotes

H_e : number of heterozygotes

Sampling variance of the allele frequencies are given by

$$V_{(p)} = V_{(q)} = \frac{pq}{2N}$$

2.7.2 Heterozygosity (H_L)

Considering a population at Hardy-Weinberg equilibrium where several alleles are observed at an individual locus. Let x_{im} be the frequency of the m-th allele at a locus in the i-th subpopulation then gene identity in the total population (J_T) is computed as follows:

$$J_T = \sum_{m=1}^{r} \bar{x}_m^2 \qquad \text{(Nei and Chakravarti, 1977)} \quad . \qquad (1)$$

Where $\overline{x_m}$ is $\frac{\sum_{m=1}^{r} x_{im}}{s}$ and s number of subpopulations, and r is the number of alleles at the locus.

$$H_L = 1 \quad J_T \qquad (2)$$

Therefore, heterozygosity expected under Hardy-Weinberg equilibrium, irrespective of actual genotype frequencies is given by

$$H_L = 1 \quad \sum \overline{x}_m^2 \qquad (3)$$

2.7.3 Wright's fixation index F_{IS}

Assuming a population divided into s subpopulations in each of which Hardy-Weinberg equilibrium does not necessarily hold. Let allele A_i (i = 1,.. ,k) have frequency p_i and fixation index F_{isi} in the ith subpopulation.

Considering only homozygotes, frequency of homozygotes for the allele, may be given by (Nei, 1977)

$$P_{ik} = p^2_{ik} + F_{isik}\, p_{ik}(1-p_{ik}) \qquad (1)$$

Thus from formula (1)

$$F_{ISik} = \frac{P_{ik} - p^2_{ik}}{p_{ik}(1 - p_{ik})} \qquad (2)$$

For all the alleles in the ith subpopulation a unified fixation index can be written by modifying formula (2):

$$F_{IS_i} = \frac{\sum_{k=1}^{n} p_{ik}(1-p_{ik})F_i}{\sum_{k=1}^{n} p_{ik}(1-p_{ik})} \quad \ldots\ldots\ldots (2b)$$

$$= \frac{\sum_{k} (P_{ik}-p_{ik}^2)}{\sum_{k} p_{ik}(1-p_{ik})} \quad (2c)$$

From formula (2c)

$$F_{IS_i} = \frac{H_s - H_o}{H_s} \quad \ldots\ldots\ldots (3)$$

where $H_{Si} = 1 \quad \Sigma_k p^2_{ik}$ and $H_{oi} = 1 - \Sigma_k P_{ik}$

are expected and observed heterozygosities, respectively

Generally, formula (3) can be written as:

$$F_{IS} = \frac{H_s \quad H_o}{H_s}$$

$$= 1 \quad \frac{H_o}{H_s} \quad \ldots\ldots (4)$$

2.7.4 Frequency of cross pollination (C)

Assuming a large random mating population where in any generation two sorts of progeny exist, those produced by crossing and those produced by self-fertilization.

Let proportion of individuals produced by crossing be C, with zero coefficient of inbreeding, F, which is equivalent to Wright's

fixation index F_{IS} (Li,1954), and proportion produced by selfing be 1-C, and their coefficient of inbreeding $F_t = \frac{1}{2}(1+F_{t-1})$ (Falconer, 1989)

where F_t and F_{t-1} are coefficients of inbreeding for the first and second generations respectively

Average coefficient of inbreeding is given by

$$F_t = \frac{1}{2}(1 + F_{t-1}) \quad (1 \quad C)$$

For constant rate of crossing, average coefficient of inbreeding reaches an equilibrium level therefore:

$$F_t = F_{t-1}.$$

Therefore, rearrangement of equation (1) gives

$$F = \frac{1 \quad C}{1 + C} \qquad (2)$$

Since coefficient of inbreeding (F) is equivalent to Wright's degree of fixation F_{IS} formula (2) can be rewritten as

$$C = \frac{1 \quad F_{IS}}{1 + F_{IS}} \quad \text{(Wright,1952)} \qquad (3)$$

2.7.5. Coefficient of genetic differentiation (G_{st})

Suppose that s subpopulations of effective size N are derived from a foundation stock and in the subsequent generations no migration occurs among the subpopulations. Assuming that the initial gene frequencies are the same for all subpopulations and random mating occurs in each subpopulation; let x_{im} be the

frequency of the m-th allele at a locus in the i-th subpopulation. Therefore homozygosity or gene identity in this subpopulation is given by Nei and Chakravarti (1977) as:

$$Ji = \sum_{m=1}^{r} x^2_{im} \qquad \ldots\ldots\ldots \qquad (1)$$

where r is the number of alleles at the locus.

Average of gene identity over subpopulations is then

$$J_s = \sum_{i=1}^{s} J_i/S \qquad (2)$$

Therefore, heterozygosity or gene diversity within subpopulations is given by

$$H_s = 1 - J_s \qquad (2b)$$

Gene identity in the total population is calculated by using the mean gene frequencies in the total population and is given by:

$$J_T = \sum_{m=1}^{r} \bar{x}^2_m \qquad (3)$$

where $\bar{x}_m$ is $\sum_i x_{im}/S$

Therefore, gene diversity in the total population is given by:

$$H_T = 1 - J_T \qquad (4a)$$

where

$$J_T = \frac{\sum_{i=1}^{s} J_i + \sum_{i \neq j} J_{ij}}{S^2} \quad \ldots \quad \ldots\ldots \quad (4b)$$

where J_{ij} is gene identity between the ith and jth subpopulations.

From formulae (2b) and (4a), coefficient of gene differentiation is given by:

$$G_{ST} = \frac{H_T - H_S}{H_T}$$

The difference

$$D_{ST} = H_T \quad H_S \quad \ldots \quad (6)$$

gives the interpopulational gene diversity.

2.7.6 Estimation of gene flow (N_m).

By the application of G_{st}, Wright (1952) suggested the formula:

$$N_m = \frac{1 - G_{st}}{4G_{st}}$$

2.7.7 Genetic identity (I)

Considering the genetic populations, X and Y, with allelic frequencies X_A, X_B, , X_n and Y_A, Y_B, ..., Y_n respectively at a locus, where N is sample size in each population.

Genetic covariance of the populations of the i-th allele could then be estimated by the formula:

$$\Sigma x_i y_i/N = \frac{X_A Y_A + X_B Y_B \ldots + X_n Y_n}{N} \qquad \ldots\ldots\ldots\ldots \quad (1)$$

genetic variance of population X is given by

$$\Sigma x^2_i/N = \frac{X^2_A + X^2_B + \ldots X^2_n}{N} \qquad \ldots\ldots\ldots\ldots \quad (2)$$

genetic variance of population Y is given by

$$\Sigma y^2_i/N = \frac{Y^2_A + Y^2_B + \ldots Y^2_n}{N} \qquad (3)$$

Nei's coefficient of genetic identity which is analogous to correlation coefficient is then calculated from formulae (1), (2) and (3), (Ferguson, 1980) as:

$$I = \frac{\sum x_i y_i}{\sqrt{Var_x Var_y}} \qquad \ldots\ldots\ldots \quad (4)$$

or $$I = \frac{covariance}{\sqrt{Var_x Var_y}}$$

2.7.8 Genetic distance (D_m)

Genetic distance between pairs was estimated by:

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

where I is the identity between two taxonomic units.

Estimates of genetic identities and distances on the basis of allelic frequency estimates have been presented in a summary form

as matrix. However, a dendrogram has been constructed for visual display of estimated phenetic relationships between the taxonomic groups studied. To define groups of related taxonomic units clustering is used by the application of numerical techniques. The approach adapted in clustering here was described as the Unweighted Paired-Group Arithmetic Average (UPGM) clustering method (Sneath and Sokal, 1973) as presented by Ferguson (1980)

2.7.9 Log likelihood of χ^2 test (G-test)

Given a very large number of samples each of size n events containing r successes and (n-r) failures, occuring according to binomial distribution, with probabilities p and q=(1-p) respectively, then the probability of obtaining r successes could be estimated by the formula.

$$C(n,r) = \frac{n!}{r!(n-r)!} p^r q^{n-r}$$

Applying this to Hardy-Weinberg distribution, let f be observed number and $\hat{f}$ be expected number for a sample size of n. Let p be observed proportions and $\hat{p}$ expected proprtions on the basis of Hardy-Weinberg Law. Generally two quantities can be computed as follows:

(a) probability of observing sampled results on the basis that $\hat{P} = P$:

$$C(n, f_i) = \frac{n!}{f_1!f_2! \ldots f_a!} p_1^{f1} p_2^{f2} \ldots p_a^{fa} \qquad (2a)$$

(b) probability of observing sampled results on the basis that expected proportions is not equal to observed:

$$C(n, f_i) = \frac{n!}{f_1!f_2! \ldots f_a!} \hat{p}_1^{f1} \hat{p}_2^{f2} \ldots \hat{p}_a^{fa} \qquad (2b)$$

Ratio (L) of formula (2a) to formula (2b) is

$$L = \frac{\frac{n!}{f_1!f_2! \ldots f_a!} p_1^{f1} p_2^{f2} \ldots p_a^{fa}}{\frac{n!}{f_1!f_2! \ldots f_a!} \hat{p}_1^{f1} \hat{p}_2^{f2} \ldots \hat{p}_a^{fa}} \qquad (3)$$

$$= \prod \left(\frac{p_i}{\hat{p}_i}\right)^{f_i} \qquad (3b)$$

From Sokal and Rohlf (1969)

$$G = 2 \ln L \qquad (4a)$$

Substituting formula (3b) into formula (4a)

$$G = \sum f_i \ln\left(\frac{P_i}{\hat{P}_i}\right) \qquad (4b)$$

since $f_i = np_i$ and $\hat{f} = n\hat{p}_i$

formula (4b) becomes

$$G = 2\sum f_i \ln\left(\frac{P_i}{\hat{P}_i}\right) \qquad \text{(Sokal and Rohlf, 1969)}$$

i.e. $G = 2 \Sigma \text{ Obs } \ln (f_i/\hat{f}_i)$

or $G = 2\sum obs \ln(\frac{obs}{Exp})$

This is equivalent to χ^2 test for goodness-of-fit when expected values are small (Ferguson,1980)

In this situation number of independent genotypes is less than N-1 and the number of degrees of freedom can be calculated as

$\frac{1}{2}(n^2-n)$ (Ferguson, 1980)

where n is the number of alleles.

2.7.10 Spearman correlation and multiple regression

These statistical analyses were computed by using SPSS/PC Statistical Software package. Spearman correlation was computed between all variables while stepwise multiple regression was used to determine whether environmental factors are associated with frequencies of allozymes and morphological variation of agronomic traits.

CHAPTER THREE

RESULTS

3.1 Morphological (Meristic) characteristics

Distribution of morphological variability in studied cowpea accessions are presented in summary form in figure 3, (see Appendices 1-13).

3.1.1 Percentage seed germination

Mean values of percentage seed germination of cowpea accessions are presented in Appendix 1. Accession 87/142 a deciduous forest type gave the highest value of 95.0±0.05% and accession 87/30 a Guinea savannah type and 87/157 gave the lowest value of 70.0±0.10% each. Among Deciduous forest zone accessions, percentage seed germination ranged from 70.0±0.07% to 95.0±0.03% with a mean of 85.0±0.21% while, among Guinea savannah zone accessions the range was 70.0±0.07 to 80.0±0.06% with a mean of 75.0±0.25%, and among Sudan savannah zone accessions the range was 75.0±0.07 to 85.0± 0.06% with a mean of 81.0±0.23%.

3.1.2 Days to flowering

Mean values for days to flowering are shown in Appendix 2 Sudan savannah type 87/83 gave the lowest mean value of 33.0±0.67 days and deciduous forest type 87/142 gave the highest value of 53.0±0.67 days. Among Deciduous forest zone accessions, number of days to flowering ranged from 50.0±1.2 to 53.0±0.67 days with a mean of 51.3±0.72 days. Among Guinea savannah zone accessions the

range was 34.0±1.57 to 39.0±1.79 days with a mean of 36.3±1.19 days while, among Sudan savannah zone accessions the range was 33.0±0.67 to 45.0± 0.67 days with a mean of 41.0±3.27 days.

3.1.3 Percentage pod set per plant

Mean values and their standard errors for this character are presented in Appendix 3. Accession 87/139 gave the highest value of 56.1±0.11 and accession 87/83 gave the lowest value of 21.1±0.09%. Percentage pod set per plant ranged from 33.7±0.11 to 56.1±0.11% with a mean of 42.8±0.29% among accessions from Deciduous forest zone. Among Guinea savannah zone accessions the range was 21.6±0.09 to 39.0±0.11% with a mean of 29.1±0.26%, while among Sudan savannah zone accessions the range was 21.1±0.09 to 45.3±0.11% with a mean of 33.7±0.27%.

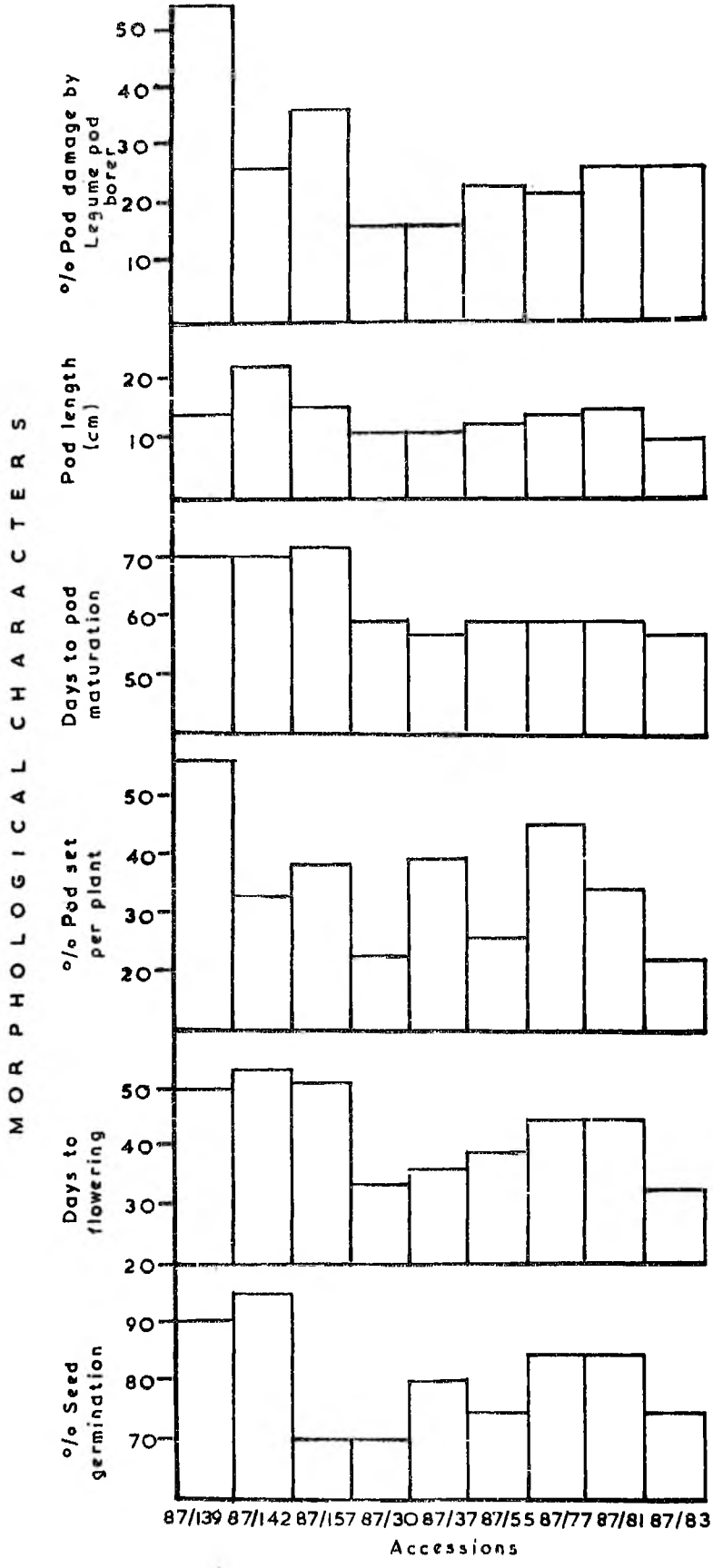
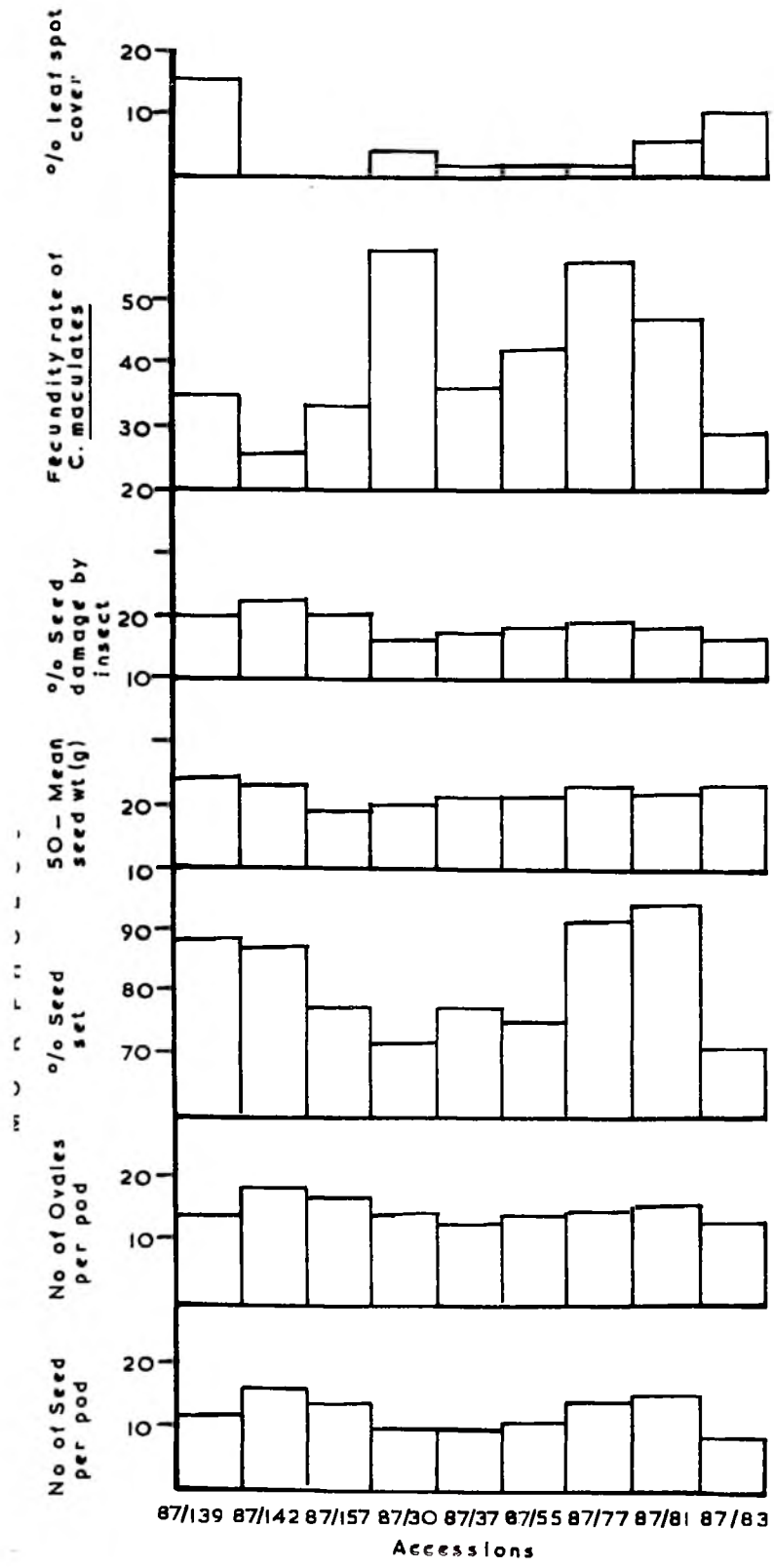


FIG. 3. Summary variability in 14 morphological characters in cowpea accessions.


% leaf spot cover
20
10
Fecundity rate of C. maculates
50
40
30
20
% Seed damage by insect
20
10
50 – Mean seed wt (g)
20
10
% Seed set
90
80
70
No of Ovules per pod
20
10
No of Seed per pod
20
10
87/139 87/142 87/157 87/30 87/37 87/55 87/77 87/81 87/83
Accessions

3.1.4 Pod maturation period

Mean values for the accessions are presented in Appendix 4. Accession 87/157 gave the highest value of 71.0±0.45 and accessions 87/37 and 87/83 gave the lowest value of 57.0±0.22 days. Among Deciduous forest zone accessions, pod maturation period ranged from 70.0±0.22 to 71.0±0.45 days with a mean of 70.3±0.27 days. Among Guinea savannah zone accessions the range was 57.0±0.22 to 59.0±0.89 days with a mean of 58.3±0.54 days while, among Sudan savannah zone accessions the range was 57.0±0.22 to 59.0± 0.0 days with a mean of 58.0±0.54 days. Pod maturation period was negatively and significantly correlated with mean sunshine.

3.1.5 Pod length

Mean values for the accessions are presented in Appendix 5. A highest value of 22.4±0.66 cm was recorded for accession 87/142 and accession 87/83 gave the lowest value of 10.93±0.37 cm.
Among Deciduous forest zone accessions, pod length ranged from 14.64±0.49 to 22.42±0.66 cm with a mean of 17.55±2.0 cm. Among Guinea savannah zone accessions the range was 11.8±0.22 to 12.58±0.89 cm with a mean of 12.12±0.19 cm, while among Sudan savannah zone accessions the range was 10.93±0.37 to 15.01± 0.29 cm with a mean of 13.51±1.06 cm.

3.1.6 Percentage pod damage by legume pod borer

Mean values of this character are presented in Appendix 6. Accession 87/139 gave the highest mean value of 54±0.11%, while

accessions 87/30 and 87/37 gave the lowest mean values of 16.0±0.10% and 16.0±0.08 respectivefully. Among Deciduous forest zone accessions, percentage pod damage by legume pod borer ranged from 26.0±0.40 to 54.0±0.95% with a mean of 38.0±0.28%, among Guinea savannah zone accessions the range was 16.0±0.03 to 23.0±0.03% with a mean of 18.3.0±0.22%, while among Sudan savannah zone accessions the range was 22.0±0.03 to 26.0± 0.04% with a mean of 24.7±0.25%

3.1.7 Number of seeds per pod

Mean values of number of seeds per pod of all accessions are presented in Appendix 7. The highest mean value of 16.0±0.39 was recorded for accession 87/142, while accession 87/83 gave the lowest mean value of 9.0±0.52. Number of seeds per pod set per plant ranged from 12.0±0.39 to 16.0±0.39 with a mean of 14.0±0.94 among accessions from Deciduous forest zone. Among Guinea savannah zone accessions the range was 10.0±0.26 to 11.0±0.52 with a mean of 10.3±0.27, while among Sudan savannah zone accessions the range was 9.0±0.52 to 15.0±0.39 with a mean of 12.7±1.52.

3.1.8 Number of ovules per pod

Mean values for all accessions of this character are presented in Appendix 8. The highest mean value of 18.0±0.39 was recorded for accession 87/142, while the lowest mean value of 13.0±0.39 was recorded for accessions 87/37 and 87/83. Among Deciduous forest zone accessions, number of ovules per pod ranged from 14.0±0.39 to 18.0±0.39 with a mean of 16.30±0.98, among Guinea savannah zone accessions the range was 13.0±0.26 to 14.0±0.13 with a mean of 13 7±0.27, while among Sudan savannah zone accessions the range was 13.0±0.39 to 15±0.39 with a mean of 14.7±0.72

3.1.9 Percentage seed set

Mean values for all accessions are shown in Appendix 9 Accession 87/83 gave a lowest mean value of 71.9±0.11%, while accession 87/81 gave the highest mean value of 94.0±0.01% Among Deciduous forest zone accessions, percentage seed set ranged from 77.1±0.01 to 88.3±0.01% with a mean of 84.4±0.21%, among Guinea savannah zone accessions the range was 72.0±0.01 to 77.1±0.05% with a mean of 74.9±0.25%, while among Sudan savannah zone accessions the range was 71.9±0.11 to 94.0±0.01% with a mean of 85.8±0.20%

3.1.10 50-seed mean weight

Mean values for all accessions are shown in Appendix 10. A highest mean value of 14.59±0.43 g was recorded for accession 87/139, while accession 87/157 recorded the lowest mean value of

9 76±0.09 g. Among Deciduous forest zone accessions, 50-mean seed weight ranged from 9.76±0.09 to 14.59±0.43 g with a mean of 12.76±1.23 g, among Guinea savannah zone accessions the range was 10.46±0.23 to 11.97±0.16 g with a mean of 11.39±0.38 g, while among Sudan savannah zone accessions the range was 12.54±0 47 to 13.85±0.31 g with a mean of 13.19±0.31 g.

3.1.11 Percentage seed damage by insect

Mean values for all accessions are shown in Appendix 11. Accession 87/142 gave the highest mean value of 12.0±0.01%, while accessions 87/30 and 87/83 gave the lowest mean value of 6.0±0.01% Among Deciduous forest zone accessions, percentage seed damage by insect ranged from 10.0±0.01 to 12.0±0.01% with a mean of 10.7±0.18%, among Guinea savannah zone accessions the range was 6.0±0.00 to 8.0±0.01% with a mean of 7.0±0.26%, while among Sudan. savannah zone accessions the range was 6.0±0.01 to 9.0±0.00% with a mean of 7.7±0.0.24%.

3.1.12 Fecundity rate of *Callosobruchus maculatus* infestation of seed

Mean values are given in Appendix 12. A highest mean value of 58.2±0.11% was recorded for accession 87/30, while accession 87/142 gave the lowest mean value of 26.0±0.06%. Among Deciduous forest zone accessions, fecundity rate of *C. maculatus* infestation of seed ranged from 26.0±0.06 to 35.4±0.06% with a mean of 31.5±0.27%, among Guinea savannah zone accessions the range was 36±0.061 to 42.3±0.063% with a mean of 45.5±0.29%, while among Sudan savannah zone accessions the range was 34.9±0.06 to 56.4± 0.06% with a mean of 46.3±0.28%.

3.1.13 Percentage leaf spot cover

Mean values for percentage leaf spots cover are presented in Appendix 13. Deciduous forest type 87/139 gave the highest value of 16.6±0.05%, while accession 87/142 gave the lowest value of 0.01±0.00% Among Deciduous forest zone accessions, percentage leaf spots cover ranged from 0.01±0.00 to 16.6±0.05% with a mean of 5.55±0.29%, among Guinea savannah zone accessions the range was 2.2±0.02 to 4.8±0.03% with a mean of 3.07±0.27%, while among Sudan savannah zone accessions the range was 2.0±0.02 to 10.9± 0.04% with a mean of 5.98±0.28%

3.2 Ecogeographical(environmental) Data.

Ecogeographical data for collection sites of cowpea accessions were collected from Ghana Meteorological Services Department and

are presented in Table 2.

ECOGEOGRAPHICAL DATA FOR COLLECTION SITES FOR COWPEA ACCESSIONS

ACCESSION NO.	LOCALITY	LONG.	LAT.	MEAN TEMP°C MAX.	MEAN TEMP°C MIN.	MONTHLY RAINFALL (MM)	NO.OF RAINY DAYS	RELATIVE HUMIDITY 1500Hrs	RELATIVE HUMIDITY 0600Hrs	MEAN SUNSHINE	VAPOUR PRESSURE 1500Hrs	VAPOUR PRESSURE 0600Hrs	MEAN MONTHLY TOTAL CLOUD (Knot)
87/139	Akora Darko	00°24'W	06°22'N	31.3	21.7	112	11	66	96	5.5	27.5	25.8	5.5
87/142	Akora Darko	00°24'W	06°22'N	31.3	21.7	112	11	66	96	5.5	27.5	25.8	5.5
87/157	Abene	00°34'W	06°38'N	28.1	18.4	110	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	45.4	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	72.8	7.9	20.0	20.5	3.9

3.3 Starch Gel Electrophoretic Data (Genetic)

3.3.1 Proteins scored

Nine soluble proteins involving 22 loci were assayed from cowpea leaf crude etracts.The proteins (all enzymes), their code numbers and loci are presented in Table 3. Figure 4 shows their allozymic expressions.

3.3.1.1 Adenlylate kinase (AK)

Two loci were resolved and designated AK1* and AK2* Four alleles were scored for AK1*, while three alleles were scored for AK2* (fig.4)

3.3.1.2 Fumarate hydratase (FH)

One locus was scored and designated as FH1* A total of three alleles were scored at its locus(fig.4)

3.3.1.3 Hexokinase (HK)

Two loci were scored and designated as HK1* and HK2*. A total of four and three alleles were scored at HK1* and HK2* loci respectively (fig.4)

TABLE 3

PROTEIN ENZYMES STUDIED, THEIR CODE NUMBERS AND NUMBER OF LOCI SCORED

NAME OF ENZYME	E.C. NUMBER	ABBREVIATION	NUMBER OF LOCI SCORED
Adenylate kinase	2.7.4.3	AK	2
Fumarate hydratase	4.2.1.2	FH	1
Hexokinase	2.7.1.1	HK	2
Isocitrate dehydrogenase	1.1.1.42	IDHP	2
Malate dehydrogenase	1.1.137	MDH	4
Malic Enzyme ($NADP^+$)	1.1.1.40	MEP	2
6-Phosphogluconate dehydrogenase	1.1.1.44	PGDH	3
Phosphoglucoisomerase	5.3.1.9	PGI	4
Phosphoglucomutase	5.4.2.20	PGM	2

3.3.1.4 Isocitrate dehydrogenase (IDHP)

Plate 1 shows a zymogram of isocitrate dehydrogenase. Two loci were scored in all accessions. The most cathodal locus designated IDHP1* had six alleles, while the anodal locus IDHP2* had five different alleles (see fig.4)

3.3.1.5 Malate dehydrogenase (MDH)

A sample of malate dehydrogenase allozymes is shown in Plate 1. Four loci were scored in all accessions and designated MDH1*, MDH2*, MDH3*, and MDH4* The loci showed a six, nine, seven and seven alleles respectively (fig.4).

3.3.1.6 Malic enzyme (MEP)

Two loci were scored and designated ME1* and ME2* (fig.4) A total of seven different alleles each were resolved at each locus.

3.3.1.7 6-phosphoglucose dehydrogenase (PGDH)

A sample of 6-phosphoglucose dehydrogenase zymogram is shown in Plate 1. Three loci were scored, and designated PGDH1*, PGDH2* and PGDH3* (fig.4) A total of five, eight and five alleles were resolved at these three loci respectively

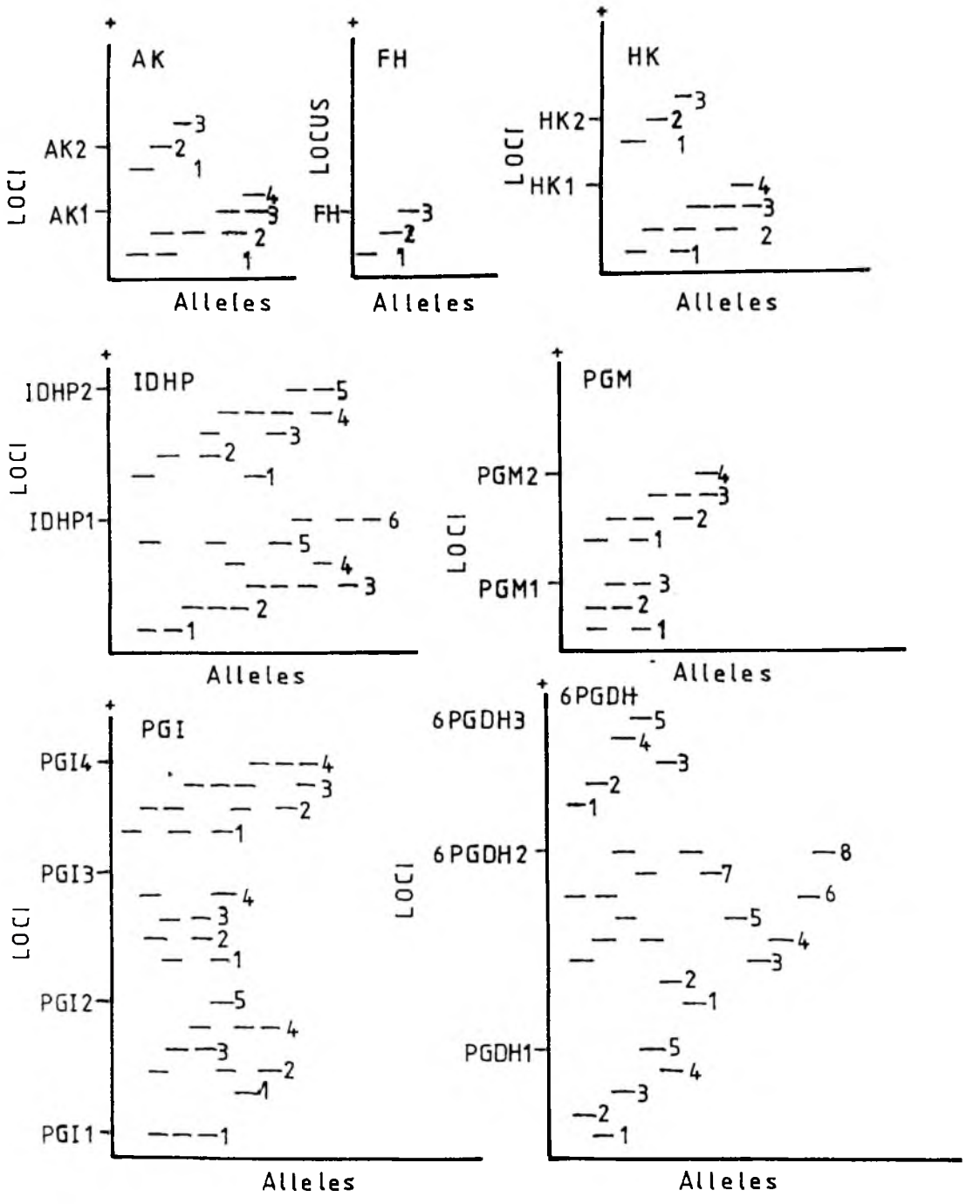
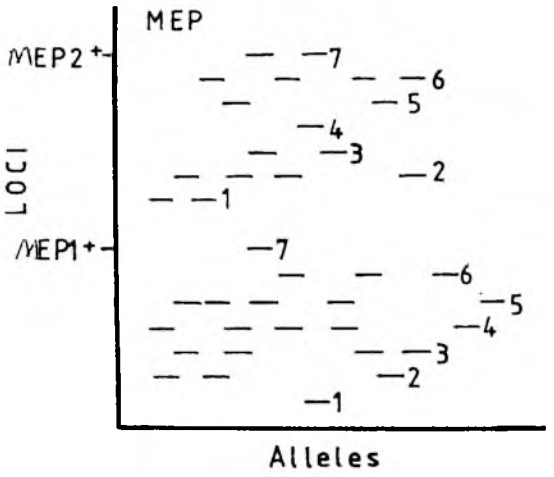


Fig. 4 ALLOZYME EXPRESSION AT 22 LOCI IN COWPEA ACCESSIONS


MEP
MEP2+
MEP1+
LOCI
Alleles

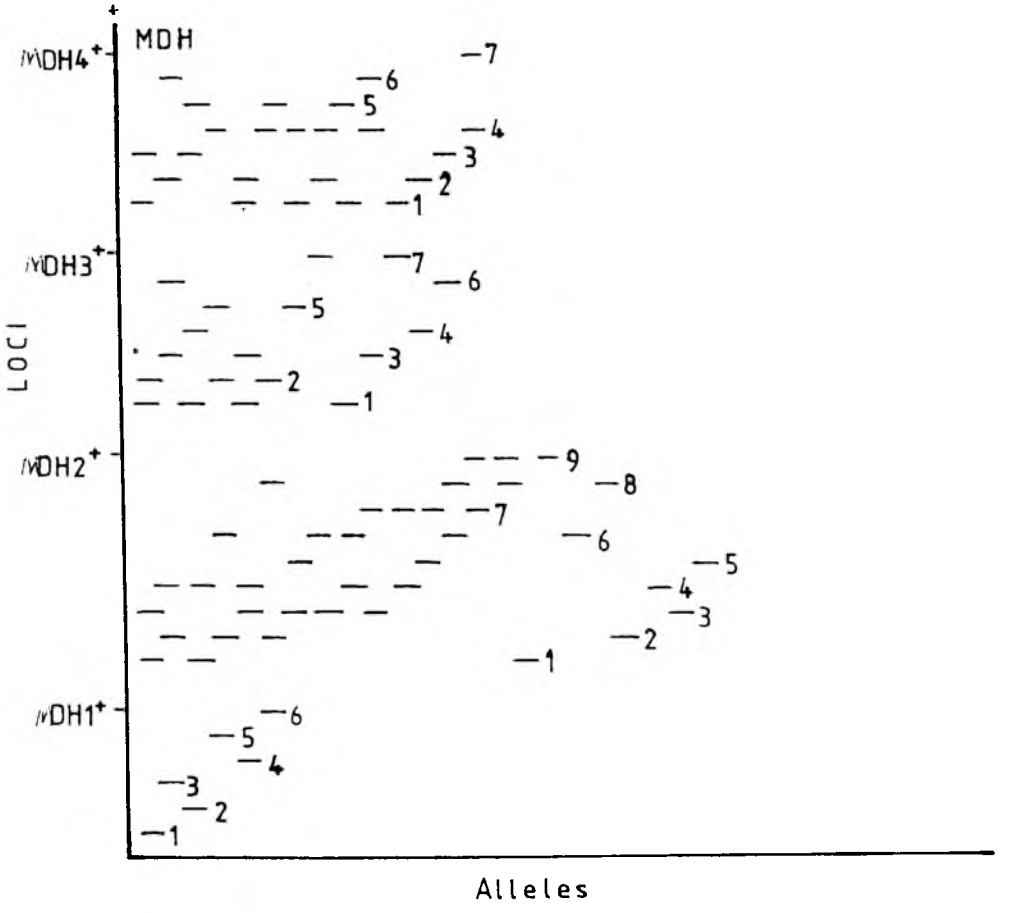

MDH
MDH4+
MDH3+
MDH2+
MDH1+
LOCI
Alleles

Fig. 4. Cont'd

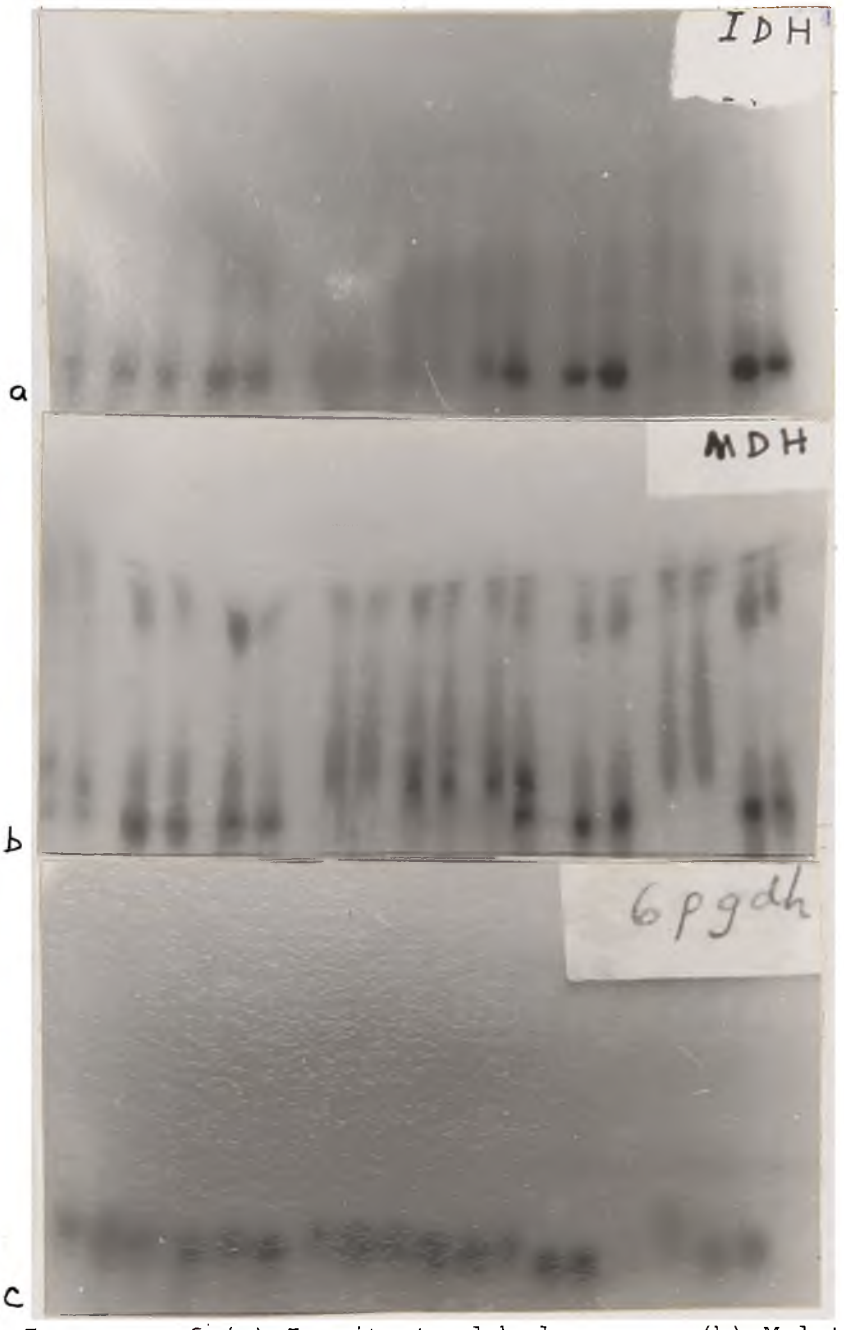


Plate 1. Zymogram of (a) Isocitrate dehydrogenase (b) Malate dehydrogenase and (c) 6-phosphoglucose dehydrogenase

3.3.1.8 Phosphoglucoisomerase (PGI)

Four loci were scored, and designated PGI1*, PGI2*, PGI3* and PGI4* (fig.4) A total of one, five, four and four alleles were resolved at these loci respectively

3.3.1.9 Phosphoglucomutase (PGM)

Two loci were scored and designated PGM1* and PGM2* A total of three and four alleles were scored at each locus respectively (fig.4)

3.4 Genetic Variability Cowpea Accessions

3.4.1 Mean number of alleles per locus

Mean number of alleles per locus for cowpea accessions are shown in Table 4. A total of 110 different alleles were distributed among the 22 loci for an average of 5.00 alleles per locus.

3.4.2 Discriminating loci

Table 5 shows taxonomically important loci among pairs of cowpea accessions from the same agroecological zones. Discriminating loci are loci at which an allele was absent in one of pairs of accessions. Among Deciduous forest accessions, 87/139 was discriminated from 87/142 and 87/157 by seven different loci, while accessions 87/142 and 87/157 were different at eight different loci. Among the Guinea savannah

accessions, accession 87/30 could be discriminated from accessions 87/37 and 87/55 at seven different loci, while accessions 87/37 and 87/55 were different at six different loci. Among the Sudan Savannah accessions, accession 87/77 was different from accessions 87/81 and 87/83 at ten and eight different loci respectively, while accessions 87/81 and 87/83 were different at six different loci.

3.4.3 Genotype frequency

Frequency of genotype frequencies at enzyme loci studied in the different cowpea accessions are shown in Appendix 15.

Table 4

Mean number of alleles per locus and percentage polymorphic loci in cowpea accessions for 22 loci

Loci	Number of alleles per locus	% polymorphic loci
AK1*	4	100
Ak2*	3	100
FH1*	3	100
HK1*	4	100
HK2*	3	100
IDHP1*	6	100
IDHP2*	5	100
MDH1*	6	100
MDH2*	9	100
MDH3*	7	100
MDH4*	7	100
MEP1*	7	100
MEP2*	7	100
PGDH1*	5	100
PGDH2*	8	100
PGDH3*	5	100
PGI1*	1	1
PGI2*	5	100
PGI3*	4	100
PGI4*	4	100
PGM1*	3	100
PGM2*	4	100
Mean	5.00 + o.47	95.5 + 0.0

TABLE 5

Discriminating loci among pairs of cowpea accessions (No. of loci screened 22)

Between accessions	Discriminating loci
87/139 and 87/142	AK2*;MDH2*; MEP1*, MDH1* PGDH2* PGDH3*; PGI2*
87/139 and 87/157	IDHP1*; MDH1*, MDH2*; MEP1*; MEP2*; PGDH2*; PGI2*
87/139 and 87/30	IDHP2*; MDH1*, MDH2*; PGDH2*; PGM2*; PGDH3*; PGI1*
87/139 and 87/37	1DHP2*; MDH2*; MDH3*; MDH4* PGDH1*; PGDH2*; PGDH3*; PGI1* PGM2*
87/139 and 87/55	1DHP2*, MDH1*; MDH2*; PGDH1*; PGDH2*; PGDH3*; PGI1*; PGM2*
87/139 and 87/77	HK1*; HK2*; MDH2*; MEP1*; MDH1* PGDH2*; PGDH3*; PGI1*; PGM2*
87/139 and 87/81	HK1*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*; PGI1*; PGM2
87/139 and 87/83	AK1*; FH1*; HK1*; 1DHP1*; 1DHP1*; 1DHP2*; MDH1*, MDH2*; MDH3*; MDH4*; MEP2*; PGDH2*; PGDH3*; PGI1*; PGM2*
87/142 and 87/157	AK2*; 1DHP1*; 1DHP2*; MDH1*; MDH3*; MEP2*; PGDH2*; PGDH3*
87/142 and 87/30	AK2*; 1DHP2*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH2*; PGDH3*; PGM2*
87/142 and 87/37	AK2*; 1DHP1*; 1DHP2*; MDH1*; MDH2*; MDH3*; MDH4*; MEP1*; PGDH1*; PGDH3*; PGM2*
87/142 and 87/55	AK2*; 1DHP2*; MDH1*; MDH2*; MDH2*; MDH3*; MEP1*; PGDH1*; PGDH2*; PGDH3*; PGM2*

Table 5 cont'd

87/142 and 87/77	AK1*; AK2*; HK1*; HK2*; 1DHP2*; MDH1*; MDH2*; MDH3*; MEP1*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/142 and 87/81	AK2*; FH1*; HK1*; 1DHP2*; MDH1*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2* PGDH1*; PGDH3*; PGM2*
87/142 and 87/83	AK2*; FH1*; HK1*; 1DHP2*; MDH1*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH2*; PGDH3*; PGM2*
87/157 and 87/30	1DHP1*; 1DHP2*; MDH1*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH2*; PGDH3*; PGM2*
87/157 and 87/37	1DHP1*; 1DHP2*; MDH1*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*; PGM2*.
87/157 and 87/55	1DHP1*; 1DHP2*; MDH1*; MDH2*; MDH3*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/157 and 87/77	AK1*; HK1*; HK2*; 1DHP1*; MDH1*; MDH2*; MDH3*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/157 and 87/81	FH1*; HK1*; 1DHP1*; 1DHP2*;MDH1*;MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*;PGDH3*; PGM2*.
87/157 and 87/83	FH1*; HK1*; 1DHP1*; 1DHP2*;MDH1*;MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH2*; PGDH3*; PGM2*
87/30 and 87/37	1DHP1*; MDH1*; MDH2*; MDH4*; MEP2*; PGDH1*;PGDH2*;
87/30 and 87/55	MDH1*; MDH2*; MDH3*; MDH4*; MEP2*; PGDH1*;PGDH2*;
87/30 and 87/77	AK1*; HK1*; HK2*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGM2*

Table 5 cont,d

87/30 and 87/81	FH1*; HK1*; 1DHP2*; MDH2*; MDH3*; MEP1*;MEP2*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/30 and 87/83	FH1*; HK1*; 1DHP1*;1DHP2*; MDH2*; MDH3*;MEP1*; MDH4*; MEP1*; PGDH2*; PGDH3*;PGM2*
87/37 and 87/55	IDHP1*;MDH1*; MDH2*; MDH3*; MDH4*; PGDH2*;
87/37 and 87/77	HK1*;1DHP2*; MDH1*; MDH2*; MDH3*; MDH4*;MEP1*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/37 and 87/81	FH1*; HK1*; 1DHP2*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*; PGM2*.
87/37 and 87/83	FH1*; HK1*; 1DHP1*;1DHP2*; MDH1*; MDH2*;MDH3*; MDH3*; MDH4*; MEP1*; MEP2*;PGDH1*; PGDH2*; PGDH3*; PGM2*
87/55 and 87/77	HK1*;1DHP2*; MDH1*; MDH2*; MEP1*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/55 and 87/81	FH1*; HK1*; 1DHP2*; MDH1*; MDH2*; MDH3*;MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*;PGDH3*; PGM2*
87/55 and 87/83	FH1*; HK1*; 1DHP1*;1DHP2*; MDH1*; MDH2*;MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*; PGM2*
87/77 and 87/81	FH1*; 1DHP2*; MDH2*; MDH3*; MDH4*; MEP1*; MEP2*; PGDH1*; PGDH2*; PGDH3*
87/77 and 87/83	FH1*; 1DHP2*; MDH2*; MDH3*; MDH4*; MEP1*; PGDH1*; PGDH2*
87/81 and 87/83	MDH2*; MDH4*; MEP2*; PGDH1*; PGDH2*; PGDH3*

3.4.4 Genetic polymorphism

Results on genetic polymorphism are shown in Table 4. Locus PGI1* was monomorphic, while 21 loci were polymorphic. Mean percentage polymorphic loci was 95.5%.

3.4.5 Allelic frequency and heterozygosity

Allele frequencies, expected and observed heterozygosities for the 22 different enzyme loci of cowpea accessions are shown in Table 6. Mean expected heterozygosity ranged between 0.549 to 0.599 with a mean of 0.561±0.005. Accession 87/55 scored the highest value of 0.599, while accession 87/149 scored the lowest of 0.549 Mean observed heterozygosity ranged from 0.240 to 0.303 with a mean of 0.264±0.007

3.4.6 Hardy-Weinberg analysis, Wright's fixation index (F_{IS}) and frequency of cross-pollination.

Deviations from Hardy-Weinberg equilibria are shown in Table 7 Wright's fixation index and frequency for cross-pollination are presented in Table 8. Between 16 to 19 loci deviated significantly from Hardy-Weinberg equilibrum law. Wright's fixation index ranged from -0.658 to 1.000, with an average value of 0.573±0.096 Frequency of cross-pollination ranged from 0.00 to 0.949, with an average value of 0.244±0.064.

3.5 Genetic Diversity of Cowpea Accessions

3.5.1 Genetic Differentiation within Cowpea accessions.

Statistics for genetic differentiation within cowpea accessions are shown in Table 8. Relative genetic differentiation ranged from 0.005(PGM1*) to 1.000(PGI1*)
with a mean value of 0.134*0.042.

3.5.2 Meristic distance and cluster analysis (dendrogram)

Meristic distance between accessions are shown in Table 9, and overall relationship is shown by a dendrogram in Fig.5 Among Deciduous forest accessions meristic distance ranged from 0.063 to 0.129, while among Guinea savannah zone accessions the range was 0.047 to 0.197. Among Sudan savannah zone accessions the meristic distance ranged from 0.010 to 0.057 All the formed one main cluster.

3.5.3 Genetic distance and cluster analysis (dendrogram)

Genetic distance between accessions are shown in Table 10, and overall relationship is shown by a dendrogram in Fig.6. The genetic distance among Deciduous forest zone accessions ranged from 0.130 to 0.167, while among Guinea savannah the range was 0.101 to 0.190. Among Sudan savannah zone the genetic dsistance ranged from 0.163 to 0.285 Accessions from Guinea savannah and Sudan savannah zones formed one cluster, while Deciduous forest accessions formed a different cluster.

TABLE 6

Allele frequencies, expected heterozygosity (H_1) and observed heterozygosity (H_{obs}) values of electrophoretically assessed accessions. Number of individuals scored is given in brackets.

COWPEA ACCESSION		87/139	87/142	87/157	87/30	87/37	87/55	87/77	87/81	87/83
AGROECOLOGICAL ZONE		DECIDUOUS FOREST			GUINEA SAVANNAH			SUDAN SAVANNAH		
LOCUS	ALLELE									
AK1*-	4	.060	.033	.054	.043	.060	.068	.000	.063	.048
	3	.080	.033	.054	.043	.060	.068	.048	.146	.161
	2	.400	.433	.357	.392	.320	.364	.387	.458	.419
	1	.460	.500	.535	.522	.560	.500	.565	.333	.371
	H_1	.618	.560	.580	.570	.577	.608	.529	.654	.659
	Hobs	.600	.533	.536	.522	.440	.591	.516	.542	.419
		(25)	(30)	(28)	(23)	(25)	(22)	(31)	(24)	(31)
AK2*-	3	.000	.130	.000	.000	.000	.000	.000	.000	.000
	2	.200	.070	.130	.130	.200	.130	.070	.310	.180
	1	.800	.800	.870	.870	.800	.870	.930	.690	.820
	HI	.320	.338	.226	.226	.320	.226	.130	.428	.095
	Hobs	.000	.000	.000	.000	.000	.000	.000	.000	.000
		(15)	(15)	(15)	(15)	(15)	(15)	(21)	(16)	(17)
FH1*-	3	.150	.136	.050	.100	.166	.318	.095	.670	.440
	2	.300	.091	.150	.850	.792	.636	.524	.330	.560
	1	.550	.773	.800	.050	.042	.046	.381	.000	.000
	HI	.585	.376	.335	.265	.343	.492	.571	.442	.493
	Hobs	.000	.000	.000	.000	.000	.000	.000	.000	.000
		(20)	(22)	(20)	(20)	(24)	(22)	(21)	(21)	(25)
HK1*-	4	.000	.000	.000	.000	.000	.000	.057	.065	.107
	3	.220	.207	.060	.119	.109	.071	.135	.174	.196
	2	.460	.483	.540	.500	.391	.619	.423	.370	.322
	1	.320	.310	.400	.381	.500	.310	.385	.391	.375
	HI	.638	.628	.545	.591	.585	.516	.651	.676	.706
	Hobs	.600	.690	.600	.524	.435	.476	.654	.696	.714
		(25)	(29)	(25)	(21)	(23)	(21)	(26)	(23)	(28)

Table 6 cont'd

COWPEA ACCESSION		87/139	87/142	87/157
AGROECOLOGICAL ZONE		DECIDUOUS FOREST		
LOCUS	ALLELE			
HK2*-	3	.187	.105	.133
	2	.250	.105	.267
	1	.563	.709	.600
	HI	.586	.354	.551
	Hobs	.000	.000	.000
		(16)	(19)	(15)
IDHP2*-	6	.000	.000	.025
	5	.102	.198	.350
	4	.159	.271	.088
	3	.693	.500	.513
	2	.045	.031	.012
	1	.000	.000	.012
	HI	.482	.636	.606
	Hobs	.023	.042	.100
		(44)	(48)	(40)
IDHP2*-	5	.095	.024	.000
	4	.351	.095	.450
	3	.000	.000	.050
	2	.500	.643	.300
	1	.048	.238	.200
	HI	.611	.520	.665
	Hobs	.333	.048	.400
		(21)	(21)	(20)
MDH1*-	6	.033	.000	.048
	5	.000	.095	.143
	4	.033	.048	.047
	3	.800	.429	.524
	2	.034	.190	.000
	1	.100	.233	.238
	HI	.347	.712	.644
	Hobs	.000	.000	.000
		(30)	(21)	(21)

87/30 GUINEA	87/37 SAVANNAH	87/55 SAVANNAH	87/77 SUDAN	87/81 SAVANNAH	87/83 SAVANNAH
.118	.125	.158	.000	.263	.118
.235	.125	.105	.125	.211	.235
.647	.750	.737	.875	.526	.647
.512	.406	.421	.219	.610	.512
.000	.000	.000	.000	.000	.000
(17)	(16)	(19)	(16)	(19)	(17)
.000	.000	.000	.000	.000	.043
.208	.314	.526	.271	.394	.181
.283	.372	.211	.143	.030	.010
.453	.314	.237	.557	.485	.702
.056	.000	.026	.029	.091	.064
.000	.000	.000	.000	.000	.000
.668	.664	.622	.595	.060	.468
.057	.000	.000	.028	.121	.277
(53)	(35)	(38)	(35)	(33)	(47)
.000	.000	.080	.048	.050	.050
.275	.125	.350	.214	.200	.275
.100	.150	.150	.119	.100	.100
.575	.575	.375	.548	.650	.575
.050	.150	.125	.071	.000	.000
.581	.609	.699	.632	.525	.581
.050	.050	.100	.190	.100	.250
(20)	(20)	(20)	(21)	(20)	(20)
.028	.047	.130	.028	.071	.030
.057	.048	.130	.200	.143	.200
.000	.000	.000	.000	.000	.000
.743	.667	.435	.629	.500	.600
.086	.048	.000	.029	.036	.130
.086	.190	.305	.114	.250	.030
.429	.512	.684	.550	.661	.581
.000	.000	.000	.000	.000	.000
(35)	(21)	(23)	(35)	(28)	(30)

TAble 6 cont'd

COWPEA ACCESSION		87/139	87/142	87/157
AGROECOLOGICAL ZONE		DECIDUOUS		FOREST
LOCUS	ALLELE			
MDH2*-	9	.000	.000	.000
	8	.000	.000	.000
	7	.161	.114	.046
	6	.129	.045	.094
	5	.129	.136	.172
	4	.226	.205	.234
	3	.065	.000	.141
	2	.161	.182	.141
	1	.129	.318	.172
	HI	.843	.790	.835
	Hobs	.323	.409	.406
		(31)	(22)	(32)
MDH3*-	7	.000	.000	.000
	6	.017	.022	.000
	5	.069	.043	.120
	4	.138	.000	.080
	3	.190	.196	.320
	2	.448	.217	.220
	1	.138	.522	.260
	HI	.720	.640	.761
	Hobs	.034	.217	.240
		(29)	(23)	(25)
MDH4*-	7	.000	.000	.000
	6	.000	.000	.000
	5	.029	.088	.094
	4	.029	.029	.031
	3	.059	.177	.156
	2	.324	.177	.156
	1	.559	.529	.563
	HI	.577	.649	.625
	Hobs	.235	.588	.500
		(17)	(17)	(16)

87/30	87/37	87/55	87/77	87/81	87/83
GUINEA	SAVANNAH		SUDAN	SAVANNAH	
.020	.000	.000	.000	.040	.000
.080	.025	.250	.014	.160	.000
.100	.125	.045	.054	.120	.031
.200	.125	.159	.243	.100	.088
.180	.175	.045	.081	.000	.088
.100	.125	.114	.378	.200	.294
.180	.425	.228	.027	.120	.132
.140	.000	.159	.122	.140	.250
.000	.000	.000	.081	.000	.117
.849	.741	.818	.766	.860	.804
.480	.350	.273	.568	.720	.735
(25)	(20)	(22)	(37)	(25)	(34)
.000	.000	.000	.000	.095	.048
.021	.087	.048	1.000	.048	.048
.375	.130	.095	.071	.048	.095
.083	.087	.143	.014	.095	.095
.271	.522	.286	.357	.333	.286
.250	.174	.190	.329	.286	.380
.000	.000	.238	.129	.095	.048
.716	.665	.794	.732	.776	.749
.125	.000	.000	.314	.000	.095
(24)	(23)	(21)	(35)	(21)	(21)
.000	.000	.000	.000	.000	.031
.133	.000	.000	.000	.088	.000
.033	.000	.167	.029	.088	.000
.234	.000	.027	.088	.118	.250
.133	.177	.278	.177	.147	.313
.367	.600	.389	.324	.412	.344
.100	.233	.139	.382	.147	.062
.764	.554	.723	.709	.758	.716
.733	.467	.556	.647	.765	.500
(15)	(15)	(18)	(17)	(17)	(16)

Table 6 cont'd

COWPEA ACCESSION		87/139	87/139	87/157
AGROECOLOGICAL ZONE		DECIDUOUS		FOREST
LOCUS	ALLELE			
MEP1*-	7	.000	.000	.000
	6	.024	.000	.000
	5	.098	.184	.216
	4	.110	.071	.243
	3	.622	.612	.365
	2	.146	.092	.041
	1	.000	.041	.135
	HI	.570	.576	.741
	Hobs	.049	.222	.054
		(41)	(49)	(37)
MEP2*-	7	.000	.000	.000
	6	.155	.048	.083
	5	.026	.048	.000
	4	.000	.000	.000
	3	.616	.333	.500
	2	.123	.476	.271
	1	.115	.095	.146
	HI	.577	.649	.648
	Hobs	.282	.190	.167
		(39)	(21)	(24)
PGDH1*-	5	.000	.000	.000
	4	.029	.077	.108
	3	.171	.038	.081
	2	.657	.693	.676
	1	.143	.192	.135
	HI	.518	.476	.507
	Hobs	.000	.000	.000
		(35)	(26)	(37)

87/30	87/37	87/55	87/77	87/81	87/83
GUINEA		SAVANNAH		SUDAN	SAVANNAH
.000	.000	.000	.015	.000	.000
.024	.077	.071	.059	.062	.087
.366	.442	.322	.162	.146	.141
.098	.077	.089	.044	.000	.000
.378	.327	.321	.529	.667	.381
.134	.077	.197	.044	.125	.391
.000	.000	.000	.147	.000	.000
.695	.680	.742	.665	.514	.675
.146	.192	.179	.118	.083	.239
(41)	(26)	(28)	(34)	(24)	(46)
.000	.000	.000	.000	.060	.000
.036	.045	.048	.053	.060	.037
.036	.090	.048	.053	.220	.315
.000	.000	.000	.000	.200	.000
.750	.455	.333	.157	.380	.148
.178	.205	.523	.526	.260	.500
.000	.205	.048	.211	.000	.000
.403	.699	.609	.649	.692	.628
.071	.091	.095	.105	.200	.111
(28)	(22)	(21)	(19)	(25)	(27)
.000	.000	.000	.000	.057	.000
.114	.364	.522	.323	.286	.181
.057	.000	.000	.000	.028	.016
.743	.500	.358	.548	.543	.754
.086	.136	.130	.129	.086	.049
.424	.599	.582	.579	.612	.396
.000	.000	.000	.000	.000	.000
(35)	(22)	(23)	(21)	(35)	(61)

Table 6 cont'd

COWPEA ACCESSIONS		87/139	87/142	87/157
AGROECOLOGICAL ZONE		DECIDUOUS		FOREST
LOCUS	ALLELE			
PGDH2*-	8	.049	.000	.000
	7	.012	.026	.029
	6	.269	.053	.176
	5	.317	.342	.147
	4	.085	.211	.353
	3	.171	.105	.059
	2	.073	.263	.118
	1	.024	.000	.118
	HI	.782	.755	.791
	Hobs	.073	.000	.059
		(41)	(38)	(34)
PGDH3*-	5	.406	.333	.120
	4	.000	.000	.000
	3	.125	.000	.080
	2	.375	.630	.440
	1	.094	.037	.360
	HI	.670	.491	.656
	Hobs	.000	.000	.000
		(32)	(27)	(25)
PGI1*-	1	1.000	1.000	1.000
	HI	.000	.000	.000
	Hobs	.000	.000	.000
		(29)	(28)	(28)
PGI2*-	5	.015	.017	.043
	4	.091	.150	.100
	3	.030	.300	.043
	2	.788	.533	.814
	1	.076	.000	.000
	HI	.364	.603	.324
	Hobs	.212	.333	.286
		(33)	(30)	(35)

87/30	87/37	87/55	87/77	87/81	87/83
	GUINEA	SAVANNAH		SUDAN	SAVANNAH
.000	.000	.115	.032	.000	.020
.000	.043	.038	.000	.138	.041
.189	.174	.135	.306	.155	.204
.271	.218	.077	.226	.103	.102
.351	.261	.308	.161	.311	.245
.027	.130	.173	.177	.190	.306
.108	.174	.154	.000	.000	.000
.054	.000	.000	.097	.100	.082
.752	.805	.813	.788	.803	.786
.000	.087	.115	.032	.172	.163
(37)	(23)	(26)	(31)	(29)	(49)
.031	.091	.116	.000	.000	.000
.188	.091	.077	.200	.000	.032
.031	.091	.269	.040	.045	.097
.375	.545	.269	.400	.728	.548
.375	.182	.269	.360	.227	.323
.681	.645	.764	.669	.416	.585
.000	.000	.000	.000	.000	.000
(32)	(22)	(26)	(25)	(22)	(31)
1.000	1.000	1.000	1.000	1.000	1.000
.000	.000	.000	.000	.000	.000
.000	.000	.000	.000	.000	.000
(42)	(34)	(31)	(35)	(38)	(30)
.064	.017	.017	.020	.017	.011
.026	.067	.138	.019	.019	.012
.269	.133	.207	.071	.071	.048
.641	.783	.638	.893	.893	.929
.000	.000	.000	.000	.000	.000
.512	.364	.531	.202	.202	.134
.179	.167	.310	.071	.071	.048
(39)	(30)	(29)	(28)	(28)	(42)

Table 6 cont'd

COWPEA ACCESSIONS		87/139	87/142	87/157
AGROECOLOGICAL ZONE		DECIDUOUS FOREST		
LOCUS	ALLELE			
PGI3*-	4	.106	.033	.071
	3	.394	.467	.429
	2	.121	.267	.300
	1	.379	.233	.200
	HI	.675	.655	.681
	Hobs	1.000	1.000	1.000
		(33)	(30)	(35)
PGI4*-	4	.119	.091	.050
	3	.143	.182	.267
	2	.548	.432	.550
	1	.190	.295	.138
	HI	.628	.685	.606
	Hobs	.571	.636	.700
		(21)	(22)	(30)
PGM1*-	3	.125	.120	.115
	2	.375	.380	.385
	1	.500	.500	.500
	HI	.594	.591	.589
	Hobs	1.000	1.000	1.000
		(24)	(25)	(26)
PGM2*-	4	.000	.000	.000
	3	.000	.000	.000
	2	.519	.276	.286
	1	.481	.724	.714
	HI	.499	.400	.408
	Hobs	.038	.000	.000
		(26)	(29)	(28)
MEAN	HI	.558	.549	.560
Standard error		(.037)	(.037)	(.041)
MEAN	Hobs.	.244	.269	.270
Standard error		(.066)	(.069)	(.068)

GRAND M

87/30	87/37	87/55	87/77	87/81	87/83
GUINEA SAVANNAH			SUDAN SAVANNAH		
.139	.121	.185	.096	.212	.143
.361	.379	.315	.404	.288	.357
.278	.328	.426	.385	.327	.381
.222	.172	.074	.115	.173	.119
.724	.705	.680	.666	.735	.693
1.000	1.000	1.000	1.000	1.000	1.000
(36)	(29)	(27)	(26)	(26)	(42)
.060	.058	.091	.037	.174	.119
.240	.250	.273	.482	.587	.173
.400	.519	.409	.222	.196	.789
.300	.173	.227	.259	.043	.019
.689	.635	.698	.650	.585	.347
.680	.692	.636	.889	1.000	1.000
(25)	(26)	(22)	(27)	(23)	(26)
.136	.113	.114	.107	.225	.154
.432	.432	.357	.393	.350	.404
.432	.455	.500	.500	.424	.422
.608	.594	.602	.584	.646	.618
1.000	1.000	1.000	1.000	1.000	1.000
(22)	(22)	(21)	(28)	(20)	(26)
.000	.000	.000	.055	.041	.058
.053	.109	.107	.056	.063	.173
.518	.587	.572	.556	.542	.423
.429	.304	.321	.333	.354	.346
.545	.551	.558	.574	.575	.668
.321	.304	.214	.037	.167	.115
(28)	(23)	(28)	(27)	(24)	(26)
.555	.557	.599	.550	.580	.550
(.042)	(.037)	(.040)	(.044)	(.041)	(.043)
.268	.240	.241	.282	.261	.303
(.070)	(.067)	(.067)	(.074)	(.072)	(.075)

EAN H_L = 0.561±0.005 Hobs = 0.264±0.007

TABLE 7

Loci that are significantly out of Hardy-Weinberg proportions in cowpea accessions. (P is probability of deviation from Hardy-Weinberg proportions)

:cession	Locality	Loci	P
7/139	Akora Darko	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI2*, PGI3*, PGM1* PGM2*.	< 0.05 -0.001
7/142	Akora Darko	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI2*, PGI3*, PGI4* PGM1* PGM2*	< 0.05 -0.001
7/157	Abene	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI3*, PGI4*, PGM1* PGM2*.	< 0.05 -0.001
7/30	Botęrly	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3* MDH4* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI2*, PGI3*, PGI4* PGM1*	< 0.05 -0.001
7/37	Zan	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH4* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI2*, PGI3*, PGM1*	< 0.05 -0.001
7/55	Limoh	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3*,MDH4* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI2*, PGI3*	< 0.05 -0.001

Table 7 cont'd

Accession	Locality		P
37/77	Buoti	FH1*, HK1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3*,MDH4* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI3*, PGI4*, PGM1*,PGM2*	< 0.05 -0.001
37/81	Buoti	AK1*, AK2*, FH1*, HK2*, 1DHP1* 1DHP2*, MDH1*, MDH3*, MDH4* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI3*, PGI4*, PGM1* PGM2*	< 0.05 -0.001
87/83	Nandom	AK1*, AK2*, FH1*,HK1*,HK2*, 1DHP1* 1DHP2*, MDH1*, MDH2*, MDH3*,MDH4* MEP1*, MEP2*, PGDH1*, PGDH2* PGDH3*, PGI3*, PGM1*,PGM2*.	< 0.05 -0.001

TABLE 8

Measures of genetic diversity within and among cowpea accessions for 22 loci.

H_s = expected mean heterozygosity;
H_t = total panmictic heterozygosity; G_{st} = relative genetic differentiation;
D_{st} = interpopulation gene diversity; C = frequency of cross pollination
F_{is} = Wright's fixation index

Locus	C	H_s	H_t	D_{st}	G_{st}	F_{is}
AK1*	0.781	0.596	0.605	0.009	0.015	0.123
AK2*	0.000	0.279	0.289	0.010	0.035	1.000
FH1*	0.000	0.434	0.568	0.134	0.236	1.000
HK1*	0.949	0.615	0.630	0.015	0.024	0.026
HK2*	0.000	0.463	0.483	0.020	0.041	1.000
1DHP1*	0.067	0.594	0.643	0.049	0.076	0.874
1DHP2*	0.163	0.603	0.635	0.032	0.050	0.719
MDH1*	0.000	0.569	0.600	0.001	0.052	1.000
MDH2*	0 411	0.812	0.865	0.043	0.061	0.417
MDH3*	0.082	0.728	0.781	0.053	0.068	0.849
MDH4*	0.698	0.675	0.747	0.072	0.096	0.178
MEP1*	0.122	0.651	0.699	0.048	0.069	0 782
MEP2*	0.134	0.617	0.697	0.080	0.115	0 763
PGDH1*	0.000	0.521	0.564	0.043	0.076	1.000
PGDH2*	0.052	0 786	0.825	0.039	0.047	0.901
PGDH3*	0.000	0.620	0.683	0.043	0.092	1.000
PGI1*	0.000	0.000	1.000	1.000	1.000	0.000
PGI2*	0.329	0.371	0.388	0.017	0.044	0.504
PGI3*	0.368	0.690	0.710	0.020	0.028	0.449
PGI4*	0.869	0.614	0.662	0.048	0.073	-0.070
PGM1*	0.206	0.603	0.606	0.003	0.005	-0.658
PGM2	0.144	0.531	0.572	0.041	0.072	0.749
Means	0.244 (.064)	0.561± (.037)	0.648 (.032)	0.083 (.043)	0.134 (.042)	0.573± (.096)

TABLE 9

Matrix of meristic identity (above diagonal) and meristic 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		.939	.889	.876	.948	.770	.927	.932	.946
87/142	063		.879	.897	.968	.812	.926	.943	.951
87/157	.118	129		.827	.876	.685	.898	.925	.845
87/30	.132	.109	.0190		.954	.821	.964	.954	.958
87/37	053	.033	.131	.047		.333	.982	.975	.969
87/55	.261	.208	.378	.197	.183		.814	.807	.847
87/77	076	.077	.108	.037	.018	.206		.990	.945
87/81	.070	.059	.078	.047	.025	.214	.010		.955
87/83	056	.050	.168	.043	.031	.166	.057	.046	

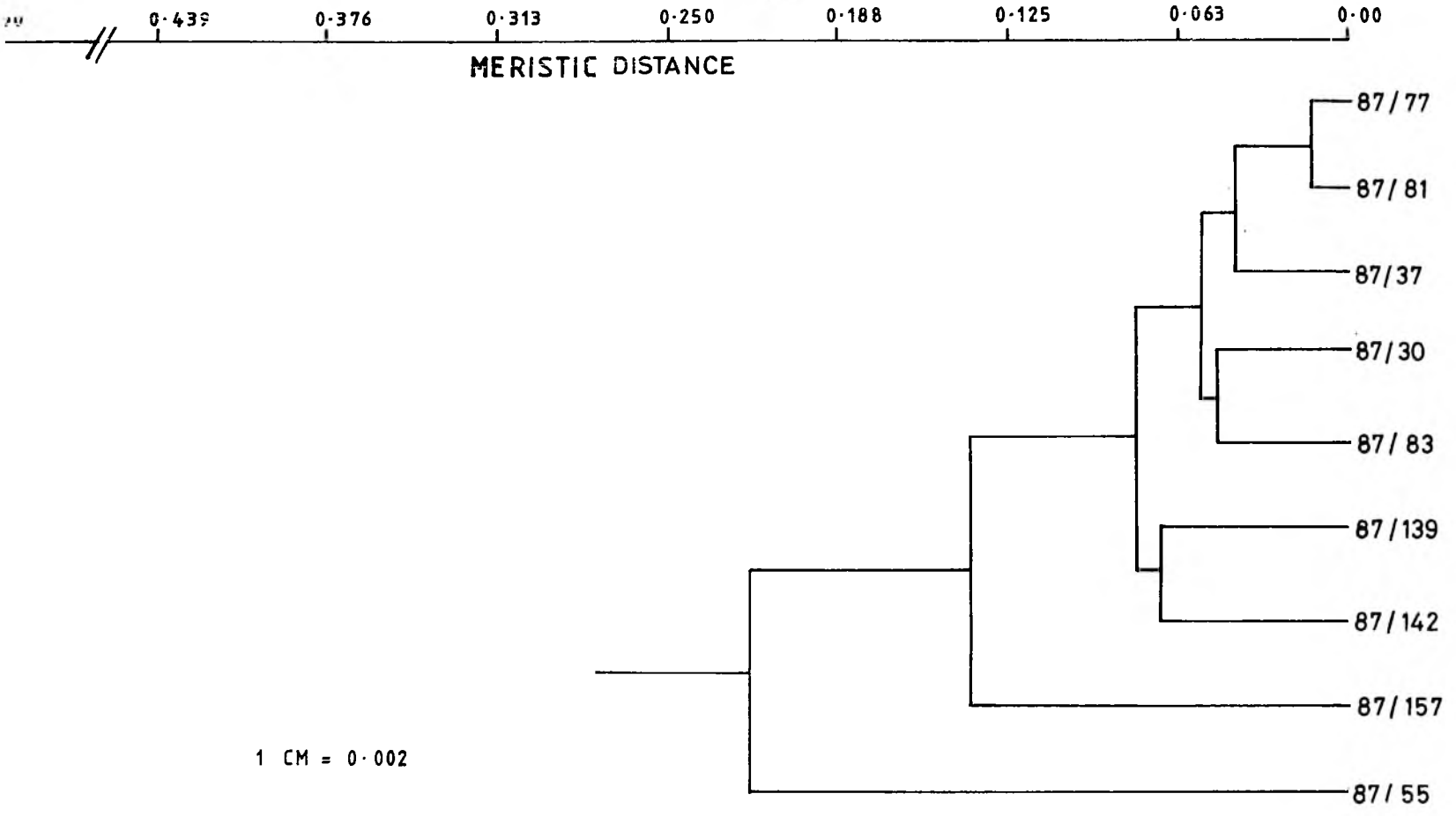


Fig. 5. Cluster analysis for meristic data of cowpea accession (UPGMA)

TABLE 10

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		.846	878	.814	.776	.695	751	746	.775
87/142	.167		.877	.702	.695	.679	.724	.674	.685
87/157	.130	.131		.774	752	.743	.762	.707	.736
87/30	.206	.354	.256		.904	.827	.762	775	.812
87/37	.254	.364	.285	.101		.879	.792	.804	.812
87/55	.364	.387	.297	.190	.129		.772	792	.791
87/77	.286	.323	.272	.272	.233	.259		.752	.774
87/81	.293	.395	.347	.255	.218	.233	.285		.850
87/83	.255	.378	.307	.208	.208	.234	.256	.163	

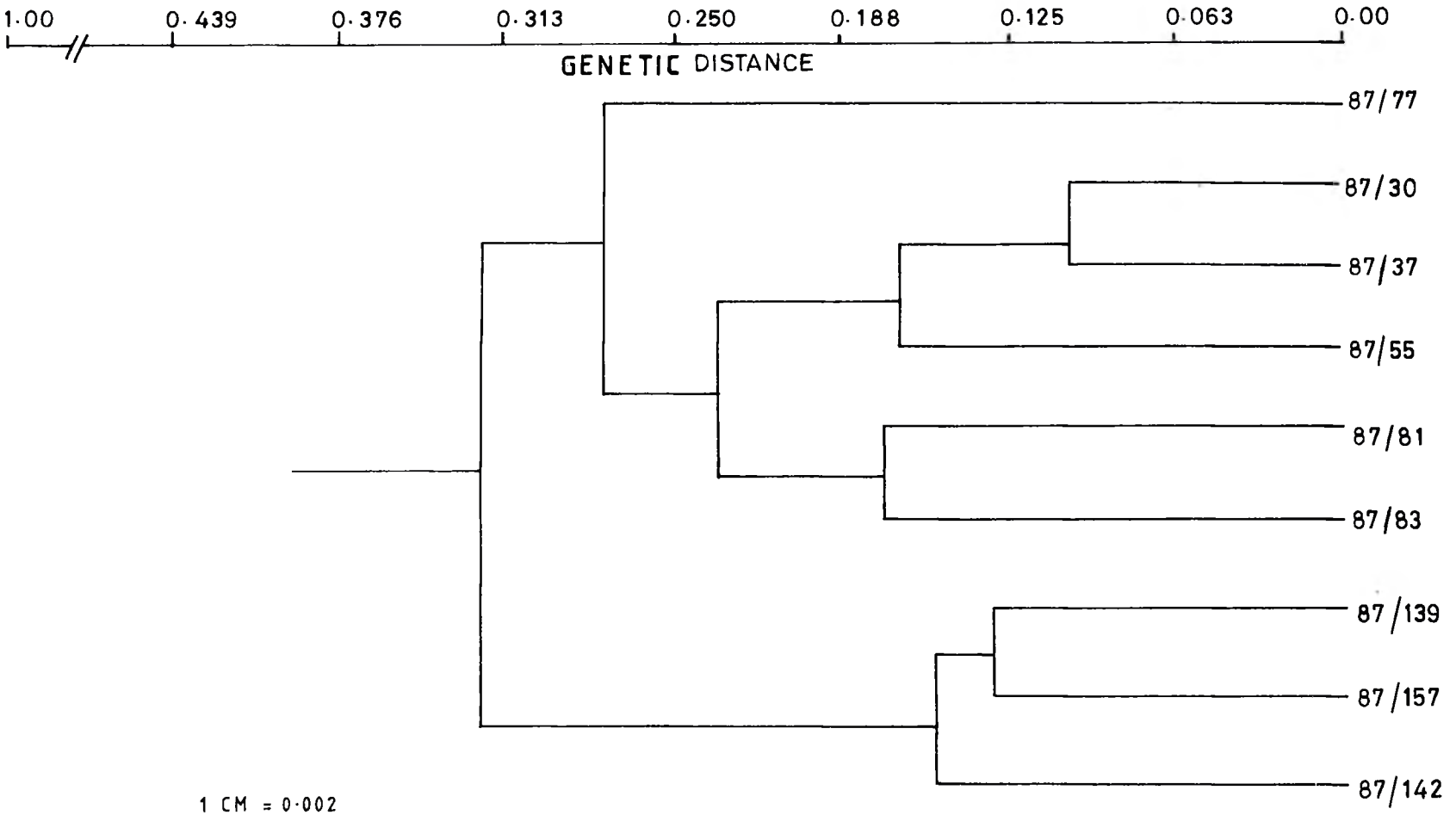


Fig.6. Cluster analysis for allozyme data of cowpea accession (UPGMA)

3.6 Correlation Analysis

3.6.1 Environmental correlates of variation

In an attempt to identify environmental correlates of allozymic and morphological variation the following variables were used:

(a) Geographical (longitude (L_n) and latitude (L_t)

(b) Climate:

(i) temperature (mean monthly maximum (T_{max}), mean monthly minimum (T_{min}), mean monthly cloudiness (Cl) and mean monthly sunshine (S_m)

(ii) moisture (mean monthly rainfall (R_m), number of rainy days per year (R_d), relative humidity per year at 1500hrs (Rh1500) and 0600hrs (Rh0600), mean monthly vapour pressure at 1500hrs (P1500) and 0600hrs (P0600)

3.6.2 Environmental, morphological and allozyme correlates

Correlations for environmental factors, morphological and allozyme variables are presented in Appendices 16,17, and 18.

3.6.3 Environmental correlates of allozymic variation

Environmental correlates of allozyme variation are shown in Table 11. Allozyme frequencies were observed to be significantly correlated with geographical, temperature and moisture factors. For instance, alleles HK1*-4, MDH1*-5, PGDH2*-3, PGDH2* 1, MEP1*-4, and IDHP1*-4 were positively and significantly correlated with longitude. Alleles IDHP2*-3, MEP1*-6, and PGDH3*-1 were positively

and significantly correlated with latitude, while allele MDH1*-4 and latitude were negatively and significantly correlated. Allele PGDH3*-1 was positively and significantly correlated with minimum temperature. Alleles PGDH1*-1, PGDH2*-5 were positively and significantly correlated with annual rainfall, while alleles HK1*-4, IDHP2*-3, MDH1*-5 and PGDH3*-1 were negatively and significantly correlated with annual rainfall.

TABLE 11

Environmental correlates of allozymic variation (only $|r_s| > 0.40$)

LOCI	LONG	LAT.	T_{max}	T_{min}	M THLY.RAIN	RAIN DAY	RH1500	RH0600	MEAN SUN	P1500	P0600	CL
AK1*-3	.7281	.5216			-.6633	-.5075	- 4322	-.4774	.4629	-.4889	-.5143	-.5941
AK1*-2	.4107											
AK1 -1	-.5518				.4319							
AK2*-3		-.6180	-.6115	-.4714	.5215	.6255	.6276	.6123	-.6306	.6241	.6050	.5768
AK2*-H	.7005				-.6048							-.4485
FH1*-3	.5905	.5329	.4615	.4900	-.6019	-.5232	-.5044	-.5309	.5115	-.5240	-.5398	-.5518
FH1*-2		.6035	.6068		-.4099	-.6339	-.6293	-.5662	.6436	-.6174	-.5489	-.5020
FH1*-1	-.5252	-.8103	-.7650	-.5234	.7108	.8266	.8106	.7808	-.8261	.8148	.7738	.7466
HK1*-4	.9586**	.7141	.4756	4642	-.8483*	-.6891	-.6076	-.6763	.6365	-.6738	-.7240	-.8220
HK1*-3		- 4647	-.6535	-.4936		4966	.5697	.4916	- 5510	.5098	.4361	
HK1*-2	-.4835											.5261
HK1*-1		.4894	.4581			-.5064	-.4904	- 4511	.4991	- 4879	-.4608	-.4673
HK1*-H	5859											
IDHP1*-6	.7007				-.6050							-.4487
IDHP1*-5		4910	.6936	6751		-.5122	-.5968	-.5409	.5700	-.5365	-.4839	
IDHP1*-4	-.8424*	- 4582		-.4949	.7033	.4261		.4562		.4274	.4901	.5614
IDHP1*-2	.6181				-.4660							
IDHP2*-3	.8184	.8757*	.6196	-.6168	-.8420*	-.8668*	- 8139	.8662*	-.8414*	-.7861		-.7078
IDHP2*-1	- 7199	- 5672	-.4303	- 4617	.6550	.5477	.5055	.5558	-.5185	.5441	.5831	.6354
MDH1 -6			.5331	.5560			-.4539	- 4002	.4361	- 4038		

Table 11 cont'd

LOCI	LONG.	LAT	T_{max}	T_{min}	M'THLY.RAIN
MDH1*-5	.8802*	.7641	.6536	.7166	-.8792*
MDH1*-4	-.6024	-.9352**	-.9254*	-.7134	.7891
MDH1*-2			-.5572	-.5442	
MDH1*-1	- 4099				
MDH2*-9			4158	.4910	
MDH2*-8			.5143	6248	
MDH2*-7	- 7162	-.6388	- 5395	- 5527	.8035
MDH2*-6		4250	5562	.5784	
MDH2*-5	-.6253	- 5543	-.6076	- 8490*	.6652
MDH2*-4	5846				
MDH2*-2	5264				
MDH2*-1		- 6774	- 7764	-.5873	.4632
MDH3*-7	6205	5213	4178	4425	-.5545
MDH3*-6		.4012	.4545	.5030	
MDH3*-3		.5861	.6311		
MDH3*-1		- 6320	- 5454		4638
MDH3*-H_L	.6142	.5837	.6106	.7685	-.7151
MDH4*-7	.7007				
MDH4*-6			4158	4910	
MDH4*-4	9221*	5702			-.7955
MDH4*-3	.5720	.5337	4034		-.6886
MDH4*-2		4606	5136		

RAIN DAY	RH1500	RH0600	MEAN SUN	P1500	P0600	CL
-.7472	-.7182	-.7629	7283	-.7492	-.7782	-.8011
.9466**	.9497**	.9265*	-.9543**	.9445**	.9155*	.8729
	.4588	.4179	-.4276	.4021		
		-.4082			-.4058	
	-.4178					
.6389	.6055	.6174	-.6260	.6316	.6192	.6207
-.4264	-.4880	-.4748	.4613	-.4502	-.4465	
.5374	.5764	.6103	-.5568	.5657	.5937	.5439
						-.4014
.6962	.7392	.6924	-.7278	.7042	.6614	.5753
-.5018	-.4732	-.5197	.4791	-.5024	-.5437	-.5863
	-.4217	-.4438		-.4075	-.4407	-.4170
-.6102	-.6254	-.5673	.6242	-.6011	-.5556	-.5119
.6395	.6067	.5947	-.6229	.6229	.6088	.6281
-.5783	-.6012	-.6148	.5944	-.5953	-.5952	-.5435
						-.4487
		-.4082			-.4058	
-.5470	-.4456	-.5153	.4851	-.5238	-.5676	-.6835
-.5449	-.4916	-.4831	.5251	-.5238	-.4872	-.4995
-.4903	-.5049	-.4365	.5072	-.4788	-.4186	

Table 11 cont'd

LOCI	LONG	LAT.	T_{max}	T_{min}	M'THLY.RAIN
MDH4*-1	-.6534	-.8190	-.7310	-.5436	.8130
MDH4*-H_t	.7787	.6229	.6029	.8254	-.7900*
MDH4*-H_{obs}	4606	.5963	6613	7226	-.5101
MEP1*-7			.4158	.4910	
MEP1*-6	6007	.8675*	.7857	.5145	- 7749
MEP1*-5	- 407				
MEP1*-4	- 8587*	- 7309	- 5481	- 5097	.7786
MEP1*-2	.5978				-.5571
MEP2*-7			4158	.4910	
MEP2*-6	- 4073	- 5928	-.5468		.5270
MEP2*-5	7904	6060			-.7207
MEP2*-4			.4158	.4910	
MEP2*-3	- 7663	- 6432	- 5092	-.5225	7582
MEP2*-2	4894			.4204	-.5237
MEP2*-1	- 4848				.4356
MEP2*-H_t		.4606	.4927		
MEP2*-H_{obs}		-.6630	- 6491		.5474
PGDH1 -5			.4158	4910	
PGDH1*-4		.6354	7974	.6633	-.4590
PGDH1 -3		-.6980	-.7118	-.5281	.5648
PGDH1 -2			- 5687	-.5249	
PGDH1*-1	- 8559*	-.7622	- 5611	-.4573	.8534*

RAIN DAY	RH1500	RH0600	MEAN SUN	P1500	P0600	CL
.8311	.7990	.7827	-.8215	.8165	.7819	.7717
-.6032	- 6128	-.6584	6059	-.6223	-.6550	-.6369
-.5850	-.6262	-.6434	.6040	-.6081	-.6317	-.5865
		- 4082			-.4058	
-.8829*	-.8523*	-.8269	8740*	-.8660*	-.8270	-.8158
.7089	.6500	.7073	-.6688	.6998	.7458	.8194
		-.4082			-.4058	-.3846
.6027	.5863	5695	-.5989	.5932	.5680	.5561
-.5934	-.4976	-.5424	.5374	-.5657	-.5890	-.6901
		-.4082			-.4058	
.6305	.5877	6253	-.6051	.6244	.6451	.6822
-.4670	-.4830	-.4611	.4739	-.4667	-.4619	- 4451
.6850	.6779	.6344	- 6913	.6731	.6204	.5805
		- 4082			-.4058	
-.6615	-.7281	-.6640	.7108	-.6761	-.6134	-.4859
7113	7205	6919	- 7227	7098	.6776	.6322
	4317					
.7533	.6813	7153	-.7128	.7338	.7468	.8104

Table 11 cont'd

LOCI	LONG.	LAT.	T_{max}	T_{min}	MTHLY.RAIN
PGDH1*-H			.5266	.4736	
PGDH2*-7			.4172		
PGDH2*-5	-.6791	-.8090	-.7752	-.7075	.8462*
PGDH2*-4		.5546	.5948	.4616	-.4761
PGDH2*-3	.8661*	.5663			-.8013
PGDH2*-2	-.7879	-.6608	-.5392	-.5862	.7136
PGDH2*-1	.8385*	.6827	.5594	.6400	-.7207
PGDH2*-H		.6288	.7438	.5690	-.4465
PGDH2*-HOB*	.5954	.6083	.4980	.4015	-.6616
PGDH3*-4		.4983	.5817	.4793	
PGDH3*-1	.8074	.9065*	.8413*	.7764	-.9074*
PGI2*-4	-.7434	-.7513	-.5896	-.4529	.6732
PGI2*-3	-.4835	-.4181	-.2915	-.2299	.4060
PGI2*-2	.7044	.7003	.5453	.4219	-.6468
PGI2*-1		-.6180	-.6115	-.4714	.5215
PGI2*-HL	-.7735	-.7143	-.5328	-.4261	.6913
PGI2*-H	-.7500	-.7452	-.5760	-.4393	.6760
PGI3*-4	.4573	.6414	.6814	.6518	-.6122
PGI3*-3	-.4573	-.6414	-.6814	-.6518	.6122
PGI3*-2	.5745	.7931	.7947	.6909	-.7605
PGI3*-1	-.5745	-.7931	-.7947	-.6909	.7605
PGI3*-H		.5614	.5435		

RAIN DAY	RH1500	RH0600	MEAN SUN	P1500	P0600	CL
	- .4092	- .4159	.4002	-.4025	-.4134	
.8168	.8102	.7999	- .8209	.8152	.7875	.7502
-.5719	-.5890	-.5521	.5904	-.5721	-.5287	-.4681
-.5521	-.4564	-.5074	.4979	-.5272	-.5489	.6431
.6366	.6043	.6620	-.6106	.6390	.6893	.7368
-.6507	-.6214	-.6941	.6226	- .6577	-.7270	-.7838
- .6525	- .6997	-.6432	.6893	-.6603	-.6047	-.5050
-.6099	-.5702	-.5768	.5914	-.5971	-.5871	-.6049
-.5050	-.5442	-.5227	.5287	- .5168	-.5032	-.4442
-.9033*	-.8892*	-.9018	.8974*	-.9036*	-.9034*	-.8921*
.7326	.6811	.7273	-.6956	.7227	.7663	.8364
.4029					.4295	.4894
- .6840	-.6338	-.6754	.6492	- .6737	- .7108	-.7752
.6255	.6276	.6123	-.6306	.6241	.6050	.5768
.6945	.6347	.6857	- .6532	.6826	.7270	.8052
.7264	.6717	.7188	- .6876	.7154	.7591	.8327
-.6479	-.6712	-.6568	.6664	-.6569	-.6357	-.5771
.6479	.6712	.6568	-.6664	.6569	.6357	.5771
- .8041	-.8108	-.7904	.8157	- .8050	-.7729	-.7217
.8041	.8108	.7904	- .8157	.8050	.7729	.7217
-.5653	-.5629	- .5534	.5642	-.5621	-.5561	-.5479

Table 11 cont'd

LOCI	LONG.	LAT.	T_{max}	T_{min}	M'THLY.RAIN
PGI4*-3	.4023	.6432	.7698	.8328	-.4528
PGI4*-2			-.4441	-.6175	
PGI4*-1	-.6463	-.5489			.6060
PGI4*-H_e	-.7381	-.4334			.6184
PGI4*-H_{obs}	.9029*	.8213	.6531	.6054	-.8386*
PGM1*-3	.4718				
PGM1*-2				-.4412	
PGM1*-1	-.5883	-.5987	-.4032		.5643
PGM1*-H_e	.5280	4661		4153	-.5120
PGM2*-4	9303*	.7517	.5708	.5916	-.8286
PGM2*-3	.6206	.7333	.5687		- 7554
PGM2*-2	.0498	.5000	.6468	.4958	
PGM2*-1	-.4674	-.8133	-.8196	-.6004	.6564
PGM2*-H_e	.8132	.8533*	.6925	.5280	-.8803*
PGM2*-H_{obs}		.4490	.5188		
APL	.7912	.5903	.4923	.6416	-.6850
%PL	.4158	.6631	.6894	.5785	-.5722
H_{obs}	.8237	.4407			.6898
H_e			.4475	.5470	

APL = Alleles per locus

%PL = Percentage loci polymorphic

*=P<0.01 **=P<0.001

RAIN DAY	RH1500	RH0600	MEAN SUN	P1500	P0600	CL
-.6288	-.6983	-.7151	.6606	-.6630	-.6971	-.6308
.5406	.4645	.4958	-.4965	.5175	.5327	.6109
.4199					.4066	.5432
-.7993	-.7479	-.8038	.7631	-.7931	-.8396*	-.9038
					-.4039	-.4355
.5941	.5197	.5411	-.5509	.5686	.5788	.6555
-.4542	- 4347	-.4646	.4404	-.4549	-.4775	-.4983
-.7241	-.6691	-.7378	.6844	-.7198	-.7772	-.8516
-.7489	-.6810	-.6630	.7206	-.7183	-.6764	-.7038
-.5176	-.5792	-.5306	.5579	-.5322	-.4945	
.8277	.8346*	.8041	-.8383*	.8249	.7903	.7438
-.8529*	-.7955	-.8091	.8238	-.8347*	-.8291	-.8645
- 4843	-.5038	-.4252	.5082	- 4734		
-.5593	-.5395	-.6100	.5379	-.5703	-.6351	-.6774
-.6723	-.6884	-.6681	.6866	-.6761	-.6516	-.6019
.4160		.4008		.4462		.5483

3.6.4 Allozyme correlates of morphological variation

Isozyme correlates of morphological variation are shown in Table 12. Pod maturation period was significantly and positively correlated with the following alleles FH1*-1 (P<0.01), MDH2*-1 (P<0.01),MDH1*-4 (P<0.001),MDH4*-1 (P<0.01), PGM2*-1 (P<0.001) but negatively correlated with the alleles FH1*-2 (P<0.001), IDHP2*-3 (P< 0.001), MDH4*-2 (P<0.01), MEP1*-6 (P<0.01) PGM2*-3 (P< 0.001) Pod length was significantly and positively correlated with AK2*-3 (P<0.001) , FH1*-1 (P< 0.01), MDH1*-4 (P< 0.01), MDH2*-1 (P<0.01), MDH3*-1 (P< 0.001), PGM2*-1 (P<0.01) but negatively correlated with FH1*-2 (P<0.01) Days to flowering was significantly and positively correlated with MEP1*-3 (P<0.01) but negatively correlated with PGDH3*-1 (*P*<0.01) Percentage seed germination was significantly and positively correlated with MEP1*-3 (P<0.01) and PGI2*-1 (P<0.01), and negatively correlated with PGDH3*-1 (P<0.01), 50-mean seed weight was significantly and positively correlated with HK1*-3 (P<0.01), but negatively correlated with PGDH2*-4 (P<0.001) Percentage seed set was significantly and positively correlated with MEP1*-3 (P<0.001) Percentage seed damage by insect was significantly correlated negatively with PGDH2* 1 (P< 0.01), Percentage pod damage by legume borer was significantly and positively correlated with MEP2*-6 (P<0.01) and PGDH1*-4 (P<0.001), and fecundity rate of *Callosobruchus maculatus* infestation of seed was significantly and positively correlated with MDH2*-6 (P<0.01) and PGDH3*-4 (P< 0.01). Number of seeds per pod was significantly and negatively correlated with FH1*-2 (P<0.01) and number of ovules

per pod was significantly and positvely correlated with MDH3* 1 ($P<0.01$) and PGM2*-1 ($P<0.01$) but negatively correlated with FH1*-2 ($P<0.01$) Percentage pod set was significantly and positively correlated with MEP2*-6 ($P<0.01$).

TABLE 12

Allozymic correllates of morphological variation (only $|r_s| > 0.04$)

	PSG	PPS	PSS	PSI	PPD	FRM	DTF	DPM	PL	MSW	NSP	NOP	LS
AK1^1-3													.5234
AK1^1-2	.4579		.5061				.5137		.4105	.4675	.4443		
AK2^1-3	.6142					-.6127	.5862	.4476	.8775**		.5500		.6614
AK2^2-2													.4444
FH1^1-3													
FH1^1-2	-.5099	-.4143	-.5496		-.6221	.6080	-.5022	-.8398*	-.7988*		-.7993*	-.8195*	
FH1^1-1		.5218			.5552	-.5343		.8874**	.7605*		.6418		.7294
HK1^1-4													
HK1^1-3	.7250		.4664	.5703			.6580			.8091*			.6348
HK1^1-2								.4161					
HK1^1-1				-.4388	-.4399		-.5235	-.6076	-.4391	-.4335			
HK1^1-H$_1$	.4361		.4094							.6209			.5087
HK2^1-2				-.4849	.5097								.5296
HK2^1-1					-.5281								-.4719
IDHP1^1-6	-.4553		-.4957				-.5783						
IDHP1^1-5										-.4553			-.5819
IDHP1^1-3					.5919			.4077		.4806			.6651
IDHP1^2-2													.4514
IDHP2^1-5				.5127	.5470		.4906			.6761			.6203
IDHP2^1-4	-.5799			-.4773	.5214		-.4468			-.4005			
IDHP2^1-3	-.5181	-.4184			-.7250	.5594	-.5334	-.8960**	-.7063		-.4989		-.5780
IDHP2^1-2	.5409			.5019			.4484			.4837			
IDHP2^1-1						-.5946		.4186	.6155			.5726	-.6580

Table 12 Cont'd.

	PSG	PPS	PSS	PSI	PPD	FRM
MDH1$_t$-6						
MDH1$_t$-5						
MDH1$_t$-4					.6080	-.7081
MDH1$_t$-3						
MDH1$_t$-2				.4554		-.4294
MDH1$_t$-1						.4755
MDH2$_t$-9						
MDH2$_t$-8						
MDH2$_t$-7	.5944	.5191	.4543			.8702*
MDH2$_t$-6						
MDH2$_t$-5			-.4599			
MDH2$_t$-4			.4348			
MDH2$_t$-3	-.4870		-.5558		-.4399	
MDH2$_t$-2						
MDH2$_t$-1	.4861					-.7334
MDH3$_t$-7						
MDH3$_t$-6			.4151			.5045
MDH3$_t$-5	-.6357	-.4657	-.6124			.5211
MDH3$_t$-4						
MDH3$_t$-3					-.5494	
MDH3$_t$-2					.6435	
MDH3$_t$-1	.4743					-.6362
MDH4$_t$-7		-.4671	-.4252			
MDH4$_t$-6		-.4072				.6257
MDH4$_t$-5						
MDH4$_t$-4	-.4130	-.6405				.4566
MDH4$_t$-3		-.6877	-.4912		-.4807	
MDH4$_t$-2					-.4315	
MDH4$_t$-1	.4790	.7467	.4449		.6519	-.4965
MEP1$_t$-7			.4242			.4996
MEP1$_t$-6					-.4106	
MEP1$_t$-5	-.4819		-.5850		-.6639	
MEP1$_t$-4				-.5733		
MEP1$_t$-3	.8240*	.5044	.9013*		.4444	
MEP1$_t$-2		-.5453	-.4567			

DTF	DPM	PL	MSW	NSP	NOP	LS
		-.4091				
	.9170**	.7541*		.5596		.7146
		-.4699		-.4992	-.5867	.6314
		.4488				
				.4999	.5107	-.6138
.5765						
		-.4702			-.4182	
			.4986	-.6535		
-.5865	-.6736	-.6153	-.5202	-.6219		-.5804
	.4124					
.4277	.7568*	.8099*		.5342	.6727	.7826*
-.5408		-.4292	-.6197	-.5128		
		-.6219		-.5604	-.5756	.5414
-.4539	-.7308	-.4577				
			.6227			.8747
.5218	.7041	.8909**		.6967		.8046*
				-.5000	-.4016	.4120
				.4135	.4782	-.4431
		-.4335		-.4303		
.4073	-.4461					
	-.8699*	-.6503		-.5668		-.7422
.4671	.8179*	.6857		.6135		.6045
	-.8615*	-.6910		-.5472		-.7234
-.4957	-.5561		-.6165	-.4763		-.4794
	.5776		-.5746			
.8528*	.4328	.6151	.6649	.7037		.4894
		-.4502		-.6042	-.5235	.5401

Table 12 Cont'd.

	PSG	PPS	PSS	PSI	PPD	FRM
MEP1^{t}-1				-.5865		
MEP2^{t}-7			.5326			
MEP2^{t}-6		.8010*			.9480**	
MEP2^{t}-5		-.4313				
MEP2^{t}-4			.5326			
MEP2^{t}-3						
MEP2^{t}-2						
MEP2^{t}-1		.6833				
PGDH1^{t}-5			.5326			
PGDH1^{t}-4					-.5415	
PGDH1^{t}-3		.5543			.8774**	
PGDH1^{t}-2						
PGDH1^{t}-1	.5738	.5243				.5065
PGDH1^{t}-H		.4755	.5347			
PGDH2^{t}-8						
PGDH2^{t}-7						
PGDH2^{t}-6		.4849				.5438
PGDH2^{t}-5	.5594	.4390		.4041		
PGDH2^{t}-4	-.7415	-.7035	-.5245	-.5669	-.5436	
PGDH2^{t}-3						
PGDH2^{t}-2						-.5773
PGDH2^{t}-1				-.7517*		
PGDH3^{t}-5	.6020	.5403		.4063	.6895	-.6495
PGDH3^{t}-4						
PGDH3^{t}-3						
PGDH3^{t}-2	.4125		.4022			
PGDH3^{t}-1	-.8226*	-.4140	-.4467	-.5717		.6759
PGDH3^{t}-H	-.4955		-.5298			
PGI2^{t}-5	-.6053		-.4163	-.4336		.4391
PGI2^{t}-4						-.6654
PGI2^{t}-3		-.4914		.4257	-.5259	
PGI2^{t}-2				-.4048		
PGI2^{t}-1	.8581*		.4436	.4476		.4939
PGI3^{t}-4						.5216
PGI3^{t}-3						-.5216

DTF	DPM	PL	MSW	NSP	NOP	LS
				.5072	.4970	-.4630
				.4000		
	.6178					.6256
	-.4735					
				.4000		
			-.4411			
				.4000		
	-.6817	-.4053				
	.7098					.6217
.5849	.5286	.7487		.5686		.5538
		-.4970			-.4813	.4949
.5572	.4123	.5185				
-.6915			-.8951**			-.5693
			.5904		-.4086	.5081
		.5135				-.4702
.6259	.7078	.5840	.4400			
			-.6005	.7857*	-.5232	-.4072
		-.4500	-.4379			-.4595
				.4128		
-.7592*		-.6012	-.5929			
		-.4949		-.5483		-.5019
-.4776			-.7409			
	.5466	.5687				.4309
						-.4898
		-.4765				
	.8009					
	-.5926	-.6320			-.4919	
	.5926	.6320				.4919

Table 12 Cont'd.

	PSG	PPS	PSS	PSI	PPD
PGI3^{1}-2	-.4145	-.5787			-.7115
PGI3^{1}-1	.4145	.5787			.7115
PGI3^{1}-H$_{1}$	-.4210				
PGI4^{1}-4					
PGI4^{1}-3			.5751		
PGI4^{1}-2			-.6558		
PGM1^{1}-2			-.5646		-.4481
PGM1^{1}-1		.5719			.4276
PGM1^{1}-H^{1}		-.4200			
PGM2^{1}-4					
PGM2^{1}-3		-.5814	-.4978		-.5128
PGM2^{1}-2					
PGM2^{1}-1					

FRM	DTF	DPM	PL	MSW	NSP	NOP	LS
	-.4285	-.6073					-.5340
	.4285	.6073					.5340
.5036	-.4164	-.5368	-.5307	-.4642			
							.5061
.5773					.4537		
-.5551	-.4288				-.5846		.4202
	-.4921	-.4268			-.5849		-.4536
	.4059	.6043	.5053		.4412		.4058
	-.4939	-.8183*	-.7078		-.7040		-.7145
.6600		-.7137	-.6529		-.4438		-.7167
-.5975		.8701*	.7655*		.5734		.8027*

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

3.6.5 Environmental correlates of morphological variation

Environmental correlates of morphological variation are presented in Table 13 There were 156 correlates between morphological and environmental factors out of which 10 were signifiant.

TABLE 13

Environmental correlates of morphological variation

	PSG	PPS	PSS	FRM	DTF	DPM	PL	MSW	NSP	NOP	PSI	LS	PPD
LONG	-.5162	-.5146	-.1364	.4922	-.4827	-.3664	-.4240	-.0523	-.1880	-.2071	-.5824	.1241	- 0.2433
LAT	-.7076	-.4517	-.1824	.7300	-.6937	-.8100	-.6356	-.5342	-.2795	-.3803	-.8479*	-.2167	- 0.6114
T_{MAX}	-.6341	-.3271	-.0438	.8298	-.5601	-.7832	-.5556	-.6177	-.1353	-.2826	-.8316	-.3775	- 0.6385
T_{MIN}	-.4218	-.2551	.2074	.8624*	-.2341	-.4126	-.3064	-.3335	.1520	-.0041	-.6756	-.3167	- 0.3995
AN.RAIN	.7177	.6023	.2759	-.5900	.6479	.5682	.5748	.3218	.3084	.3347	.6295	.0278	0.4157
RAINDAY	.7287	.4598	.2106	-.7223	.7154	.8321	.6525	.5664	.3039	.4015	.8397*	.2300	0.6282
RH1500	.6974	.4092	.1409	-.7786	.6573	.8208	.6189	.5946	.2345	.3547	.8472*	.2959	0.6401
RH0600	.6627	.4060	.0973	-.7857	.6236	.7750	.5908	.5223	.1972	.3191	.8606*	.2555	0.6020
MEAN SUN	-.7237	-.4397	-.1864	.7510	-.6939	-.8339*	-.6423	-.5952	-.2771	-.3850	-.888*	-.2709	- 0.6410
P1500	.7100	.4407	.1722	-.7495	.6845	.8169	.6332	.5616	.2666	.3739	.8467*	.2479	0.6246
P0600	.6541	.4122	.1021	-.7653	.6312	.7687	.5915	.4887	.2077	.3242	.8684*	.2201	0.5878
CL	.6315	.4296	.1256	-.6966	.6448	.7394	.5871	.4041	.2389	.3366	.8611*	.1300	0.5425

*=P<0.01

3.7 Multiple Regression Analysis

Test of multiple regression was conducted to find the best environmental predicting factors for gene diversity and cowpea agronomic characters. Independent variables were environmental factors for the three agroecological zones and dependent variables were allele frequencies, gene diversity indices and cowpea agronomic characters.

3.7.1 Environmental predictors of genetic differentiation in cowpea

Significant R^2 (coefficient of multiple regression) values for environmental predictors of genetic differentiation are presented in Table 14. The R^2 values between the allozymes IDHP1*-6, MDH4*-7 and the combination of the variables cloudiness, monthly minimum temperature and longitude were 0.959 and 0.959 respectively The R^2 values between the four variable combination of monthly cloudiness, minimum temperature, monthly annual rainfall and longitude, and variation in some allozymes were as follows: HK1*-3 (0.977) and HK1*-2 (0.985) HK1*-1 (0.999) and 1DHP2*-3 (0.996) MDH1*-4 (0.977), MDH2*-2 (0.994) MDH3*-3 (0.998), 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) MDH1*-4 (0.977), MEP1*-6 (0.975), MEP1*-5 (0.979)

3.7.2 Environmental predictors of cowpea morphological factors

Significant R^2 (coefficient of multiple regression) values for environmental predictors of cowpea morphological factors are presented in Table 14. The R^2 values between the variable combination of annual rainfall, mean monthly total cloud, longitude

and mean monthly minimum temperature and variation in three agronomic characters were as follows: days to flowering (0.993), pod maturity period (1.000) and percentage pod set (0.996).

TABLE 14

Coefficients of multiple regression (R^2) of percentage polymorphic loci, selected allele frequencies and cowpea morphological factors as dependent variables and environmental factors as independent variables (R_m= monthly rainfall, Cl=monthly cloudiness, L_n=longitude and T_{min}=minimum temperature)

Dependent variable	ClL_nT_{min}	$R_mClL_nT_{min}$
AK2*-H_L		1.0000***
HK1*-4		0.999**
HK1*-3		0.977*
HK1*-2		0.985*
HK1*-1		0.999**
HK1*-H_L		0.992*
IDHP1*-6	0.960*	
IDHP2*-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

Table 14 cont'd

PGDH3*-5

PGI4*-2

PGM2*-3

% POD SET

DAYS TO FLOWERING

POD MATURATION PERIOD

% LOCI POLYMORPHIC

% SEED GERMINATION

*=P<0.01 **=P<0.001

0.992*

0.986*

0.999

0.996*

0.993*

1.000***

1.000***

0.981

***=P<0.0001

CHAPTER FOUR

DISCUSSIONS

4.1 Morphological (Meristic) Variability

Meristic variation is a continuous variation which can be either genetic or environmental. Its usefulness in breeding programmes depends generally on its degree of heritability.

4.1.1 Percentage seed germination

Cowpea accessions from Deciduous forest zone gave the highest mean percentage seed germination, while those from the savannah zones gave the lowest. For seeds to maintain their viability over a certain period of time, among other factors, they should have low moisture content. Therefore, cowpea seeds of the accessions studied from the Deciduous forest zone must have relatively low moisture content as compared to those of the savannah accessions which possibly have relatively high moisture content. Therefore, cowpea seeds from savannah accessions kept in genebank should be regenerated within a reasonable period of time before they lose their viability Percentage seed germination was positively and significantly correlated with pod length and days to flowering. Thus, breeding for cowpea varieties with high percentage seed germination will lead to selection for long pods and high number of days to flowering.

4.1.2 Number of days to flowering

Savannah zones accessions were the earlier to flower Monthly

rainfall and number of rainday per year decrease from Deciduous forest zone to the savannah zones (Table 2). Therefore savannah zone accessions should device a mechanism whereby they can complete their life-cycle before drought. Early flowering is therefore adaptive and advantageous to the continued existing of savannah cowpea accessions studied.

Assuming the factor for early flowering in the accessions studied are under strong genetic control, then savannah accessions will serve as a good source of breeding materials for cowpea improvement.

4.1.3 Percentage pod set per plant

Mean percentage pod set per plant in the savannah accessions were lower than those of the Deciduous forest. Amount of rainfall can affect the yield performance of crops. Therefore, the differences in mean percentage pod set per plant between accessions from the two ecogeographical zones might be due to difference in their amount of rainfall(see Table 2) Coefficient of variation in the ecogeographical zones were 0.68% (Deciduous forest zone), 0.90% (Guinea savannah zone) and 0.81% (Sudan savannah zone) Coefficient of variation of savannah accessions was higher than that of Deciduous forest accessions, ie. there was an increase from Deciduous forest zone to savannah zones.

4.1.4 Pod maturation period

In the savannah zones mean sunshine was higher than in the

Deciduous forest zone (Table 2) The correlation implies that cowpea accessions with shorter pod maturation period are restricted to ecogeographical zones with high mean sunshine. The savannah zones have low amount of rainfall (Table 2) Therefore, for the savannah accessions to be successful, they must evolve an early pod maturation period mechanism. Thus, early pod maturation period in the cowpea accessions studied might be adaptive.

4.1.5 Pod length

The savannah zone accessions had shorter pods than those of the Deciduous forest zone. Deciduous forest zone accessions under the influence of high rainfall (Table 2) have adequate resources to produce long pods, while savannah accessions on the other hand, under influence of low rainfall have relatively little resources and thus produce short pods. Again, with a shorter pod maturation period, it is advantageous for the savannah accessions to produce short pods.

4.1.6 Percentage pod damage

Mean percentage pod damage in the cowpea accessions studied was higher in the Deciduous forest zone accessions. This might be due to the fact that accessions from Deciduous forest zone were genetically susceptible to the attack by legume pod borer, while accessions from Savannah zones showed some degree of resistance. Another possible reason is that Deciduous forest accessions have relatively longer pod maturation period which might mean late pod

set coinciding with legume pod borer activity; on the other hand, savannah accessions have short pod maturation period thus an early pod set before commencement of legume pod borer activity In the light of this argument, mean percentage pod damage was higher in the Deciduous forset accessions. Assuming percentage pod damage is genetic, then the savannah accessions studied could serve as a good source of breeding materials for cowpea improvement.

4.1.7 Number of seeds per pod

Mean number of seeds per pod was lower in the savannah accessions than in the Deciduous accessions. With low rainfall pattern in the savannah zones, accessions collected from this zone might have evolved an early maturation period mechanism, which might possibly account for the low number of seeds per pod. However, with long maturation period in the Deciduous forest accessions, they possibly have enough time to accumulate resources to produce large number of seeds per pod.

With their high number of seeds per pod, Deciduous forest accessions could serve as good source of breeding materials for cowpea improvement, assuming this character is under genetic control.

4.1.8 Number of ovules per pod

Mean number of ovules per pod was lower in the savannah accessions. Number of ovules per pod was positvely and significantly correlated with long pod and high number of seeds per

pod (Appendix 17). Therefore breeding for long pod could result in selection for increase in ovule number and high number of seeds per pod.

4.1.9 Percentage seed set

Mean percentage seed set was higher in both Sudan savannah and Deciduous forest zones accessions. This character does not seem to follow any specific pattern so far as ecogeographical factors are concerned.

4.1.10 50-mean seed weight

Seed weight in the cowpea accessions studied did not follow any pattern so far as ecogeographical factors are concrened. Coefficient of variation was 9.64% (Deciduous forest zone), 3.34% (Guinea savannah zone) and 2.35% (Sudan savannah zone) There is therefore a gradual decrease in variation from Deciduous forest zone to Sudan savannah zone.

Therefore, coefficient of variation in the cowpea accessions studied could be adaptive.

4.1.11 Percentage seed damage by insect

Percentage seed damage by insect was positively and significantly correlated with number of rainday per year, relative humidity, pressure and cloud cover, but negatively and significantly correlated with latitude and mean sunshine (Table 13) Mean percentage seed damage by insect decreased from the

Deciduous forest zone to the savannah zones. This possibly suggests that percentage seed damage by insect is adaptive. It is also possible that the insect prefers habitats where there is high rainday, high humidity, high pressure, high cloud cover, low latitude and low mean sunshine as prevalent in Deciduous forest zone (Table 2) Thus Deciduous forest zone accessions registered high mean percentage seed damage by insect.

4.1.12 Fecundity rate of *Callosobruchus maculatus* infestation of seed

Fecundity rate of *C. maculatus* infestation of seed, a post-harvest factor, is positively and significantly correlated with minimum temperature. Mean fecundity rate of *C. maculatus* infestation of seed was higher in the savannah accession. It is therefore possible that fecundity rate of *C. maculatus* infestation of seed in the cowpea accessions studied has some selective advantage since there was an increase in fecundity rate from the Deciduous forest zone to the Savannah zones. There is also the possibility that, *C. maculatus* thrives better under savannah conditions, thus the savannah accessions registered higher mean values of fecundity rate.

4.1.13 Percentage leaf spots cover

Percentage leaf spots cover distribution in the cowpea accessions studied did not follow any definite pattern so far as ecogeographical factors were concerned.

4.2 Genetic Variability in Cowpea Accessions

Genetic variation of a population enables it to withstand drastic and lethal environmental changes. It is recognised as one of the important factors in the process of species adaptation. Selection can only be accomplished successfully if genetic variation for the character to be improved is available (Balagtas and Ramirez, 1991) The magnitude of the variability within and among populations is extremely important for breeding, and populations with higher genetic variability, other conditions being the same, are superior source materials (Geric *et al.*, 1989) The nature and structure of genetic variation, when viewed together with other features of crops may assist with the development of efficient strategies for collecting and conserving germplasm (Bretting and Goodman,1989)

4.2.1 Mean number of alleles per locus

Mean number of alleles per locus is one of several measures used to quantify diversity of genetic variation (Hayward,1984) Some workers have used it to characterize genetic variation in some crops. For example, Morden *et al.*,(1989) reported a mean value of 1.81 alleles per locus for over 49 different alleles in *Sorghum bicolor* (a self pollinating species) Also Brown and Munday reported a mean value of 2.38 alleles per polymorphic locus in barley (a self pollinating species)

In the present work, a total of 110 alleles were distributed

among 22 loci for a mean of 5.00 alleles per locus. This value is higher than those for *Sorghum bicolor* and barley. It is considered higher than what is expected of per polymorphic locus in selfing plants (2.38) and average expected (1.48) for other annuals (Hamrick and Godt,1990). However the mean value of 5.00 alleles per locus was considered lower than the average of 7.09 reported for maize (Doebley *et al.*,1985) but it was higher than the average of 2.5 reported for open-pollinated populations of Yugoslav maize collections (Geric *et al.*, 1989). Differences observed between the mean number of alleles per locus (5.00) for cowpea and those of other crop species could be attributed to differences in number of analyzed accessions, number of analyzed individuals per accessions, mechanism of pollination, propagation, degree of cross-pollination, type and number of enzyme systems studied.

4.2.2 Discriminating loci

Discriminating loci are loci at which an allele was absent in one of pairs of accessions. Among Deciduous forest accessions, 87/139 was discriminated from 87/142 and 87/157 by seven different loci, whilst accessions 87/142 and 87/157 were different at eight different loci (see Table 5). Among the Guinea savannah accessions, accession 87/30 could be discriminated from accessions 87/37 and 87/55 at seven different loci, while accessions 87/37 and 87/55 were different at six different loci (see Table 5) Among the Sudan Savannah accessions, accession 87/77 was different from accessions 87/81 and 87/83 at ten and eight different loci respectively, while

accessions 87/81 and 87/83 were different at six different loci (see Table 5). Treating accessions from each ecogeographical zone as unit groups, Deciduous forest zone accessions (87/139, 87/142 and 87/157) could be discriminated from Guinea savannah zone accessions (87/30, 87/37 and 87/55) at locus IDHP2*. Deciduous forest accessions as a unit group were discriminated from Sudan savannah accessions (87/77, 87/81 and 87/83) as a unit group at loci HK1*, and PGDH3*. Guinea savannah zone accessions as a unit group were discriminated from Sudan savannah accessions as unit group at loci MEP1*, PGDH2* and PGM2*

From the results no two accessions were similar in terms of the presence or absence of some alleles. Differences were observed among accessions within the same agroecological zones and also between accessions from different agroecological zones. There is an indication that there are both microclimatic and macroclimatic factors differences favouring certain genotypes, leading to the loss of certain alleles. It is also likely that there are different evolutionary forces operating within the cowpea accessions.

4.2.3 Genetic polymorphism

Using isozyme technique to study genetic diversity in *Vigna unguiculata* Panella and Gepts (1992) observed 14 out of 24 (58.00%) loci to be polymorphic in wild *V. unguiculata* and one out of 24 (4.00%) loci to be polymorphic in cultivated species. In another study they observed that 21 out of 24 loci (88.00%) in five related *Vigna* species were polymorphic. In a study of 70 accessions of *V.*

unguiculata in Philippine collections, Balgatas and Ramirez observed five polymorphic loci. However they did not state the number of loci that they screened. Vaillancourt *et al.*(1993) studied 112 accessions of cultivated cowpea (*V. unguiculata* subsp. *unguiculata*) and found that only six out of 26 (23.00%) loci were polymorpic; in 43 accessions of wild cowpea (*V. dekindtiana*) 19 out of 26 (73.00%) loci were observed to be polymorphic.

However in the present work 21 out of 22 (95.5%) loci were found to be polymorphic. Polymorphism as a variable to quantify genetic diversity is dependent on sample size, therefore the difference between 95.5% polymorphic loci in the present work and those of other workers might be due to differences in sample size. Other attributes could be differences in number and type of enzyme systems studied as well as number and type of accessions used for the study.

4.2.4 Expected heterozygosity

Heterozygosity is often considered an adaptive strategy for a species to exploit heterogeneous environments, and genetic variability is therefore expected to be higher in unstable environments than in stable ones (Levin, 1963, in Lewontin, 1974) It has been observed that enzyme polymorphism differs according to the functions of the enzymes. In a survey of nine *Drosophila* species, Latter (1981), observed that one functional group of 12 enzymes gave an average heterozygosity of 0.100, while another group of 13 enzymes gave an average of 0.230. Balagatas and Ramirez

(1991) in a study of Philippine cowpea collections, observed an expected heterozygosity of 0.360. Vaillancourt *et al.*, (1993) also observed a mean expected heterozygosity of 2.90 in 112 accessions of cultivated cowpea (*V. unguiculata*) and 0.017 in 43 accessions of wild cowpea.

In the present work, the range of expected heterozygosity was 0.549-0.599 with a mean of 0.561±0.037 (Table 6) This value was higher than those of previous workers, it was also higher than the average expected 0.149±0.016 for selfing species (Hamrick and Godt,1990). Landraces are distinct local types, adapted to many variants and interactions of natural and cultural environments to which crop species were gradually exposed (Harlan,1975), and residual genetic variability in them depends largely on the degree of outcrossing, selective value of heterozygotes and frequency dependent advantage of specific genotypic combinations (Breese,1989). Therefore in landraces, heterogeneity and heterozygosity may either be reduced or augmented by local practices and preferences. Conscious selection by local farmers of landraces may be for uniformity or heterogeneity In this context high genetic diversity in Ghanaian cowpea landraces of the present work could be attributed to an array of forces including, human selection, natural selection, seed exchange among local cowpea farmers and occassional outcrossing.

Mean expected heterozygosity for the present work was also higher than those observed in *Sorghum bicolor* (0.008) (Morden *et al.*,1989), barley (0.082) (Morden *et al.*,1989), mungbean (0.360)

and ricebean (0.340) (Balagtas and Ramirez,1991)

This high level of electrophorectically detectable genetic diversity observed in Ghanaian cowpea landraces suggests that collection of Ghanaian cowpea germplasm seeds should be disproportionately represented in germplasm banks (Breeting and Goodman,1989)

4.2.5 Hardy-Weinberg equilibrium ,Wright's fixation index F_{IS} and frequency of cross pollination(C)

Hardy-Weinberg equilibrium law and Wright's fixation index are useful for assessing population sructure and breeding system which are important practical approaches to collection of plant genetic resources. By predicting genotype frequencies from allele frequencies under random mating, Hardy-Weinberg law can be used to determine whether such a system exists. Deviation from equilibrium may be due to selfing or possible selection advantage of any one or more of the genotype classes and can indicate the extent of any variant population structure (Hayward,1987) Positive F_{IS} values suggest heterozygote deficiencies as a result of non-random mating, selection, genetic drift, mutation, or limited gene flow (Gehring and Lindhart,1992)

In the present work, 165 out of 198 analyzed at the most of the 21 polymorphic loci deviated significantly from Hardy-Weinberg proportions (Table 7) Nineteen loci gave positive F_{IS} values and 24.4% individuals were produced by outcrossing. Landraces are usually heterogeneous and show varying degrees of outbreeding which

may vary with season and location. The only population that may be regarded a priori as strictly homozygous and homogeneous are breeders line produced by repeated and controlled selfing (Breese,1989).In this context, it is possible that the 24.4% crossing suggests that Ghanaian cowpea landraces show varying degrees of outbreeding and heterozygote deficiencies observed could result from combined forces of natural selection and some degree of inbreeding. Twenty-four per cent rate of outcrossing is large enough to generate considerable heterozygosity in Ghanaian cowpea landraces, therefore during regeneration of Ghanaian cowpea landraces it may be necessary for curators to treat them as outcrossers so as to maintain the integrity, gene and genotype constitution and hence preserve the differences between accessions (Breese,1989)

4.3 Diversity among cowpea accessions

4.3.1 Genetic differentiation in cowpea accessions

Genetic differentiation in populations may be due to a lot of causes, among which are the following suggested by Falconer (1988)

(i) random genetic drift occurring independently in different sub-populations

(ii) uniformity within sub-populations due to progressive reduction in genetic variation within each sub-population

(iii) geographical or ecological causes under natural conditions or from controlled breeding in domesticated or laboratory breeding

(iv) genetic drift (Mettler and Gregg,1969),occurring in

(a) populations that become small periodically (i.e. "bottle-neck effect") and

(b) populations established by a few emigrants ("founders principle") carrying a small sample of genetic variation from a large population

Amount of genetic diferrentiation among a genetic population of plants is a useful guide to what proportion of accessions and individual seeds a curator can represent in a plant germplasm bank.

Hamrick and Godt (1990) observed 35.7% genetic differentiation among populations of annuals and 51.0% among populations of self pollinated species. Nevo *et al*. (1991) also observed 19.9% genetic differentiation among populations of wild emmer wheat. In the present study an overall 13.4% genetic differentiation was observed among cowpea accessions. This value was lower than values estimated for other annuals and self-pollinated species (Hamrick and Godt,1990) On the average the greatest percentage of total genetic diversity of Ghanaian cowpea landraces reside within accessions on the basis that 86.6% genetic differentiation was observed within accessions. This suggests important levels of gene flow linking accessions. Overall gene flow estimate within accession was 1.62 This value which is greater than 1.0 indicates that there is sufficient gene flow to prevent local differentiation due to genetic drift (Slatkin, 1987) Therefore, the differences in allele frequencies of Ghanaian cowpea landraces could be due to selection.

If all factors are equal, absence of local genetic differentiation in this study suggests that genetic variation within Ghanaian cowpea landraces may be adequately preserved in a germplasm bank by incorporating relatively few but more seeds of individual accessions.

4.3.2 Meristic distance (D_m) and cluster analysis (dendrogram)

D_m values based on meristic data are closest between accessions 87/81 and 87/77 (D=0.010) both from Sudan Savannah zone; and farthest between accessions 87/55 (a Guinea Savannah type) and 87/157 (a Deciduous Forest type) (D_m =0.378) (Table 9 and fig.5) Based on meristic data, genetic divergence among Deciduous Forest accessions ranged from 0.063 to 0.129 (mean D_m=0.103±0.017); that among Guinea Savannah accessions ranged from 0.047 to 0.190 (mean D_m=0.123±0.034 and among Sudan Savannah accessions D_m ranged from 0.010 to 0.057 (mean D_m=0.038±0 012)

Dendrogram for meristic data shows only one cluster Guinea and Sudan Savannah accessions show greater similarity Accession 87/55 (a Guinea Savannah type) is most diverse. All savannah accessions were fairly close in distance, this suggests that these accessions bear some meristic resemblance. This resemblance might be due to the fact that the accessions from the savannah zones have the same extent environmental influence. Generally there were no distinct differences among the accessions. This observation might be due to the fact that the 14 morphological markers used to characterize the accessions have little genetic components.

4.3.3 Genetic distance (D_m) and cluster analysis (dendrogram)

D_m values based on allozyme data were closest between 87/37 and 87/30 (D_m=0.101) from Guinea savannah zone; and farthest between 87/81 (Sudan savannah accession) and 87/142 (Deciduous forest accession) (D_m=0.397) Genetic divergence among Deciduous forest accession ranged from 0.130 to 0.167 (mean D_m=0.143±0.010); that among Guinea savannah accession ranged from 0.101 to 0.190 (mean D_m=0.140±0.021) and that among Sudan savannah accession ranged from 0.163 to 0.285 (mean D_m=0.235±0.030)

The dendrogram for allozyme data (Fig.6) shows two main clusters. Accessions from Guinea and Sudan savannahs are closer and form one cluster, whilst Deciduous forest zone accessions form a separate cluster. Accession 87/77 (a Sudan savannah type)is most highly diverse. However it cannot be concluded that it is the genetic source for the other eight accessions, since the dendrogram is unrooted. Diversity within Sudan savannah accessions is the greatest. It is evident that accessions from the Sudan savannah zone should serve as a good source for improvement of the sum of genetic variability in materials used in cowpea breeding. The Sudan savannah zone should therefore receive serious consideration during collection of Ghanaian cowpea germplasm.

4.4 Correlation Analysis

4.4.1 Environmental correlates of allozymic variation

Genetic diversity within a population of plants may be created

and maintained by several mechanisms. One of the most common mechanisms is differential habitats and micro-habitats (life-zone) within the area occupied by the population (Noy-Meir *et al.*, 1991). In most terrestrial environments there is a certain degree of local heterogeneity in at least some soil and vegetation variables, which could conceivably result in differential selection pressures within plant populations. However, the hypothesis that such factors are indeed responsible for the genetic polymorphism observed within a particular population must be supported by positive specific evidence (Noy-Meir *et al.*, 1991). If protein and other molecular polymorphisms 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 and

(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).

In a study of microgeographical variation in allozyme frequencies in *Avena barbata*, Hamrick and Allard (1972) found that allele frequencies of various enzymes had similar patterns of variation over the geographical area studied. They concluded that the pattern of genetic variability of the species were best explained by Neo-Darwinian evolutionary models in which selection plays a predominant role. In a study of natural selection of allozyme polymorphism in wild emmer wheat, Nevo *et al.*(1991) observed that allozyme patterns in wild Emmer wheat were structured in accordance with ecological heterogeneity in space and secondarily over time of the test site. In a different study of protein polymorphism in wild soyabean *Glycine soja* in China, Xu Bao *et al.*, (1993) observed that there was significant correlation between protein polymorphism and longitude.

In the present study some allozyme frequencies were observed to be significantly correlated with geographical, temperature and moisture factors. For instance, alleles HK1*-4, MDH1*-5, PGDH2*-3, PGDH2*-1, MEP1*-4, and IDHP1*-4 were positively and significantly correlated with longitude (see Table 11) By partial correlation analysis allele HK1*-4 and longitude were correlated by removing the effects of alleles MDH1*-5 ($r=0.83$, d.f.= 199, $P<0.01$), PGDH2*-3 ($r=0.84$, d.f.= 199, $P<0.01$), PGDH2*-1 ($r= 0.85$, d.f.=199, $P<0.01$), MEP1*-4 ($r=0.84$, d.f.=199, $P<0.01$) and IDHP1*-4 ($r=85$, d.f.=199, $P<0.01$) By partial correlation analysis allele MDH1*-4 and longitude were correlated by removing the effects of alleles HK1*-4 ($r=0.38$, d.f.= 199, $P<0.01$), PGDH2*-3 ($r=0.45$, d.f.= 199, $P<0.01$),

PGDH2*-1 (r= 0.50, d.f.=199, P<0.01), MEP1*-4 (r=0.46, d.f.=199, P<0.01) and IDHP1*-4 (r=49, d.f.=199, P<0.01) By partial correlation analysis allele PGDH2*-3 and longitude were correlated by removing the effects of alleles HK1*-4 (r=0.28, d.f.= 199, P<0.01), MDH1*-5 (r=0.45, d.f.= 199, P<0.01), PGDH2*-1 (r= 0.52, d.f.=199, P<0.01), MEP1*-4 (r=0.49, d.f.=199, P<0.01) and IDHP1*-4 (r=0.51, d.f.=199, P<0.01) By partial correlation analysis allele PGDH2*-1 and longitude were correlated by removing the effects of alleles MDH1*-5 (r=0.33, d.f.= 199, P<0.01), PGDH2*-3 (r= 0.37, d.f.=199, P<0.01), MEP1*-4 (r=0.38, d.f.=199, P<0.01) and IDHP1*-4 (r=0.41, d.f.=199, P<0.01), however the correlation between these two variables was not real after removing the effect of allele HK*1-4 (r=0.09, d.f.=199, P>0.01). By partial correlation analysis allele MEP1*-4 and longitude were correlated by removing the effects of alleles HK1*-4 (r=0.23, d.f.= 199, P<0.01), MDH1*-5 (r=0 42, d.f.= 199, P<0.01), PGDH2*-3 (r= 0.44, d.f.=199, P<0.01), PGDH2*-1 (r= 0.49, d.f.=199, P<0.01), and IDHP1*-4 (r=0.49, d.f.=199, P<0.01) By partial correlation analysis allele IDHP1*-4 and longitude were correlated by removing the effects of alleles MDH1*-5 (r=0.36, d.f.= 199, P<0.01), PGDH2*-3 (r= 0.38, d.f =199, P<0.01), PGDH2*-1 (r= 0 40, d.f.=199, P<0.01), MEP1*-4 (r=0.40, d.f.=199, P<0.01) and IDHP1*-4 (r=0.49, d.f.=199, P<0.01) There was no real correlation between IDHP1*-4 and longitude by removing the effect of allele HK1*-4 (r=0.12, d.f.=199, P>0.01) Generally there was real correlation between alleles HK1*-4, MDH1*-5, PGDH2*-3, MEP1*-4, and longitude, however association between alleles

PGDH2*-1, IDHP1*-4 and longitude was not real but was the result of their common association with the other alleles. Alleles IDHP2*-3, MEP1*-6, and PGDH3*-1 were positively and significantly correlated with latitude, while allele MDH1*-4 and latitude were negatively and significantly correlated (see Table 11) By partial correlation analysis allele IDHP2*-3 and latitude were correlated by removing the effects of alleles MDH1*-4 (r=0.39, d.f.=199, P<0.01), MEP1*-6 (r=0.47, d.f.=199, P<0.01), PGDH3*-1, (r=0.44, d.f.=199, P<0.01) By partial correlation analysis allele MDH1*-4 and latitude were correlated by removing the effects of alleles IDHP2*-3 (r=0 74, d.f.=199, P<0.01), MEP1*-6 (r=0 72, d.f.=199, P<0.01) and PGDH3*-1, (r=0.99, d.f.=199, P<0.01) By partial correlation analysis allele MEP1*-6 and latitude were correlated by removing the effects of alleles IDHP2*-3 (r=0.42, d.f.=199, P<0.01), MDH1*-4 (r=0.35, d.f.=199, P<0.01) and PGDH3*-1, (r=0.41, d.f.=199, P<0.01) By partial correlation analysis allele PGDH3*-1 and latitude were correlated by removing the effects of alleles IDHP2*-3 (r=0.62, d.f.=199, P<0.01), MDH1*-4 (r=0.56, d.f =199, P<0.01), MEP1*-6 (r=0.63, d.f.=199, P<0.01) There is therefore a real association between alleles IDHP2*-3, MEP1*-6, PGDH3*-1 MDH1*-4 and latitude. Allele PGDH3* 1 was positively and significantly correlated with minimum temperature (Table 11) Alleles PGDH1*-1, PGDH2*-5 were positively and significantly correlated with annual rainfall, while alleles HK1*-4, IDHP2* 3, MDH1*-5 and PGDH3*-1 were negatively and significantly correlated with annual rainfall (see Table 11) By partial correlation analysis allele HK1*-1 and annual rainfall were

correlated by removing the effects of alleles IDHP2*-3 (r=0.45, d.f.=199, P<0.01), MDH1*-5 (r=0.38, d.f.=199, P<0.01), PGDH1*-1 (r=0.43, d.f.=199, P<0.01), PGDH2*-5 (r=0.45, d.f.=199, P<0.01) and PGDH3*-1 (r=0.31, d.f.=199, P<0.01) By partial correlation analysis allele IDHP2*-3 and annual rainfall were correlated by removing the effects of alleles HK1*-1 (r=0.41, d.f.=199, P<0.01), MDH1*-5 (r=0.35, d.f.=199, P<0.01), PGDH1* 1 (r=0.40, d.f.=199, P<0.01), PGDH2*-5 (r=0.43, d.f.=199, P<0.01) and PGDH3*-1 (r=0.28, d.f.=199, P<0.01) By partial correlation analysis allele MDH1*-5 and annual rainfall were correlated by removing the effects of alleles HK1*-1 (r=0.55, d.f.=199, P<0.01), IDHP2*-3 (r=0.56, d.f.=199, P<0.01), PGDH1*-1 (r=0.55, d.f.=199, P<0.01), PGDH2*-5 (r=0.57, d.f =199, P<0.01) and PGDH3*-1 (r=0.46, d.f.=199, P<0.01) By partial correlation analysis allele PGDH1*-1 and annual rainfall were correlated by removing the effects of alleles HK1*-1 (r=0.46, d.f.=199, P<0.01), IDHP2*-3 (r=0.47, d.f.=199, P<0.01), MDH1*-5 (r=0.40, d.f.=199, P<0.01), PGDH2*-5 (r=0.47, d.f.=199, P<0.01) and PGDH3*-1 (r=0.34, d.f.=199, P<0.01) By partial correlation analysis allele PGDH2*-5 and annual rainfall were correlated by removing the effects of alleles HK1*-1 (r=0.43, d.f =199, P<0.01), IDHP2*-3 (r=0.44, d.f.=199, P<0.01), MDH1*-5 (r=0.37, d.f.=199, P<0.01), PGDH1*-1 (r=0 42, d.f.=199, P<0.01) and PGDH3*-1 (r=0.30, d.f.=199, P<0.01) By partial correlation analysis allele PGDH3*-1 and annual rainfall were correlated by removing the effects of alleles HK1*-1 (r=0.66, d.f.=199, P<0.01), IDHP2* 3 (r=0.66, d.f.=199, P<0.01), MDH1*-5 (r=0.62, d.f.=199, P<0.01), PGDH1* 1

(r=0.65, d.f.=199, P<0.01) and PGDH2*-5 (r=0.67, d.f.=199, P<0.01) Therefore from partial correlation analysis it could be concluded that there was real association between the alleles HK1*-4, IDHP2*-3, MDH1*-5, PGDH1*-1, PGDH2*-5, PGDH3*-1 and annual rainfall. Most different alleles were found to be associated with different local populations. Repeatability of certain allozymes within and between populations of agroecological zones was evident (eg. IDHP1*-6, MDH1*-2, PGDH2*-8, PGDH2* 1) (see Table 6). Different alleles were characteristic of different agroecological zones (eg. HK1*-4, 1DHP2*-5, MDH2*-8, MDH3*-7, PGM2*-4, PGM2*-3) (see Table 6) There was evidence of cline effect, for example, frequencies of alleles AK1*-3, MDH3*-6, MDH4*-3, MDH4*-4 and PGM1*-3 showed clinal effects from Deciduous forest zone to Sudan savannah zone the widest ecogeographical area materials studied were collected (see Table 6). Frequencies of alleles MEP2*-1, PGDH1*-5 and PGI2*-5 showed clinal effect from Sudan savannah zone to Deciduous forest zone.

In summary there was evidence here to suggest that genetic diversity in Ghanaian cowpea landraces is related to ecogeographic parameters and was, for example, partially attributed to geographical, temperature and moisture factors. The findings of this work therefore, do not favour the neutralist theory of selection.

Natural selection may operate through differential viabilities, survival and fecundities of genotypes in a population and since relative performance of genotypes in these respects varies with environment (Breese,1989), multiplication and

regeneration of Ghanaian cowpea landraces by curators and cowpea breeders should be carried out as near to the region of natural distribution as possible, and factors causing genetic shifts should be minimized so as to maintain the integrity of heterozygous and heterogeneous populations of cowpea landraces.

4.4.2 Morphological correlates of allozymic variation

Selfing species experience slower "decay" of linkage disequilibrium, increase in the variance of coancestry and direct gene expression due to increased homozygosity all these factors are believed to show increased associations among isozyme and phenotypic variation (Brown *et al.*, 1990) Price *et al* (1984) compared estimates of population differentiation based on allozyme polymorphism and morphometric traits in the self-pollinated species *Avena barbata, Hordeum vulgare* and *H. jubation* and an outcrossing species, *Clarkia williamsonii.* They found that rank correlations between morphometric and allozyme distance measures were significant for the selfing species. In this study, correlations between the morphometric and some allozyme frequencies were highly significant for cowpea accessions. From the results cowpea breeders can screen for the following cowpea agronomic characters at the accompanying loci. percentage seed germination (PGI2*), days to pod maturity (IDHP2*, MDH1* and MDH4*), pod length (AK2* and MDH3*), 50-mean seed weight (HK1* and PGDH2*), percentage pod damage by legume pod borer (PGDH1*), fecundity rate of *Callosobruchus maculatus* infestation of seed (PGDH3*) and percentage seed set

(MEP1*)

It is quite evident that the use of isozyme as gene markers for quantitative traits in cowpea breeding is possible and can lead to the discovery of new genes for desirable agronomic traits.

4.4.3 Environmental correlates of morphological variation

Out of 156 correlates between morphological and environmental factors 10 (6.4%) were significant ($r=0.8397-0.8684$; $P<0.01$) (Table 13) Percentage seed damage by insect showed significantly negative correlation with geographical factors (L_t), and showed significantly positive correlation with moisture factors (R_d), Rh1500, Rh0600, P1500, P0600) and temperature factors (Cl, S_m) Fecundity rate of *Callosobruchus maculatus* infestation of seed showed significantly positive correlation with temperature factor (T_{min}) Days to pod maturity showed significant correlation with temperature factor (S_m) Percentage seed damage by insect was scored as a pre-harvest factor. From the experimental results it is low at relatively high altitudes but high at lower altitudes (Table 13) To produce healthy seeds and thus increase yield cowpea farmers at low altitudes must employ measures to control insect activities during the time that the plants are forming pod. However, cowpea breeders should breed for cowpea varieties that are tolerant to insect infestation for farmers' use. One important source of post-harvest loss in cowpea is *C. maculatus* activity From the experimental observations high temperatures are likely to

promote *C. maculatus* activity. Therefore to reduce post-harvest loss as a result of *C. maculatus* activity cowpea seeds must be stored under cold conditions after harvest. An alternative measure is that cowpea breeders must breed for cowpea varieties which are tolerant to *C. maculatus* infestation.

4.5 Multiple Regression Analysis

4.5.1 Environmental predictors of genetic differentiation in cowpea

The present study demonstrates that the following macro-climatic factors are associated with allozymic differentiation: (i) geographic (ii) temperature (iii) moisture.

Over all accessions the three variable combination of cloudiness, monthly minimum temperature and longitude explains significantly variation in the following allozymes: IDHP1*-6 (0.959) and MDH4*-7 (0.959). the four variable combination of monthly cloudiness, minimum temperature, mean monthly rainfall and longitude explains significantly variation in the following allozymes (Table 14) HK1*-3 (0.977) and HK1*-2 (0.985) HK1*-1 (0.999) and 1DHP2*-3 (0.996) MDH1*-4 (0.977), MDH2*-2 (0.994) MDH3*-3 (0.998), 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); however explanation of variation in the following allozymes approached significance: MDH1*-4 (0.977), MEP1*-6 (0.975), MEP1*-5 (0.979) Expected heterozygosity variation at the following loci was also explained significantly AK2* (1.000) and HK1* (0.992),

these variables also explained significantly the variance of percentage loci polymorphic(1.000). On the basis of the above evidence it can be concluded that among ecological factors, the critical ones for genetic differentiation in Ghanaian cowpea landraces appear to be those with impact on geographical distribution, temperature and moisture.

4.5.2 Environmental predictors of cowpea morphological characters

Days to flowering, pod maturation period and percentage pod set in cowpea may be directly selected as monthly cloudiness, monthly minimum temperature, annual rainfall and longitude. Days to flowering and pod maturation period in the accessions studied decrease with annual rainfall and mean monthly total cloud but decreases with increase in longitude and monthly temperature. Over all accessions, the variable combination of mean monthly rainfall, mean monthly total cloud, longitude and mean monthly minimum temperature explain significantly variation in these three agronomic characters (Table 14): days to flowering (0.993), pod maturity period (1.000) and percentage pod set (0.996) Effect of longitude, suggests a photoperiodic effect on flowering patterns of cowpea; it can also be suggested that early flowering in cowpea probably evolved with low rainfall patterns.

In conclusion, longitude, monthly total cloud, mean monthly minimum temperature and annual rainfall in combination may

adaptively affect flowering time, maturation time and percentage seed set in cowpea.

4.6 Integrative Discussion

A record of phenotypes and their variation patterns in space and time is an integral and important part of the inventory in conservation of germplasm. Cowpea phenotypes of the quantitative characters studied showed a lot of variation. In all the characters studied, the accessions fell into at least two separate phenotypic populations. The phenotypic variation reflects the high genetic polymorphism [21 out of 22 loci (99.5%) were found to be polymorphic] and high genetic mean heterozygosity (0.561 $\pm$ 0.04) observed. Accessions therefore showed high heterozygosity and heterogeneity Therefore Ghanaian cowpea germplasm seeds should be disproportionately represented in germplasm banks in relation to phenotypic population types. Preservation of the largest number of phenotypes would therefore add to the variation sampled by the biochemical methods and would guarantee conservation of greater diversity

Twenty-four percent outcrossing was observed in the studies. This rate of outcrossing is considered large enough to generate considerable heterozygosity in Ghanaian cowpea used for the studies. This mating mechanism, among other factors, possibly accounts for the level of phenotypic heterogeneity observed in the cowpea accession used for the study Therefore during regeneration of Ghanaian cowpea landraces it may be necessary for curators and

plant breeders to treat them as outcrossings so as to maintain the integrity, gene, genotype and phenotype constitution and hence preserve the differences between accessions.

Based on allozyme data, accessions from the Savannah zones formed one separate cluster from those of the Deciduous forest zone. Based on meristic data, though one cluster was formed by all accessions, the Savanna zone accessions were grouped together, while the Deciduous forest zone accessions were also closer together By the cluster analysis Savanna accessions, 87/55 and 87/77, were most diverse based on meristic and allozyme data respectively Therefore it is evident that accessions from the Savannah zones should serve as good source for improvement of the sum of genetic variability in materials used in cowpea breeding. It is also possible that the Savannah zones of Ghana are among the centres of genetic diversity of cowpea germplasm and should receive serious consideration during collection of Ghanaian cowpea germplasm.

In the studies both some allozyme frequencies and morphological factors were observed to be significantly correlated with geographical, temperature and moisture factors. For instance, alleles HK1*-4, MDH1*-5, PGDH2*-3, PGDH2*-1, MEP1*-4, IDHP1*-4 and PGM2*-3 and percentage seed damage by insect were significantly correlated with longitude, positively and negatively respectively There were highly significant correlations between the morphometric and allozyme frequencies. For example, pod maturation period was significantly correlated positively with alleles FH1*-1, MDH2*-1,

MDH1*-4, MDH4*-1 and PGM2*-1. This shows that both phenotypic and allozymic variations follow a similar pattern. However they both vary dynamically with ecogeographical parameters attributed to geographical, temperature and moisture factors. Therefore multiplication and regeneration of Ghanaian cowpea landraces by curators and cowpea breeders should be carried out as near to the region of natural distribution as possible, and factors causing genetic shifts should be minimized so as to maintain the integrity of heterozygous and heterogeneous populations of cowpea land races.

EXTENDED SUMMARY

Nine cowpea landraces (accessions) representative from three agroecological zones of Ghana namely, Deciduous forest, Guinea savannah, and Sudan savannah were used for the study. The accessions were 87/139, 87/142, 87/157 (from Deciduous forest zone), 87/30, 87/37, 87/55 (from Guinea savannah zone) and 87/77, 87/81 and 87/83 (from Sudan savannah zone) The study was undertaken to study genetic structure and diversity among these cowpea landraces.

A study of morphological variability was undertaken based on the following 13 characters: percentage seed germination, days to flowering, percentage pod set, pod maturation period, pod length, percentage pod damage by legume pod borer, number of seeds per pod, number of ovules per pod, seed weight, percentage seed set, percentage seed damage by insect, fecundity rate of *Callosobruchus maculatus* infestation of seed and percentage leaf spots cover

Highest percentage seed germination of 95.0±0.03% was registered in accession 87/142 of Deciduous forest type ,while the lowest value of 70.0±0.07% was registered in accessions 87/157 and 87/30 (Deciduous forest and Guinea savannah types)

The lowest value of 33.0±0.67 for the number of days to flowering was registered in accession 87/83 a Sudan savannah type, while the highest value of 53.0±0.67 was registered in accession 87/142 a Deciduous forest type.

Accession 87/139 gave the highest value of 56.1±0.11% for percentage pod set per plant, while the lowest value of 21.1±0.09% was given by accession 87/83.

Accession 87/157 gave the highest value of 71.0±0.45 for pod maturation period, while accessions 87/37 and 87/83 gave the lowest value of 57.0±0.22.

Accession 87/142 gave the highest value of 22.43±0.66 cm for pod length, while accession 87/83 gave the lowest value of 10.93±0.37 cm.

Accession 87/139 gave the highest mean value of 54.0±0.95% for percentage pod damage by legume pod borer, while accessions 87/30 and 87/37 gave the lowest value of 16.0±0.03% respectively

The highest mean value of 16.0±0.39 for the number of seeds per pod was registered in accession 87/142 ,while accession 87/83 gave the lowest mean value of 9.0±0.52

Highest mean value of 18.0±0.39 for number of ovules per pod was recorded for accession 87/142, while the lowest mean value of 13.0±0.39 was recorded for accessions 87/37 and 87/83

Accession 87/81 gave the highest mean value of 94.0±0.01% for percentage seed set, while accession 87/83 gave the lowest mean value of 71.0±0.11%.

Accession 87/139 gave the highest mean value of 14.59±0 43 g for 50- seed mean weight, while accession 87/157 gave the lowest mean value of 9 76.0±0.09 g.

Accession 87/142 gave the highest mean value of 12.0±0.01% for percentage seed damage by insect, while accessions 87/30 and 87/83

gave the lowest mean value of 6.0.0±0.01%.

Accession 87/30 gave the highest mean value of 58.20±0.06% for fecundity rate of *Callosobruchus maculatus* infestation of seed, while accession 87/142 gave the lowest mean value of 26.0±0.06%

Accession 87/139 gave the highest mean value of 16.6±0.05% for percentage leaf spot cover, while accession 87/142 gave the lowest mean value of 0.01±0.00%.

Soluble proteins of 14 to 30 day-old leaves of a minimum of 15 individuals from each accession were subjected to starch gel electrophoresis. The following nine soluble cowpea proteins were assayed: Adenylate kinase, Fumarate hydratase, Hexokinase, Isocitrate dehydrogenase, Malate dehydrogenase, Malic enzyme, 6-phosphoglucose dehydrogenase, Phosphoglucoisomerase and phosphoglucomutase.

Twenty-two loci were encoded by the nine enzyme systems. A total of 110 different alleles were distributed among the 22 loci for an average of 5.00 alleles per locus. 21 out of 22 (95.5%) loci were found to be polymorphic. Expected heterozygosity ranged from 0.549-0.599 with an overall mean of 0.561±0.005. Mean observed heterozygosity was 0.264±0.007

Discriminating loci were also studied. Among Deciduous forest accessions, 87/139 was discriminated from accessions 87/1423 and 87/157 at six different loci, among accessions from Guinea savannah, accession 87/30 was discriminated from accessions 87/37 and 87/55 at seven different loci, while accessions 87/37 and 87/55 were discriminated from each other at six different loci; and among

accessions from Sudan savannah, accessions 87/77 was discriminated from accessions 87/81 and 87/83 at ten different loci respectively, while, accessions 87/81 and 87/83 were discriminated from each other at six different loci. Treating accessions from each ecogeographical zone as unit groups, Deciduous forest zone accessions (87/139, 87/142 and 87/157) could be discriminated from Guinea savannah zone accessions (87/30, 87/37 and 87/55) at locus IDHP2* Deciduous forest accessions as a unit group were discriminated from Sudan savannah accessions (87/77, 87/81 and 87/83) as a unit group at loci HK1*, and PGDH3* Guinea savannah zone accessions as a unit group were discriminated from Sudan savannah accessions as unit group at loci MEP1*, PGDH2* and PGM2*

Most of the 21 polymorphic loci deviated significantly from Hardy-Weinberg proportions and 19 loci gave positive values for Wright's fixation index with an average of 0.573 ± 0.096.
An average rate of 0.244 ± 0.064 outcrossing was observed; heterozygote deficiencies were observed and attributed to natural selection and some degree of inbreeding.
A proportion of 0.867 of total genetic variation was found to reside within accessions of Ghanaian cowpea and proportion of total genetic variation due to genetic differentiation among accessions was 0.133. An overall gene flow estimate of 1.62 was found within cowpea accessions. Panmictic heterozygosity of 0.622 was lowest in Deciduous forest accessions but a highest value of 0.632 was found in Sudan savannah accessions.

Cluster analysis for allozyme data showed two main clusters. Accessions from Guinea savannah and Sudan savannah were closer and formed one cluster, while accessions from Deciduous forest zone formed a separate cluster.

Genetic distance among Deciduous forest accessions ranged from 0.130 to 0.167 with a mean genetic distance of 0.143±0.10. Based on meristic data it ranged from 0.063 to 0.129 with a mean meristic distance of 0.103±0.017

Genetic distance among accessions from Sudan savannah zone ranged from 0.163 to 0.285 with a mean genetic distance of 0.140±0.021. Based on meristic data, meristic divergence ranged from 0.10 to 0.057 with a mean distance of 0.037±0.012.

Genetic distance among accessions from Sudan savannah zone ranged from 0.163 to 0.285 with a mean genetic distance of 0.235±0.010. Based on meristic data, meristic divergence ranged from 0.010 to 0.057 with a mean distance of 0.038±0.012.

Accession 87/77 (Sudan savannah type) was most diverse.

Dendrogram for meristic data showed one main cluster and accession 87/55 (a Guinea savannah type) was most diverse.

DEDUCTIONS AND RECOMMENDATIONS

Twenty-four per cent rate of outcrossing observed in the study is large enough to generate considerable heterozygosity in Ghanaian cowpea landraces, therefore during regeneration of Ghanaian cowpea landraces it may be necessary for curators to treat them as outcrossers so as to maintain the integrity, gene and genotype constitution and hence preserve the differences between accessions (Breese,1989).

Amount of genetic diferrentiation among genetic population of plants is a useful guide to what proportion of accessions and individual seeds a curator can represent in a plant germplasm bank. From the study, greatest percentage of total genetic diversity of Ghanaian cowpea landraces on the average, reside within accessions on the basis that 86.6% genetic differentiation was observed within accessions. The absence of local genetic differentiation in this study suggests that genetic variation within Ghanaian cowpea landraces may be adequately preserved in a germplasm bank by incorporating relatively few but more seeds of individual accessions.

Absence of differentiation among accessions further suggests important levels of gene flow (1.62) linking accessions. This value which is greater than 1.0 indicates that there was sufficient gene flow to prevent local differentiation due to genetic drift (Slatkin, 1987) Therefore, the differences in allele frequencies of Ghanaian cowpea landraces could be due to selection.

Genetic diversity in cowpea accessions was in accordance

with natural selection and was affected by climatic and humidity factors. Alleles HK1*-4, MDH1*-4, PGDH2*-3, PGDH2*-1 and PGM2*-4 had high frequencies at higher longitudes while 1DHP1*-4 and PGDH4*-1 had higher frequencies at lower longitudes. Alleles IDHP2*-3, MEP1*-6 AND PGDH3*-1 were of higher frequencies at higher latitudes while MDH1*-1 had lower frequency at lower latitude. Alleles MEP1*-6 and PGDH3*-1 had higher frequencies at high mean sun days while MDH1*-4 and PGM1*-2 had lower frequencies at low mean sun days. Allele PGDH3*-1 had higher frequency at high maximum temperature while allele MDH2*-5 had high frequency at low maximum temperature. Alleles PGDH1*-1 and PGDH2*-5 had higher frequencies at high mean monthly rainfall, while alleles HK1*-4, 1DHP2*-3, MDH1*-5 and PGDH3*-1 were of high frequencies at low annual rainfall; while alleles 1DHP2*-3, MEP1*-6 and PGDH3*-1 were of high frequencies at low rainday; alleles MDH1*-4 and PGM2*-1 had high frequencies at high relative humidity at 1500hrs while, alleles PGDH1*-1 and PGDH3*-1 had low frequencies at low relative humidity at 1500hrs. Alleles 1DHP2*-3 and MDH1*-4 had high frequencies at high relative humidity at 0600hrs. Allele MDH1*-4 had high frequency high pressure 1500hrs, while allele MEP1*-6 had high frequency at low pressure at 1500hrs. Alleles MDH1*-4 and PGDH3*-1 had high frequencies at low pressure at 0600hrs. Allele PGDH3*-1 had low frequency at low cloud.

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.

Diversity within Sudan savannah accessions is the greatest. It is evident that accessions from the Sudan savannah zone should serve as a good source for improvement of the sum of genetic variability in materials used in cowpea breeding. The Sudan savannah zone should therefore receive serious consideration during collection of Ghanaian cowpea germplasm.

Allozymic loci can be used by cowpea breeders to screen for the accompanying cowpea agronomic traits: AK2* and MDH3* (long pods) HK1* and PGDH2* (high seed weight); 1DHP2* and MDH4* (early pod maturation time) MDH1* (late pod maturation time) PGI2* (high percentage seed germination, high seed viability); PGDH1* (resistance to legume pod borer attack) and PGDH3* (susceptibility to *Callosobruchus maculatus* infestation of seeds) and MEP1* (high percentage seed set)

Appendix 1

Summary variability in percentage seed germination among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	90.0±0.05
87/142	95.0±0.03
87/157	70.0±0.07
87/30	70.0±0.07
87/37	80.0±0.06
87/55	75.0±0.07
87/77	85.0±0.06
87/81	85.0±0.06
87/83	75.0±0.07

Appendix 2

Summary variability in number of days to flowering among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	50.0 ± 1.12
87/142	53.0 ± 0.67
87/157	51.0 ± 0.89
87/30	34.0 ± 1.57
87/37	36.0 ± 1.79
87/55	39.0 ± 1.79
87/77	45.0 ± 1.12
87/81	45.0 ± 0.67
87/83	33.0 ± 0.67

Appendix 3

Summary variability in percentage pod set per plant among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	56.1 ± 0.11
87/142	33 7 ± 0.11
87/157	38.6 ± 0.11
87/30	21.6 ± 0.09
87/37	39.0 ± 0.11
87/55	26.6 ± 0.10
87/77	45.3 ± 0.11
87/81	34.8 ± 0.11
87/83	21.1 ± 0.09

Appendix 4

Summary variability in pod maturation period among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	70.0 ± 0.22
87/142	70.0 ± 0.22
87/157	71.0 ± 0.45
87/30	59.0 ± 0.22
87/37	57.0 ± 0.22
87/55	59.0 ± 0.89
87/77	59 0 ± 0.00
87/81	59.0 ± 0.00
87/83	57.0 ± 0.22

Appendix 5

Summary variability in pod length(cm) among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	14.64 ± 0.49
87/142	22.42 ± 0.66
87/157	15.59 ± 0.16
87/30	11.97 ± 0.29
87/37	11.80 ± 0.22
87/55	12.58 ± 0.40
87/77	14.58 ± 0.29
87/81	15.01 ± 0.29
87/83	10.93 ± 0.37

Appendix 6

Summary variability in percentage pod damage by legume pod borer among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	54.0 ± 0.95
87/142	26.0 ± 0 40
87/157	36.0 ± 0.04
87/30	16.0 ± 0.03
87/37	16.0 ± 0.03
87/55	23.0 ± 0.04
87/77	22.0 ± 0.04
87/81	26.0 ± 0.04
87/83	26.0 ± 0.04

Appendix 7

Summary variability in number of seeds per pod among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	12.0 ± 0.39
87/142	16.0 ± 0.39
87/157	14.0 ± 0.39
87/30	10.0 ± 0.39
87/37	10.0 ± 0.26
87/55	11.0 ± 0.52
87/77	14.0 ± 0.39
87/81	15.0 ± 0.39
87/83	9.0 ± 0.52

Appendix 8

Summary variability in number of ovules per pod among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	14.0 ± 0.39
87/142	18.0 ± 0.39
87/157	17.0 ± 0.26
87/30	14.0± 0.13
87/37	13.0 ± 0.26
87/55	14.0 ± 0.39
87/77	15.0 ± 0.39
87/81	16.0 ± 0.39
87/83	13.0 ± 0.39

Appendix 9

Summary variability in percentage seed set among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	88.3 ± 0.01
87/142	87.9 ± 0.01
87/157	77.1 ± 0.01
87/30	72.0 ± 0.01
87/37	77.1 ± 0.05
87/55	75.6 ± 0.01
87/77	91.5 ± 0.01
87/81	94.0 ± 0.01
87/83	71.9 ± 0.11

Appendix 10

Summary variability in 50-mean seed weight among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	14.59 ± 0.43
87/142	13.92 ± 0.61
87/157	9 76 ± 0.09
87/30	10.46 ± 0.23
87/37	11.74 ± 0.38
87/55	11.97 ± 0.16
87/77	13.85 ± 0.31
87/81	12.54 ± 0 47
87/83	13.19 ± 0.68

Appendix 11

Summary variability in percetage seed damage by insect among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	10.0 ± 0.01
87/142	12.0 ± 0.01
87/157	10.0 ± 0.01
87/30	6.0 ± 0.00
87/37	7.0 ± 0.01
87/55	8.0 ± 0.01
87/77	9.0 ± 0.00
87/81	8.0 ± 0.00
87/83	6.0 ± 0.01

Appendix 12

Summary variability in fecundity rate of *Callosobruchus maculatus* infestation of seed among cowpea accessions studied.

ACCESSIONS	Mean ± standard error
87/139	35.4 ± 0.06
87/142	26.0 ± 0.06
87/157	33.0 ± 0.06
87/30	58.2 ± 0.06
87/37	36.0 ± 0.06
87/55	42.3 ± 0.06
87/77	56.4 ± 0.06
87/81	47 7 ± 0.06
87/83	34.9 ± 0.06

Appendix 13

Summary variability in percentage leaf spots cover among cowpea accessions studied.

ACCESSIONS	Mean ± standard error %
87/139	16.6 ± 0.05
87/142	0.01 ± 0.00
87/152	0.04 ± 0.00
87/30	4.8 ± 0.03
87/37	2.4 ± 0.02
87/55	2.0 ± 0.02
87/77	2.0 ± 0.02
87/81	5.0 ± 0.03
87/83	10.9 ± 0.04

APPENDIX 14

A. STAINING RECIPES

1. AK

40mg	Glucose
30mg	Adenosine diphosphate (ADP)
7.5mg	Nicotinamide adenine dinucleotide phosphate (NADP)
20mg	Magnesium Chloride ($MgCl_2$) Hexokinase/Glucose-6-phosphate dehydrogenase (HK/G6PDH)
30mls	Tris-HCl (0.2M) pH 8.0
Trace	MTT
Trace	PMS

2. FH

30mg	Nicotinamide adenine dinucleotide (NAD)
200mg	Fumic acid
30mls	Tris-HCl (0.2M) pH 7 0
50μl	Malate dehydrogenase (MDH)
Trace	MTT
Trace	PMS

3. HK

60mg	Glucose
100mg	$MgCl_2$
15mg	Adenosine triphosphate (ATP)
5mg	NADP
30mls	Tris-HCl (0.2M) pH 7.0
20μl	G6PDH
7mg	MTT
Trace	PMS

Appendix 14 cont'd

STAINING RECIPES

4. IDHP

45mg	Sodium isocitric acid
10mg	$MgCl_2$
10mg	NADP
30mls	Tris-HCl (0.2M) pH 8.0
7mg	MTT
Trace	PMS

5. MDH

150mg	L-Malic acid
10mg	NAD
600mg	Tris
30mls	Distilled water
6mg	MTT
Trace	PMS

6. ME

60mg	DL-Malic acid
10mg	$MgCl_2$
10mg	NADP
30mls	Tris-HCl (0.2M) pH 8.0
7mg	MTT
Trace	PMS

Appendix 14 cont'd

STAINING RECIPES

7 PGDH

30mg	6-phosphogluconate
10mg	$MgCl_2$
10mg	NADP
30mls	Tris-HCl (0.2M) pH 8.0
7mg	MTT
Trace	PMS

8. PGI

20mg	Fructose-6-phosphate
10mg	$MgCl_2$
10mg	NADP
30mls	Tris-HCl (0.2M) pH 8.0
50μl	G6PDH
7mg	MTT
Trace	PMS

9 PGM

50mg	Glucose-1-phosphate
70mg	$MgCl_2$
3mg	NADP
30mls	Tris-HCl (0.2M) pH 8.0
50μl	G6PDH
7mg	MTT
Trace	PMS

Appendix 14 cont'd

B: BUFFER SOLUTIONS

1. 0.2m Continuous-Tris-Citrate (CTC)

30.29g Tris

11.98g Citric acid

Distilled water to make 1 litre and pH adjusted to 8.0.

2. 0.2m Tris-HCl

24.228g Tris

Distilled water to make 1 litre and pH adjusted down with conc. HCL to 8.0 or 7.0

3. **STARCH GEL FIXING SOLUTION**

10 parts Acetic acid

20 parts Methanol

70 parts Distilled Water

APPENDIX 15

GENOTYPE FREQUENCIES IN COWPEA ACCESSIONS

COWPEA ACCESSION		87/139	87/142	87/157	87/30	87/37	87/55	87/77	87/81	87/83
	LOCALITY	DECIDUOUS FOREST				GUINEA SAVANNAH			SUDAN SAVANNAH	
AK1* -	3/3	.000	.000	.000	.000	.000	.000	.000	.083	.064
	2/2	.160	.200	.143	.174	.160	.131	.129	.250	.258
	1/1	.240	.267	.321	.304	.400	.273	.355	.125	.258
	3/4	.120	.066	.107	.087	.120	.131	.097	.125	.097
	2/3	.040	.000	.000	.000	.000	.000	.000	.000	.097
	1/2	.440	.467	.429	.435	.320	.455	.419	.417	.226
		(25)	(30)	(28)	(23)	(25)	(22)	(31)	(24)	(31)
AK2*	3/3	.000	.133	.000	.000	.000	.000	.000	.000	.000
	2/2	.200	.067	.133	.133	.200	.133	.067	.312	.176
	1/1	.800	.800	.867	.867	.800	.867	.933	.688	.824
		(15)	(15)	(15)	(15)	(15)	(15)	(15)	(16)	(17)
FH*	3/3	.150	.136	.050	.100	.167	.318	.095	.667	.440
	2/2	.300	.091	.150	.850	.792	.637	.524	.333	.560
	1/1	.550	.773	.800	.050	.041	.045	.381	.000	.000
		(20)	(22)	(20)	(20)	(24)	(22)	(21)	(21)	(25)
HK1* -	3/3	.080	.068	.000	.000	.000	.000	.000	.000	.000
	2/2	.160	.129	.240	.238	.174	.381	.154	.087	.072
	1/1	.160	.103	.160	.238	.392	.143	.192	.217	.214
	3/4	.000	.000	.000	.000	.000	.000	.115	.130	.214
	2/3	.280	.276	.120	.238	.217	.143	.154	.217	.179
	1/2	.320	.414	.480	.286	.217	.333	.385	.349	.321
		(25)	(29)	(25)	(21)	(23)	(21)	(26)	(23)	(28)
HK2* -	3/3	.187	.105	.133	.118	.125	.158	.000	.263	.118
	2/2	.250	.105	.267	.235	.125	.105	.125	.211	.235
	1/1	.563	.790	.600	.647	.750	737	.875	.526	.647
		(16)	(19)	(15)	(17)	(16)	(19)	(16)	(19)	(17)
IDHP1*	6/6	.000	.000	.025	.000	.000	.000	.000	.000	.000
	5/5	.091	.188	.325	.208	.314	.526	.257	.333	.085
	4/4	.159	.270	.076	.283	.372	.211	.143	.030	.064
	3/3	.682	.500	.500	.453	.314	.237	.544	.425	.575
	2/2	.045	.021	.000	.056	.000	.026	.028	.091	.000
	4/6	.000	.000	.000	.000	.000	.000	.000	.000	.021
	3/6	.000	.000	.000	.000	.000	.000	.000	.000	.064
	3/5	.023	.000	.025	.000	.000	.000	.028	.121	.191
	2/5	.000	.021	.000	.000	.000	.000	.000	.000	.000
	2/4	.000	.000	.025	.000	.000	.000	.000	.000	.000
	1/5	.000	.000	.025	.000	.000	.000	.000	.000	.000
		(44)	(48)	(40)	(52)	(35)	(38)	(35)	(33)	(47)

Appendix 15 cont'd

COWPEA ACCESSION		87/139	87/142	87/157	87/30	87/37	87/55	87/77	87/81	87/83
	LOCALITY	DECIDUOUS FOREST				GUINEA SAVANNAH		SUDAN SAVANNAH		
IDHP2*	5/5	.095	.000	.000	.000	.000	.000	.048	.050	.038
	4/4	.191	.095	.300	.250	.100	.300	.143	.150	.077
	3/3	.000	.000	.000	.100	.150	.150	.005	.100	.346
	2/2	.333	.619	.150	.550	.550	.350	.476	.600	.346
	1/1	.048	.238	.150	.050	.150	.100	.048	.000	.000
	2/5	.000	.048	.000	.000	.000	.000	.000	.000	.000
	2/4	.333	.000	.300	.500	.050	.100	.143	.100	.193
	1/4	.000	.000	.000	.000	.000	.100	.000	.000	.000
	1/3	.000	.000	.100	.000	.000	.000	.047	.000	.000
		(21)	(21)	(20)	(20)	(20)	(20)	(21)	(20)	(26)
MDH1*	6/6	.033	.000	.047	.028	.047	.130	.028	.071	.033
	5/5	.000	.095	.143	.057	.047	.130	.200	.143	.200
	4/4	.033	.048	.047	.000	.000	.000	.000	.000	.000
	3/3	.801	.429	.525	.743	.668	.435	.630	.500	.601
	2/2	.033	.190	.000	.086	.048	.000	.028	.036	.133
	1/1	.100	.238	.238	.086	.190	.304	.114	.250	.033
		(30)	(21)	(21)	(35)	(21)	(23)	(35)	(28)	(30)
MDH2*	8/8	.000	.000	.000	.000	.000	.182	.000	.080	.000
	7/7	.097	.045	.000	.038	.050	.045	.000	.080	.000
	6/6	.097	.045	.115	.155	.100	.091	.108	.000	.029
	5/5	.129	.136	.192	.077	.100	.000	.054	.000	.088
	4/4	.161	.000	.077	.077	.050	.091	.163	.080	.059
	3/3	.000	.000	.077	.115	.350	.182	.000	.000	.000
	2/2	.064	.091	.077	.077	.000	.136	.027	.040	.088
	1/1	.129	.273	.193	.000	.000	.000	.081	.000	.000
	8/9	.000	.000	.000	.023	.000	.000	.000	.000	.000
	7/9	.000	.000	.000	.000	.000	.000	.000	.080	.000
	6/8	.000	.000	.000	.077	.050	.136	.027	.160	.000
	5/7	.000	.000	.038	.077	.000	.000	.054	.000	.000
	4/7	.000	.136	.077	.038	.150	.000	.054	.000	.059
	4/6	.000	.000	.000	.000	.000	.000	.189	.040	.088
	3/7	.129	.000	.000	.000	.000	.000	.000	.000	.000
	3/6	.000	.000	.000	.000	.000	.000	.054	.000	.029
	3/5	.000	.000	.000	.115	.150	.091	.000	.000	.000
	3/4	.000	.000	.154	.000	.000	.000	.000	.000	.000
	2/8	.000	.000	.000	.038	.000	.000	.000	.000	.000
	2/6	.064	.000	.000	.000	.000	.000	.000	.000	.000
	2/4	.129	.183	.000	.077	.000	.046	.189	.200	.325
	1/4	.000	.091	.000	.000	.000	.000	.000	.000	.000
	1/3	.000	.000	.000	.000	.000	.000	.000	.240	.235
		(31)	(22)	(26)	(26)	(20)	(22)	(37)	(25)	(34)

Appendix 15 cont'd

COWPEA ACCESSION		87/139	87/142	87/157	87/30	87/37	87/55	87/77	87/81	87/83
	LOCALITY	DECIDUOUS FOREST			GUINEA SAVANNAH			SUDAN SAVANNAH		
MDH3*	7/7	.000	.000	.000	.000	.000	.000	.000	.095	.048
	6/6	.000	.000	.000	.000	.087	.048	.000	.048	.000
	5/5	.068	.043	.120	.333	.130	.095	.057	.048	.095
	4/4	.138	.000	.080	.083	.087	.143	.000	.095	.095
	3/3	.172	.130	.280	.250	.522	.286	.257	.333	.238
	2/2	.448	.175	.200	.208	.174	.190	.285	.286	.381
	1/1	.138	.435	.200	.000	.000	.238	.086	.095	.381
	6/7	.000	.000	.000	.000	.000	.000	.000	.000	.048
	3/6	.034	.043	.000	.043	.000	.000	.200	.000	.000
	2/5	.000	.000	.000	.083	.000	.000	.029	.000	.094
	1/4	.000	.000	.000	.000	.000	.000	.029	.000	.000
	1/3	.000	.087	.080	.000	.000	.000	.000	.000	.000
	1/2	.000	.087	.040	.000	.000	.000	.057	.000	.000
		(29)	(23)	(25)	(24)	(23)	(21)	(35)	(21)	(21)
MDH4*	4/4	.000	.000	.000	.000	.000	.000	.000	.000	.062
	3/3	.050	.000	.000	.067	.000	.111	.000	.059	.313
	2/2	.235	.059	.062	.200	.537	.278	.294	.176	.125
	1/1	.471	.354	.438	.000	.000	.056	.059	.000	.000
	4/7	.000	.000	.000	.000	.000	.000	.000	.000	.062
	4/6	.000	.000	.000	.267	.000	.000	.000	.118	.000
	4/5	.050	.000	.000	.000	.000	.000	.000	.000	.000
	3/5	.000	.176	.188	.066	.000	.333	.000	.176	.000
	2/6	.000	.000	.000	.000	.000	.000	.000	.059	.000
	2/4	.000	.059	.062	.200	.000	.056	.000	.118	.313
	1/5	.000	.000	.000	.000	.000	.000	.059	.000	.000
	1/4	.000	.000	.000	.000	.000	.000	.176	.000	.000
	1/3	.000	.176	.125	.067	.333	.000	.353	.000	.000
	1/2	.176	.176	.125	.133	.133	.166	.059	.294	.125
		(17)	(17)	(16)	(15)	(15)	(18)	(17)	(17)	(16)
MEP1*	6/6	.025	.000	.000	.024	.038	.000	.059	.041	.000
	5/5	.073	.102	.216	.293	.385	.321	.118	.125	.109
	4/4	.096	.041	.216	.098	.077	.036	.029	.000	.000
	3/3	.610	.531	.352	.341	.231	.286	.530	.625	.283
	2/2	.146	.081	.027	.098	.077	.179	.147	.125	.370
	1/1	.000	.041	.135	.000	.000	.000	.000	.000	.000
	5/7	.000	.000	.000	.000	.000	.000	.029	.000	.000
	4/6	.000	.000	.000	.000	.000	.071	.000	.000	.000
	4/5	.025	.021	.000	.000	.000	.000	.000	.000	.000
	3/6	.000	.000	.000	.000	.077	.071	.000	.042	.174
	3/5	.025	.143	.000	.073	.115	.000	.000	.042	.021
	3/4	.000	.020	.027	.000	.000	.000	.000	.000	.000
	2/5	.000	.000	.000	.073	.000	.000	.059	.000	.043
	2/4	.000	.020	.027	.000	.000	.036	.029	.000	.000
		(41)	(49)	(37)	(41)	(26)	(28)	(34)	(24)	(46)

Appendix 15 cont'd

COWPEA ACCESSION		87/139	87/142	87/157	87/30	87/37	87/55	87/77	87/81	87/83
	LOCALITY	DECIDUOUS FOREST			GUINEA SAVANNAH			SUDAN SAVANNAH		
MEP2*	6/6	.000	.000	.000	.000	.000	.000	.000	.040	.000
	5/5	.000	.000	.000	.036	.091	.048	.052	.200	.297
	3/3	.615	.333	.458	.750	.455	.333	.158	.320	.148
	2/2	.103	.429	.250	.143	.182	.524	.474	.240	.444
	1/1	.000	.048	.150	.000	.182	.000	.211	.000	.000
	4/7	.000	.000	.000	.000	.000	.000	.000	.040	.000
	3/7	.000	.000	.000	.000	.000	.000	.000	.080	.000
	3/6	.000	.000	.083	.000	.000	.000	.000	.040	.000
	2/6	.000	.000	.042	.071	.045	.000	.105	.000	.074
	2/5	.051	.095	.000	.000	.000	.000	.000	.040	.037
	1/6	.231	.095	.042	.000	.045	.095	.000	.000	.000
		(39)	(21)	(24)	(28)	(22)	(21)	(19)	(25)	(27)
PGDH1*	5/5	.000	.000	.000	.000	.000	.000	.000	.057	.000
	4/4	.029	.077	.108	.114	.364	.522	.323	.286	.181
	3/3	.171	.038	.081	.057	.000	.000	.000	.029	.016
	2/2	.657	.692	.676	.743	.500	.348	.548	.543	.754
	1/1	.143	.193	.135	.086	.136	.130	.129	.085	.049
		(35)	(26)	(37)	(35)	(22)	(23)	(31)	(35)	(61)
PGDH2*	8/8	.024	.000	.000	.000	.000	.115	.032	.000	.020
	7/7	.000	.026	.000	.000	.043	.038	.000	.133	.000
	6/6	.268	.053	.194	.189	.130	.077	.290	.069	.163
	5/5	.294	.342	.161	.270	.217	.077	.227	.103	.102
	4/4	.073	.211	.355	.352	.217	.270	.161	.277	.204
	3/3	.171	.105	.065	.027	.130	.154	.161	.138	.265
	2/2	.073	.263	.032	.108	.174	.154	.000	.000	.000
	1/1	.024	.000	.128	.054	.000	.000	.097	.103	.082
	5/8	.049	.000	.000	.000	.000	.033	.000	.103	.000
	4/7	.024	.000	.065	.000	.087	.077	.000	.069	.082
	4/6	.000	.000	.000	.000	.000	.000	.000	.000	.000
	3/6	.000	.000	.000	.000	.000	.000	.032	.000	.082
		(41)	(38)	(31)	(37)	(23)	(26)	(31)	(29)	(49)
PGDH3*	5/5	.419	.333	.120	.031	.091	.115	.000	.000	.000
	4/4	.000	.000	.000	.188	.091	.078	.200	.000	.030
	3/3	.097	.000	.080	.031	.091	.269	.040	.045	.097
	2/2	.387	.630	.440	.375	.545	.269	.400	.727	.548
	1/1	.097	.037	.360	.375	.182	.269	.360	.228	.322
		(31)	(27)	(25)	(32)	(22)	(26)	(25)	(22)	(31)
PGI1*	1/1	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
		(34)	(31)	(35)	(38)	(30)	(29)	(28)	(28)	(42)

Appendix 15 cont'd

COWPEA ACCESSION		87/139	87/142	87/157	87/30	87/37	87/55	87/77	87/81	87/83
	LOCALITY	DECIDUOUS FOREST			GUINEA SAVANNAH				SUDAN SAVANNAH	
PGI2*	3/3	.030	.300	.029	.256	.133	.207	.071	.071	.048
	2/2	.758	.367	.686	.564	700	.483	.857	.857	.904
	3/4	.000	.000	.029	.026	.000	.000	.000	.000	.000
	2/5	.030	.033	.085	.128	.034	.034	.036	.036	.024
	2/4	.030	.300	.171	.026	.133	.276	.036	.036	.024
	1/4	.162	.000	.000	.000	.000	.000	.000	.000	.000
		(31)	(30)	(35)	(39)	(30)	(29)	(28)	(28)	(42)
PGI3*	2/4	.030	.033	.114	.250	.207	.333	.154	.154	.095
	2/3	.212	.500	.486	.307	.448	.519	.615	.500	.667
	1/4	.182	.033	.029	.026	.034	.037	.038	.269	.190
	1/3	.576	.434	.371	.417	.311	.111	.193	.077	.048
		(33)	(30)	(35)	(36)	(29)	(27)	(26)	(26)	(42)
PGI4*	4/4	.000	.000	.000	.000	.000	.000	.000	.087	.000
	3/3	.000	.000	.000	.000	.000	.091	.111	.391	.038
	2/2	.286	.182	.233	.240	.269	.182	.000	.000	.654
	1/1	.095	.182	.067	.080	.038	.091	.000	.000	.000
	3/4	.000	.000	.000	.000	.000	.000	.000	.043	.000
	2/4	.239	.182	.100	.120	.115	.182	.074	.130	.038
	2/3	.190	.227	.466	.120	.308	.182	.297	.262	.231
	1/3	.095	.136	.067	.360	.192	.182	.444	.087	.039
	1/2	.095	.091	.067	.080	.077	.090	.074	.000	.000
		(25)	(30)	(28)	(23)	(25)	(22)	(31)	(24)	(31)
PGM1*	2/3	.000	.000	.000	.136	.091	.000	.000	.150	.115
	1/3	.250	.240	.231	.136	.136	.286	.214	.300	.192
	1/2	750	.760	.769	.728	.773	714	.786	.550	.693
		(24)	(25)	(26)	(22)	(22)	(21)	(28)	(20)	(26)
PGM2*	3/3	.000	.000	.000	.000	.043	.000	.000	.000	.115
	2/2	.500	.276	.286	.357	.435	.464	.556	.500	.423
	1/1	.462	724	.714	.321	.218	.321	.333	.333	.347
	3/4	.000	.000	.000	.000	.000	.000	.111	.083	.115
	2/3	.000	.000	.000	.107	.130	.215	.000	.042	.000
	1/2	.038	.000	.000	.215	.174	.000	.000	.042	.000
		(26)	(29)	(28)	(28)	(23)	(28)	(27)	(24)	(26)

APPENDIX 16

CORRELATION OF ECOGEOGRAPHICAL FACTORS

	LONG.	LAT.	T_{MAX}	T_{MIN}	M.RAIN	RAINDAY	RH 1500	RH 0600	MEAN SUN	P 1500	P 0600	CL
LONG.	1.0000											
LAT.	.7875	1.0000										
T_{MAX}	.5971	.9424**	1.0000									
T_{MIN}	.6366	.7912	.8856*	1.0000								
M .RAIN	-.9495**	-.9017*	- 7716	- 7528	1.0000							
RAINDAY	-.7663	-.9990**	-.9466**	- 7823	.8921*	1.0000						
RH 1500	-.7051	-.9881**	-.9821**	-.8381*	.8532*	.9904**	1.0000					
RH 0600	-.7645	-.9934**	-.9682**	-.8507*	.8847*	.9914**	.9948**	1.0000				
MEAN SUN	.7280	.9942**	.9691**	.8127	-.8711*	-.9967**	-.9981**	-.9941**	1.0000			
P 1500	- 7583	-.9982**	-.9604**	-.8120	.8871*	.9986**	.9955**	.9966**	-.9987**	1.0000		
P 0600	- 7967	-.9964**	-.9482**	-.8253	.8987*	.9926**	.9869**	.9973**	-.9895**	.9951**	1.0000	
CL	-.8596*	-.9833**	-.8805*	- 7478	.9184*	.9756**	.9480**	.9700**	-.9587**	.9716	.9852	1.0000

*=$P<0.01$

**=$P<0.001$

APPENDIX 17

Morphological Correlate of variation (only $|r_s| > 0.4$).

	PSG	PPS	PSS	PSI	PPD	FRM	DTF	DMP	PL	MSW	NSP	NOP	LS
PPS	.5971	.6167	0.846		.6065		.5852			.5451			
PSS	7621	.6167					.8028	.4759	.5804	4902	.8706	.6741	
PSI					.4846	-.6925		.6771		.4250			
PPD	.4308	6065		4846			.5039	7266		7081			.8320
FRM				.6925			-.4499	- 4729					
DTF	.9429**	.5852	.8028		.5039		.8615*	.8596*		.6830	.8456*	.7965	
DMP	7768		4759	.6771	7266	- 4499	8615*		.7363	.8041	.5539	.6058	
PL	.8730*		.5804			-.4729	.8596	7363		4904	.8510*	.9432**5	
MSW	.6854	.5451	4902	4250	7081		.6820	.8041	4904				.4739
NSP	7967		8706				.8456*	.5539	.8510*			.9434**	
NOP	7695		.6741				7965	.6058	.9432				
LS					.8320					4739			

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

APPENDIX 18

Samples of allozymic correlates of variation (only significant correlates are shown)

	AK1'-4	AK1'-3	AK1'-2	AK1'-1	AK2'-3	AK2'-2	AK2'-1
AK1'-3				-.9006*			
FH1'-3		.8586*		-.8831*			
HK2'-3	.8466						
HK2'-1						.8449*	-.8554
1DHP1'-2			.8618*	-.9379*			
MDH3'-7		.8531		-.8871*			
MDH3'-6	-.8599*						
MDH3'-5			-.8661*				
MDH2'-1					.8703*		
PGDH1'-1		-.9175					

Appendix 18 cont'd

	FH1'-3	FH1'-2	FH1'-1	HK1'-4
HK2'-1				
1DHP1'-5				
1DHP1'-3				
1DHP2'-3		.8821*		
MDH1'-4			.9066*	
MDH2'-1			.8474	
MDH3'-7	.9430*			
MDH3'-5		.9248*		
MDH3'-3				
MDH4'-4				.9609**
MDH4'-1			.9451**	
MEP1'-6		.8610*	-.9408**	
MEP1 -4				-.9134*
MEP1'-3		.8642*		
MEP2'-5				.8640
PGDH1'-4				
PGDH1'-2				
PGDH1'-1				.8542

HK1'-3	HK1'-2	HK1'-1	HK2'-3	HK2'-2	HK2'-1
.8378*					
.8608					
		.9407**			
	-.9659**				
	.9073*				

ACKNOWLEDGEMENT

This work was supported financially by a 36 month fellowship awarded me by Unilever, Ghana Limited and a grant from National Agricultural Research Project (NARP). I am therefore indebted to both organizations.

I am deeply grateful to my main supervisor Professor E. Laing who suggested the topic and gave useful criticisms in the course of the work. I am also grateful to Dr. E.K. Abban who introduced me to isozyme techniques and supervised the laboratory work. Dr. (Mrs.) Jumah, a member of the supervisory committee also merits thanks for her useful correction of the manuscript.

Acquisition of cowpea accessions used for the work could not have been possible without the assistance of Dr. S.O. Bennet Lartey, Plant Genetic Resource Centre, Council for Scientific and Industrial Research (CSIR) Bunso. I am very grateful to him.

The Director of the Institute of Aquatic Biology (I.A.B) CSIR, deserves special thanks for allowing me space in the Institute's Chemistry Laboratory, where the work was done, and granting me the use of other miscellaneous facilities.

The technical staff of the Fish Genetic Unit of I.A.B., Messrs M.K. Abakah and E. Amedume also deserve my gratitude for their technical support.

Finally, I would like to thank Mr. Stephen Eghan who did the computer analysis.

REFERENCES

1. Amoatey, H.M. 1987.
Genetic studies in some cowpea (*Vigna unguiculata)* (L) Walp varieties in Ghana. M.Sc. Thesis University of Ghana, Legon.

2. Asante, I.K. 1991.
Inheritance and genetical linkage in the cowpea *(Vigna unguiculata)* (L) Walp) M.Phil Thesis University of Ghana, Legon.

3. Asiedu, R. 1992.
Isozyme analysis and its application in plant breeding in *Biotechnology enhancing research on tropical crops in Africa.* Thottappilly G., Monti, L.M., Mohan Raj, D.R. and Moore, W. (eds) Ibadan, Nigeria CTA/11TA 261-265.

4. Asiedu, R., Fisher J.M., Driscoll, C.J. 1990.
Resistance to *Heterodera avenae* in the rye genome of triticale. *Theoretical and Applied Genetics* 49: 97-108.

5. Baht, K.V. Bhat, S.R. and Chandel, K.P.S. 1992.
Survey of Isozyme polymorphism for clonal identification in *Musa* 1. Esterase, acid phosphatase and catalase. *Journal of Horticultural Science 67:00-00.*

6. Balagtas, G.E. and Ramirez, D.A. 1991.
Genetic variation in Philippine collections of mungbean (Vigna radiata (L) Wilczek), ricebean (*Vigna umbellata* (L) Thumb and Ohwi) and cowpea (*Vigna unguiculata* (L) Walp) *The Philippine Agriculturalist 74 (1): 103-119.*

7. Bennet Lartey, S.O. 1991.
Variability and heterosis in cowpea (*Vigna unguiculata*) (L) Walp) accessions from four main regions of Ghana. Ph.D. Thesis , University of Ghana, Legon.

8. Breese, L. 1989.
Multiplication and regeneration of germplasm in *Scientific management: Characterization evaluation and enhancement.* Stalker, H.T. and Chapman, C. 1989 (eds.) IBPGR Rome with Department of Crop Science North Carolina State University 41-54.

9. Bretting, P.K. and Goodman, M.M. 1989.
Genetic variation in crop plants and management of germplasm collections in *Scientific management: Characterization, evaluation and enhancement.* Stalker, H.T and Chapman, C. 1989 (eds.) IBPGR Training Course: Lecture Series 2. IBPGR Rome with Department of Crop Science North Carolina State University 41-54.

10. Brown, A.H.D., Clegg, M.T. Kahler, A.L., and Bruce, S.W. (eds.) 1990
Plant population genetics, breeding and genetic resources. Sinauer Associates Inc. Sunderland, Massachusetts.

11. de Mpoy, B.E. 1985.
Variability of different characterization in Botswana cowpea germplasm. *Tropical Grain Legume Bulletin* 31: 1-4.

12. Doebley, F.F., Goodman, M.M. and Stuber, C.W. 1985.
Isozyme Variation in the races of maize from Mexico. *American Journal of Botany* 72: 629-639

13 Doku, E.V. 1970.
Variability in local and exotic varieties of cowpea (*Vigna unguiculata* (L) Walp) in Ghana. *Ghana Journal of Agricultural Science* 3 139-143.

14. Dovlo, F.E. 1976.
Dietary uses of grain legumes in Ghana. Proceedings of the University - Council for Scientific and Industrial Research Symposium on grain legumes. Legon 10th-11th December 1976.

15. Falconer, D.S. 1988.
Introduction to quantitative genetics 3rd edition. English Language Book Society Longman. England.

16. Faris, D.G. 1965.
The origin and evaluation of the cultivated forms of *Vigna sinensis*. *Canadian Journal of Genetic Cytology* 7: 433-452.

17 Ferguson, A. 1980.
Biochemical systematics and evaluation. Blackie, Glasgow and London.

18. Gehring, J.L. and Linhart, Y.B. 1992.
Population structure and genetic differentiation in nature and introduced populations of *Deschampsia caespitosa (Poaceaee)* in the Colorado Alpine. *American Journal of Botany* 79 (12): 1337-1343.

19. Geric, I., Zlokolica, M. and Geric, C. 1989.
Races and populations of maize in Yugoslavia: Isozyme variation and genetic diversity International Board for Plant Genetic Resources. Rome.

20. Ghana Grains Development Project 1990
Maize and cowpea production guide. Ghana/CIDA Grains Development Project. January 1990

21. Hamrick, J.L. and Godt, J.W. 1990.
Allozyme diversity in plant species in *Plant population genetics, breeding and genetic resources*. Brown, A.H.D., Clegg, M.T., Kahler, A.L., and Weir, B.S (eds.) 1990. Sinauer Association Inc. Sunderland, Massachesetts.

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

23. Harlan, J.R. 1975.
Our vanishing genetic resources. *Science* 188: 618-621

24. Hayward, M.D. 1984.
Isoenzymes as genetic markers : In *Characterization of variability in collection, characterization and utilization of genetic resources of temperate forage grass and clover*. Tyler B.F. 1987 (ed.) IBPGR Training Course: Lecture series 1 IBPGR Rome 1987 54-57.

25. Hunter, R and Market, C.L.1957.
Histochemical demonstration of enzymes separated by zone electrophoresis. *Science* 125: 1294-1295

26. IITA, 1983. Cowpea germplasm collections, conservation and utilization. A special proposal for funding to the Government of Italy. Submitted by IITA, Ibadan, Nigeria. January 1983.

27. Kimura, M. 1982.
The neutral theory of molecular evolution. Cambridge University Press. London. New York New Rochelle. Melbourne. Sydney.

28. Latter, B.D.H. 1981. The distribution of heterozygosity in temperate and tropical species of *Drosophila*. *Genetical Research* 38: 137-156

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

30. Li, C.C. 1954.
Population Genetics. The University of Chicago Press Chicago.

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

32. Mettler, L.E. and Gregg, T.G. 1969.
Population genetics and evolution. Prentice-Hall Inc. Eaglewood Cliffs. New Jersey.

33. Morden, C.W. Doebley, J.F. and Schertz, K.F 1989.
Variation in old World races of *Sorghum bicolor (Poaceae) American Journal of Botany* 76 (2): 247-255.

34. Moore, G.A. and Collins, G.B. 1983.
New challenges confronting plant breeders in *Isozymes in plant genetics and breeding, Part A.* Tanksley, S.D. and Orton, J.A. (eds.) 1983 Elsevier Science. Amsterdam.

35. Nei, M 1977
F-statistics and analyis of gene diversity in subdivided populations. *Annals of Human Genetics, London* 41: 225-233.

36. Nei, M and Chakravarti, A. 1977
Drift variances of F_{ST} and G_{ST} statistics obtained from a finite number of isolated populations. *Theoretical Population Biology* 11: 307-325.

37 Nevo, E., Noy-Meir, I., Beiles, A. Krugman, T and Agami, M. 1991.
Natural selection of allozyme polymorphisms. Micro-geographical and temporal ecological differentiations in Wild Emmer wheat. *Israel Journal of Botany* 40 (5-6) 419-449

38. Ng, N.Q. and Marechal, R. 1985.
Cowpea taxonomy, origin and germplasm In *Cowpea research, production and utilization.* Singh, S.R. and Rachie, K.O. (eds) John Wiley and Sons. Chichester

39. Noy-Meir, Agami, Cohen, E. and Anikster, Y. 1991.
Floristic and ecological differentiation of habitats within a wild wheat population at Ammiad. *Israel Journal of Botany* Vol. 40 363-384.

40. Onwueme, I.C. and Sinha, T.D. 1991.
Field crop production in Tropical Africa: Principle and Practice. Technical Centre for Agricultural and Rural Co-operation. Netherlands.

41. Panella, L. and Gepts, P 1992.
Genetic relationships within *Vigna unguiculata* (L) Walp based on isozyme analysis. *Genetic Resources and Crop Evolution* 39: 71-88.

42. Pollock, C.J. Stoddart, J.L., Howard, T and Jones, T.W.A. 1984.
Protein electrophoresis in *Isoenzymes as genetic markers in collection, characterization and utilization of genetic resources of temperate forage grass and clover* Tyler, B.F 1987 (ed.) IBPGR. Rome.

43. Porter, W.M., Rachie, K.O. Rawal, K.M., Wien, H.C., William, R.J and Luse, R.A. 1974.
Cowpea germplasm catalog IITA, Ibadan.

44. Price, S.C., Schumaker, K.N., Kahler, A.L., Allard, R.W. and Hill, J.E. 1984.
Estimates of population differentiation obtained from enzyme polymorphisms and quantitative characters. *Journal of Heredity* 75: 141 142.

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

46. Rachie, K.O. and Rawal, K.M. 1976.
Integrated approaches to improving cowpeas *Vigna unguiculata* (L) Walp. Technical Bulletins. International Institute for Tropical Agriculture, Ibadan, Nigeria.

47 Ramanatha Rao, V and Riley, K.W. 1994.
The use of biotechnology for conservation and utilization of plant genetic resources. Plant Genetic Resources Newsletter 1994 97· 3-20 Singapore.

48. Rawal, K.M. 1973.
Systematic germplasm collection of grain legumes in West Africa. Proceedings of the first IITA Grain legumes Improvement Workshop, 29 October 2 November, 1973 IITA, Ibadan. Nigeria.

49. Singh, S.R., Jackai, L.E. N., 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*. Thottappilly G. Monti, L.M. Mohan Raj, D.R. and Moore, A.W. (eds.) Ibadan, Nigeria CTA/IITA.

50. Slatkin, M. 1987
Gene flow and geographic structure of natural population. *Science* 236: 787-792.

51. Sneath, P.H.A and Sokal, R.R. 1973
Numerical Taxonomy The principle and practice of numerical classification. W.H. Freeman and Company San Francisco.

52. Sokal, R.R. and Rohlf, F.J. 1969.
Biometry, the principles and practice of statistics in biological research. W.H.

Freeman and Company. San Francisco.

53. Steele, W.M. 1976.
Cowpeas in *Evolution of crop plants*. Simmonds, N.W. (ed.) pp 183-185. Longman. London.

54. Vaillancourt, R.E., Weeden, N.F and Barnard, J 1993.
Isozyme diversity in the cowpea species complex. *Crop Science* 33 (3) 606-613.

55. Wright, S. 1952.
The theoretical variance within and among subdivisions of a population that is in a steady state. *Genetics* 33: 312-2

56. Xu Bao, Zhuang Bingchang, Xu Hang, Li Fushan Lu Quihua and Wang Yumin, 1993
Polymorphism and geographical distribution of protein content of Wild soyabean (*Glycine soja*) in China I Plant Genetic Resources Newsletter 94/95. FAO Rome.