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GERMPLASM EVALUATION OF BOTSWANA COWPEA
(*Vigna unguiculata* (L.) Walp.)
LANDRACES

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Barbara E. deMooy

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GERMPLASM EVALUATION OF BOTSWANA COWPEA (*Vigna unguiculata* (L.) Walp.) LANDRACES

By

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ABSTRACT

GERMPLASM EVALUATION OF BOTSWANA COWPEA
(*Vigna unguiculata* (L.) Walp.) LANDRACES

By

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Germplasm of cowpea (*Vigna unguiculata*) landraces collected in Botswana was evaluated during 1982-84. Ninteen quantitative and fifteen qualitative traits were examined. All plant characters were polymorphic in nature. Dividing the country into 5 clusters based on geographically isolated collection sites allowed for comparison of diversity within and between regions. The Shannon-Weaver diversity index was used to calculate phenotypic diversity indices. Results indicate that some selection for various plant traits may be taking place within regions. The highest diversity indices were obtained for plant growth habit and seed color. A screening experiment for nodulation capacity using local cowpea varieties and introductions indicates that considerable variability exists within these populations. Five high and four low nitrogen-fixing genotypes were provisionally identified based on a stable performance across two environments and

two sampling periods for nodule number, nodule fresh weight and shoot fresh weight. No differences in nodulation capacity were observed between local varieties and introductions.

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TABLE OF CONTENTS

Page

LIST OF TABLES

Chapter 1

LIST OF FIGURES

Chapter 1

Appendices

INTRODUCTION

Genetic Diversity of *Vigna unguiculata* Landraces in Botswana

Vigna unguiculata L. Walp., commonly called cowpea, is a tropical and subtropical legume crop widely grown in Africa. Cowpeas can tolerate a large range of environmental conditiions which accounts for their cultivation under humid as well as semiarid farm production sytems (Blackhurst and Miller, 1980). The green pods, ripened seed and green leaves are all utilized as a source of high quality protein (Summerfield et al., 1974).

In Botswana, cowpea is the main grain legume grown and is often a main source of protein served at meals. Most farmers still grow traditional landraces and a single field plot may be comprised of several diverse plant types. As with all field crops, this genetic diversity provides insurance against total crop failures in the event of environmental adversities. Of interest to both the farmer and plant breeder is the type and amount of genetic diversity harboured within these landraces since primitive cultivars can provide a wealth of new genetic material for

incorporation into plant breeding programs. The objectives of this study were (1) to collect germplasm samples of Vigna unguiculata from the arable portions of Botswana across diverse soil and climatic environments and (2) evaluate this collection for various characteristics which may be of importance in a plant breeding program.

Origin, Spread and Domestication of Vigna

The center of origin of cowpea has been debated for several years. Some suggested an Indian center (Vavilov, 1950) while others favored an African center (Piper, 1913). Recent studies have suggested West or Central Africa as the center of origin of *Vigna* (Faris, 1965; Rawal, 1975).

Faris (1965) collected samples of *Vigna* world-wide in order to determine the species geographic range and diversity. Morphological characteristics were measured and 170 species were delineated. Of these, 120 had been reported in Africa. He concluded that due to the high diversity found there, cowpea most likely originated in Africa. In addition, the wild relatives of *V. unguiculata* have only been reported in Africa - often growing around fields of the cultivated form. Two wild forms exist, *V. unguiculata* subsp. *dekindtiana* and subsp. *mensensis*, and are identifiable by their small leaves, seeds and explosive dehiscence (Lush and Evans, 1981). Attempts at interspecific crosses between common x wild cowpea resulted in a greater than 40% success. This was higher than percentages obtained for any other interspecific crosses

attempted (Faris, 1965). This evidence suggested not only an African origin but that the prototype of domesticated cowpea is most likely the wild subspecies dekindtiana found only in Africa (Lush and Evans, 1981).

Based on sparse archeological findings, linguistic evidence and geographic distribution, an advanced agricultural civilization in the Mande subfamily of the Nigritics was identified by Murdock (1959). These people are thought to have been centered in Western Sudan near the headwaters of the Niger river. Evidence suggests they were domesticating several crop plants before 4500 b.c., including cowpea.

Cowpea was probably one of the commodities moved along the trade route between Africa and India (Sauer, 1952). It is thought to have reached Europe quite early since the Greeks and Romans grew cowpea under the name of Phaseolus (Burkill, 1953). Introduction into the New World occurred in the late 17th century (Mackie, 1946; Wight, 1970; Corley, 1966).

In comparing wild and domesticated subspecies of cowpea, Lush and Evans (1981) noted several changes which occurred in Vigna during domestication. They suggest that the wild subspecies dekindtiana was first cultivated in the humid tropics where its pods dehisce slowly. Eventually, changes in the structure of pod valves and seed coats reduced pod dehiscense and seed hardness. Through

selection by farmers, seed and pod size increased along with increases in the rate of dry weight accumulation. Domestication had no effect on the maximum photosynthetic rate of leaves although the duration of their photosynthetic activity has increased. Variability in testa color has increased in domesticated cowpea as compared to wild types (Smartt, 1976) with the highest diversity occurring in African landraces (Faris, 1965).

Studies on the effects of environment on adaptation of wild and domesticated cowpea have led to the conclusion that these adaptations are associated with several different developmental processes (Lush et al., 1980). Wild accessions of cowpea from semi-arid environments showed the timing of nearly all developmental stages to be dependent on environmental conditions. Those accessions derived from sub-humid environments however, showed environmental sensitivity only during time of germination. Cultivated cowpea gave a range of responses and in the most extreme cases, development was controlled almost entirely by time of planting. The results of domestication, therefore, have been accompanied by a reduction of environmantal influences on the life cycle of the plant.

Taxonomy of Cultivated Cowpea

The genus Vigna is a member of the Leguminoseae, subfamily Papilionoideae and the tribe Phaseoleae. Because of its close relationship to Phaseolus, much confusion existed in the literature on classification and nomenclature (Sellschop, 1962). Piper (1913) divided the genus into three species based on seed and pod characteristics: Vigna sinensis (common cowpea), Vigna catjang or cylindrica and Vigna sesquipedalis (yard long bean). This classification proved unsatisfactory since all "species" crossed readily, thus demonstrating free gene exchange between them (Smartt, 1976; Faris, 1965). In a recent nomenclature change, the three forms of cultivated Vigna have veen combined into one species Vigna unguiculata (L.) Walp. and are now designated as subspecies unguiculata (common), cylindrica (catjang) and sesquipedalis (yard long) (Gunn, 1973). All subspecies were found to have 2n=22 chromosomes (Sen and Bhowal, 1960; Faris, 1964) despite previous reports of 2n=24 chromosomes (Mackie, 1946).

Subspecies unguiculata is found mostly in Africa where it is cultivated largely as a grain legume. This resulted

in selection pressure being greatest for seed size so that of all three cultivated subspecies, unguiculata has the greatest seed weight. Lush and Wien (1980) demonstrated the advantage of large seed size in the evolution of cowpea by comparing emergence rates and seedling vigor with wild relatives. Larger seeds of the cultivated cowpea has better emergence and establishment under agricultural conditions due to the greater size of plants arising from them. Subspecies sesquipedalis and cylindrica are rare in Africa but common in India where they are thought to have evolved from subsp. unguiculata (Faris, 1965). In India, cowpea preference has been for green pod consumption and selection pressure for increased pod length resulted in the yard long bean (subsp. sesquipedalis). Cowpea is also used as a forage crop in India, mainly the subsp. catjang. As no selection was applied for grain or pod size in this subspecies, reproductive structures remain small.

Importance of Cowpea in Botswana

Marketing and Local Consumption

Cowpea is the third most important crop in Botswana after maize (Zea mays) and sorghum (Sorghum bicolor). Seed

scarcities are common on the commercial market and varieties are often imported for distribution to agricultural cooperatives in some parts of the country This seed is usually not field tested for adaptability and yield before distribution. Due to this lack of widespread seed availability, most subsistence farmers continue to produce their own seed from traditional landraces. Although quanitites may be limited, almost all farmers plant cowpea, even if their stock amounts to only a few grams. Despite the importance of the crop in Botswana, production problems remain and yields per hectare are often low, averaging between 200-300 kg/ha. Cowpea research into breeding of new improved varieties for Botswana's agriculture began only recently with the inception of the Botswana Bean/Cowpea Collaborative Research Support Program in 1982. Investigations are underway to select landraces with suitable genotypes as a tri-purpose grain-forage-leafy vegetable crop. Consumption of green, immature pods is unknown in the country although the immature pods may be shelled and the green seeds cooked, as with lima beans.

Under Botswana's semi-arid conditions, intensified by the recent drought plaguing the area, forage grass for cattle consumption is scarce. Cowpea crop residues are extremely important in supplying cattle feed. Fields are normally "fenced" for protection against grazing animals by placing thorn bushes around the plots. These are removed

after harvest and cattle then feed on what remains. In such a situation, the architecture of a genotype is an important consideration in a plant breeding program since large, branching, leafy plants would provide more forage.

Dietary Importance

Nutritionally, cowpea provides 23% seed protein and 5% leaf protein. It is highest in the essential amino acid lysine but also contains high amounts of tryptophan (FAO, 1977). In his studies among the Tlokwa tribe of Eastern Botswana, Grivetti (1978) found cowpea to play an important role in the diets of pregnant and lactating women. Although the consumption of the seed is prohibited during this period, women may eat the stewed green cowpea leaves (morogo). As a grain legume, cowpea is eaten with maize, sorghum and peanut. Stone ground legume flour is often served as a porridge substitute. And cowpea leaves are sometimes served with meals as relishes. Among the Tlokwa, cowpea was found to be stored in the pods. The grain is prepared for consumption by boiling. Afterwards, the pods are split open and the seeds eaten.

Creation of Genetic Diversity

Genetic variability arises through mutation and natural crossing and favorable gene combinations are selected by natural or artificial means. An understanding of the mechanisms by which genetic diversity is created helps plant breeders in combining characteristics which may not occur naturally. Although cowpea is a self-pollinating species, outcrossing frequently occurs and, no doubt, contributes significantly to the creation of genetic diversity. Flowers produced on *Vigna* form a nectary around the base of the ovary which secretes sugars and attracts various types of bees. Visiting bees may trip the stigma and facilitate cross pollination (Ojehoman, 1968).

Rawal (1975) studied the occurrence of natural hybridization among wild, weedy and cultivated *Vigna unguiculata*. Evidence of introgressive hybridization between weedy and cultivated cowpea was discovered and zones of natural hybridization corresponded to cultivated areas. Weedy forms of cultivated cowpea exist all over Africa and two specific races were identified based on geographic distribution. One race grows in the sub-humid and humid areas of Africa and is difficult to distinguish

morphologically from the wild subspecies. It is a perennial and produces abundant seeds. Gene exchange between this weedy race and wild or cultivated cowpea is difficult to detect. The second weedy race is found in semi-arid environments. The plants are annuals and may be prostrate or climbing in growth habit. The prostrate race hybridizes freely with wild and cultivated cowpea. Weedy forms are found only in disturbed areas such as a farmer's field. They are similar in developmental stages to cultivated cowpea except for dehiscense of the mature pods. In Botswana, plants of weedy Vigna may be found growing among cultivated plants, although they are usually rogued by farmers, and are identifiable by their dark pod color. Rawal (1975) presented the following hypothesis based on his studies, to account for the movement of genes between wild, weedy and cultivated cowpea: (1) the wild subspecies of sub-humid and humid Africa is the progenitor of both the weed and cultivated forms of cowpea; (2) the wild form at one time existed in a wider geographic range than at present and the colonizer form now growing in the sub-humid and semi-arid regions evolved from the wild form; (3) the natural colonizer was domesticated in West Africa; (4) the interaction between the colonizer and cultivated plants gave rise to the companion weed observed in the fields, and introgression with cultivated V. unguiculata.

Collections of introgression types of cowpea held at

IITA in Nigeria represent all gradations of characteristics from the weedy form to the cultivated genotypes (Rawal, 1975; Rawal et al., 1976). In his analysis of characteristics of wild subspecies dekindtiana, Lush (1979) also reported intergradation between wild and common cowpea.

Results reported by Faris (1965) showed an almost complete inability to obtain interspecific crosses between Vigna unguiculata and other Vigna species. Often only small pods formed and seeds aborted before development. However, attempts at crosses between all Vigna unguiculata cultivated species and wild species indicated that successful crosses are easy to obtain. Hybridization was most successful between wild x common cowpea and least successful between wild x sesquipedalis and clyndrica x sesquipedalis.

Indications of a developing barrier to gene exchange between cultivated and wild cowpea was noted in studies involving crosses between wild, weedy and domesticated genotypes (Rawal et al., 1976). Seed weights of resultant F1's were consistently lower when crosses were made between truly wild accessions and elite cultivars. No F1 seed weight reduction was observed, however, when wild x weedy or cultivated x weedy crosses were made.

Two outcrossing mechanisms found to occur in breeding plots at IITA were reported by Rachie et al

(1975). The first mechanism, genetic male sterility, is controlled by a simple recessive gene designated ms2 ms2. These mutants have facilitated artificial hybridization without emasculation and resulted in higher fruit setting than crosses using normal fertiles. Four other genetic male steriles have since been identified. All are inherited as simple recessives and have been designated as ms3 through ms6.

The second outcrossing mechanism reported by Rachie et al (1975) is mechanical in nature and involves a constriction of the petals. This provides an opening for the pistil to emerge at a pre-receptive stage while simultaneously restricting stamen development. This characteristic is also inherited as a simple recessive and has been designated cp cp for constricted petal. Unfortunately, seed set was poor in these mutants. The authors suggest the flowers may be unattractive to insect pollinators due to their appearance and lack of fragrance. In addition, the constricted petals pose a mechanical difficulty to insects in trying to probe the nectaries inside. However, ants were observed to play a role in pod setting in plants with constricted petal under greenhouse conditions although their contribution under field conditions is not known.

Extent of Known Diversity in Cowpea

Evaluation of World Collections

In order to determine the distribution of morphological characteristics in *Vigna unguiculata*, Faris (1965) collected over 380 samples world wide and measured several plant and seed variables. Only small differences were detected in the six plant characteristics measured (leaf length to width ratio, base angle, width, position; trifoliate leaf number; plant color) for the populations derived from Africa and India. However, in comparison of seed characteristics (length, depth, width and seed coat color, pattern and texture) large differences were detected between the two continents with Africa displaying the greatest range of variability.

Seven hundred and fifty accessions of cowpea from world collections were evaluated in India (Singh et al., 1971). As expected, special types were peculiar to India, such as small seeded *cylindrica* and long and narrow seeded *sesquipedalis*, reconfirming the directional selection pressure applied to these subspecies for different

purposes. In a second study, Mehra et al (1970) screened a world collection of cowpea for suitable forage types. After each cutting, existing varieties needed to be resown because of poor regrowth. Selection was based on finding genotypes capable of high yields under a multiple cutting regime. Of the more than 1000 accessions screened, fifty were selected for further genetic studies. Compared with African material, Indian genotypes were found to be more variable with respect to plant height, spread and degree of branchiness.

The world germplasm collection of Vigna unguiculata is maintained at the International Institute of Tropical Agriculature (IITA) in Nigeria. Over 10,000 accessions are contained within the cowpea world collection at IITA, a large portion of these being derived from within West Africa (IITA Germplasm Catalogue, 1974). Only a few of the accessions originated from Botswana. Evaluation of the lines for various morphological and physiological characteristics as well as insect and disease incidence demonstrated a wide range of variability for all characteristics. A comparison of the variability contained within this world collection and that observed in Botswana would be of interest (1) in detecting the type of genetic variability selected for, through both natural and artificial means, under semi-arid conditions and (2) to detect any differences due to crop usage and culture in

frequency distributions for various variables.

Evaluation of National Collections

Despite the importance of cowpea in many African nations, literature concerning the collection, evaluation and use of local landraces is scarce. Even with an abundance of adapted material at hand, many national programs look to the introduction of exotic plant material for crop improvement. This approach has been followed in Botswana as well. Although selections from exotic material may result in an increase in production, an evaluation of both local and exotic germplasm should be the first step in any crop improvement program.

In Ghana, Doku (1970) examined 39 varieties of native and introduced cowpea for various yield and physiological characteristics. Genotypic and phenotypic coefficients of variation and heritability estimates for the characters measured were shown to vary markedly. The author was able to identify differences in susceptibility to virus between cultivars. Local cultivars were found to be more sensitive to cowpea mosaic virus. Yet other favorable characteristics must account for the persistennce of these genotypes in the farmer's field. Such studies allow the

breeder to identify which genotypes to combine in a plant breeding program so as to produce a cultivar suitable to growers.

Evaluation of local collections of Vigna for use as a green-pod vegetable in India resulted in identification of 6 genotypes which offered a combination of three most important characterisitcs, ie photo-insensitivity, pod length and earliness (Mital et al, 1980). Another genotype was identified as carrying resistance to Macrophomina wilt and others were desirable donor parents for various characters of agronomic importance.

Genetic Vunerability

Improvements in agriculture and communications increase linkages between farmers and experiment stations, thus increasing the flow of technology to rural sectors. As the movement of introduced or improved varieties will be included in this 'information-flow', the local germplasm base may be replaced. In some cases, however, the local varieties are not replaced but, instead, mixing occurs (Knowles, 1969). Such a 'mixed-crop' may actually lead to the enhancement of the germplasm pool. The introduction of 'California Ramshorn Blackeye' into Botswana several years ago did not result in the displacement of local cultivars

in favor of this introduction. Instead, today one will find that a farmers' seed stock may consist of a variety of seed types, among which may be found a few seeds of 'Blackeye'. Through many years of adaptation and gene exchange, variations in 'Blackeye' seed size, eye size and shape and testa texture have arisen, giving evidence of germplasm exchange.

The threat remains, however, that economic pressure to increase yields may give the farmer no alternative but to abandon the planting of landraces in favor of introductions which may be higher yielding. This has not yet happened in Botswana, and the germplasm base of *Vigna unguiculata* landraces is still rich and varied in all plant and seed characteristics.

Diversity Studies

Sampling Natural Diversity

The fact that both wild and domesticated species of self-pollinated plants contain large reserves of genetic variablitiy has been demonstrated through population and ecological studies (Imam and Allard, 1965; Jain, 1969). This genetic variability has been shown to exist between

geographic areas as well as within populations of specific environmental sites. Often genetic variability will follow ecological clines, such as changes in plant height which may be correlated with altitude (Allard, 1970). Also, Imam and Allard (1965) demonstrated that genetic variability may be expressed in a 'mosaic pattern' which is superimposed on patterns of differentiation associated with geographical areas. Sampling such extensive diversity becomes a problem since any colleciton would obviously comprise but a portion of the total variation.

There has been much discussion on the best sampling procedures and collection methods which would result in maximum representative variability with minimum sample size (Allard,1970; Hawkes, 1981; Bennett, 1970). Yet the sampling problem arises because the collector may have no a priori knowledge of the extent of variability existing in an area and is therefore unsure whether large collections may not contain many duplicate samples or if small collections exclude extremes. These doubts may be somewhat eased by the study of topographical, vegetation, rainfall and soil maps of the areas to be sampled. A list of the diverse geographical areas within the collection zone may then serve as a starting point.

Basis of Diversity in Self-Pollinated Plants

Initially, self-pollinated plant populations were believed to be highly homogeneous with genotypes adapted to specific environmental niches (Stebbins, 1957). However, in their study, Allard, Jain and Workman (1968) demonstrated the complex structure inherent in self-pollinated species. Large reserves of genetic variability and heterozygosity have been shown to exist within populations of agricultural and natural species and polymorphic differences within landraces are common. The fact that this variability is due, in part, to heterozygosity may be demonstrated by (1) the segregation of progeny for prominent polymorphic characters and (2) the response to selection for (+) and (-) characters of a quantitative nature (Allard, 1970). The combination of genotypic differences coupled with phenotypic plasticity, which allows self-pollinated species to occupy micro-niches, results in the wide diversity observed in these plant types (Imam and Allard, 1965).

The persistence of such genetic variability, particularly for agricultural crops under heavy inbreeding, can only be understood in lignt of a number of complex, interacting factors such as migration, mating systems, population size, local and yearly fluctuations in selection pressures and seed densities in the soil (Imam and Allard

1965) in combination with several human, cultural factors.

Diversity Measurements

The diversity found within a species may be used for character improvement in a plant breeding program (Doku, 1970; Mehra et al., 1970), to study the nature of inheritance of certain plant traits (Ojomo, 1971; Brittingham, 1950; Roy and Richharia, 1948) or to evaluate genetic distances within or between populations which gives an estimate of relatedness between individuals (Jain et al, 1975; Lewontin, 1972). The two former uses are of immediate, practical importance to plant breeding programs while the latter yields important information as to the extent of genetic homogeneity within a population or between populations. In terms of genetic vulnerability, a population which is highly homogeneous or populations which are closely related may be at a greater genetic risk (Adams, 1977).

Several multivariate techniques are currently used to study genetic diversity (Cooley and Lohnes, 1971; Reyment et al, 1984). Among the techniques most often used in crop species are principal components analysis (Adams, 1977; Adams and Wiersma, 1977; Moore, 1975), discriminant analysis (Riggins et al., 1977; Ellis et al., 1971),

Mahalanobia's D2 statistic (Chandra, 1977; Narayan and Macefield, 1976; Lee and Kaltsikes, 1973) and various multivariate clustering methods (Edye et al.,1970; Scott and Knott 1979; Maronna and Jacovkis, 1974; Broich and Palmer, 1980; DeWet and Hukabay, 1967).

In order to evaluate genetic distances between or within populations, several diversity indices have been formulated and are currently used in ecological and population studies (Pielou, 1966). The Shannon-Weaver diversity index (H') is one such statistic that has been particularly favored among ecologists (Poole, 1974). Orginating from information theory, the index was developed to measure the amount of entropy, or randomness, associated with the generation of information messages (Shannon and Weaver, 1964). As it relates to the biological sciences, the index gives an estimate of genetic diversity based on frequency data. Maximum diversity in a population is achieved if all genotypic classes or species are equally represented as a percent of the total. Likewise, H' is lowest when only a few classes/species are common and others non-existent or rare. However, diversity is not solely dependent on relative abundance but depends on the number of classes/species as well. Therefore, changes (increases) in H' occur as more classes/species are added to a collection such as occurs through mutation or natural hybridization. The Shannon-Weaver diversity index is

favored because it takes both number and frequency of genotypic classes or species into account and expresses them on a common scale (Lloyd and Ghelardi, 1964).

Succussful use of this index for distinguishing geographical patterns in a world colleciton of Durum wheat has been demonstrated by Jain et al (1975). More than 3000 accessions were examined for various phenotypic traits, some of them polymorphic in nature. Estimations of H' were calculated for individual countries as well as regions. The highest value obtained was derived from analysis of Ethiopian accessions, one of the reputed centers of diversity for Durum wheat.

Poiarkova and Blum (1983) also used the index to measure variability among wheat landraces in Israel. Compared with Jain's estimates of phenotypic diversity from Middle Eastern seed samples (H' = 0.54), they found the diversity originating from Israel was even higher (H' = 0.77) than that found in the total Middle Eastern component of the world wheat collection.

Rationale for the Present Work

This thesis work was undertaken in an attempt to define the extent of genetic variability present in Botswana landraces of cowpea. It was felt that the

establishment of a germplasm collection is a necessary foundation on which to construct a plant breeding program.

MATERIALS AND METHODS

In October, 1982 a series of germplasm collection trips was initiated with the objective of sampling the diversity of local cowpea landraces in Botswana. Collections continued over a two year period culminating in a total of four expeditions and over 600 seed samples. A total of 34 villages was visited and several farmers per village were randomly selected for germplasm sampling (Appendix A & B). Farmers were visited in various districts and also out at the 'lands' - field plots located some distance from the village where planting is done. Collection trips conducted in September-October were most successful because this is the time of year when farmers are preparing their seeds for sowing in anticipation of November-December rains. Following each colleciton trip, samples were brought to the Sebele Agricultural Station and fumigated for storage weevils (Callosobruchus sp). Each sample was separated according to seed type and assigned an accession number. Similar seed types producing different plant growth habits were treated as separate accessions. All accessions were stored in glass or metal containers and put into cold storage.

Germplasm Evaluation

Random samples were removed from storage for field evaluation during two successive years. During the first year (1982), 180 accessions were planted for field evaluation at the Sebele Agriculture Research Station while 157 were field evaluated in 1983. For each experiment, one five-meter row of each accession was planted. The rows were spaced 1.5 meters with 20 cm plant spacing within the row. Phosphate was applied pre-plant as superphosphate (P_2O_5 = 10.5%) at the rate of 250 kg/ha. No nitrogen fertilizer was applied. Insecticides were used as needed to control flower thrips (*Megalurothrips* ssp) or pod-sucking bugs.

Description of the Evaluation Sites

The Agriculture Research Station is located at Sebele about 10 Km north of Gaborone, the capital city. The station is located at latitude 24°34' (South) and longitude 25°57' (East) at an altitude of 994 meters. The soils of the research station are underlain by Gaborone Granite. They are moderate to well drained with a slope of <10% and a depth of 90 cm or more. Soil pH varies between profiles

from acid (pH 5.1 - 6.0) topsoil to neutral or mildly alkaline (pH 6.6 - 7.8) subsoil. During the 1982 evaluation, accessions were planted on a Chromic Cambisol. In 1983, a Eutric Cambisol was used for the evaluation.

Rainfall in Botswana is restricted to the months of October through April and is highly variable. Distributions for 1982-83 and 1983-84 are shown in Appendix C & D. Mean maximum/minimum monthly air temperatures, total hours of sunshine, relative humidity, solar radiation and soil evaporation for the two growing seasons are listed and compared in Appendix E.

Discriptors and Coding Used for Field Evaluations

Seed samples were evaluated on the basis of various morphologic and agronomic characteristics. Both quantitative and qualitative traits were recorded. Qualitative traits such as growth habit and seed coat color are coded in numeric form for ease in data analysis.

1. Plant Growth Habit

   1 - Acute erect: branches form acute angle to main stem

   2 - Erect: branches are less acute than 1

   3 - Semierect: lower branches are perpendicular to main stem but do not touch the ground

   4 - Intermediate: some lower branches touch the ground

5 - Semiprostrate: main stem reaches 20 cm or more above the ground and lower branches spread to 1 meter

6 - Prostrate: plants lie flat on the ground

7 - Climbing

2. Twining Habit

1 - No twining tendency

2 - Moderate

3 - Pronounced

3. Pod Attachment to Peduncle - recorded during green pod stage

1 - Erect pod attachment to peduncle

2 - Angle between pod and peduncle is 90^{o} or less

3 - Pendant pods

4. Number of main branches: values represent averages of three plants at 42 days after planting

5. Number of nodes per main stem: values represent averages of three plants recorded 28 days after planting

6. Peduncle length: measured when peduncles were full length (cm)

7. Raceme position: recorded when peduncles were fully expanded

1 - Pods are produced above canopy

2 - Pods are formed in upper leaf layers of canopy

3 - Pods are formed in all layers of crop canopy

8. Days to 50% first flower: recorded before 10:00 a.m. when flowers were fully open; values represent the number of days from planting until 50% of the plants in a plot have their first open flower

9. Days to 95% ripe pod: the number of days from planting until 95% of the plants in a single row have their first mature pod

10. Pod formation period: the number of days from flowering until pods are fully mature

11. Determinacy

    1 - determinate

    2 - indeterminate

12. Vigor index: a measure of the height and width of plant foliage in each plot; values represent an average of three plants measured 28 days after planting (cm)

13. Flower pigmentation: recorded when flowers are fully open and before 10:00 a.m.

    1 - flowers are white; no pigmentation

    2 - wing has pigmented margin and standard has pigmented "V"

    3 - wing and standard have pigmented margin

    4- wing has pigmented margin; standard lightly pigmented

    5 - both wing and standard are completely pigmented

14. Plant pigmentation: recorded 56 days after planting

    1- no pigmentation on stem, branches, petioles or peduncles

    2 - moderate pigmentation on stem, branches, petioles, and/or peduncles

    3 - extensive pigmentation on stem, branches, petioles, and/or peduncles

    4 - Solid pigmentation on stem, branches, petioles and/or peduncles

15. Pod pigmentation: recorded when pods are still green

    1 - pods are solid green with no pigmentation

    2 - pods have a pigmented tip only

    3 - pods have pigmented suture and tip

    4 - pods have pigmented valves but sutures are green

5 - pods have spotted pigmentation

6 - pods are uniformally pigmented

16. Pod shape: recorded at the green pod stage

1 - straight

2 - curved

3 - coiled

17. Pod length: values represent the average of 3 pod measurements in mm of fully matured pods

18. Pod width: values represent the average of same three pods as above

19. Terminal leaflet shape: recorded 42 days after planting

1 - globose: width is greatest near leaflet tip

2 - subglobose: width is greatest near petiole

3 - hastate: leaflet is wide near petiole but narrows near tip, "T" shaped

4 - subhastate: width of leaflet is greatest across center

5 - strip: leaflet is similar to hastate but narrower

20. Terminal leaflet length: one leaflet measured from base of petiole to leaf tip in mm

21. Terminal leaflet width: same leaflet as above measured at widest point in mm

22. Pod shattering:

1 - non-shattering

2 - shattering

23. 10 seed weight: weight in grams of 10 randomly selected seeds

24. Seed length: a measurement in mm of seed length from end to end of one randomly selected seed

25. Seed width: a measurement in mm of seed width perpendicular to length of one randomly selected seed

26. Seed thickness: a measurement in mm of seed thickness perpendicular to length across hilum; same seed as above

27. Seed crowding: a visual estimate of the amount of seed compression in fully mature pods; values represent average of three pods

    1 - no seed compression

    2 - slight compression

    3 - marked compression

    4 - highly compressed; width is greater than length

28. Testa texture:

    1 - smooth

    2 - rough

    3 - wrinkled

    4 - loose

    5 - split

29. Seed eye pattern: the eye refers to the pigmented area around the hilum

    1 - eye covers the entire seed; self-colored group

    2 - eye encircles the micropylar end of the hilum in a clear line widening at the sides and covers the non-micropylar portion of the seed with an indistinct margin; watson group

    3 - eye encircles the hilum with a distinct margin but the margin may widen at the sides or extend out the non-micropylar end of the hilum to varying degrees; holstein group

    4 - like holstein, showing a distinct margin, but smaller; small eye group

    5 - similar to small eye group except that the eye spills out in front of the hilum for a short

distance and has an indistinct margin; narrow eye group

6- narrow eye with some speckling and/or mottling on the body; kabba group

30. Eye color:

1 - black eye group

2 - blue eye group

3 - purple eye group

4 - speckled/mottled eye group

5 - brown/tan eye group

6 - red eye group

7 - green eye group

31. Pods per peduncle: average of three observations

32. Locules per pod: average of three observations

33. Seeds per pod: average of three observations

34. Pods per plant: total number of pods produced per single-row plot divided by the number of plants

35. 100 seed weight: weight in grams of 100 randomly selected seeds

Shannon-Weaver Diversity Index

Data was analyzed on a per-character basis by simple calculations of means, ranges, standard deviations and frequency distributions. This yielded information of genetic diversity for both quantitative and qualitative data on a national (geographic) level. Afterwards, five

regions within the country were delineated as separate collection sites (see appendix A) based on geographical clustering and actual distances. Qualitative traits were class-coded for frequency analysis. Traits of a quantitative nature were divided into discrete categories and coded so that class frequencies could be obtained. Divisors differed depending on the range of the character under analysis, but were constant across all regions for each character.

During the actual collection, differences were noted between sampling sites for such characteristics as seed color and size. Analysis of the diversity contained within each of these sites was accomplished by using the Shannon-Weaver diversity index (H'). Individual indices were calculated for each site and for all descriptors. Only 219 of the 336 accessions were included in the latter analysis because of uncertainty regarding origin of some seed samples. Proper use of the Shannon-Weaver diversity index is based on random samples from a larger population. As collection sites were chosen at random and farmers within those sites were also chosen at random, it is felt that the data recorded meet that requirement.

The equation for diversity measurement is given by the formula

$$H' = -\sum_{i=1}^{s} pi \log pi \qquad (1)$$

where; s = the number of genotypic classes or species

pi = the proportion of the total number of individuals comprising the ith class or species

and, $\sum pi = 1$

Since the log of any value <1 is negative, the formula is preceded by a negative constant (usually 1) which results in a positive value of H'. The base of logarithms is open to choice but in most cases the base e should be used (Poole, 1974).

H' is an upward biased estimate and is correctable providing the true value of s is known, since the magnitude of the bias depends on it (Pielou, 1966). However, this value is rarely known. When using natural logarithms, the expected value of H' may be found by the formula

$$E(H') = \left| -\sum_{i=1}^{s} pi \ln pi \right| - \left| \frac{s - 1}{2N} \right| \qquad (2)$$

where; N = the size of the sample

The variance of the estimate of H' is found by

$$var(H') = \frac{\sum_{i=1}^{s} pi \ln^2 pi - (\sum_{i=1}^{s} pi \ln pi)^2}{N} + \frac{s - 1}{2 N^2} \qquad (3)$$

In large samples, the first term is usually sufficient. The approximate standard error of H' is given by

$$s.e. = \sqrt{\frac{\sum_{i=1}^{s} pi \ln^2 pi - (\sum_{i=1}^{s} pi \ln pi)^2}{N}} \quad (4)$$

When H' has been estimated for two or more collections, pair-wise comparisons can be made with a t-test where

$$t = \frac{H_1' - H_2'}{[var(H_1') + var(H_2')]^{1/2}} \quad (5)$$

and the null hypothisis is Ho: $H_1' \neq H_2'$. The approximate degrees of freedom of the test is calculated by

$$df = \frac{[var(H_1') + var(H_2')]^2}{var(H_1')^2 / N_1 + var(H_2')^2 / N_2} \quad (6)$$

where; N1 = the number of individuals in the first sample
N2 = the number of individuals in the second sample

Cluster Analysis

Further analysis of the data was performed using the Clustan program for multivariate data analysis (Program Library Unit, Edinburgh University, 1978). Nineteen variables were used for the clustering procedure and 213 accessions were included in the analysis. Accessions having missing values for any of the variables were excluded. Ward's method, based on error sum of squares, was

chosen as the hierarchy option. After analysis, the number of clusters selected for data interpretation was based on eigenvalues greater than two.

RESULTS AND DISCUSSION

Analysis of Genetic Variability

Both quantitative and qualitative traits showed a broad range of variabiliity on a nation-wide basis (Tables 1 and 2). Examination of the genetic variability present throughout Botswana reveals similarities, for most traits, to the extent of diversity previously found in the world collection of *Vigna unguiculata* held at IITA in Nigeria (IITA, 1974). Such a wide range of diversity in a small geographic area the size of Botswana (582,139 sq. km) suggests a large amount of gene exchange and recombination. Many factors undoubtedly contribute to this diversity, including natural hybridization, natural and artificial selection and gene exchange between one geographic area and another.

Analysis on a regional basis reveals the predominance of certain plant traits over others (Tables 3 and 4) which, quite possibly, results from farmer preferance for certain genotypes. Farmers do intentionally select certain seed and plant types over others for propagation for specific purposes. Throughout our collection trips, farmers were

Table 1. Number of accessions, means and ranges for quantitative characters.

Character	Accessions	$\bar{X}$	Range
No. main branches	218	3.50 ± 1.62	1 - 9
Nodes per main stem	219	4.73 ± 1.32	2 - 9
Peduncle length (cm)	218	14.37 ± 5.84	3.00 - 36.33
Days to 50% first flower	215	87.50 ± 39.40	39 - 168
Days to 95% ripe pod	213	139.32 ± 44.93	64 - 189
Pod forming period	213	53.27 ± 35.95	12 - 147
Plant height (cm)	219	13.80 ± 3.54	6.66 - 30.00
Plant width (cm)	219	20.48 ± 6.10	4.66 - 42.00
Pod length (mm)	214	129.34 ± 32.12	37.50 - 208.66
Pod width (mm)	214	7.79 ± 1.66	4.00 - 11.67
Leaflet length (mm)	219	89.54 ± 1.70	54 - 160
Leaflet width (mm)	218	53.79 ± 1.13	28 - 100
10 seed weight (g)	219	1.55 ± .50	5.00 - 3.50
Seed length (mm)	219	7.28 ± 1.54	4 - 12
Seed width (mm)	219	6.19 ± 1.25	4 - 9
Seed thickness (mm)	219	4.48 ± 0.93	3 - 7
Pods per peduncle	214	1.82 ± 0.74	1 - 4
Locules per pod	214	13.95 ± 2.24	5 - 21
Seeds per pod	216	10.26 ± 3.02	2 - 16

Table 2. Number of accessions and ranges for qualitative characters.

Character	Accessions	Range
Growth habit	219	1 - 7
Twining habit	219	1 - 3
Pod attachment	215	1 - 3
Raceme position	218	1 - 3
Determinacy	215	1 - 2
Flower pigmentation	211	1 - 6
Plant pigmentation	219	1 - 4
Pod pigmentation	215	1 - 6
Pod shape	217	1 - 3
Leaflet shape	219	1 - 4
Pod shattering	217	0 - 1
Seed crowding	219	1 - 4
Testa texture	219	1 - 5
Eye pattern	219	1 - 6
Eye color	219	1 - 7

Table 3. Number of accessions, means and ranges for quantitative characters by regions.

Character	Region 1			Region 2			Region 3			Region 4			Region 5		
	Accessions	$\bar{X}$	Range	Accessions	$\bar{X}$	Range	Accessions	$\bar{X}$	Range	Accessions	$\bar{X}$	Range	Accessions	$\bar{X}$	Range
No. main branches	13	3.85	2-6	102	2.94	1-7	66	3.42	1-7	24	5.33	3-9	13	4.62	3-6
Nodes per main stem	13	5.62	3-8	103	4.50	2-8	66	4.45	2-8	24	5.58	3-9	13	5.46	3-7
Peduncle length (cm)	13	12.80	6.66-23.33	103	15.94	3.66-36.33	65	13.12	4.66-25.33	24	12.16	3.00-18.33	13	13.86	4.66-23.00
Days to 50% first flower	13	92.62	54-148	102	71.15	41-158	63	97.00	39-168	24	116.50	54-158	13	111.08	60-158
Days to 95% ripe pod	13	166.08	76-189	102	124.41	66-189	63	139.73	64-188	22	169.77	84-188	13	176.00	94-188
Pod forming period	13	81.15	26-132	102	53.26	15-147	63	44.11	12-127	22	56.27	21-132	13	64.69	15-124
Plant height (cm)	13	13.17	7.66-17.00	103	14.06	7.00-30.00	66	13.25	6.66-20.66	24	14.36	10.00-23.33	13	14.16	9.00-21.66
Plant width (cm)	13	22.05	12.33-32.00	103	19.29	4.66-33.33	66	19.58	7.33-42.00	24	25.47	15.33-32.67	13	23.73	11.00-32.00
Pod length (mm)	13	128.05	81.67-199.66	103	122.84	65.00-200.33	64	135.63	37.50-208.66	21	136.91	72.00-185.66	13	139.06	89.00-180.33
Pod width (mm)	13	8.23	6.00-11.67	103	7.32	4.00-11.33	64	8.16	5.33-11.66	21	8.34	6.00-11.00	13	8.47	5.00-11.33
Leaflet length (mm)	13	93.54	56-110	103	87.32	54-127	66	85.27	55-140	24	99.42	75-125	13	106.62	83-160
Leaflet width (mm)	12	57.17	37-80	103	53.24	33-82	66	50.30	28-83	24	60.17	45-79	13	61.00	35-100
10 seed weight (g)	13	1.86	1.0-3.4	103	1.39	.70-3.5	66	1.69	.60-3.0	24	1.66	.90-3.3	13	1.67	5-24
Seed length (mm)	13	7.46	4-12	103	6.94	4-12	66	7.61	4-11	24	7.46	5-11	13	7.85	6-11
Seed width (mm)	13	6.54	5-8	103	5.66	4-8	66	6.71	4-9	24	6.42	5-9	13	7.00	5-9
Seed thickness (mm)	13	5.00	4-7	103	4.39	3-6	66	4.67	3-7	24	4.17	3-7	13	4.38	3-6
Pods per peduncle	13	1.38	1-2	102	2.05	1-4	64	1.73	1-3	22	1.41	1-2	13	1.54	1-3
Locules per pod	13	13.62	10-16	103	13.89	8-18	64	13.66	9-21	21	15.10	11-18	13	14.31	12-18
Seeds per pod	13	9.77	5-14	103	10.27	3-16	65	10.46	2-16	22	10.05	4-15	13	10.08	6-13

Table 4. Number of accessions and ranges for qualitative characters by regions.

Character	Region 1		Region 2		Region 3		Region 4		Region 5	
	Accessions	Range	Accessions	Range	Accessions	Range	Accessions	Range	Accessions	Range
Growth habit	13	2 - 5	103	1 - 7	66	1 - 6	24	3 - 5	13	2 - 6
Twining habit	13	1 - 3	103	1 - 3	66	1 - 3	24	1 - 3	13	1 - 2
Pod attachment	13	1 - 3	102	1 - 3	64	1 - 3	23	1 - 3	13	1 - 3
Raceme position	13	2 - 3	103	1 - 3	66	1 - 3	24	2 - 3	12	1 - 3
Determinacy	13	1 - 2	103	1 - 2	62	1 - 2	24	1 - 2	13	1 - 2
Flower pigmentation	13	1 - 6	101	1 - 6	61	1 - 6	24	1 - 6	12	2 - 6
Plant pigmentation	13	1	103	1 - 3	66	1 - 4	24	1 - 3	13	1 - 4
Pod pigmentation	13	1	102	1 - 6	65	1 - 6	23	1 - 5	12	1 - 5
Pod shape	13	1 - 3	102	1 - 2	65	1 - 3	24	1 - 2	13	1 - 2
Leaflet shape	13	2 - 3	103	1 - 4	66	2 - 4	24	2 - 3	13	2 - 4
Pod shattering	13	0 - 1	103	0 - 1	65	0 - 1	23	0	13	0
Seed crowding	13	1 - 4	103	1 - 4	66	1 - 4	24	1 - 4	13	1 - 4
Testa texture	13	1 - 5	103	1 - 4	66	1 - 4	24	1 - 4	13	1 - 4
Eye pattern	13	1 - 6	103	1 - 6	66	1 - 6	24	1 - 6	13	1 - 6
Eye color	13	1 - 6	103	1 - 6	66	1 - 7	24	1 - 7	13	4 - 6

asked why they grew a particular mixture and what special attributes were possessed by the components of the mixtures which made them desirable. Almost invariably, the farmers could identify particular seed types, often calling them by name, and cite the characteristics of these genotypes which contributed to its significance in the mixture (i.e. good drought tolerance, aphid resistance, resistance to pod bugs, etc.).

Correlation coefficients between metrical traits among cowpea landraces are shown in Table 5. Correlations were noted between days to 50% first flower, pod forming period and most other metrical traits. A range of 12 to 147 days for pod formation found among the accessioons reflects the variability present in the landraces for growth habit, since erect, determinate types often mature quickly while prostrate, indeterminate types require several weeks to complete their reproductive phase. The observed variability in reproductive period and consequent correlation with other plant traits is one genetic aspect of cowpea which may be related to the social and ecological structure of the country. Mixtures of fast maturing determinate varieties with late maturing, indeterminate types provides some insurance to farmers that some yields will be obtained, either in leaf or grain harvest, even if only scant seasonal rainfall is received. Indetermiate types supply a source of leaf vegetable over

Table 5. Matrix of correlation coefficients between quantitative traits of cowpea landraces.

Character		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Main branches	1		0.586**	-0.035	0.187**	0.207**	0.047	0.248**	0.558**	0.242**	0.281**	0.292**	0.317**	0.123	0.123	0.227**	0.077	-0.121	0.296**	0.163*
Nodes on main stem	2			-0.098	0.191**	0.183**	0.027	0.455**	0.670**	0.135	0.231**	0.425**	0.472**	0.141*	0.067	0.217**	0.141*	-0.175*	0.228**	0.136
Peduncle length	3				-0.309**	-0.346**	-0.127	0.125	-0.196**	0.075	-0.251**	-0.208**	-0.129	-0.206**	-0.136	-0.296**	-0.126	0.357**	0.164*	0.204**
Days to 50% flowering	4					0.640**	-0.289**	-0.095	0.243**	0.405**	0.519**	0.371**	0.128	0.436**	0.280**	0.469**	0.131	-0.248**	0.137	0.134
Days to 95% ripe pod	5						0.525**	-0.130	0.313**	0.346**	0.500**	0.424**	0.313**	0.399**	0.179*	0.372**	0.205**	-0.234**	0.131	0.104
Pod forming period	6							-0.075	0.123	0.019	0.073	0.133	0.235**	0.030	-0.070	-0.038	0.100	-0.041	0.031	0.000
Plant height	7								0.585**	0.050	0.092	0.306**	0.379**	0.116	0.110	0.098	0.136	0.108	0.030	-0.007
Plant width	8									0.202**	0.387**	0.640**	0.575**	0.267**	0.223**	0.333**	0.263**	-0.160*	0.143	0.032
Pod length	9										0.569**	0.287**	0.110	0.518**	0.413**	0.310**	0.203**	-0.013	0.563**	0.593**
Pod width	10											0.545**	0.404**	0.697**	0.437**	0.579**	0.477**	-0.227**	0.143*	0.192**
Leaflet length	11												0.749**	0.372**	0.241**	0.417**	0.329**	-0.206**	0.158*	0.082
Leaflet width	12													0.287**	0.138	0.252**	0.282**	-0.137	0.022	-0.059
10 seed weight	13														0.583**	0.683**	0.603**	-0.249**	0.094	0.138
Seed length	14															0.549**	0.395**	-0.151*	-0.018	-0.023
Seed width	15																0.624**	-0.344**	0.035	0.062
Seed thickness	16																	-0.215**	-0.013	0.068
Pods per peduncle	17																		0.058	0.134
Locules per pod	18																			0.734**
Seeds per pod	19																			

*,**Significant at the 0.05 and 0.01 probability levels, respectively.

an extended period of time, as well as grain. In addition, they provide a wealth of forage material for cattle, which is the main source of revenue to Botswana farmers.

Shannon-Weaver Diversity Measurements

Phenotypic diversities, as measured by the Shannon-Weaver index (H'), for fifteen qualitative plant characters are presented in Table 6. Differences among the five regions were obtained for all traits under investigation. Generally, index values were quite high, and for only a few plant characters (ie. plant and pod pigmentation in region 1 and pod shattering in regions 4 and 5) was monomorphism expressed. Averages of H' values over all characters within areas showed region 2 and 3 to contain the highest amounts of phenotypic diversity (1.0196 and 0.9550, respectively) while region 1 had the lowest value (0.7180).

Diversity index values were used to calculate expected values of H' (E(H')) and variances (Var(H')) shown in Table 7. From this information, pairwise comparisons were calculated between H' values of different regions for all characters studied. The resultant t-tests (Table 8) indicate which regions differ significantly with respect to phenotypic diversity. Although index values obtained for growth habit were high for all regions, significant

Table 6. Shannon-Weaver diversity indices for qualitative characters.

Character	Region 1	2	3	4	5
Growth habit	1.2657	1.5542	1.5901	1.0775	1.4126
Twining habit	0.2712	0.6469	0.3064	0.7924	0.4292
Pod attachment	1.0123	0.8652	0.9960	0.6324	0.9251
Raceme position	0.6902	1.0695	0.8881	0.6365	1.0397
Determinacy	0.4293	0.6927	0.6674	0.6616	0.5402
Flower pigmentation	1.0101	1.6401	1.4143	1.2817	0.9830
Plant pigmentation	0	0.7542	0.8955	0.6750	0.8587
Pod pigmentation	0	1.0363	0.7830	0.4703	1.0984
Pod shape	1.0101	0.6220	0.8484	0.6365	0.6663
Leaflet shape	0.4292	0.8412	0.8798	0.3768	0.9110
Pod shattering	0.2711	0.5542	0.1394	0	0
Seed crowding	1.3060	1.1373	1.3059	1.2678	1.3517
Testa texture	0.6870	0.7772	0.7694	0.6161	0.6870
Eye Pattern	1.0101	1.5835	1.2999	0.8602	0.6902
Eye color	1.3777	1.5194	1.5408	1.5373	1.0101
$\overline{H}'$	0.7180	1.0196	0.9550	0.7681	0.8402

Table 7. Shannon-Weaver diversity indices, expected values and variances for qualitative traits.

Character	N*	S**	H'	E (H')	Var (H')
			Region 1		
Growth habit	13	7	1.2657	1.0349	0.0138
Twining habit	13	3	0.2712	0.1943	0.0337
Pod attachment	13	3	1.0123	0.9354	0.0109
Raceme position	13	3	0.6902	0.6133	0.0005
Determinacy	13	2	0.4293	0.3908	0.0291
Flower pigmentation	13	6	1.0101	0.8178	0.0137
Plant pigmentation	13	4	0	-	-
Pod pigmentation	13	6	0	-	-
Pod shape	13	3	1.0101	0.9332	0.0137
Leaflet shape	13	5	0.4292	0.2754	0.0291
Pod shattering	13	2	0.2711	0.2326	0.0337
Seed crowding	13	4	1.3060	1.1906	0.0116
Testa texture	13	5	0.6870	0.5332	0.0482
Eye pattern	13	6	1.0101	0.8178	0.0137
Eye color	13	7	1.3777	1.1469	0.0327
			Region 2		
Growth habit	103	7	1.5542	1.5251	0.0047
Twining habit	103	3	0.6469	0.6372	0.0067
Pod attachment	102	3	0.8652	0.8554	0.0042
Raceme position	103	3	1.0695	1.0598	0.0005
Determinacy	103	2	0.6927	0.6878	0
Flower pigmentation	101	6	1.6401	1.6302	0.0028
Plant pigmentation	103	4	0.7542	0.7369	0.0043
Pod pigmentation	102	6	1.0363	1.0118	0.0111
Pod shape	102	3	0.6220	0.6122	0.0013
Leaflet shape	103	5	0.8412	0.8218	0.0076
Pod shattering	103	2	0.5542	0.5493	0.0023
Seed crowding	103	4	1.1373	1.1227	0.0042
Testa texture	103	6	0.7772	0.7529	0.0061
Eye pattern	103	6	1.5835	1.5592	0.0005
Eye color	103	7	1.5194	1.4903	0.0016

Table 7. (Continued).

Character	N*	S**	H'	E (H')	Var (H')
			Region 3		
Growth habit	66	7	1.5901	1.5446	0.0035
Twining habit	66	3	0.3064	0.2912	0.0099
Pod attachment	64	3	0.9960	0.9804	0.0031
Raceme position	66	3	0.8881	0.8729	0.0052
Determinacy	62	2	0.6674	0.6513	0.0008
Flower pigmentation	61	6	1.4143	1.3733	0.0113
Plant pigmentation	66	4	0.8955	0.8728	0.0044
Pod pigmentation	65	6	0.7830	0.7445	0.0203
Pod shape	65	3	0.8484	0.8330	0.0007
Leaflet shape	66	5	0.8798	0.8495	0.0060
Pod shattering	65	2	0.1394	0.1317	0.0055
Seed crowding	66	4	1.3059	1.2822	0.0022
Testa texture	66	6	0.7694	0.7315	0.0060
Eye pattern	66	6	1.2999	1.2670	0.0069
Eye color	66	7	1.5408	1.4953	0.0045
			Region 4		
Growth habit	24	7	1.0775	0.9525	0.0017
Twining habit	24	3	0.7924	0.7509	0.0139
Pod attachment	23	3	0.6324	0.5889	0.0264
Raceme position	24	3	0.6365	0.5948	0.0044
Determinacy	24	2	0.6616	0.6408	0.0025
Flower pigmentation	24	6	1.2817	1.1775	0.0091
Plant pigmentation	24	4	0.6750	0.6125	0.0225
Pod pigmentation	23	6	0.4703	0.3616	0.0323
Pod shape	24	3	0.6365	0.5948	0.0044
Leaflet shape	24	5	0.3768	0.2935	0.0173
Pod shattering	23	2	0	-	-
Seed crowding	24	4	1.2678	1.2053	0.0076
Testa texture	24	5	0.6161	0.5328	0.0258
Eye pattern	24	6	0.8602	0.7560	0.0148
Eye color	24	7	1.5373	1.4123	0.0153

Table 7. (Continued).

Character	N*	S**	H'	E (H')	Var (H')
			Region 5		
Growth habit	13	7	1.4126	1.1818	0.0257
Twining habit	13	3	0.4292	0.3523	0.0291
Pod attachment	13	3	0.9251	0.8482	0.0250
Raceme position	12	3	1.0397	0.9564	0.0100
Determinacy	13	2	0.5402	0.5017	0.0198
Flower pigmentation	12	6	0.9830	0.7747	0.0589
Plant pigmentation	13	4	0.8587	0.7433	0.0262
Pod pigmentation	12	6	1.0984	0.8901	0.0801
Pod shape	13	3	0.6663	0.5894	0.0040
Leaflet shape	13	5	0.9110	0.7572	0.0175
Pod shattering	13	2	0	-	-
Seed crowding	13	4	1.3517	1.2363	0.0048
Testa texture	13	5	0.6870	0.5332	0.0482
Eye pattern	13	6	0.6902	0.4979	0.0005
Eye color	13	7	1.0101	0.7793	0.0137

*N = number of accessions evaluated; **S = number of phonotypic classes per character.

Table 8. Student's t values for pairwise comparisons by regions of H' values for qualitative plant characters.

Region	1	2	3	4	5
			Growth Habit		
1		2.1211*	2.4664*	1.5117	0.7391
2			0.3964	5.9588**	0.8121
3				7.1085**	1.0387
4					2.0244
5					
			Twining Habit		
1		1.8692	0.1686	2.3889*	0.5856
2			2.6428**	1.0137	1.1506
3				3.1503**	0.6218
4					1.7515
5					
			Pod Attachment		
1		1.1971	0.1378	1.9670	0.4602
2			1.5309	1.3308	0.3505
3				2.1170*	0.4230
4					1.2910
5					
			Raceme Position		
1		11.9945**	2.6212*	0.7671	3.4108**
2			2.4027*	6.1857**	0.2908
3				2.5679*	1.2296
4					3.3600**
5					
			Determinacy		
1		1.5438	1.3770	1.3068	0.5015
2			0.8895	0.6209	1.0835
3				0.1010	0.8862
4					0.8129
5					
			Flower Pigmentation		
1		4.9045**	0.5623	1.7987	0.1006
2			1.9016	3.2854**	2.6454*
3				0.9284	1.6278
4					1.8257
5					

Table 8. (Continued).

Region	1	2	3	4	5
			Plant Pigmentation		
1		-	-	-	-
2			1.5149	0.4838	0.5984
3				1.3441	0.2104
4					0.8324
5					
			Pod Pigmentation		
1		-	-	-	-
2			1.4295	0.27169**	0.2056
3				1.3634	0.9954
4					1.8735
5					
			Pod Shape		
1		3.1688**	1.3475	2.7769*	2.5842*
2			5.0625**	0.1921	0.6085
3				2.9672**	2.6562*
4					0.3251
5					
			Leaflet Shape		
1		2.1506*	2.3848*	0.2433	2.2319*
2			0.3310	2.9430**	0.4406
3				3.2953**	0.2035
4					2.8636**
5					
			Pod Shattering		
1		1.4921	0.6652	-	-
2			4.6967**	-	-
3				-	-
4					-
5					
			Seed Crowding		
1		1.3421	0.0009	0.2757	0.3569
2			2.1075*	1.2013	2.2600*
3				0.3849	0.5474
4					0.7534
5					

Table 8. (Continued).

Region	1	2	3	4	5
			Testa Texture		
1		0.3871	0.3539	0.2606	-
2			0.0709	0.9020	0.3871
3				0.8597	0.3539
4					0.2602
5					
			Eye Pattern		
1		4.8119**	2.0191*	0.8879	2.6845*
2			3.2968**	5.8475**	28.2486**
3				2.9849**	7.0876**
4					1.3744
5					
			Eye Color		
1		0.7651	0.8456	0.7308	1.7065
2			0.2740	0.1389	4.1174**
3				0.0251	3.9338**
4					3.1120**
5					

*,**Significant at the .05 and .01 level respectively.

differences in H' were obtained between most regions except 5, whose value (H'=1.4126) was intermediate between the two extremes, region 4 (H'=1.0775) and region 3 (H'=1.5901). Such differences in diversity are noted by a lower degree of polymorphism for a given trait, as in region 4, or a high degree of phenotypic diversity with some alleles expressed more frequently than others, as in region 3. Figures 1 and 1a demonstrate the relative frequencies of the various classes of plant growth habit occurring on a country-wide and regional basis. The position of cowpea racemes, and consequently pod production, is an important plant breeding characteristic in developing varieties for commercial production. Botswana landraces of cowpea show considerable variability both within and between regions for this trait. Significant differences were obtained between nearly all regions (Table 8), with region 2 displaying the greatest variability (Figures 2 and 2a). Other plant characters contributing significantly to differences among regions were pod shape (Table 8 and Figures 3 and 3a) and leaflet shape (Table 8 and Figures 4 and 4a). No differences in H' values were found for determinacy, plant pigmentation or testa texture, indicating the similarities in frequency distributions for these characters across all regions (Figures 5 and 5a).

Interesting regional differences were obtained among

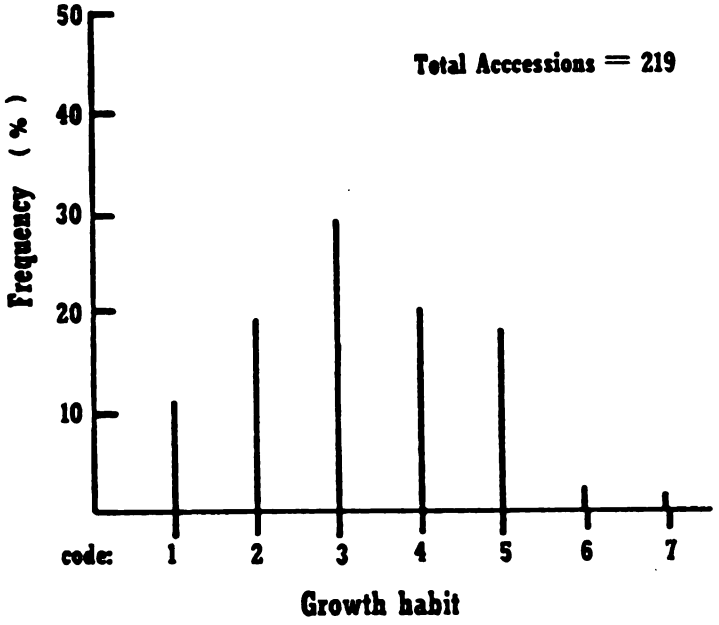

Figure 1. Dispersion of cowpea growth habit among all acessions.

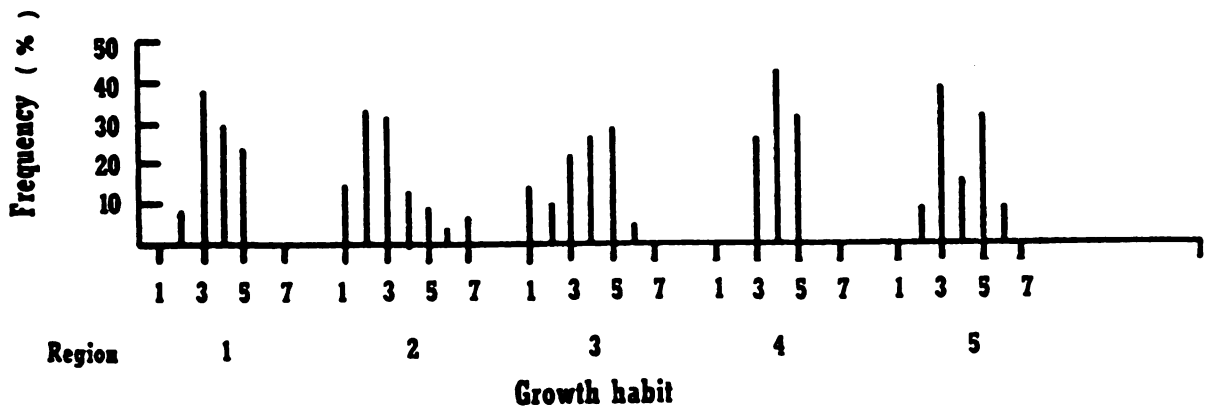

Figure 1a. Cowpea growth habit distributions by regions.

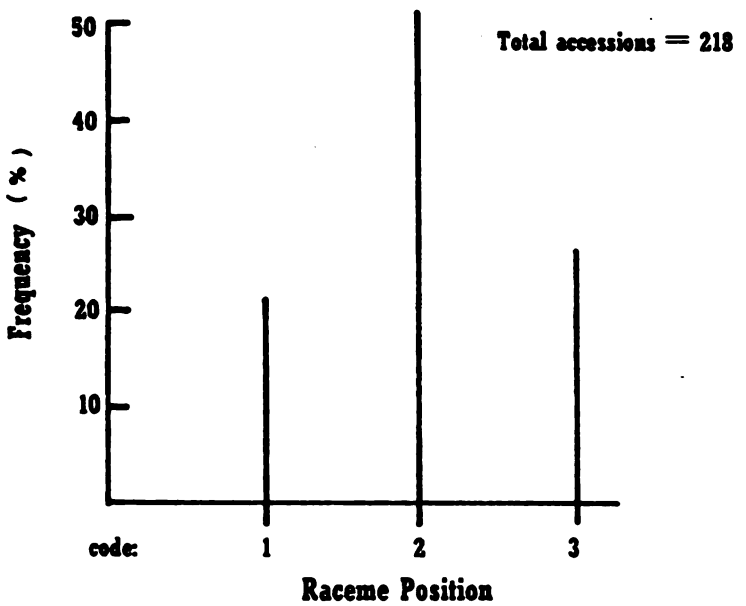

Figure 2. Distribution of raceme position for all cowpea accessions.

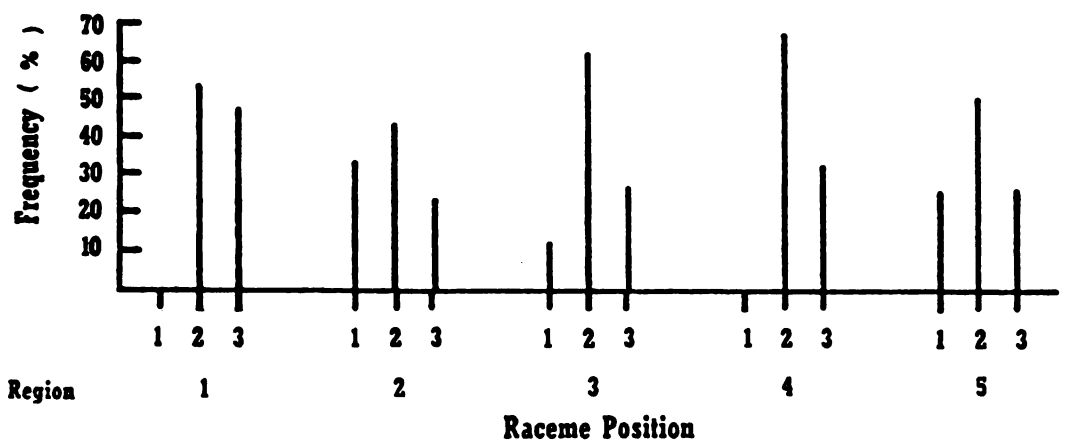

Figure 2a. Distribution of raceme position by regions.

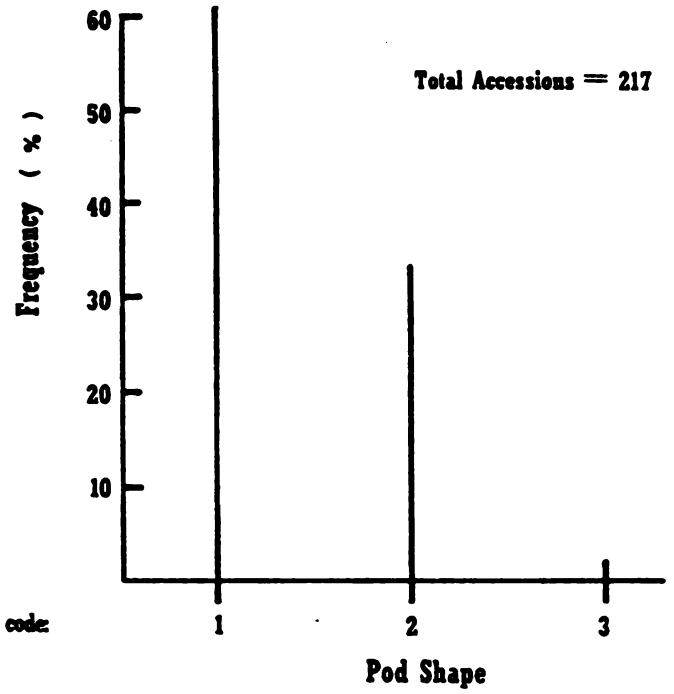

Figure 3. Frequencies of the various classes of pod shapes observed among cowpea accessions.

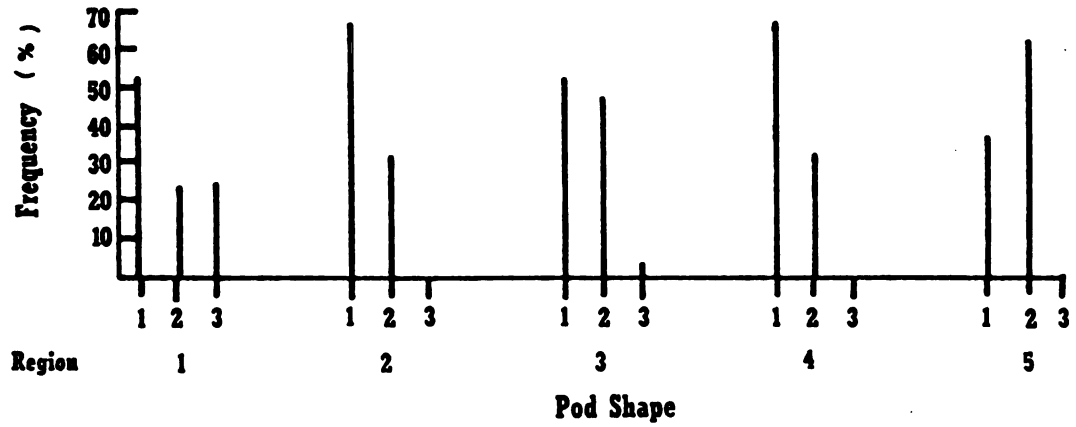

Figure 3a. Distribution of pod shape classes according to regions.

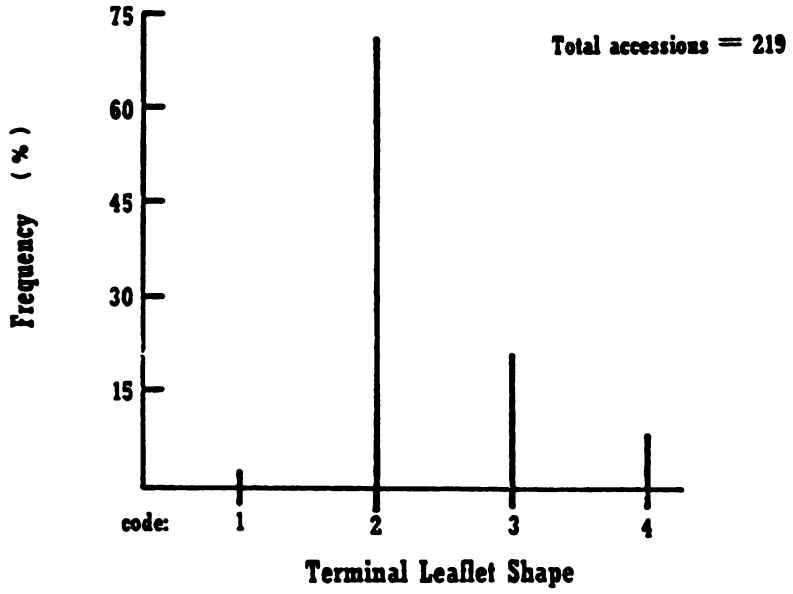

Figure 4. Observed variability in terminal leaflet shape of Botswana cowpea landraces.

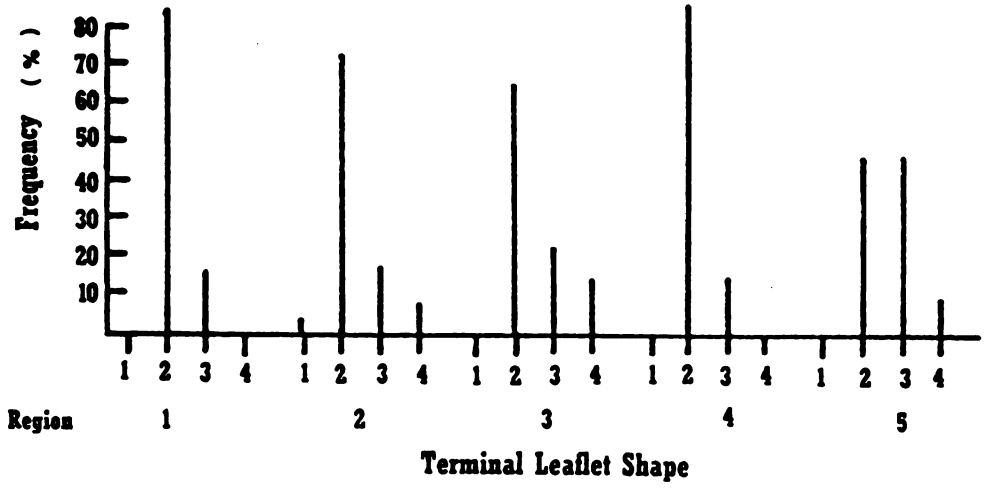

Figure 4a. Distribution of leaflet shape according to regions.

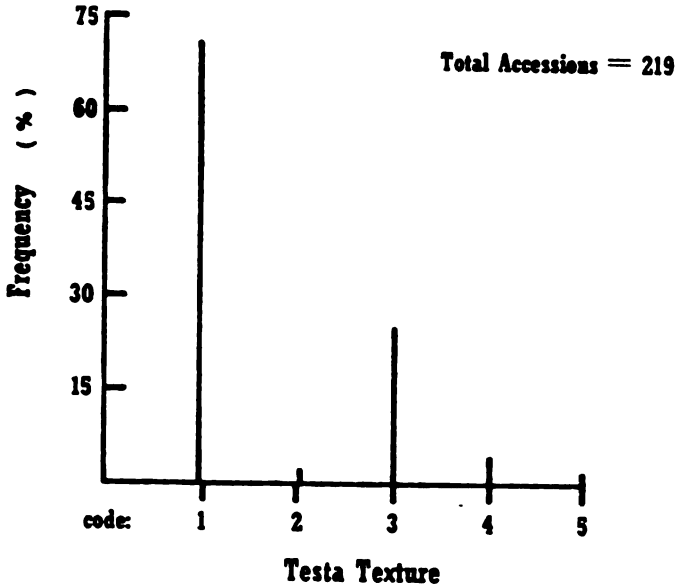

Figure 5. Distribution of the various classes of seed coat texture for all accessions of cowpea.

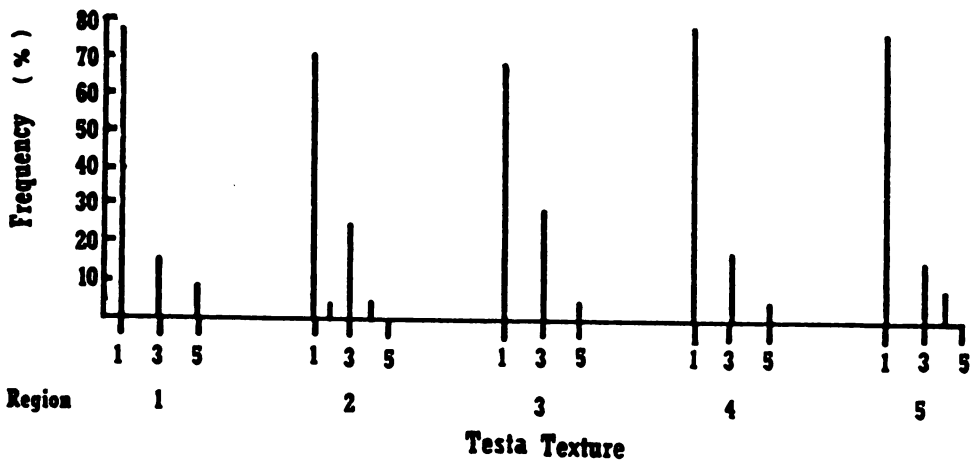

Figure 5a. Similarities in the distribution of seed coat texture throughout all regions.

H' values for seed eye pattern (Table 8 and Figures 6 and 6a). Almost all regions were significantly different from each other, with region 5 displaying the lowest (H'=0.6902) and region 2 the highest (H'=1.5835) diversity. Among all regions, eye patterns 1 and 6 appeared to be most prevelant (Figure 6a) corresponding to solidly pigmented and red hilumed seed, respectively. Because of the distinct pigmentation of the hilum, as opposed to the seed body, detection of "preferred seed types" in cowpea becomes complicated, especially among landraces where a multitude of combinations exist. This is further reflected in Table 8 and Figures 7 and 7a. All regions show high diversity and are not significantly different from one another, except for region 5 (H'=1.0101) which differs from regions 2,3 and 4. Only 3 of the seven color classes known to exist in Botswana landraces are found in region 5 (Figure 7a). Further investigations would be needed to determine if the lower diversity of eye color found in this region is a result of direct selection by farmers for preferred seed types, since no such trends are evident in any other regions.

For the nineteen qualitative cowpea characters under investigation, the highest average H' value was obtained for region 3 (H'=1.0893) while region 4 had the lowest value (H'=0.8673, Table 9). Calculations of E(H') and Var(H') (Table 10) and corresponding t-tests (Table 11)

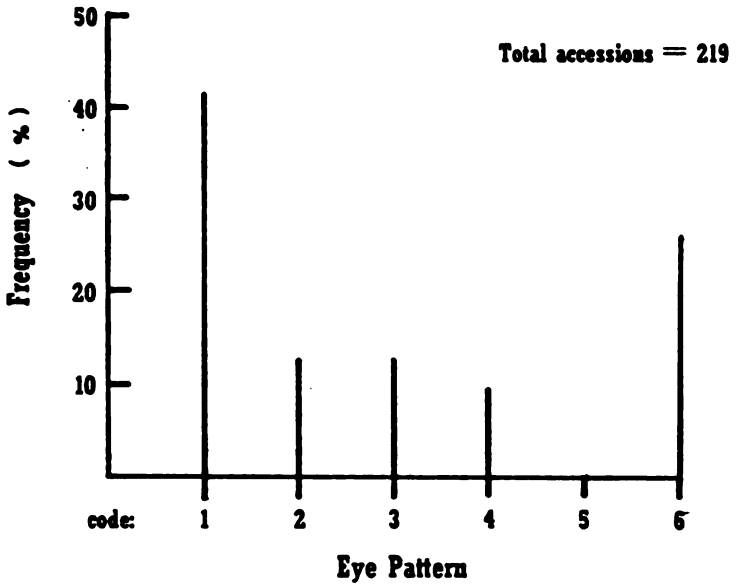

Figure 6. Distribution of seed eye pattern in cowpea landraces.

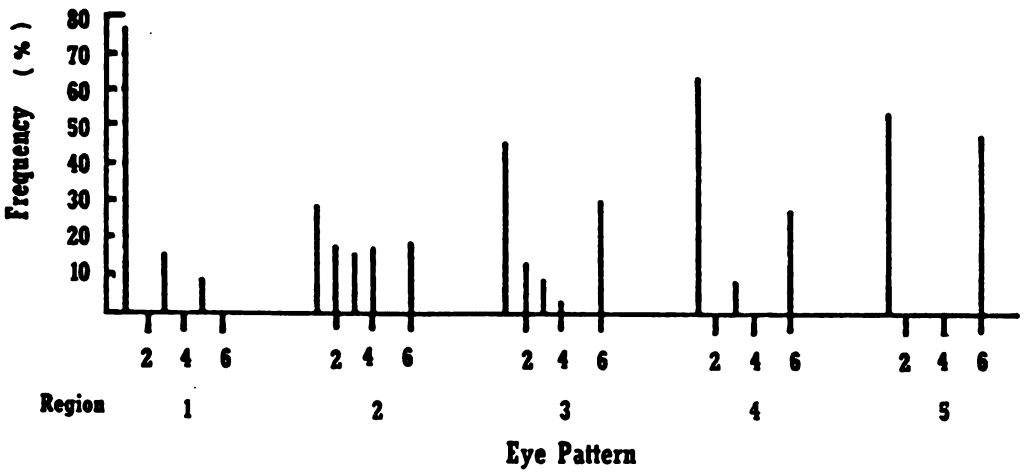

Figure 6a. Distribution of seed eye pattern by regions.

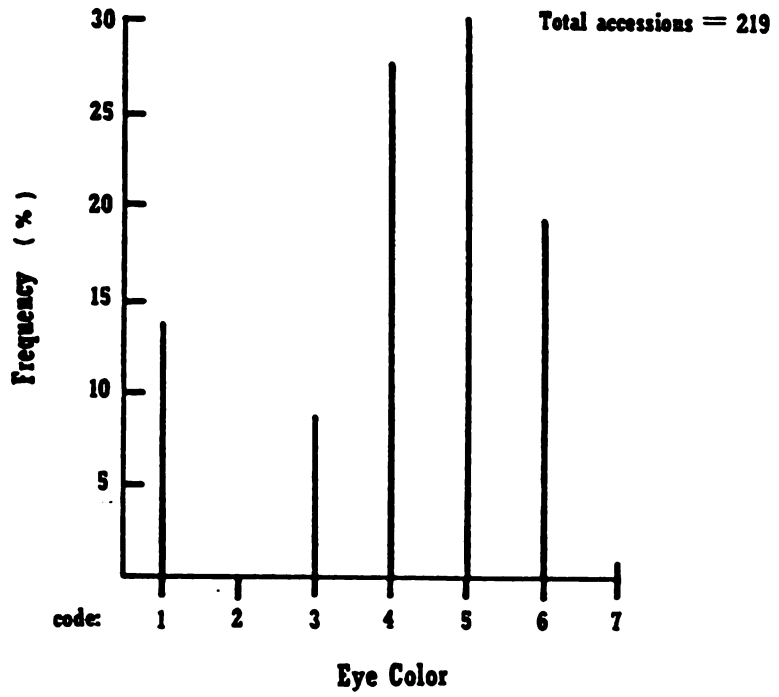

Figure 7. Seed eye color frequencies of Botswana cowpea landraces.

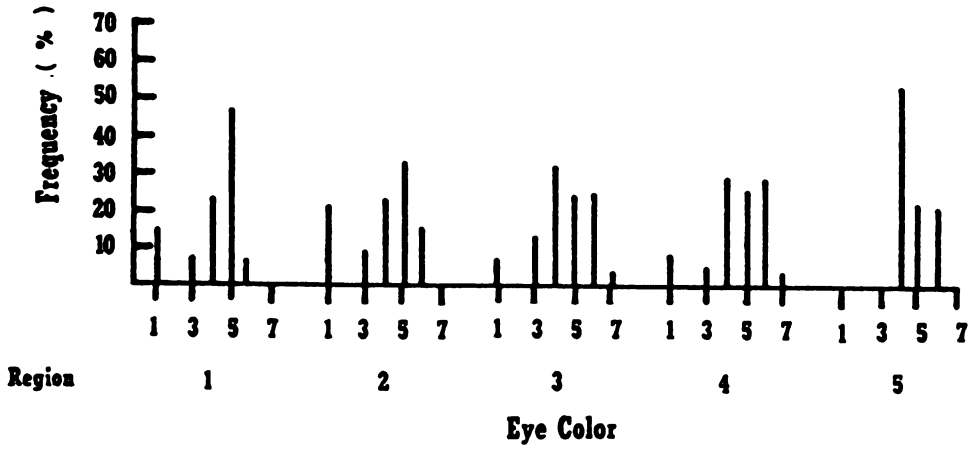

Figure 7a. Distribution of eye color by regions.

Table 9. Shannon-Weaver diversity indices for quantitative characters.

Character	Region 1	2	3	4	5
No. main branches	0.8981	1.0331	1.1590	1.0378	0.6902
Nodes on main stem	1.0579	0.9115	1.0836	0.8152	1.0928
Peduncle length	0.6870	1.1871	0.9337	0.6365	0.8587
Days to 50% first flower	0.8587	0.6673	1.1546	0.9582	1.0579
Days to 95% ripe pod	0.9370	1.1810	1.3335	0.8961	0.2711
Pod forming period	1.2202	1.0484	0.8874	0.9297	1.1569
Plant height	0.6663	1.1204	0.9949	0.8294	1.0123
Plant width	0.9110	0.9376	1.0236	0.6792	0.7903
Pod length	0.9251	0.9913	1.2867	1.0671	0.8981
Pod width	0.9839	1.2417	1.1927	0.9990	1.2047
Leaf length	0.8587	1.1727	1.2791	1.0506	0.8981
Leaf width	0.9184	0.8280	0.8621	0.5117	0.8587
10 seed weight	0.9110	0.8047	1.1153	0.9181	1.0101
Seed length	0.6870	0.9082	0.8451	0.8366	0.5402
Seed width	0.6902	0.8814	1.0147	0.7781	0.9839
Seed thickness	1.2658	1.1584	1.3202	1.1524	1.2711
Pods per peduncle	0.6663	1.1235	1.0005	0.6765	0.8981
Locules per pod	0.6663	0.8043	0.9813	0.6365	0.4292
Seeds per pod	1.0579	1.1669	1.2279	1.0702	0.6197
$\overline{H}'$	0.8877	1.0088	1.0893	0.8673	0.8706

Table 10. Shannon-Weaver diversity indices, expected values and variances for quantitative traits.

Character	N*	S**	H'	E (H')	Var (H')
			Region 1		
No. main branches	13	5	0.8981	0.7443	0.0198
Nodes per main stem	13	4	1.0579	0.9425	0.0804
Peduncle length	13	5	0.6870	0.5332	0.0482
Days 50% first flower	13	4	0.8587	0.7433	0.0262
Days 95% ripe pod	13	4	0.9370	0.8216	0.0590
Pod forming period	13	4	1.2202	1.1048	0.0199
Plant height	13	5	0.6663	0.5125	0.0040
Plant width	13	4	0.9110	0.7956	0.0175
Pod length	13	5	0.9251	0.7713	0.0250
Pod width	13	4	0.9839	0.8685	0.0157
Leaflet length	13	6	0.8587	0.6664	0.0262
Leaflet width	13	4	0.9184	0.8030	0.0192
10 seed weight	13	4	0.9110	0.7956	0.0175
Seed length	13	3	0.6870	0.6101	0.0482
Seed width	13	3	0.6902	0.6133	0.0005
Seed thickness	13	5	1.2658	1.1120	0.0137
Pods per peduncle	13	4	0.6663	0.5509	0.0040
Locules per pod	13	5	0.6663	0.5125	0.0040
Seeds per pod	13	4	1.0579	0.9425	0.0062
			Region 2		
No. main branches	102	5	1.0331	1.0135	0.0029
Nodes per main stem	103	4	0.9115	0.8969	0.0054
Peduncle length	103	5	1.1871	1.1677	0.0039
Days 50% first flower	102	4	0.6673	0.6526	0.0065
Days 95% ripe pod	102	4	1.1810	1.1663	0.0032
Pod forming period	102	4	1.0484	1.0337	0.0063
Plant height	103	5	1.1204	1.1010	0.0011
Plant width	103	4	0.9376	0.9230	0.0050
Pod length	103	5	0.9913	0.9719	0.0039
Pod width	103	4	1.2417	1.2271	0.0024
Leaflet length	103	6	1.1727	1.1484	0.0032
Leaflet width	103	4	0.8280	0.8134	0.0023
10 seed weight	103	4	0.8047	0.7901	0.0056
Seed length	103	3	0.9082	0.8985	0.0023
Seed width	103	3	0.8814	0.8717	0.0024
Seed thickness	103	5	1.1584	1.1390	0.0041
Pods per peduncle	102	4	1.1235	1.1088	0.0032
Locules per pod	103	5	0.8043	0.7849	0.0046
Seeds per pod	103	4	1.1669	1.1523	0.0033

Tab

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Table 10. (Continued).

Character	N*	S**	H'	E (H')	Var (H')
			Region 3		
No. main branches	66	5	1.1590	1.1287	0.0022
Nodes per main stem	66	4	1.0836	1.0609	0.0046
Peduncle length	65	5	0.9337	0.8952	0.0034
Days 50% first flower	63	4	1.1546	1.1308	0.0025
Days 95% ripe pod	63	4	1.3335	1.3097	0.0017
Pod forming period	63	4	0.8874	0.8636	0.0098
Plant height	66	5	0.9949	0.9646	0.0030
Plant width	66	4	1.0236	1.0009	0.0053
Pod length	64	5	1.2867	1.2555	0.0057
Pod width	64	4	1.1929	1.1695	0.0029
Leaflet length	66	6	1.2791	1.2412	0.0050
Leaflet width	66	4	0.8621	0.8394	0.0042
10 seed weight	66	4	1.1153	1.0926	0.0035
Seed length	66	3	0.8451	0.8299	0.0065
Seed width	66	3	1.0147	0.9995	0.0025
Seed thickness	66	5	1.3202	1.2899	0.0074
Pods per peduncle	64	4	1.0005	0.9771	0.0024
Locules per pod	64	5	0.9813	0.9501	0.0127
Seeds per pod	65	4	1.2279	1.2048	0.0047
			Region 4		
No. main branches	24	5	1.0378	0.9545	0.0193
Nodes per main stem	24	4	0.8152	0.7527	0.0364
Peduncle length	24	5	0.6365	0.5532	0.0044
Days 50% first flower	24	4	0.9582	0.8957	0.0095
Days 95% ripe pod	22	4	0.8961	0.8279	0.0167
Pod forming period	22	4	0.9297	0.8615	0.0328
Plant height	24	5	0.8294	0.7461	0.0107
Plant width	24	4	0.6792	0.6167	0.0011
Pod length	20	5	1.0671	0.9671	0.0032
Pod width	21	4	0.9990	0.9276	0.0076
Leaflet length	24	6	1.0506	0.9464	0.0038
Leaflet width	24	4	0.5117	0.4492	0.0122
10 seed weight	24	4	0.9181	0.8556	0.0242
Seed length	24	3	0.8366	0.7949	0.0100
Seed width	24	3	0.7781	0.7364	0.0211
Seed thickness	24	5	1.1524	1.0691	0.0136
Pods per peduncle	22	4	0.6765	0.6083	0.0015
Locules per pod	21	5	0.6365	0.5413	0.0051
Seeds per pod	22	4	1.0702	1.0020	0.0024

Table 10. (Continued).

Character	N*	S**	H'	E (H')	Var (H')
			Region 5		
No. main branches	13	5	0.6902	0.5364	0.0005
Nodes per main stem	13	4	1.0928	0.9774	0.0009
Peduncle length	13	5	0.8587	0.6664	0.0262
Days 50% first flower	13	4	1.0579	0.8656	0.0062
Days 95% ripe pod	13	4	0.2711	0.1557	0.0337
Pod forming period	13	4	1.1569	1.0415	0.0315
Plant height	13	5	1.0123	0.8585	0.0109
Plant width	13	4	0.7903	0.6749	0.0362
Pod length	13	5	0.8981	0.7443	0.0198
Pod width	13	4	1.2047	1.0893	0.0229
Leaflet length	13	6	0.8981	0.7058	0.0198
Leaflet width	13	4	0.8587	0.7433	0.0262
10 seed weight	13	4	1.0101	0.8947	0.0137
Seed length	13	3	0.5402	0.4633	0.0198
Seed width	13	3	0.9839	0.9070	0.0157
Seed thickness	13	5	1.2711	1.1173	0.0180
Pods per peduncle	13	4	0.8981	0.7827	0.0198
Locules per pod	13	5	0.4292	0.2754	0.0291
Seeds per pod	13	4	0.6172	0.5018	0.0108

*N = number of accessions evaluated; **S = number of phenotypic classes designated per character.

Table 11. Student's t values for pairwise comparisons by regions of H' values for quantitative plant characters.

Region	1	2	3	4	5
			Number of Main Branches		
1		0.6111	1.759	0.7065	1.4592
2			1.7630	0.0315	5.8807**
3				0.8266	9.0221**
4					2.4703*
5					
			Nodes Per Main Stem		
1		0.4896	0.0882	0.7101	0.1224
2			1.7210	0.4710	2.2842*
3				1.3255	0.1241
4					1.4374
5					
			Peduncle Length		
1		2.1910*	1.0860	0.2202	0.6295
2			2.9658**	6.0436**	1.8929
3				3.365**	0.4359
4					1.2702
5					
			Days to 50% First Flower		
1		1.0584	1.7466	0.5266	1.1067
2			5.1366**	2.2998*	3.4660**
3				1.7929	1.0367
4					0.7957
5					
			Days to 95% Ripe Pod		
1		0.9784	1.6093	0.1596	2.1871*
2			2.1786*	2.0196	4.7368**
3				3.2246**	5.6466**
4					2.7840*
5					
			Pod Forming Period		
1		1.0614	1.9311	1.2654	.2792
2			1.2689	0.5995	0.5581
3				0.2046	1.3261
4					0.8960
5					

Table 11. (Continued).

Region	1	2	3	4	5
			Plant Height		
1		6.3587**	3.9275**	1.3452	2.8345**
2			1.9600	2.6789*	0.9858
3				1.4140	0.1476
4					1.2445
5					
			Plant Width		
1		0.1773	0.7457	1.6996	0.5209
2			0.8474	3.3085**	0.7257
3				4.3050**	1.1452
4					0.5753
5					
			Pod Length		
1		0.3894	2.0638	0.8456	0.1276
2			3.0149**	0.8996	0.6054
3				2.3278*	2.4335*
4					1.1144
5					
			Pod Width		
1		1.9432	1.5325	0.0989	1.1238
2			0.6703	2.4270*	0.2471
3				1.8923	0.0735
4					1.1778
5					
			Terminal Leaflet Length		
1		1.8313	2.3800*	1.1079	0.1837
2			1.1750	1.4594	1.8107
3				2.4358*	2.4194*
4					0.9927
5					
			Terminal Leaflet Width		
1		0.6165	0.3680	2.295*	0.2802
2			0.4230	2.6267*	0.1819
3				2.7362**	0.0195
4					1.7708
5					

Table 11. (Continued).

Region	1	2	3	4	5
			10 Seed Weight		
1		0.6994	1.4098	0.0384	0.5610
2			3.2560**	0.6569	1.4785
3				1.1849	0.8021
4					0.4726
5					
			Seed Length		
1		0.9843	0.6760	0.6201	0.5630
2			0.6726	0.6456	2.4754*
3				0.0662	1.8801
4					1.7170
5					
			Seed Width		
1		3.5505**	5.9245**	0.5981	2.3075*
2			1.9043	0.6739	0.7619
3				1.5401	0.2290
4					1.0728
5					
			Seed Thickness		
1		0.8050	0.3745	0.6863	0.0298
2			1.5088	0.0451	0.7581
3				1.1579	0.3081
4					0.6677
5					
			Pods Per Peduncle		
1		1.0946	4.1775**	0.1375	1.5025
2			1.6437	6.5202**	1.4862
3				5.1882**	0.5770
4					1.5184
5					
			Locules Per Pod		
1		1.4881	2.4375*	0.3124	1.3032
2			1.3457	1.7038	2.0433*
3				2.5844*	2.7004*
4					1.1210
5					

Table 11. (Continued).

Region	1	2	3	4	5
			Seeds Per Pod		
1		1.1183	1.6283	0.1326	3.3800**
2			0.6820	1.2808	4.6293**
3				1.8716	4.9053**
4					3.9429**
5					

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indicate significant differences between region 2 and most other regions in the number of days required from planting until 50% of the plants within a plot produced their first flowers. Region 2 had the lowest diversity for this character, reflected in a higher frequency of early-maturing genotypes (Figure 8a).

The number of days required from flowering until plants reach physiological maturity (95% ripe pod) is related to the determinate status of cowpea (Figures 9, 9a, 10, 10a). Extended pod-production period is a useful plant characteristic under Botswana's semi-arid conditions with erratic and scant rainfall. On a nationwide scale, most landraces (59.6%) require more than 128 days to complete their reproductive period (Figure 9). Genotypes derived from region 5 show a particularly strong tendency towards indeterminate types with extended pod production periods and have a low diversity index of H'=0.2711, which is significantly different from all other regions. Other quantitative characters found to have less variability in specific regions were leaflet width in region 4 (Figures 11 and 11a), and seeds per pod in region 5 (Figures 12 and 12a) which differed from all other regions (Table 11).

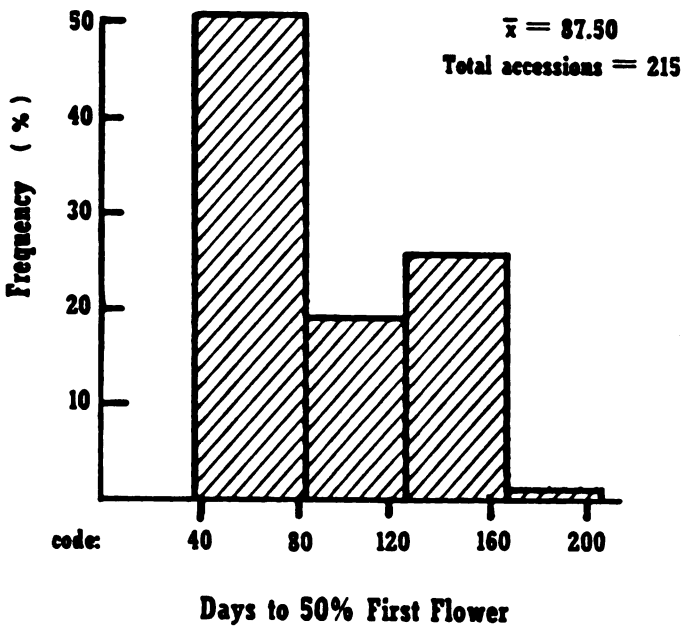

Figure 8. Distribution of the days to 50% first flower among cowpea landraces.

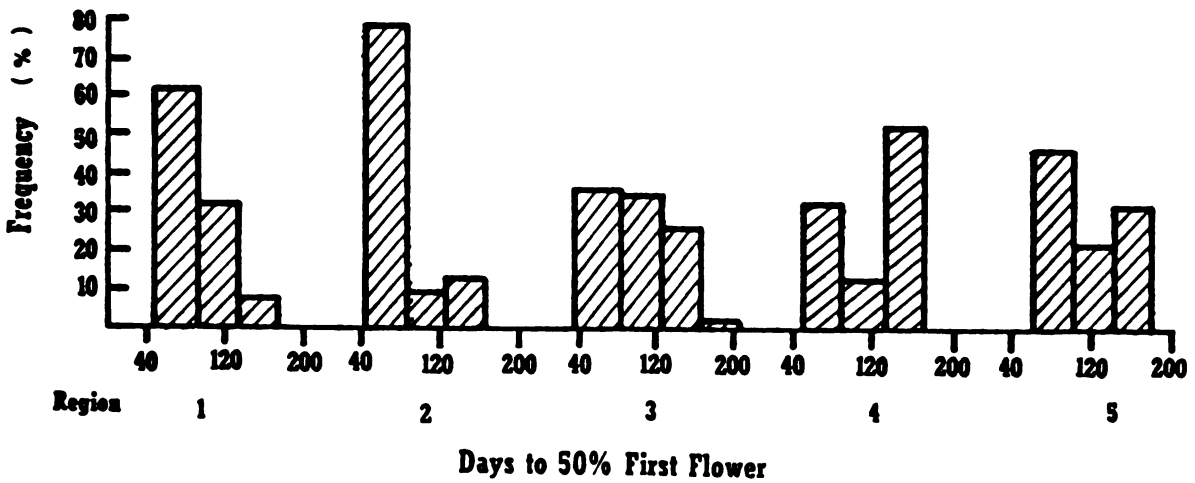

Figure 8a. Regional distribution of the days to 50% first flower.

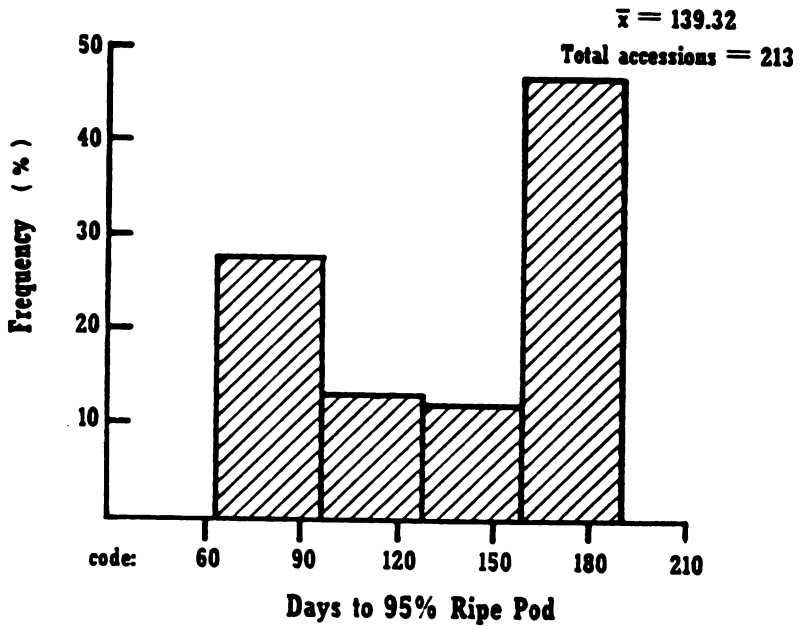

Figure 9. Frequency distribution of pod production period for all accessions.

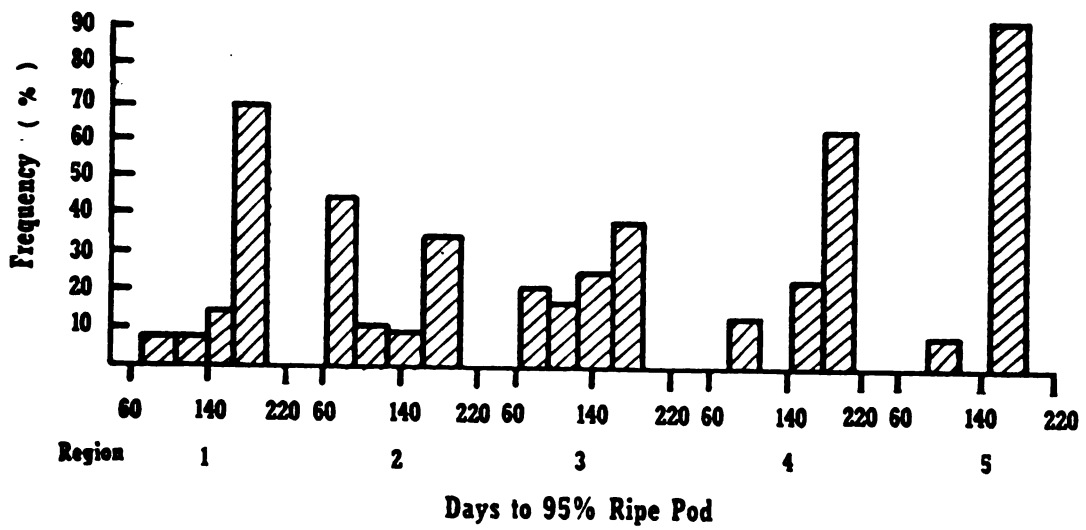

Figure 9a. Distribution of accessions based on days required to complete reproduction (by regions).

Figure

Frequency (%)

Re

Figure

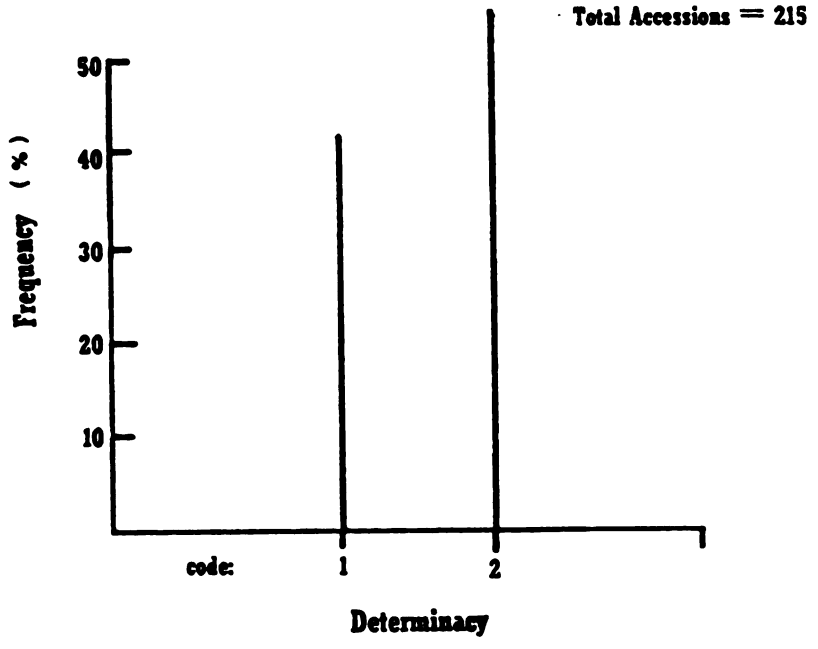

Figure 10. Determinate growth patterns observed among cowpea landraces.

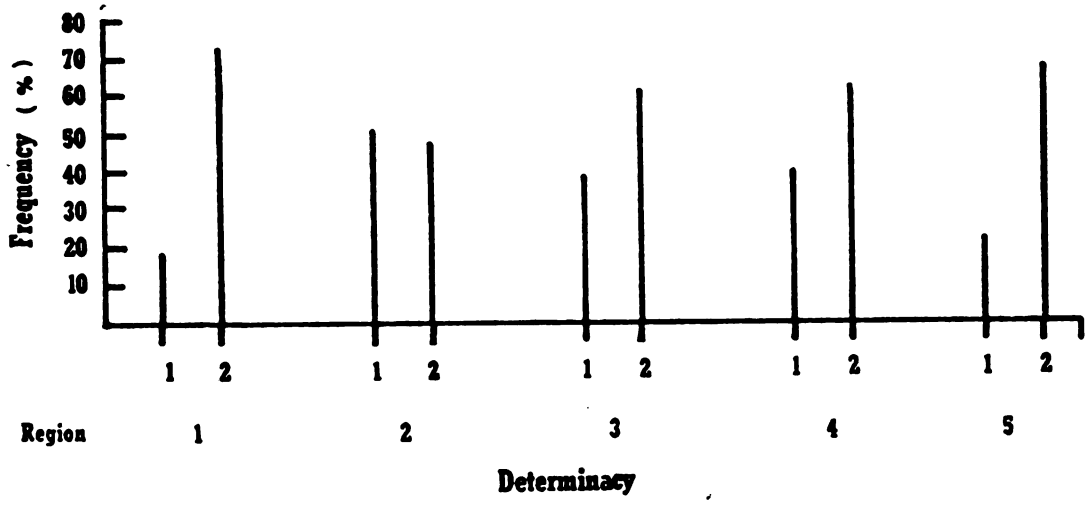

Figure 10a. Distribution of determinate classes by regions.

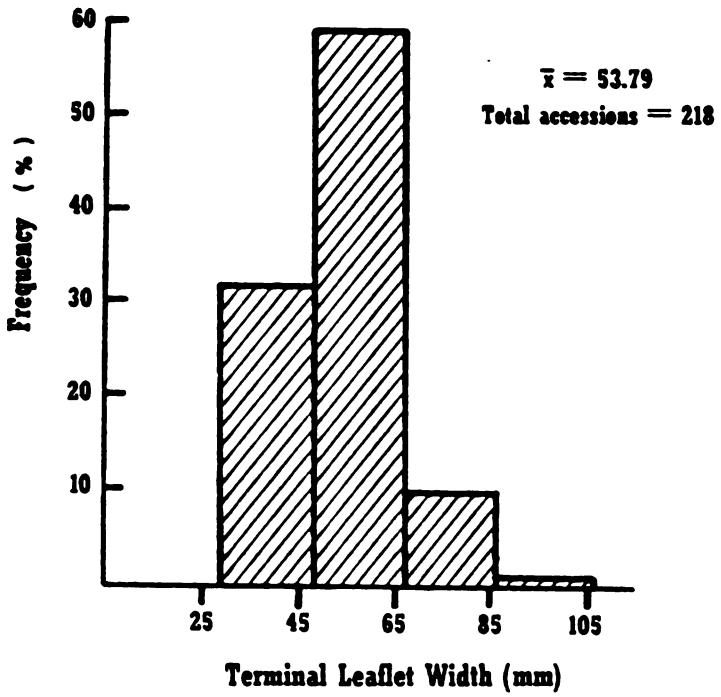

Figure 11. Range of variability in leaf width measured among Botswana cowpea.

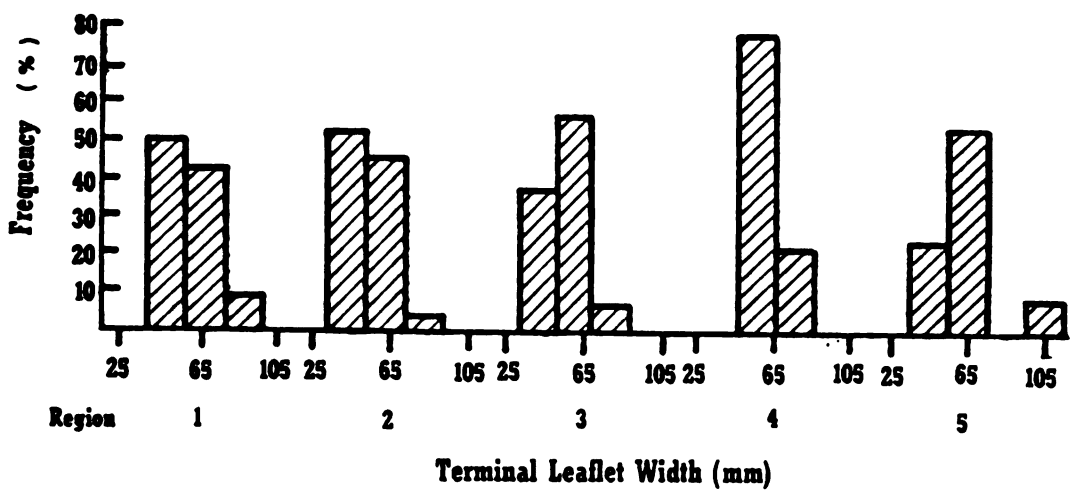

Figure 11a. Distribution of leaflet width among cowpea landraces.

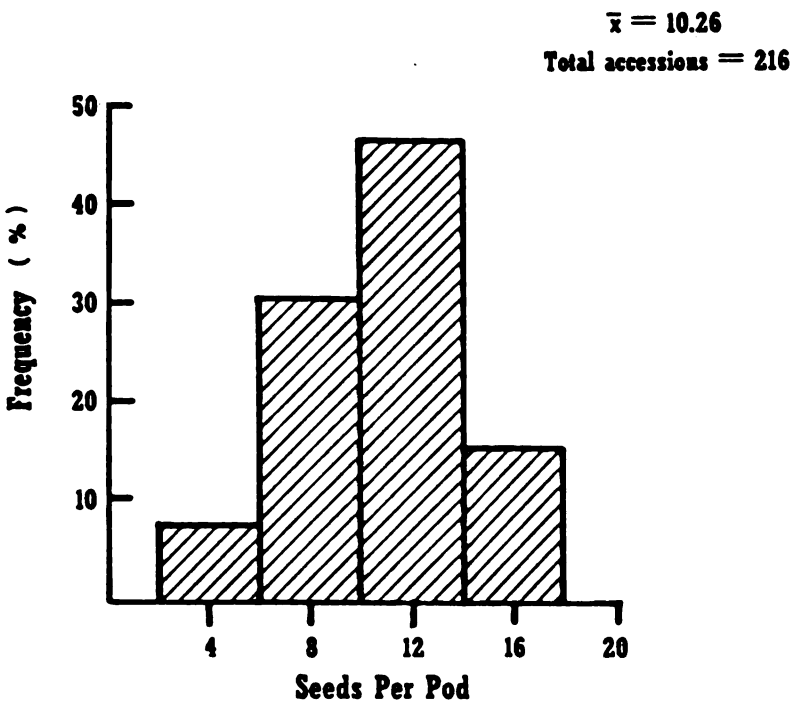

Figure 12. Variability in the number of seeds per pod formed in cowpea landraces.

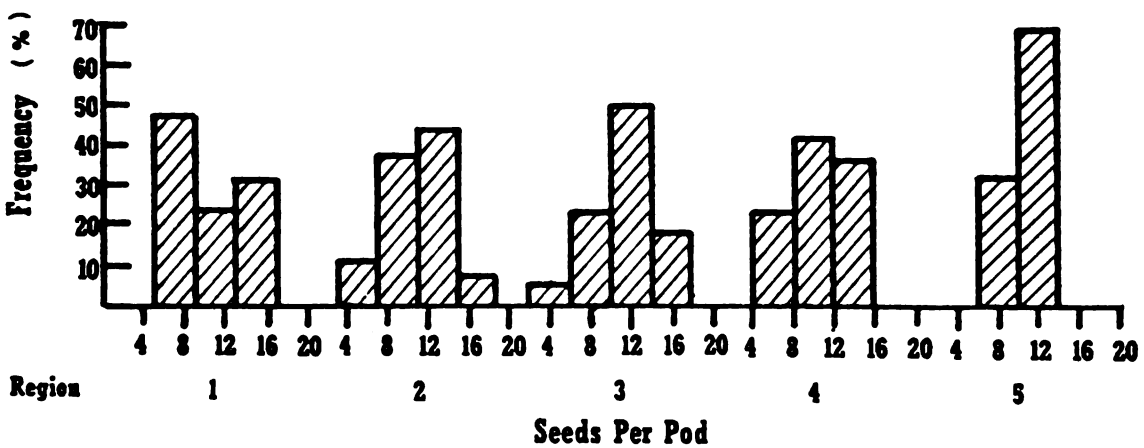

Figure 12a. Distribution of seeds per pod by regions.

Cluster Analysis

A cluster analysis was used to determine the pattern of genetic similarities between individuals within the germplasm collection of cowpea landraces. Three main clusters were identified (correlation of similarity = 21.732, Figure 13). Geographically, these clusters overlapped in their distribution (Figure 14), and between cluster dissimilarities were not correlated with latitude, rainfall or soil type. Table 12 lists the most common attributes of each cluster. Variables were ranked for each cluster according to the size of their F-ratios. Small F-ratios are good diagnostics for indicating similarity between individuals within a specific cluster for a given variable. Thus, pod width and 10 seed weight distinguished clusters 2 and 3 and the number of days to 95% ripe pod was found to be an important variable in distinguishing between cluster 1 and 2. However, it is the set of variables and their ordering of importance which gives each cluster its unique properties. Cluster groupings were also not found to be correlated with a particular seed color, although variables related to seed size and number did appear to be important in cluster identification.

The wide diversity of landraces grown throughout Botswana reflects local farmer preferences and natural

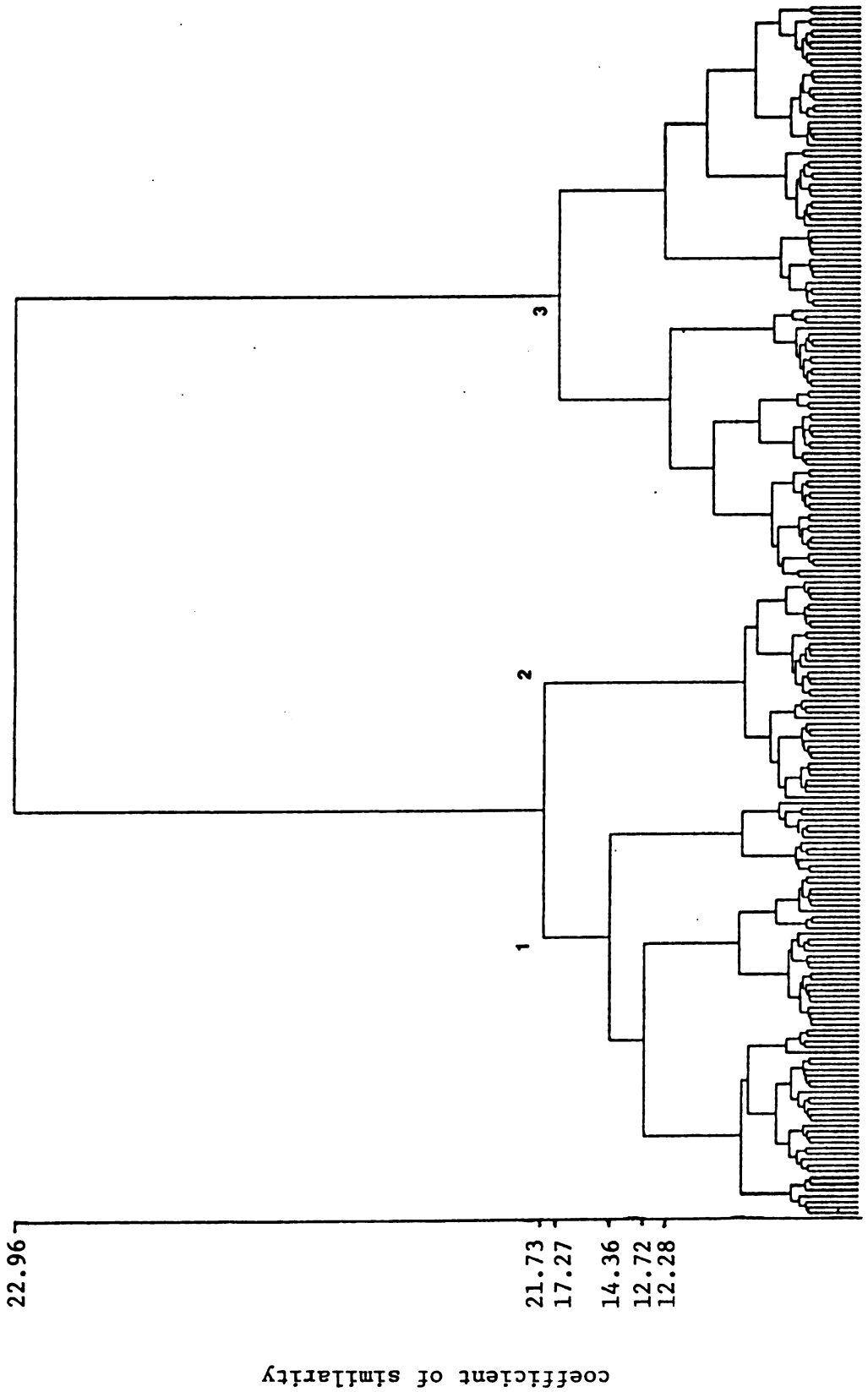

Figure 13. Dendrogram of Botswana cowpea landraces based on 19 quantitative variables.

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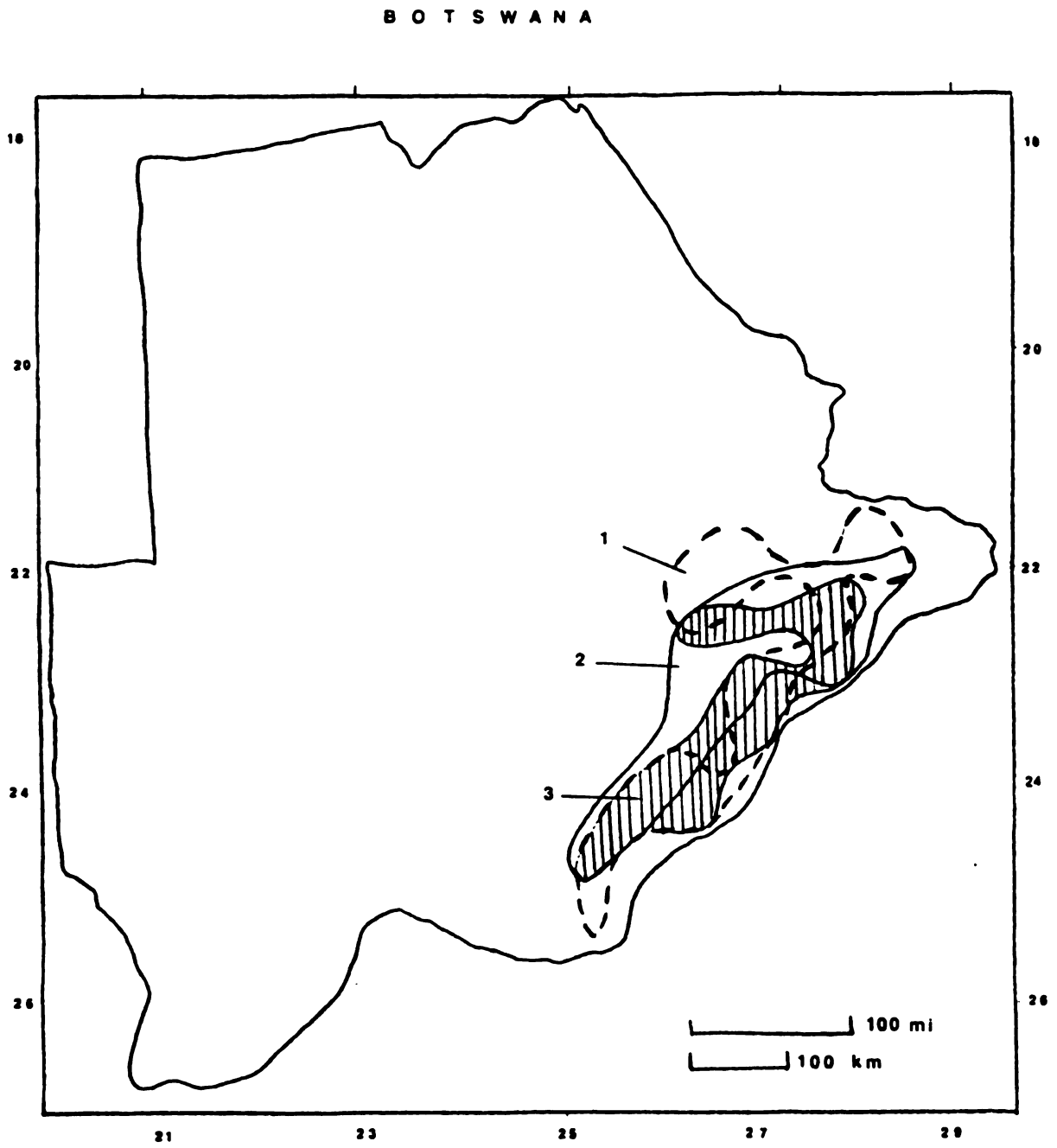

Figure 14. Geographic clustering of cowpea landraces.

Table 12. Cluster diagnosis of means, standard deviations and F-ratios for the 4 most similar variables per cluster.

Cluster Number	Variable	Mean	Standard Deviation	F-Ratio
1	Days to 95% ripe pod	170.09	24.19	0.2903
	Peduncle length (cm)	13.14	4.14	0.5030
	Plant width (cm)	25.13	4.83	0.6442
	Pods per peduncle	1.47	0.62	0.6861
2	Days to 95% ripe pod	165.12	25.67	0.3270
	Plant height (cm)	11.45	2.04	0.3402
	10 seed weight (g)	1.88	0.34	0.3728
	Pod width (mm)	9.02	1.05	0.3896
3	Days to 50% first flower	58.71	15.37	0.3270
	10 seed weight (g)	1.20	0.35	0.3402
	Pod width (mm)	6.67	1.08	0.3728
	Seed thickness (mm)	4.10	0.67	0.3896

selection for genotypes most adapted to the many variable, and sometimes unfavorable, environmental conditions. The reasons that farmers continue to grow landraces under such conditions become evident. The greater adaptability of landraces and the protection afforded them by the mixture of components is essential to farmers faced with nutrient-poor soils, low rainfall and many disease and insect problems. The special gene combinations that have evolved under such systems of cultivation in Botswana may confer, to the population as a whole, greater stability. This is in contrast to elite cultivars bred for commercial production where stability is based on a single genotype.

It is no wonder, then , that such variation still exists in Botswana, despite the cultivation of commercial cowpea purelines in neighboring Zimbabwe and South Africa. Farming remains a low-technology practice in terms of the application of fertilizer, irrigaiton or pesticides. There is, perhaps, no better protection against the vagaries of nature then to plant a range of genotypes throughout the environment, each possessing various levels of resistance to the array of possible difficulties, such as insects, drought and disease, quite capable of eliminating populations of pure line varieties.

The question of why individual farmers use mixtures of differing levels of diversity remains unanswered. If extremely diverse and unpredictable environments are only

manageable by planting highly heterogeneous mixtures, then one would expect a correlation between environmental instability and landrace mixture diversity. One would also expect such mixtures to evolve at a greater rate, since the presence of many more genes increases the probability of mutation, drift or natural selection which change population allelic frequencies and presents opportunities for genetic recombination. Such questions need far more study than they have heretofore received in order to provide for a more complete understanding of the dynamics of landrace populations.

The degree of polymorphism exhibited by this collection for all characters studied is quite large. Only upon examination on a regional basis were differences, presumably preferences, for particular plant traits evident. Although artifical selection is practiced for certain desirable seed and plant traits, the extent of this selection is not known. It is also not known if farmers deliberatly select against certain seed types appearing in the mixtures, such as off-types resulting from natural hybridization. Such genotypes were found in most samples collected and are presumed to originate as a result of outcrossing which, under experimental conditions in Botswana, range from 0.5 to 2.0%. If these rarer genotypes are not selected against by farmers, then their incorporation into a landrace mixture depends on the many

environmental adversities with which they must contend.

In terms of plant traits which displayed the largest variability, growth habit ranked high with an H' index value of 1.380. Through their history of cultivation, cowpea landrace mixtures have evolved a gamut of plant types, each with some special property enabling it to exploit the micro environment in which it is found. This was no where as well pronounced as in Pelotshetlha and Kanye in southeastern Botswana where some samples collected from farmers' fields contained seed all of a beige color, yet plant types were quite diverse. However, selection for a particular seed color was not observed. In fact, seed color had the highest H' value of all qualitative traits (1.397).

The information concerning genetic properties of these cowpea landraces may serve as a useful basis in establishing a national plant breeding program in Botswana. Foremost, the amount of diversity for each of the many quantitative and qualitative traits measured provides knowledge to the breeder as to the extent of variation in the available gene pool. In this case, a satisfactory basis has been defined for a breeding program based on plant architecture or yield components. Further evaluations would be needed in order to identify the extent of resistance genes harboured within the collection to the many insects and diseases which threaten cowpea production.

In addition, regional analysis of the collection proved useful in detecting any preferences for gene combinations which might have resulted through generations of cultivation of cowpea by agriculturalists. Diversity indices, such as the one used in this study, provided information as to the amount (number of phenotypic classes) and extent (frequency of each class) of diversity on a per character basis which was used to compare regions. One of the most important factors in a plant breeding program is the selection of parents. Diverse parents, when crossed, are expected to produce a greater array of, though not necessarily more desirable, segregants than parents which are closely related. Through the use of cluster anlaysis, which classified the available germplasm into relatively homogeneous groups, accessions of divergent clusters might now be crossed and the progeny screened for useful or superior recombinants.

CONCLUSIONS

An evaluation of cowpea (*Vigna unguiculata*) germplasm collected throughout the arable portion of Botswana revealed extensive genetic diversity among landraces grown by local farmers. Of the nineteen quantitative and fifteen qualitative plant characters evaluated, all were polymorphic in nature. The country was divided into five regions corresponding to geographically isolated collection sites. Analysis of genetic diversity found within and between regions indicates that some selection for various plant characters is taking place between the different regions. Calculation of diversity index values (H') for all traits averaged across all regions indicates that plant growth habit and seed color had the highest genetic variability of all qualitative characters.

A cluster analysis of all accessions on a nation-wide basis indicates that three main groups of cowpea genotypes exist. Clustering was not correlated with north-south clinal patterns associated with latitude, soil type or rainfall. Given the variable environmental and climatic conditions in Botswana, it may be that micro-environmental evolution was more important in forming the genotypes which

clustered together within these three groups. Selection of diverse parents from the three clusters may be useful in a breeding program to generate F_2 populationsfrom which new, useful recombinants might result.

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INTRODUCTION

Genetic Variability for Dinitrogen Fixation Among African Cowpea

Biological N_2 fixation is largely responsible for the supply of N needed for food crop production in the tropics (Kang et al., 1975). Among the food legumes, peanut (Arachis hypogaea), cowpea (Vigna unguiculata) and dry bean (Phaseolus vulgaris) are the most important protein sources in Africa (Sinha, 1977). Cowpea in particular probably has received the least amount of attention from researchers. Little is known of the variation in cowpea yields from farmers in Africa (Summerfield et al., 1978). Average yields are estimated to be between 100 and 300 kg/ha and total crop failures are common (Rachie and Roberts, 1974). Estimations of the amount of atmospheric nitrogen fixed by cowpea range from 73 to 240 kg/ha^{-1} a^{-1} (Sinha, 1977). The evaluation of germplasm collections of Vigna unguiculata has demonstrated the wealth of genetic variability which exists in this crop (IITA, 1974). The possibility of selecting genotypes for increased dinitrogen fixation has recently gained attention (Zary et al., 1978a; Graham, 1983). Enhancing the efficiency of symbiotic N_2

fixation through selection and breeding may provide a means for improving yields for cowpea producers in Africa.

Factors Affecting Variability in N2 Fixation in Cowpea

Defining the extent of genetic variability for dinitrogen fixation in a crop species is often complicated. This is because heterogeneity may be expressed in both the host and the symbiont. Complex interactions between host, Rhizobium and environment are known to occur (Graham, 1982). Under non-symbiotic conditions, phenotypic variation in a crop species results from a combination of genetic differences, environmental effects on plant growth and reproduction and genotype x environment (GE) interactions. When considering a host-Rhizobium relationship, additional factors must be examined such as Rhizobium x host and Rhizobium x environment interactions or even second order interactions (Summerfield et al., 1978).

Environmental Factors Limiting Symbiosis

Environmental factors often play a key role in limiting the expression of genetic variablity for N_2 fixation in cowpea. Extreme environmental conditions may

have varying effects on the symbiotic system, possibly affecting the host plant and Rhizobium in different ways (Minchin et al., 1981). Thus, the range of environments for the symbiosis may be narrower than that of the nitrogen-fed plant (Lie, 1981).

Environmental factors are thought to operate mainly through the host component of the symbiotic relationship since the host is exposed to both above and below ground environmental stresses. Adaptation to environmental changes may occur through phenotypic plasticity of the nitrogen-fixing legume, or through genetic variability present in a plant population. Some of the more important factors affecting N_2 fixation under field conditions are soil moisture, light, temperature, pH and available soil nitrogen.

Soil Moisture

Of all legumes grown in the tropics, cowpeas are highly desirable because of their ability to survive and reproduce under limited rainfall. Currently, there is much interest in the development of superior cultivar-Rhizobium combinations which are able to maximize the N_2-fixation potential of cowpea in semi-arid regions.

The effect of moisture stress on the nodulation capacity of Vigna unguiculata depends on the growth stage

at which the stress is imposed. Moisture stress imposed during the seedling stage adversely affects the production of root hairs (Lie, 1981). Since these are the normal sites of infection of the Rhizobium bacteria, symbiotic association may be decreased (Sharifi, 1984).

Repeated exposure of cowpea to water stress prior to flowering resulted in decreased nitrogenase activity and nodule weight (Summerfield et al., 1976). Ultimately, total seed weight, seed number and fruit weight per plant were reduced. Imposed water stress after flowering did not reduce yield and nitrogenase activity was less affected.

Sprent (1972) has demonstrated a high degree of correlation between soil-water content and nitrogen-fixing activity in Vicia faba. Slow drying of soil under natural field conditions resulted in a reduction of nodule activity. Root nodules need a constant supply of water in order to export the products of fixation. Therefore, the effect of soil moisture stress on nitrogen fixation is believed to be a direct one. However, reduced supplies of photosynthates from wilted leaves may also affect the process since nodules are dependent on carbohydrates produced in the leaves (Hardy et al., 1975; Lawn et al., 1974).

Soil moisture stress may also affect the differential survival rate of rhizobia present in the soil profile. Boonkerd and Weaver (1982) demonstrated that strains of

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cowpea rhizobia have different capacities for survival in soil exposed to moisture and temperature conditions commonly found in tropical regions. However, the surviving rhizobia may not necessarily be the most effective symbiotic strains. It is thought that genetic differences among rhizobia to survive in adverse soil conditions result from the geographical origin of the species (Wilkins, 1967), although Osa-Afiana and Alexander (1982) found no such evidence in their studies of cowpea rhizobia. When cowpea cultivar Calilfornia no. 5 blackeye was inoculated with different strains of rhizobia and exposed to drought, Zablotowicz et al., (1981) found some strains to be more effective in recovery of fixation ability than others.

It is evident that both the host and rhizobia genotypes are important in forming a sucessful symbiotic association under moisture stress conditions and must be considered together when breeding cowpea for semi-arid conditions.

Light

The main effect of light on the symbiosis is through photosynthesis. Carbohydrates produced in the leaves are partly used for the development and functioning of the nodules. Photosynthates are presumed to be the key

limiting factor in nitrogen fixation under field conditions (Hardy and Havelka, 1976). It has been demonstrated that improvements in photosynthesis such as increasing light intensity and/or CO_2 concentrations also improve nodulation and nitrogen fixation (Bethlenvalvay et al., 1978a).

Pate (1966) has shown that the upper leaves in field pea (Pisum arvense L.) supply assimilates to the shoot apex while most of the assimilates produced in the lower leaves move downward to the root and nodules. Under field conditions, self-shading and mutual-shading reduce the light available to lower leaves. When a closed canopy develops the effect is even more pronounced (Sprent, 1976; Hardy and Havelka, 1976). Intercropping cowpea with maize (Zea mays) and sorghum (Sorghum bicolor) is a common practice among subsistence farmers in Africa. Such cultural practices may contribute to decreases in nitrogen fixation in cowpea by shading. Reduction in light intensity of cowpea grown under experimental conditions resulted in a decrease in root dry weight (Dart and Mercer, 1965). More primary root nodules were formed under two-thirds light conditions while full light conditions induced more secondary root nodules. When a 50% light interception treatment was applied to cowpea cv. Prima, seed yields were reduced by 25% due to the production of less pods.

An additional effect of light on nodule formation has been demonstrated with phytochrome. Treatments of

alternating red and far-red light have shown that far-red light inhibits nodulation substantially while irradiation with red light decreases the inhibitory effect (Lie 1969). Under natural conopy conditions in Medicago sativa, an excess of far-red light was due to the preferential filtering of red light by chlorophyll (Robertson, 1966). This means that under field conditions biological nitrogen fixation may be reduced through shading which affects photosynthesis as well as root-nodule formation.

Temperature

High temperatures are known to affect plant metabolic processes such as respiration, photosynthesis and transpiration. In a symbiotic associaion, dinitrogen fixation may be indirectly affected via these plant processes. Studies comparing nodulated and N-fertilized cowpea cv. K2809 exposed to warm days (33°C) and cool nights (19°C) resulted in decreased dry weights and seed yields of the nodulated plants (Minchin et al., 1980). Soil temperatures of 21°C have been shown to reduce root growth and possibly root hairs, resulting in decreased infection by rhizobia (Dart et al., 1965).

The most direct effect of temperature extremes appears to be on the growth and survival of rhizobia. High soil

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temperatures inhibit the growth of rhizobia in the field. Temperatures of 35°C proved to be detrimental to the survival of two cowpea rhizobial strains TAL309 and 3281 (Boonkerd and Weaver, 1982). But differences among cowpea rhizobia in tolerance to high temperatures and dessication in soils was reported by Osa-Afiana and Alexander (1982). In their studies, temperatures between 29-35°C resulted in differential surviving ability. However, when temperatures were increased to 40°C, no bacteria survived.

At lower temperatures (15-20°C), growth of rhizobia is also inhibited (Dart et al., 1965). Pisum sativum cv. Iran was found to be "resistant" to nodulation by a large number of rhizobial strains when grown at 20°C but nodulated normally at 26°C (Lie, 1971). The effect of low temperatures appears to be due to a delay in infection, since nitrogen fixation itself is not cold-sensitive (Lie, 1981).

Recent studies indicate that elevated temperatures may also affect the nodulation process via the bacteria. Zurkowski and Lorkiewicz (1979) exposed several strains of R. trifolii to high temperatures which resulted in the reversion of some strains to non-nodulating mutants (Nod^-). Two strains of R. trifolii, 24 and T12, were examined in detail. The inability to nodulate in these mutant strains was due to the absense of plasmid pWZ2, indicating the plasmid-mediated control of nodulation in R. trifolii.

pH

Studies on nitrogen fixation in legumes have shown that the activity of the nodule bacteria is directly affected by soil pH (Mengel et al., 1978; Keyser et al., 1979). Generally, rhizobia prefer a soil pH between 6.0 and 8.0 and may fail to develop in strongly acid soils (Allen and Allen, 1950). Experiments conducted with cowpea cv. California Blackeye no. 5 produced maximum nodule and pod number per plant within the pH range of 6.6 to 7.6 (Hwan-E Joe and Allen, 1980). Nodule size decreased at a pH of 7.5 and above , and roots became more fibrous. At acid pH levels 4.2 to 5.8, inoculated plants had 83% fewer nodules than those grown in the optimum range.

Similar results of non-viability of rhizobia at acid pH levels were obtained with Medicago sativa (Munns, 1968). Acidity reduced nodule numbers at pH 5.5 and nearly prevented nodulation at pH 4.5. However, root growth and production of root hairs were not affected within this pH range. Also, a pH of 4.4 inhibited root hair infection by rhizobia and subsequent root curling was prevented, while raising the pH to 5.4 allowed these processes to continue. After root curling occurred, the pH could be lowered again to 4.4 without hindering the normal completion of the nodulation process.

Soil Nitrogen

In the tropics cowpeas are normally grown on soils deficient in nitrogen. Yet some soils may contain sufficiently high amounts of available N to affect the nodulation process. Studies on the relationship between nitrogen and nodulation in cowpea are largely based on applied fertilizer N. Generally it is the timing and amount of applied N which affects legume symbiosis (Eaglesham et al., 1983). Cowpea usually form nodules about 9-11 days after germination. Investigations on the nitrogen dependence of growing cowpea have shown that seedlings are dependent on reserves within the cotyledons (Ndunguru and Summerfield, 1975) which are normally shed within a few days after emergence. Since dinitrogen fixation is not detectable for about 15 days after planting (Summerfield et al., 1976), this means that young plants may undergo a 'nitrogen hunger' period (Minchin et al., 1980; Summerfield et al., 1976; Pate and Dart, 1961). Applications of small starter doses (20-30 mg/plant) of fertilizer N has been suggested as a means to boost N_2 fixation and yields in cowpea (Minchin et al., 1980; Eaglesham et al., 1977; Dart and Mercer, 1965; Pate and Dart, 1959). The benefits seem partly to arise from increased root production which results in an increase of

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infection sites for soil Rhizobia (Kang et al., 1975; Pate and Dart, 1961). Applications of large amounts of fertilizer N depress nodulation (Tewari, 1965; Dart and Wildon, 1970; Pate and Dart, 1961).

However, genotypic responses to applied N fertilizer vary (Eaglesham et al., 1983) and some nodulating genotypes have been found to be vegetatively equal to non-nodulated plants, even producing significantly greater seed yields (Summerfield et al., 1977; Dart et al., 1977).

Jacobsen and Feenstra (1984) used mutagenic treatment of P. sativum var. Rondo to induce variability for nodulation in this species. A monogenic and recessive mutant was discovered which nodulates efficiently in the presence of 15 mM KNO_3. This mutant nodulated abundantly in a nitrogen-free medium as well.

In Africa, where cowpea production is mostly on a subsistence level, it is not possible for the farmer to alter the many environmental factors which affect nodulation and yield. Irrigation systems are often too expensive or water sources may be limiting. Day time air temperatures may reach highs of 35°C while soil temperatures may rise above 42°C. Such conditions decrease nitrogen fixation (Dart and Mercer, 1965; Minchin et al., 1980). Soil amendments to alter pH or nitrogen status normally are not practiced among subsistence farmers. The practical solution to improving dinitrogen fixation and

yields in cowpea consists in breeding and selecting within the range of genetic variability present in landraces and cultivars.

Host Factors Affecting Dinitrogen Fixation

Although differences for N_2 fixation exist among legume host-strain symbiosis (Neves et al., 1981; Bethlenfalvay et al., 1978b; Pate and Dart, 1961), it is the host which appears to play a more important role in controlling the symbiosis (Graham, 1982). The variable nodulation responses observed within legume hosts and cross-inoculation groups have provided material for genetic studies and screening legume host genotypes for increased N_2 fixation has been accomplished using indigenous soil *Rhizobium* (Westerman and Kolar, 1978; Lie, 1981; Nangju, 1980). Cowpeas are thought to have originated in Africa and cultivated for several generations. Therefore, indigenous rhizobia-host associations are readily formed and in most cases inoculation is not needed (Rachie and Roberts, 1974).

Vorhees, in 1915, first noticed genetic differences among cultivars of soybean (*Glycine max*) for nodulation ability. Plots containing strains of *R. japonicum* were planted with six cultivars of soybeans. All cultivars except one, 'Haberlandt', nodulated normally. Thus,

selectivity for nodulation of host genotypes was demonstrated. Further studies revealed involvment of host genetic factors at various stages of the symbiotic process. For convienence, these processes are divided into initiaiton of nodules, nodule development and effectiveness of nodulation.

Initiation of the Symbiosis

Strain specificity between rhizobia and soybean genotypes was reported by Caldwell and Vest (1968). Plants grown for sucessive years in different locations were predominately nodulated by *Rhizobium japonicum* strains of specific serogroups. Nangju (1980) studied soybean nodulation response to indigenous rhizobia in Africa. Exotic cultivars from the U.S.A. failed to produce nodules unless inoculated, while those from Indonesia nodulated quite well with indigenous strains of *Rhizobium*. Specificity in nodulation has also been reported in *Lupinis* sp. (Lange 1961). A collection of indigenous Australian rhizobia revealed that *L. digitatus*, *L. albus* and *L. pilosus* grouped together on susceptibility to nodulation while *L. luteus* and *L. angustifolius* were rarely nodulated by the different sample strains.

Genetic control of nodule initiation has been

identified in various legume crops. Simple recessive genes for failure to nodulate with compatible bacteria have been reported in soybean (Weber, 1966), peas (Lie, 1971), and red clover (Nutman, 1954b). Nodulating and non-nodulating soybean isolines have been used to study the plants' role in nodulation (Weber, 1966).

Recent work by Dazzo (1980) has shown that plant lectins appear to be involved in bacterial associations. The lectin-recognition hypothesis states that infection site recognition involves binding of specific legume lectins to unique carbohydrates found only on the surface of the appropriate rhizobial symbiont (Dazzo & Hubbell, 1982). This hypothesis is being tested using the R. trifolii-T. repens (Clover) association. Recent studies have shown that mutation of certain plasmid genes of R. trifolii encoding essential nodulation functions result in alteration of the bacterial polysaccharides leading to significant loss of clover lectin-binding activity (Dazzo et al., in press).

Nodule Development

Host controlled factors affecting nodule development involve type, number, size, time to first nodule appearance, and nodulation patterns (Graham, 1982). Dart

(1975) classified nodule types into three distinct morphological forms which are determined by the host genotype. Elongated, cylindrical types normally occur on clove, peas and alfalfa and spherical types are found on soybean, cowpea and beans. Collar nodules are found in lupin and are unique in that the nodules grow around the root.

Although various environmental factors may influence the number and size of nodules, some studies have clearly demonstrated genetic control of these characteristics by the host. Nutman's (1967) investigation of varietal differences in the nodulation of subterranean clover showed high nodule number per plant to be dominant over sparse nodulation but of probable complex inheritance. Experiments using Phaseolus vulgaris demonstrated differences in number and weight of nodules produced for various Rhizobium strains tested (Graham, 1973). White clover (Trifolium repens L.) inoculated with an effective rhizobial strain produced a wide range of variation in total numbers, size and weight of nodules when grown in a nitrogen-free medium (Jones, 1962).

An inverse relationship exists between numbers of nodules and size of nodules (Nutman, 1967; 1981). It has been suggested that this is the means by which the host ensures a proper amount of nodule mass to support plant growth (Nutman, 1981). How a host regulates nodule mass is

still under investigation, but it is most likely influenced by plant hormones and environmental factors (Dart, 1975; Dart and Pate, 1959).

The time from sowing to first nodule development has been studied with the objective of selection for early nodulation genotypes. Host-controlled differences in time to first nodule appearance have been reported in T. subterraneum and Stylosanthes spp. (Graham, 1982). Crosses involving early and late-nodulating parents in subterraneum clover displayed polygenic inheritance (Nutman, 1967). Jones (1962) obtained a range of variation for nodule appearance from 4 to 16 days in Trifolium repens cv s100 Nomark when inoculated with an effective rhizobial strain. Earliness of nodule formation was significantly correlated with harvest dry weight.

Nodulation patterns on legumes have been attributed to host-regulated genes. In studies involving Lupinis, Lange and Parker (1960) distinguished three species (L. lueteus, L. mutabilis and L. digitatus) which exhibited preferential siting of nodules when inoculated with a Rhizobium strain, although a fourth species (L. angustifolius) did not. Distribution of nodules occurred either on the crown, tap root or lateral roots.

Genetic regulation of nodulation patterns in species of Phaseolus and soybean was reported by Bhandari and Sen 1966). Three distribution patterns were identified:

localized, diffused and mixed. A single gene was responsible for control of nodule siting in Phaseolus species. Crosses between V. radiata var. N.P. 28 having a localized nodulation pattern and V. trilobata with a diffuse pattern displayed dominance for the diffuse pattern in the progeny. The ability to breed genotypes with preferential siting of nodules may be very important under semi-arid conditions where surface soil moisture is limited and surface temperatures are high.

In an interesting investigation reported by Doku (1970), the effect of selfing and hybridization on the nodulation of cowpea was examined. Cowpeas are normally self-pollinated with occasional outcrossing. Upon examination of local cowpea varieties from Ghana, one variety was discovered in which a considerable degree of outcrossing occurred. Self pollination significantly increased the mean number and weight of effective nodules per plant as well as the mean weight of each effective nodule. Although the magnitude of these increases began to decrease by the S_4 generation, a large increase was again observed when two selfing lines of the S_4 generation were crossed.

Effectiveness of the Rhizobium-Host Symbiosis

The presence of root nodules does not predispose the legume host to nitrogen fixation. Nodules may be present but ineffective or inefficient. Ineffective nodulation means that infection on the root hair by rhizobium bacteria occurs and nodules are induced, however, nitrogen fixation does not occur (Caldwell and Vest, 1977).

Investigations with red clover indicate that a single recessive gene (i1, i1) is responsible for an ineffective nodulation response when inoculated with Rhizobium trifolii strain A (Nutman, 1954b). Homozygous i1 i1 plants gave an effective response when inoculated with other strains of R. trifolii. Additionally, a recessive suppressor, m, was found which restores i1 i1 plants to complete effectiveness with Rhizobium strain A.

Caldwell (1966) reported three host genes controlling ineffective nodulation in soybeans. Inoculation of the cultivar 'Hardee' with Rhizobium japonicum strains of serogroups c1 and 122 yielded stunted plants with ineffective nodules. Inheritance studies of F_1, F_2 and F_3 generations of 'Hardee' x normal nodulating genotypes indicated that ineffective nodulation is conditioned by a single dominant gene Rj2. This gene is distinguishable from Rj1 which conditions nodulating or non-nodulating response

of soybean to most all strains of R. japonicum (Caldwell and Vest, 1977). A third gene, Rj3, controls ineffective nodulation in 'Hardee' when inoculated with R. japonicum strain 33.

Inefficient nodulation is described as normal nodule formation on the legume root with subsequent lack of nitrogen fixation. One such example involves the soybean cultivar 'Peking' which, when inoculated with R. japonicum strains of serogroup 123, forms large normal-looking nodules yet nodule interiors remain white or light pink, and small, often insufficient, amounts of nitrogen are fixed. Plants of 'Peking' remain chlorotic and small, displaying typical symptoms of nitrogen deficiency (Caldwell and Vest, 1977)

Genetic Variability for N_2 Fixation

Intraspecific variability for nitrogen fixation among legume host genotypes has been reported in several seed legumes including Vigna unguiculata (L.) Walp. (Zary et al., 1978; Minchin et al., 1978; Graham and Scott, 1982), Phaseolus vulgaris (Westerman and Kolar, 1978; Rennie and Kemp, 1981; Felix et al., 1981; McFerson and Bliss, 1981), Cicer arietinum (Rupela and Dart, 1982), Pisum sativum (L.) (Bethlenfalvay and Phillips, 1979), Vicia faba (El-

sherbeeny et al., 1977), Glycine wightii (Nicholas and Haydock, 1971), and Glycine max (Wacek and Brill, 1976).

Screening of 100 cowpea genotypes, inoculated with mixed strains of Rhizobium, for variation in N_2 fixation revealed significant differences among host plant genotypes (Zary et al., 1978). Differences were obtained regardless of whether the criterion used for measurement was nodule mass, nodule number or nitrogen fixing activity measured by acetylene reduction. Consistent differences among performances of individual genotypes in replicated experiments demonstrated evidence of genetic control of the trait and the possibility of breeding for enhanced N_2 fixation in cowpea.

Graham and Scott (1982) compared 12 cowpea varieties in a time phase study for nodulation ability, dry matter production and N-accumulation under field conditions. Results indicated that when plants were completely dependent upon symbiosis for their nitrogen requirements, high and low N-fixers could be readily identified. A strong correlation was found between total plant N and nodule weight at 42 days after planting. This sampling date also represented the time of maximum dry matter accumulation.

Studies involving Vigna mungo report the existence of genetically controlled intra-cultivar variability for dinitrogen fixation (Fernandez and Miller, 1983ab). Random plant samples were selected from two cultivars and two

hybrid populations were obtained by crossing. Nodule number, weight and plant specific activity showed significant differences between F_1's for both populations indicating that the parental cultivars were not genetically pure for these variables.

In Phaseolus vulgaris at least three factors are thought to be contributing to the variability in N_2 fixation: supply of carbohydrates to the nodule, relative rates of N uptake from soil and time to flowering (Graham, 1981). Climbing cultivars appear to transport a large portion of their carbohydrates to nodules compared with plants of other growth habits. Bush bean cultivars were found to absorb soil nitrogen more rapidly than climbing types (Graham and Rosa, 1977) which might cause a decrease in carbohydrate supply to nodules, leading to lowered fixation. Maturity rates influence nitrogen fixation through competition of developing pods for photosynthates needed for nodule development. Thus, delaying flowering may be one way of increasing seasonal fixation (Hardy et al., 1973).

Felix et al. (1981) compared cultivars of common bean from different geographic locations for nitrogenase and nitrate reductase activities under field conditions. Observations indicated that, on the average, tropical cultivars have a higher level of acetylene reduction and a lower nitrate reductase activity than temperate cultivars.

The increased nitrogenase activity of the tropical cultivars was due to a higher amount of nodules. These results support the idea of breeding cultivars for improved N_2 fixation in tropical countries where the use of N-fertilizer may be limited.

Screening for enhanced levels of nitrogen fixation and seed yield in common bean has already been accomplished (McFerson and Bliss, 1981; McFerson et al., 1982). Populations were developed by the backcross-inbred method. A number of homozygous lines were developed similar in most characters to the recurrent parents. Evaluation of these lines under field conditions for nitrogen (C_2H_2) fixation and yield resulted in considerable variation for both traits, and transgressive segregation was observed.

Examination of eight varieties of Vicia faba in association with a standard strain of rhizobia and also with the application of N-fertilizer gave large differences in varieties (El-Sherbeeny et al., 1976). Dry matter production, %N and total N uptake differences were apparent between varieties, Rhizobium and mineral N treatments. Similar results were reported by Nicholas and Haydock (1971) in their studies of Glycine wightii. Variations within lines of G. wightii were measured by dry weight per nodule and time to nodule appearance.

For most legume crops, exploration into the variability of N_2 fixation and the possibility of breeding

for enhanced fixation is just beginning. Most of the studies conducted to date are based on improved cultivars. The genetic potential for dinitrogen fixation harboured within land races and wild relatives of common legume crops has yet to be investigated.

Breeding Objectives

Breeding programs involved in enhancement of dinitrogen fixation ultimately aim at improving yield. For grain legumes the desired goal is increased seed production while forage legume breeding is aimed at increased vegetative production. Since the improvement of dinitrogen fixation would involve various host characteristics, including morphological, physiological and agronomic traits, the task becomes somewhat complicated, although not impossible. Furthermore, one may proceed from either side of the symbiotic association - by improving the host or improving the bacteria. Provided that a good agronomic host is already available, searching for a strain of *Rhizobium* that will fix the greatest amount of N_2 with a particular genotype is one possibility for improving fixation (Caldwell and Vest, 1977; Nutman, 1981; Graham, 1982). However, this procedure would not be feasible for cowpea since the host is susceptible to infection by a

large range of Rhizobium sp. within the cowpea cross-inoculation group. Furthermore, control over indigenous soil Rhizobium would not be possible.

Genetic resistance to all but a select group of highly efficient Rhizobium strains has been suggested as another means of improving dinitrogen fixation. Cultivars developed by this method would have very specific adaptatibility and require the compatible strain to be present or applied as inoculum (Caldwell and Vest, 1977). Such work is now being investigated for soybean (Devine, 1977).

In Africa, breeding for favorable gene combinations that are least likely to be affected by bacterial strains seems a plausible approach. Dart (1975) has suggested several methods for improving host characteristics which affect nodulation. Endogenous plant hormones and Rhizobium-produced hormones both contribute in regulating the quantity of nodule mass produced in a symbiotic association. One approach to improving fixation is to alter this balance so more nodules are produced. Placement of nodules, which is also a host regulated trait, may be altered so that the bacteroids are not exposed to adverse soil conditions such as high surface temperatures. While plants compensate for nodule number by increasing nodule size, plants with most nodules are thought to fare best. Breeding for an expanded root system would create more

potential infection sites by soil rhizobia.

Equally important in selection for increased N_2 fixation is plant architecture. Graham (1981) described differences in growth habits of Phaseolus vulgaris which affect several aspects of the dinitrogen fixation process. For example, climbing cultivars were found to transfer a greater amount of non-structural carbohydrates to nodules than plants of other growth habits. No such investigations have yet been conducted in cowpea, but one would need to consider such differences (should they exist) in light of farmer's preference for plant types. Improvements in plant architecture which would decrease shading to lower leaves and increase photosynthate supply to nodules is another alternative.

Because of the integrative relationship between host-Rhizobium-environment, breeding for improved fixation requires special considerations and may be best solved by cooperative efforts between breeders, microbiologists and agronomists.

Selection Criteria and Methods

Time of Sampling

Unlike breeding for disease resistance or improved grain quality, the symbiotic process is progressive throughout the life of the plant and intimately connected to its many growth stages and physiological processes. Rates of N_2 fixed are dependent on the stage of plant growth (Caldwell and Vest, 1977). An understanding of nodulation ontogeny for a particular legume crop is essential in order to determine periods of maximum fixation or fixation 'stress', thereby identifying optimum selection periods or stages where breeding efforts may be diverted.

Previous studies in cowpea have identified such stages. Time phase studies have shown that fixation rates increase exponentially from the third to the sixth week at an average daily rate of 20% (Zary et al., 1978b). Maximum N_2 fixation occurs about 42 days after planting and this period correlates well with maximum plant dry matter accumulation, total N and nodule weight (Graham, 1982). After 6 weeks, activity declines rapidly through pod fill to senescence (Zary and Miller, 1978b). The best time for screening cowpea, then, would be at full flower (Zary et al., 1978ab;1980). In addition to seasonal patterns,

diurnal patterns of $N_2(C_2H_2)$ fixation have been observed in cowpea (Zary and Miller, 1980). Maximum diurnal activity was found to peak at 1200 hours when measured at both 34 and 53 days after planting. These results indicate that selection of high N-fixing genotypes by the acetylene reduction method needs to be coordinated with both seasonal and diurnal patterns of fixation.

Methods of Measurement

Selection of superior nodulating genotypes depends not only on the time of sampling but on the traits sampled and the method of measurement. Variation in N_2 fixation among host legumes has been measured by acetylene reduction (Pacovsky et al., 1984; Westerman and Kolar, 1978; Zary et al., 1978b; Lawn and Bushby, 1982), H_2 evolution (Layzell et al.,1979; Pacovsky et al.,1984), total N (Rennie and Kemp, 1981; Graham, 1982), and quantitatively viz. plant dry matter, nodule weight and nodule number (Graham and Scott, 1982; Nicholas and Haydock, 1970; Rennie and Kemp, 1981 and others). No general agreement has been reached among legume breeders as to which method is most reliable, and decisions are no doubt influenced by time and resources.

Selection of Traits

Various investigators have utilized different host characteristics in selecting genotypes with superior nodulation ability. Graham and Scott (1982) found a strong correlation between total N and shoot weight in cowpea. Presumably, varieties of a higher N-fixing ability can also accumulate more dry matter so size and vigor of plant tops may be used as a measure of greater N-fixation. Total N was also highly correlated with nodule weight. The absence of correlation between total N and nodule number suggests that it is nodule mass which is the more important criterion in assessing nodulation (Graham, 1981).

Zary et al. (1978) used four indexing criteria in defining the extent of genetic variability among 100 cowpea genotypes. Specific activity was found to be the most consistant indicator of N_2 fixation followed by nodule mass and plant top dry weight. Nodule number was found to be the least consistent. Nodule weight has also been suggested as a suitable characteristic for selection in Glycine wightii (Nicholas and Haydock, 1970).

Westerman and Kolar (1978) studied variations in dinitrogen fixation in Phaseolus vulgaris. Their results showed N_2 (C_2H_2) fixation to be related to average seasonal nodule weight and plant dry weight near physiological

maturity. Moreover, total N uptake was signigicantly related to seed yields of the cultivars and cultivars with high seed yields also had high N_2 (C_2H_2) fixations. This suggests the possibility of selection based on yields only. Evaluation of Phaseolus vulgaris under two low temperature regimes showed the amount of dinitrogen fixed to be correlated with leaf area and leaf and shoot weight (Rennie and Kemp, 1981). Similar relsults were reported in studies involving four Asiatic Vigna species (Lawn and Bushby, 1982). These studies indicate the need for measuring a number of host variables in initial screenig experiments and careful selection of the most reliable traits for further breeding purposes.

Genetic Vulnerability

Mostly all studies addressing the problem of dinitrogen fixation are based on data obtained from advanced or cultivated legumes. The number of plant introductions is usually small and those that adapt successfully to new environments are fewer yet. It is these successful introductions which are further used for breeding (Lie, 1981). The specificity of host genotypes for particular Rhizobium strains has been reported (Nangju, 1980). Narrowing of the genetic base of host species may

tend to favor certain strains of soil Rhizobium. Although the bacterium may live as a soil saprophyte, Nutman (1967) has reported that its distribution and multiplication is closely related to the presence of a compatible host. Over time, the disappearance of indigenous leguminous hosts may alter the population of soil Rhizobium. Moreover, the remaining symbiotic associations may not be those of highest dinitrogen fixing ability.

Rationale for the Present Work

Studies of genetic diversity in landraces of cowpea are lacking. Given the amount of variability found in cultivated varieties for N_2 fixation, one would expect even greater diversity in land races. This study attempts to define the range of variability for indigenous and exotic lines of cowpea currently grown in Botswana and to identify high and low nitrogen-fixers for use in future breeding programs.

MATERIALS AND METHODS

Origin of Plant Material

One hundred lines of cowpea were grown in Botswana during 1982-83 to evaluate the nodulation capacity of each (Appendix F). Forty nine percent of the accessions were local landraces collected from Botswana farmers while fifty lines were derived from breeding material and world collections held at the International Institiue of Tropical Agriculture in Nigeria and SAFGRAD in Borkina Faso. The check, Blackeye, was originally introduced into Botswana from California some time ago but has since undergone considerable adaptive changes. It has been one of the few commercial introductions of cowpea into Botswana and, until recently, the only seed source multiplied for planting.

Field Plot Design

A split plot design was used for the experiment with nitrogen treatments as the main plot and 100 varieties as sub-plots. Two replications were used and the entire

experiment was planted at two different locations in Botswana (Sebele and Mahalapye). Rows were spaced at 1 M with 20 cm within-row plant spacings. Plots consisted of a single 6 M row. Varieties were randomized within treatments and treatments were randomized within replications. A total of 2650 M^2 area was required per experiment. Phosphate was applied pre-plant as superphosphate (P_2O_5 = 10.5%) at a rate of 250 Kg/ha. Planting at Sebele began on November 4 and terminated on November 5, 1982. At Mahalapye, planting occurred on December 1, 1982. Plots were sprayed twice to control pod-sucking bugs using NOGAS. (1 ml/l H_2O ULV). No Rhizobium inoculum was applied since one objective of the experiment was to determine genetic differences in nodulation between cowpea varieties under conditions of natural Rhizobia, particularly since inoculation practices are unknown among Botswana farmers. Nitrogen was applied by side dressing in the form of lime ammonium nitrate (LAN = 28%) at a rate of 100 kg N/Ha, seven days after planting.

Description of the Experimental Sites

The two sites used for the experiment are located approximately 200 Km apart. Mahalapye (site 1) is located at longitude 26°48' and latitude 23°04'. Total rainfall

received during the 1982-83 growing season was 280 mm. Sebele (site 2) is located about 10 Km north of Gaborone, the capital city, at longitude 25°57' and latitude 24°34'. Rainfall received at Sebele during the course of this experiment exceeded 450 mm. Rainfall distributions for both sites may be found in appendices C and G.

Sampling Procedures

All varieties were sampled for nodule number, nodule fresh weight and dry weight, root weight, shoot fresh weight and dry weight at 2, 4 and 6 weeks after planting. For site 1, four random plants per variety were dug and evaluated at each of the three sampling times for both replications and for each treatment. Total values were recorded and later averages were calculated and used for analysis. At site 2, similar procedures were followed except that hardened soil conditions by the 4th week after planting prohibited the removal of plant roots without damage. Therefore, only shoot fresh weights and dry weights were recorded. By the 6th week after planting, the soils became even drier due to the lack of rainfall. Each plant which was sampled had first to be moistened around the roots by applying buckets of water. Since this entailed a great deal of extra time, only two plants per variety were

sampled. As before, total values for all six variables were recorded and later averages were calculated for use in analysis.

Since some local varieties are indeterminate in nature, harvesting continued over a period of several weeks. Five random plants per plot were harvested and the following data recorded: total number of pods per 5 plants, pod dry weight, pods per plant, total seed weight, seeds per pod, total seed number, yield per plant and 100 seed weight. Some insect damage occurred due to pod-sucking bugs despite insecticide treatments. In order to account for this damage, three measures of 100 seed weights were recorded: a random sample of damaged and non-damaged seed, a selected sample of non-damaged seed and a selected sample of damaged seed. Harvesting at site 2 was conducted in the same manner except that 10 random plants per plot were sampled for all varieties.

RESULTS AND DISCUSSION

The means and ranges for the six variables examined during the statistical analysis of the data at site 1 are summarized in Table 13. Only 60 of the varieties were included in the evaluation. Applications of nitrogen fertilizer at a rate of 100 Kg N/Ha produced no statistically significant differences from treatments with 0 Kg N/Ha. This effect carried through for all sampling periods at 2, 4 and 6 weeks after planting. The area occupied by the experiment had been sown with sorghum the previous season and soil analysis of mineral and mineralizable N were low (Appendix H). However, supression of nodule growth was visually evident, particularly by the fourth and sixth week after planting.

Genetic diversity for nodulation characteristics were expressed for all variables measured and through all sampling periods. Thus, even at two weeks after planting, it was possible to categorize genotypes with respect to their capacity for nodulation (Table 14). Significant interactions were observed between varieties and nitrogen treatments for some of the variables at different times during the course of the experiment. Although these

Table 13. Means and ranges of variables measured on cowpea accessions at 2 and 6 weeks after planting (Mahalapye).

Treatment	Nodule Number		Nodule Fresh Weight (g)[a]		Nodule Dry Weight (g)[a]		Root Weight (g)		Shoot Fresh Weight (g)		Shoot Dry Weight (g)	
	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range
	2 Weeks											
N	12.568	0.050-41.250	5.10	1.300-15.000	0.977	0.001-3.400	0.496	0.230-1.030	2.670	0.430-6.880	0.298	0.020-0.730
-N	13.705	2.500-46.250	5.70	1.200-15.000	0.942	0.001-4.200	0.415	0.100-0.950	1.622	0.680-3.100	0.203	0.010-0.400
LSD(.05) =	NS		NS		NS		NS		NS		NS	
	6 Weeks											
N	13.327	0-42.000	0.163	0-0.948	0.041	0-0.213	8.492	1.170-15.780	172.073	42.50-350.75	23.412	2.20-92.250
-N	18.219	0.750-46.500	0.755	0-3.000	0.150	0-0.595	8.433	2.770-15.780	159.738	32.75-296.00	21.017	3.800-36.830
LSD(.05) =	NS		NS		NS		NS		NS		NS	

[a]Multiplied by 100 for data at 2 week sampling.

Table 14. Analysis of variance for nodulation characteristics at 2 and 6 weeks after planting (Site 1: Mahalapye).

Source	df	Mean Squares					
		Nodule Number	Nodule Fresh Weight[a] (g)	Nodule Dry Weight[a] (g)	Root Weight (g)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)
		2 Weeks					
Replicate	1	15.025	36.504	3.577	0.182	45.605	0.377
Nitrogen (N)	1	77.463	24.066	0.077	0.384	65.982	0.545
Error a	1	520.823	115.093	1.820	0.128	90.798	0.388
Varieties (V)	59	110.941**	15.706**	0.727**	0.059**	1.045**	0.016**
N x V	59	55.854*	9.467**	0.323*	0.007	0.213	0.002
Error b	118	33.953	5.696	0.270	0.009	0.288	0.003
		6 Weeks					
Replicate	1	140.229	2.080	0.083	3.276	406.960	91.816
Nitrogen (N)	1	1388.026	20.341	0.696	0.202	8541.826	332.809
Error a	1	378.855	2.549	0.084	2.122	72.721	149.329
Varieties (V)	57	157.683**	0.211**	0.007*	12.000**	5010.518**	87.007
N x V	57	44.216	0.134	0.005	3.863	3306.727**	55.884
Error b	114	50.811	0.109	0.005	3.792	1826.690	63.131

*,**Significant at the 5 and 1% levels, respectively.

[a]Multiplied by a factor of 10^4 for data at 2 weeks.

interactions were not consistently significant, there is an indication that nitrogen treatments were having varying effects depending on the variety.

Similar results were obtained at site 2 (Table 15 and 16) where sampling was carried out at two and six weeks after planting. Again, no significant differences were obtained between nitrogen treatments although, environmentally, this site was quite different from site 1 in terms of rainfall and soils (Appendices C & G). A combined analysis of variance over the two locations indicates that the plants performed differently between the two sites for some of the measured characteristics (Table 17). Initially, this difference was mainly expressed in nodule number, fresh weight and dry weight. However, by the sixth week, differences in genotypic responses between environments began to show in plant biomass. Throughout the experiment, variety x location interactions were significant for most characters measured. In terms of the objectives of this experiment, the differences in environments allowed for the identification of superior-nodulating genotypes which displayed a stable performance, despite soil and climatic site differences.

Simple correlations were calculated between all nodulation variables for both two and six week sampling data and for both locations. As expected, nodule number was highly correlated with nodule fresh weight throughout the

Table 15. Means and ranges of variables measured on cowpea accessions at 2 and 6 weeks after planting (Sebele).

Treatment	Nodule Number		Nodule Fresh Weight (g)[a]		Nodule Dry Weight (g)[a]		Root Weight (g)		Shoot Fresh Weight (g)		Shoot Dry Weight (g)	
	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range	$\bar{X}$	Range
	2 Weeks											
N	2.427	0-12.75	0.931	0-5.000	0.228	0-1.300	0.483	0.220-0.780	1.289	0.450-2.550	0.180	0.070-0.340
-N	3.292	0-16.500	1.164	0-6.500	0.288	0-1.875	0.466	0.220-0.900	1.282	0.300-2.960	0.176	0.040-0.360
LSD(.05) =	NS		NS		NS		NS		NS		NS	
	6 Weeks											
N	8.617	0-42.000	5.623	0-46.000	2.182	0-36.000	6.260	0.430-23.170	67.932	18.500-203.000	9.934	4.100-41.500
-N	8.940	0-61.000	7.648	0-53.500	3.357	0-21.555	6.951	2.140-17.930	79.649	22.500-218.000	12.119	3.450-33.350
LSD(.05) =	NS		NS		NS		NS		NS		NS	

[a]Values are multiplied by a factor of 100.

Table 16. Analysis of variance of nodulation characteristics for Site 2 (Sebele).

Source	df	Mean Squares					
		Nodule Number	Nodule Fresh Weight[a] (g)	Nodule Dry Weight[a] (g)	Root Weight (g)	Shoot Fresh Weight (g)	Shoot Dry Weight (g)
		2 Weeks					
Replicate	1	886.266	116.431	8.201	0.026	0.784	0.002
Nitrogen (N)	1	74.204	5.384	0.359	0.030	0.005	0.001
Error a	1	153.539	4.154	0.270	0.240	1.895	<0.001
Varieties (V)	98	7.919**	1.524**	0.090**	0.051**	0.422**	0.006**
N x V	98	4.558	1.025	0.067	0.016	0.071	0.001
Error b	196	4.684	0.833	0.052	0.017	0.067	0.001
		6 Weeks					
Replicate	1	5017.041	2728.187	467.321	310.320	67981.631	697.196
Nitrogen (N)	1	10.010	298.862	136.306	45.857	13180.078	458.369
Error a	1	174.690	20.289	14.916	46.634	59086.007	993.049
Varieties (V)	95	87.230**	78.188**	15.960*	12.892**	1019.274*	25.158**
N x V	95	48.865	43.176	11.500	2.559	510.141	17.470
Error b	190	50.375	38.910	10.308	4.066	698.613	16.356

*,**Significant at the 5 and 1% levels, respectively.

[a]Multiplied by a factor of 10^4 for mean square values.

Table 17. Combined analysis of variance from Sites 1 and 2.

Source	df	Mean Squares					
		Nodule Number	Nodule Fresh Weight[a]	Nodule Dry Weight[a]	Root Weight	Shoot Fresh Weight	Shoot Dry Weight
		2 Weeks					
Location	1	12100.894*	22.195*	0.558*	0.036	84.720	0.575
Rep (L)	2	311.761	0.549	0.047	0.088	22.703	0.185
Nitrogen	1	140.916	0.292	0.001	0.294	31.487	0.278
LN	1	0.325	0.290	0.003	0.113	34.554	0.264
Error a	2	331.844	0.633	0.010	0.191	45.666	0.191
Varieties	58	74.211**	0.103**	0.005**	0.100**	1.145**	0.018**
LV	58	43.632**	0.068**	0.003**	0.015	0.248*	0.003*
NV	58	28.261*	0.049*	0.002	0.013	0.151	0.002
LNV	58	34.227**	0.060**	0.002	0.015	0.139	0.001
Error b	232	19.343	0.033	0.002	0.017	0.181	0.002
		6 Weeks					
Location	1	4303.896*	16.473	0.465	404.389*	976383.066*	14168.305*
Rep (L)	2	1663.584	1.021	0.060	100.824	17864.017	243.657
Nitrogen	1	902.236	10.267	0.398	20.924	72.552	0.968
LN	1	393.167	8.427	0.247	25.711	19376.788	597.827
Error a	2	226.991	1.127	0.039	16.813	17333.630	328.060
Varieties	56	186.648**	0.119**	0.006**	19.711**	3830.543**	59.776*
LV	56	83.118**	0.086**	0.003	7.690**	2517.945**	53.445
NV	56	36.559	0.057	0.003	3.433	1570.840	36.003
LNL	56	55.709	0.062	0.004	3.383	2422.737**	42.393
Error b	224	53.117	0.053	0.003	4.364	1363.317	41.900

[a]Values are multiplied by 100.

analysis (Tables 18 and 19). Nodule fresh weight and nodule dry weight were also highly correlated as were shoot fresh weight and shoot dry weight. Therefore, nodule number, nodule fresh weight and shoot fresh weight were chosen as useful variables for ranking genotypes. Root weight was not considered since plant extraction methods under field conditions, such as were used in this experiment, may be insufficient to remove the total root mass of deeply-rooted genotypes. Thus, fair comparisons between individuals could not be made.

The sorting of genotypes acccording to mean values of the chosen variables allowed for identification of individuals which consistently ranked among the top or botton third of all accessions. Five genotypes were selected whose performance across sampling periods, treatments and environments were considered as consistently high. Blackeye, the check variety, was among the top performing genotypes. An introduction from IITA, IT81D-985, also ranked high. The three other accessions were local landraces (Tables 20 and 21). Two poorly nodulated varieties, Vita-5 and IT82E-8, were also identified. These lines are introductions originating from Nigeria. Two other lines, IT82E-17 and TVu 3236, were found to be low ranking in site 1 and 2, respectively.

Comparison of mean values of nodule number, nodule fresh weight and shoot fresh weight for the high and low-

Table 18. Correlation coefficients between nodulation characteristics of cowpea grown with and without nitrogen (Site 1: Mahalapye)[a].

Variable	Nodule Number (NN)	Nodule Fresh Weight (NFW)	Nodule Dry Weight (NDW)	Root Weight (RW)	Shoot Fresh Weight (SFW)	Shoot Dry Weight (SDW)
	2 Weeks					
NN		0.408 (0.441)[b]	0.705 (0.526)	0.485 (0.478)	0.433 (0.720)	0.442 (0.629)
NFW			0.478 (0.520)	0.127 (0.409)	-0.162 (0.348)	-0.023 (0.371)
NDW				0.456 (0.361)	0.465 (0.419)	0.479 (0.452)
RW					0.555 (0.511)	0.581 (0.507)
SFW						0.902 (0.895)
	6 Weeks					
NN		0.797 (0.769)	0.721 (0.640)	0.341 (0.409)	0.285 (0.402)	0.192 (0.409)
NFW			0.795 (0.835)	0.206 (0.342)	0.115 (0.334)	0.097 (0.388)
NDW				0.284 (0.298)	0.184 (0.279)	0.192 (0.328)
RW					0.755 (0.726)	0.623 (0.794)
SFW						0.712 (0.887)

[a]Correlations exceeding .2732 and .3541 are significant at the 5 and 1 percent levels, respectively.

[b]Values within parenthesis represent nitrogen-free treatments.

Table 19. Correlation coefficients between nodulation characteristics of cowpea grown with and without nitrogen (Site 2: Sebele).

Variable	Nodule Number (NN)	Nodule Fresh Weight (g) (NFW)	Nodule Dry Weight (g) (NDW)	Root Weight (g) (RW)	Shoot Fresh Weight (g) (SFW)	Shoot Dry Weight (g) (SDW)
	2 Weeks[a]					
NN		0.845 (0.902)[b]	0.763 (0.887)	0.024 (0.273)	0.268 (0.310)	0.340 (0.210)
NFW			0.873 (0.926)	0.030 (0.239)	0.192 (0.251)	0.271 (0.193)
NDW				0.032 (0.211)	0.125 (0.237)	0.223 (0.161)
RW					0.372 (0.641)	0.304 (0.576)
SFW						0.922 (0.918)
	6 Weeks[a]					
NN		0.732 (0.793)	0.568 (0.801)	0.195 (0.269)	0.027 (0.363)	-0.016 (0.314)
NFW			0.619 (0.962)	0.197 (0.199)	0.071 (0.211)	0.030 (0.189)
NDW				0.064 (0.198)	-0.016 (0.215)	-0.017 (0.189)
RW					0.662 (0.758)	0.475 (0.672)
SFW						0.694 (0.879)

[a]Values exceeding 0.2050 and 0.2673 are significant at the 5 and 1% levels, respectively.

[b]Values within parenthesis represent nitrogen-free treatments.

Table 20. Ranking of selected cowpea genotypes at 2 and 6 weeks after planting (Site 1)[a].

Cowpea Accession	2 Weeks						6 Weeks					
	Nodule Number		Nodule Fresh Weight		Shoot Fresh Weight		Nodule Number		Nodule Fresh Weight		Shoot Fresh Weight	
	N	-N	N	-N	N	-N	N	-N	N	-N	N	-N
High												
IT81D-985	59	44	43	55	57	48	54	(39)[b]	59	(19)	(20)	44
Blackeye	60	49	60	59	59	48	60	53	57	56	(29)	52
B152	52	51	47	36	39	43	53	58	52	49	43	59
B006-C	(35)	48	53	43	52	45	59	60	58	54	59	56
B145	58	60	56	60	54	60	56	56	56	57	60	60
Low												
Vita 5	9	2	8	15	7	5	13	4	13	18	(32)	19
IT82E-8	(23)	14	7	10	(24)	15	1	6	2	5	15	8
IT82E-17	5	3	(49)	(56)	11	16	6	3	3	8	16	6

[a]Numbers listed indicate a genotypes' rank out of 60 total accessions measured for each nodulation characteristic.

[b]Numbers in parenthesis indicate inconsistencies in ranking.

Table 21. Ranking of selected cowpea genotypes at 2 and 6 weeks after planting (Site 2)[a].

Cowpea Accession	2 Weeks[a]						6 Weeks[b]					
	Nodule Number		Nodule Fresh Weight		Shoot Fresh Weight		Nodule Number		Nodule Fresh Weight		Shoot Fresh Weight	
	N	-N	N	-N	N	-N	N	-N	N	-N	N	-N
High												
IT81D-985	98	80	98	74	94	(62)[c]	71	68	82	71	93	78
Blackeye	75	99	(55)	99	98	94	94	94	89	93	(10)	(38)
B152	96	68	75	76	85	(54)	88	74	65	63	(7)	64
B006-C	91	76	89	89	88	79	89	95	83	84	64	79
B145	(25)	93	(12)	79	87	99	96	67	93	67	83	(57)
Low												
Vita 5	2	(47)	3	27	4	17	1	9	2	15	2	(69)
IT82E-8	5	(90)	5	(80)	31	(42)	29	(73)	18	(61)	(46)	5
TVu3236	30	5	16	3	10	7	(39)	15	(35)	20	31	25

[a]Rank out of 99 total accessions.

[b]Rank out of 96 total accessions.

[c]Numbers in parenthesis indicate inconsistancies in ranking.

ranking genotypes demonstrates the differences in nodulation capacity between these two groups (Table 22, 23 and 24). Table 25 summarizes the differences for each location and treatment.

Further investigations into the effect of the two nitrogen treatments on these individuals indicated that, for most genoytpes, nitrogen suppressed nodule formation and growth, as would be expected (Figures 15 and 16), although the effect of nitrogen on shoot fresh weight was not consistent. Since none of the landraces was identified as having a stable, poor performance, Tables 26 and 27 were constructed in order to determine if, on the average, the landrace population performed any differently than introduced varieties. In comparing treatment means between the two groups, no statistically significant differences were obtained for any of the variables examined.

An examination of the differences in grain yield between the two sites was not meaningful since yields at site 1 were severly reduced due to an infestation of pod bugs and the drought occurring at site 2 caused many of the genotypes to shed their flowers and, hence, fail to produce pods.

The inability to detect nitrogen treatment differences in this experiment may be attributable to different causes. Possibly the sensitivity of the F-test is such that the small degrees of freedom in the error term did not allow

Table 22. Mean values of nodules per plant at 2 and 6 weeks after planting for selected high and low nitrogen-fixing genotypes.

Cowpea Accession	2 Weeks				6 Weeks			
	Mahalapye		Sebele		Mahalapye		Sebele	
	N	-N	N	-N	N	-N	N	-N
High								
IT81D-985	32.3	16.1	6.4	5.0	24.1	20.0	11.0	11.0
Blackeye	37.5	20.4	3.5	11.9	29.9	28.9	18.0	29.0
B152	17.0	21.6	5.3	4.1	22.4	30.8	16.0	11.8
B006-C	12.0	19.8	4.4	4.9	29.0	37.0	17.0	33.8
B145	29.6	31.0	1.4	5.5	25.1	30.0	23.0	10.8
Low								
Vita 5	7.0	4.9	0	2.5	7.1	7.6	0	2.5
IT82E-8	9.4	9.4	0	5.8	2.3	8.5	0	9.5
TVu3236			1.6	1.0			6.5	3.3
IT82E-17	6.3	5.9			5.5	6.6		
LSD(.05)[a]	8.2		3.0		10.0		9.8	

[a]LSD is for comparing means between any two values within the same column.

Table 23. Mean values of nodule fresh weight (g) at 2 and 6 weeks after planting for high and low nitrogen-fixing genotypes.

Cowpea Accession	2 Weeks				6 Weeks			
	Mahalapye		Sebele		Mahalapye		Sebele	
	N	-N	N	-N	N	-N	N	-N
High								
IT81D-985	0.63	0.88	0.03	0.02	0.53	0.50	0.10	0.11
Blackeye	0.13	0.11	0.01	0.04	0.49	1.72	0.16	0.36
B152	0.06	0.06	0.01	0.02	0.28	1.10	0.07	0.09
B006-C	0.08	0.08	0.02	0.02	0.50	1.28	0.11	0.18
B145	0.09	0.14	<0.01	0.02	0.30	1.76	0.18	0.10
Low								
Vita 5	0.03	0.04	-	<0.01	0.08	0.50	-	<0.01
IT82E-8	0.03	0.03	-	<0.01	0.04	0.30	-	0.07
TVu3236			<0.01	<0.01			0.03	0.02
IT82E-17	0.07	0.09			0.04	0.36		
LSD(.05)[a]	0.03		0.01		0.462		0.08	

[a]LSD is for comparing means between any two values within the same column.

Table 24. Mean values of shoot fresh weight (g) at 2 and 6 weeks after planting for selected high and low nitrogen-fixing genotypes.

Cowpea Accession	2 Weeks				6 Weeks			
	Mahalapye		Sebele		Mahalapye		Sebele	
	N	-N	N	-N	N	-N	N	-N
High								
IT81D-985	3.8	2.0	1.9	1.4	142.4	196.4	118.0	91.5
Blackeye	4.0	2.5	2.0	1.9	160.8	205.8	47.8	73.3
B152	2.8	1.9	1.7	1.3	187.1	258.9	45.3	84.5
B006-C	3.3	1.9	1.8	1.6	279.3	218.3	73.8	93.0
B145	4.7	3.0	1.8	2.0	303.6	260.8	84.5	82.5
Low								
Vita 5	1.9	1.0	0.8	0.9	163.6	100.0	31.5	86.3
IT82E-8	2.6	1.3	1.1	1.2	133.3	118.1	25.3	32.0
TVu3236			0.9	0.8			58.3	68.3
IT82E-17	2.1	1.3			144.8	115.6		
LSD(.05)[a]	0.8		0.4		59.2		36.6	

[a]LSD is for comparing means between any two values within the same column.

Table 25. Group mean values of high and low nitrogen fixing genotypes for nodulation characteristics.

	Mahalapye				Sebele			
	High		Low		High		Low	
Variable	N	-N	N	-N	N	-N	N	-N
	2 Weeks							
Nodule number	25.680	21.780	7.567	6.733	4.200	6.280	0.533	3.100
Nodule fresh weight	0.198	0.254	0.043	0.053	0.018	0.024	<0.010	<0.010
Nodule dry weight	0.019	0.016	0.006	0.006	0.004	0.006	<0.001	0.002
Root weight	0.636	0.592	0.453	0.403	0.618	0.570	0.347	0.403
Shoot fresh weight	3.720	2.260	2.200	1.200	1.840	1.640	0.933	0.967
Shoot dry weight	0.424	0.286	0.240	0.157	0.234	0.214	0.140	0.137
	6 Weeks							
Nodule number	26.100	29.340	4.967	7.567	17.000	19.280	2.167	5.100
Nodule fresh weight	0.420	1.272	0.053	0.387	0.124	0.168	0.030	0.030
Nodule dry weight	0.110	0.265	0.014	0.065	0.048	0.004	0.074	0.019
Root weight	9.950	10.676	7.497	6.427	6.048	7.468	4.313	5.903
Shoot fresh weight	117.220	228.040	147.233	111.233	73.880	84.960	38.367	62.200
Shoot dry weight	28.940	19.596	20.547	16.897	11.734	14.190	6.923	12.143

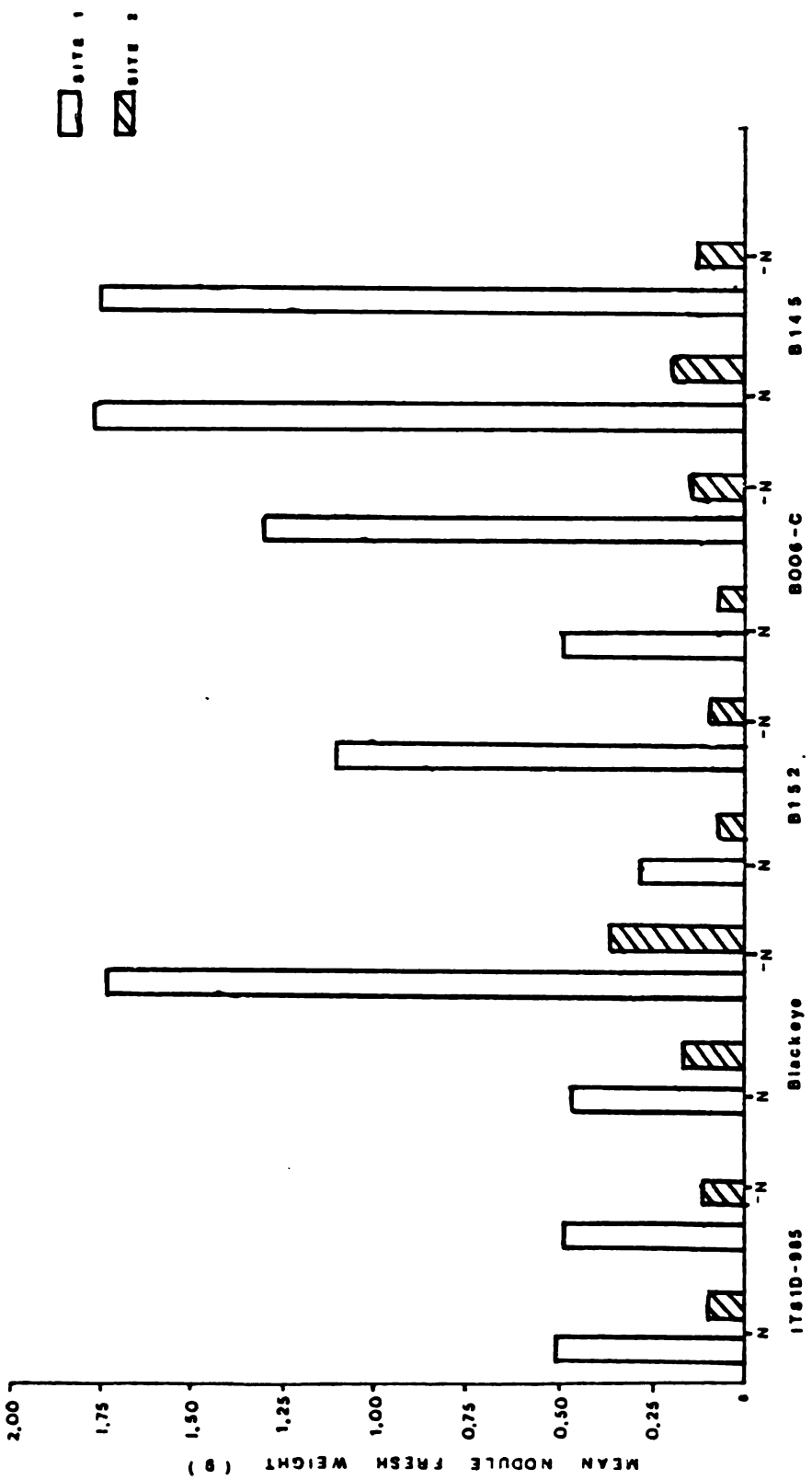

Figure 15. The effect of N-fertilizer on nodule fresh weight on selected high nitrogen fixing genotypes.

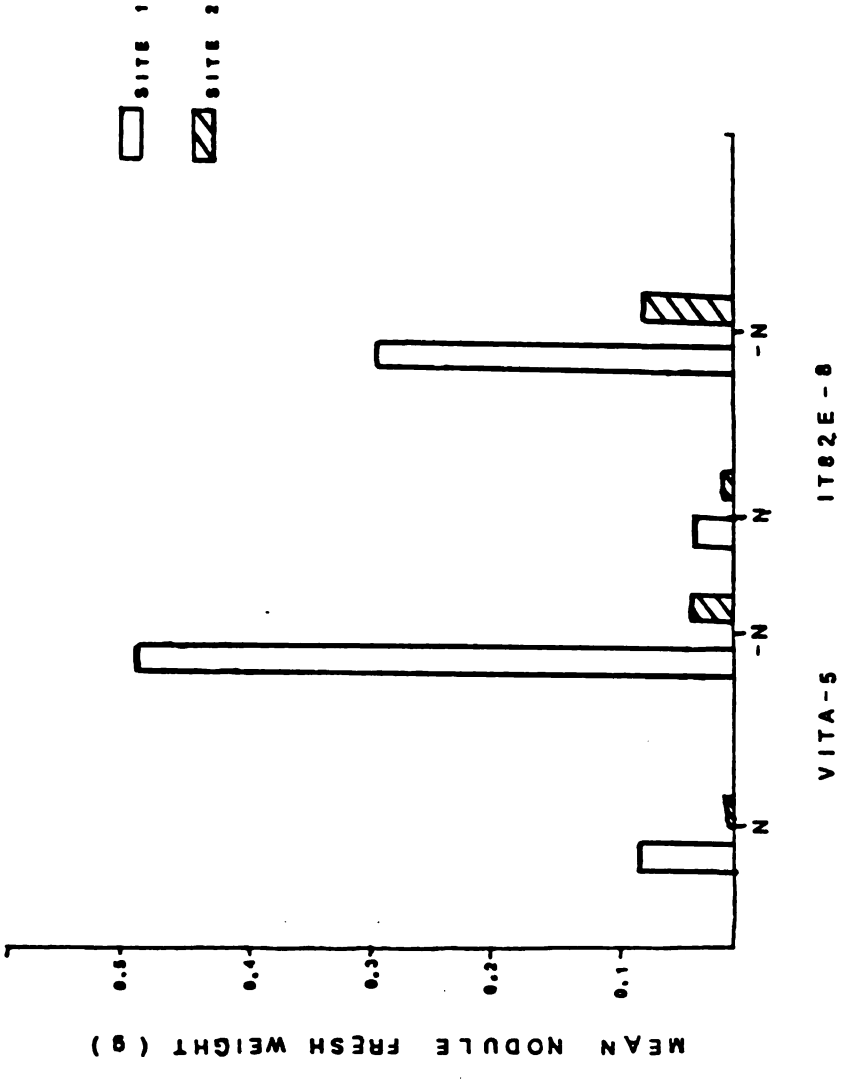

Figure 15a. The effect of N-fertilizer on nodule fresh weight on selected low nitrogen fixing genotypes.

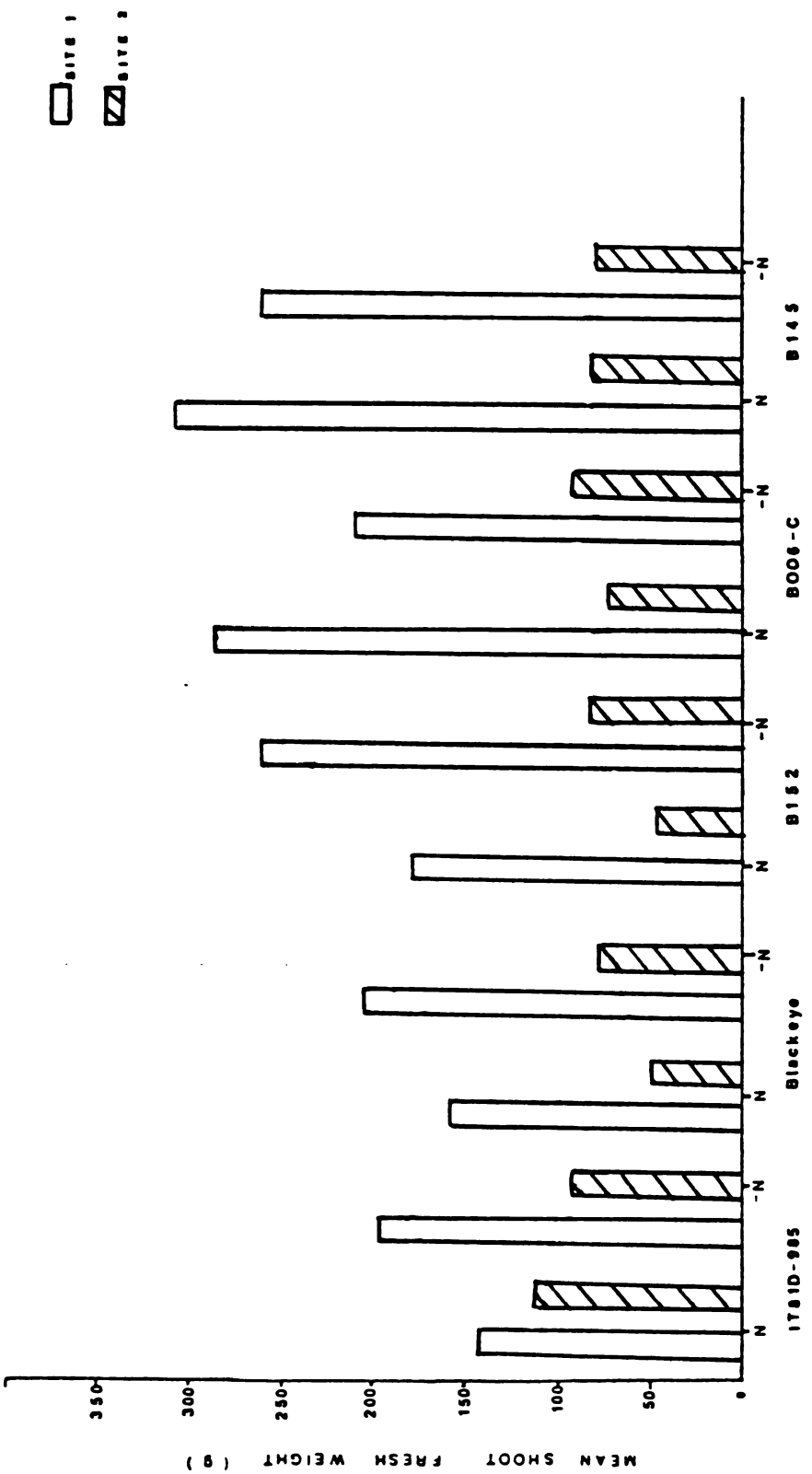

Figure 16. The effect of N-fertilizer on shoot fresh weight on selected high nitrogen fixing genotypes.

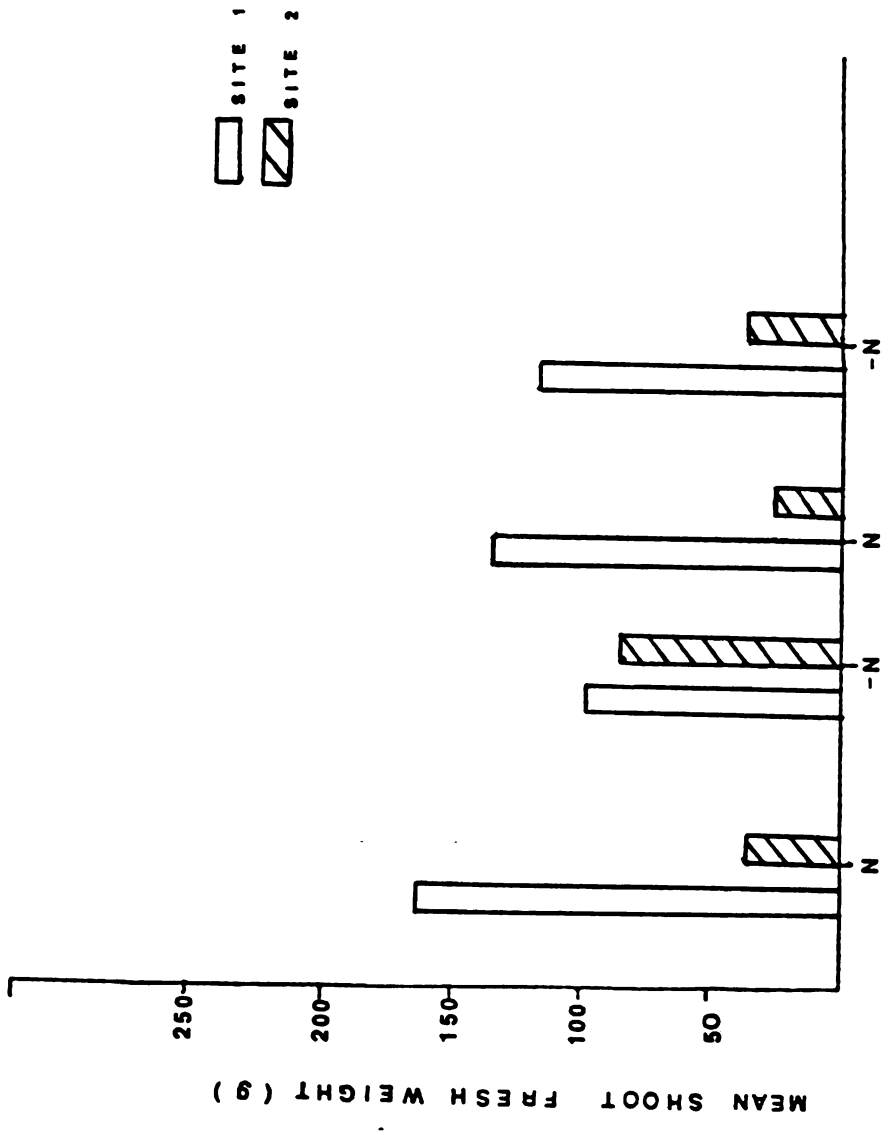

Figure 16a. The effect of N-fertilizer on shoot fresh weight on selected low nitrogen fixing genotypes.

Table 26. Treatment means for comparison between cowpea landraces and introduced varieties at 2 and 6 weeks after planting (Site 1: Mahalapye).

	Nodule Number		Nodule Fresh Weight (g)[a]		Nodule Dry Weight (g)[a]		Root Weight (g)		Shoot Fresh Weight (g)		Shoot Dry Weight (g)	
	N	-N	N	-N	N	-N	N	-N	N	-N	N	-N
	2 Weeks											
Introductions	13.47	14.176	0.530	0.580	.001	.001	0.500	0.424	2.761	1.640	0.303	0.208
Landraces	11.60	13.200	0.480	0.560	.001	.001	0.490	0.406	2.570	1.602	0.293	0.198
LSD(.05)	NS		NS		NS		NS		NS		NS	
	6 Weeks											
Introductions	12.288	16.686	0.151	0.690	0.038	0.147	8.575	8.676	171.850	158.990	22.959	21.457
Landraces	14.360	19.861	0.174	0.825	0.044	0.154	8.354	8.173	171.607	160.540	23.797	20.546
LSD(.05)[b]	NS		NS		NS		NS		NS		NS	

[a]Mean values of nodule fresh weight at 2 weeks are multiplied by a factor of 100.

[b]LSD is for comparing means between any two values within the same column.

Table 27. Treatment means for comparison between cowpea landraces and introduced varieties at 2 and 6 weeks after planting (Site 2: Sebele).

	Nodule Number		Nodule Fresh Weight (g)[a]		Nodule Dry Weight (g)[a]		Root Weight (g)		Shoot Fresh Weight (g)		Shoot Dry Weight (g)	
	N	-N	N	-N	N	-N	N	-N	N	-N	N	-N
	2 Weeks											
Introductions	2.369	2.954	0.919	0.978	0.224	0.258	0.477	0.474	1.340	1.260	0.187	0.172
Landraces	2.483	3.625	0.943	1.347	0.232	0.318	0.489	0.457	1.239	1.303	0.174	0.180
LSD(.05)	NS		NS		NS		NS		NS		NS	
	6 Weeks											
Introductions	8.030	8.015	7.426	6.515	2.602	2.841	5.988	6.755	66.913	79.491	9.729	12.126
Landraces	9.228	9.904	6.355	8.829	2.531	3.895	6.543	7.156	68.994	79.813	10.148	11.935
LSD(.05)[b]	NS		NS		NS		NS		NS		NS	

[a]Values are multiplied by a factor of 100 for 2 and 6 weeks means.

[b]LSD is for comparing means between any two values within the same column.

for detection of any significant differences between the treatments. An improvement in the sensitivity of the test might have resulted by increasing replications or changing the experimental design. As this was a preliminary evaluation, further experiments may need to be altered in their structure in order to detect any significant treatment differences. Also, because of the severe drought affecting site 2 after planting and during nodule sampling, it is possible that plants did not have the opportunity to fully utilize available mineral nitrogen sources. Although this site received more total rainfall than site 1, the distribution was uneven. In fact, six weeks after planting, some fertilizer granules were found intact in the sub-soil layers where they had been placed one week after planting. The occurrence of occasional significance in nitrogen x variety interactions indicates that, generally, nitrogen treatments had some effect on most varieties and that, perhaps, the varieties did not respond to nitrogen in the same manner.

The significant differences obtained between genotypes for all variables measured throughout the experiment indicates that selection for specific characteristics related to nodulation is possible in these cowpea genotypes. High and low-performance genotypes expressed their genetic potential for nodulation across diverse environments. These genotypes also maintained their

performance throughout their growth cycle. It should be possible, then, to screen populations at any time during the active nitrogen-fixation period, and to identify parental types for crossing.

The choice of selection variables must be considered with regard to the time and expense involved in data collection. The correlations obtained between nodule fresh weight-dry weight and shoot fresh weight-dry weight allowed for the elimination of some variables in selecting desirable genotypes. In this experiment, all three characteristics used were consistent indicators of a plants' nodulation capacity, and any one of the indicators by themselves might have been used for selection. Graham and Scott (1983) reported that strong correlations between nodule weight and total N occurred about six weeks after planting. This time coincided with maximum vegetative growth and they suggested using dry matter accumulation as a selection criterion. Considering the time involved in root extraction, selection based on shoot fresh weight or dry weight may be the most efficient screening method for large scale selection programs. Furthermore, extraction of cowpea roots under field conditions is difficult given the extreme depths which these roots may reach, and errors in nodule measurement may occur.

CONCLUSIONS

A screening experiment involving Vigna unguicualta landraces from Botswana and introductions from other plant breeding programs within Africa indicates that considerable variability exists with respect to N_2 fixation characteristics. The plant characteristics examined all revealed significant differences between genotypes. Three variables were used to rank the genotypes in order to identify individuals of high and low nitrogen-fixing potential. Five high (IT81D-985, B152, B006-C, B145 and Blackeye) and four low (Vita-5, IT82E-8, TVu 3236, and IT82E-17) nodulating genotypes were identified based on stable performance across two environments and two sampling periods. The stability of these plant characteristics suggest the possibility of using shoot fresh weight or dry weight as a selection criteria in future screening experiments in order to increase the efficiency of parental selection. Compared to introductions, landraces originating from Botswana did not perform differently with respect to their nodulation capacity.

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APPENDICES

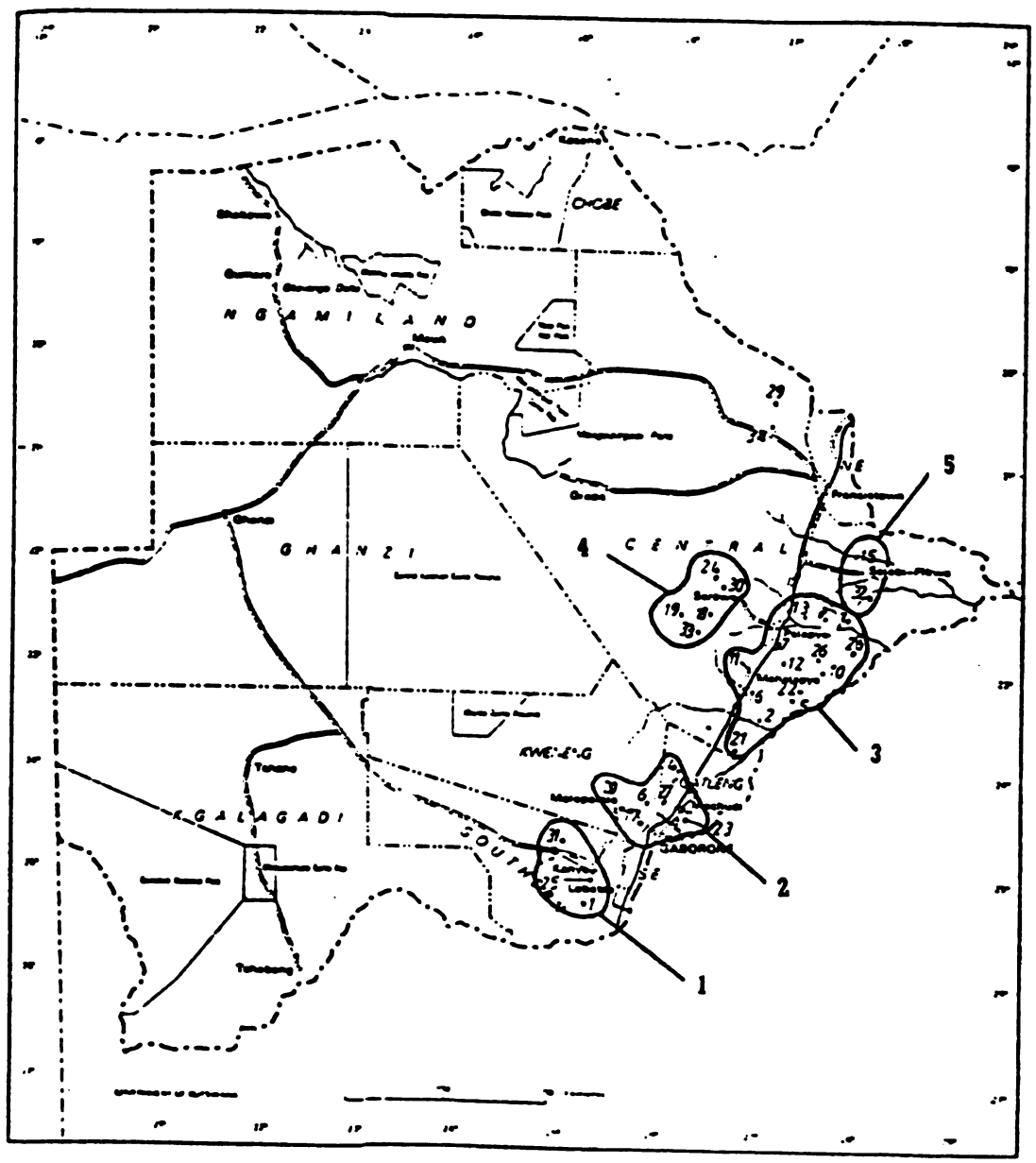

Figure A. The five cowpea collection sites in Botswana.

Table B. Places where cowpea germplasm samples were collected in Botswana.

Village		Location	
Number	Name	Long (E)	Lat (S)
1	Barolong Farms	25 21	25 32
2	Dovedale	27 00	23 29
3	Kanye	25 20	24 54
4	Kgatleng	24 32	26 00
5	Khudumetse	27 08	23 25
6	Kopong	25 54	24 28
7	Lecheng	27 13	22 40
8	Lesenepolole	27 34	22 35
9	Matabelang	26 02	24 33
10	Machaneng	27 30	23 12
11	Mahalapye	26 48	23 04
12	Mokobeng	27 40	23 00
13	Malaka	27 20	22 37
14	Maunatlala	27 37	22 36
15	Mmadinare	27 45	21 53
16	Mmaphashalala	26 50	23 39
17	Mmopane	25 52	26 34
18	Mogapinyana	25 36	22 22
19	Mogorosi	26 34	22 27
20	Molepolole	24 16	24 24
21	Mookane	26 39	23 41
22	Ngwapa	27 30	23 06
23	Oodi	26 02	23 34
24	Paje	26 47	22 17
25	Pelotshetlha	25 23	24 12
26	Radisele	26 59	22 46
27	Sebele	25 57	24 34
28	Sefhare	27 32	27 47
29	Senete	27 07	20 20
30	Serowe	26 44	22 25
31	Sesung	25 00	24 42
32	Tamasane	27 23	22 23
33	Tlhabala	26 23	22 30
34	Tutume	20 16	20 29

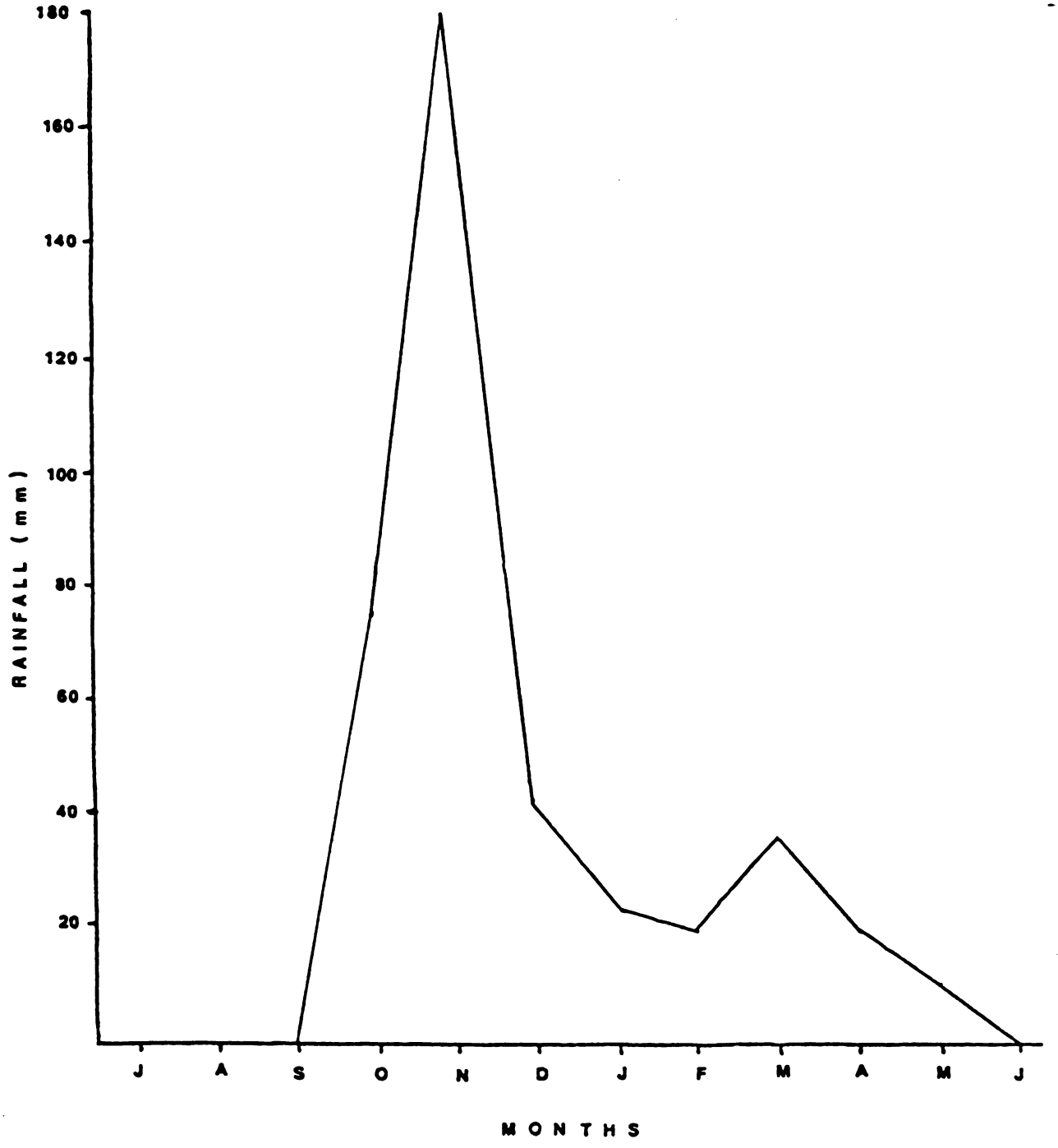

Figure C. Rainfall during 1982-83 growing season (Sebele).

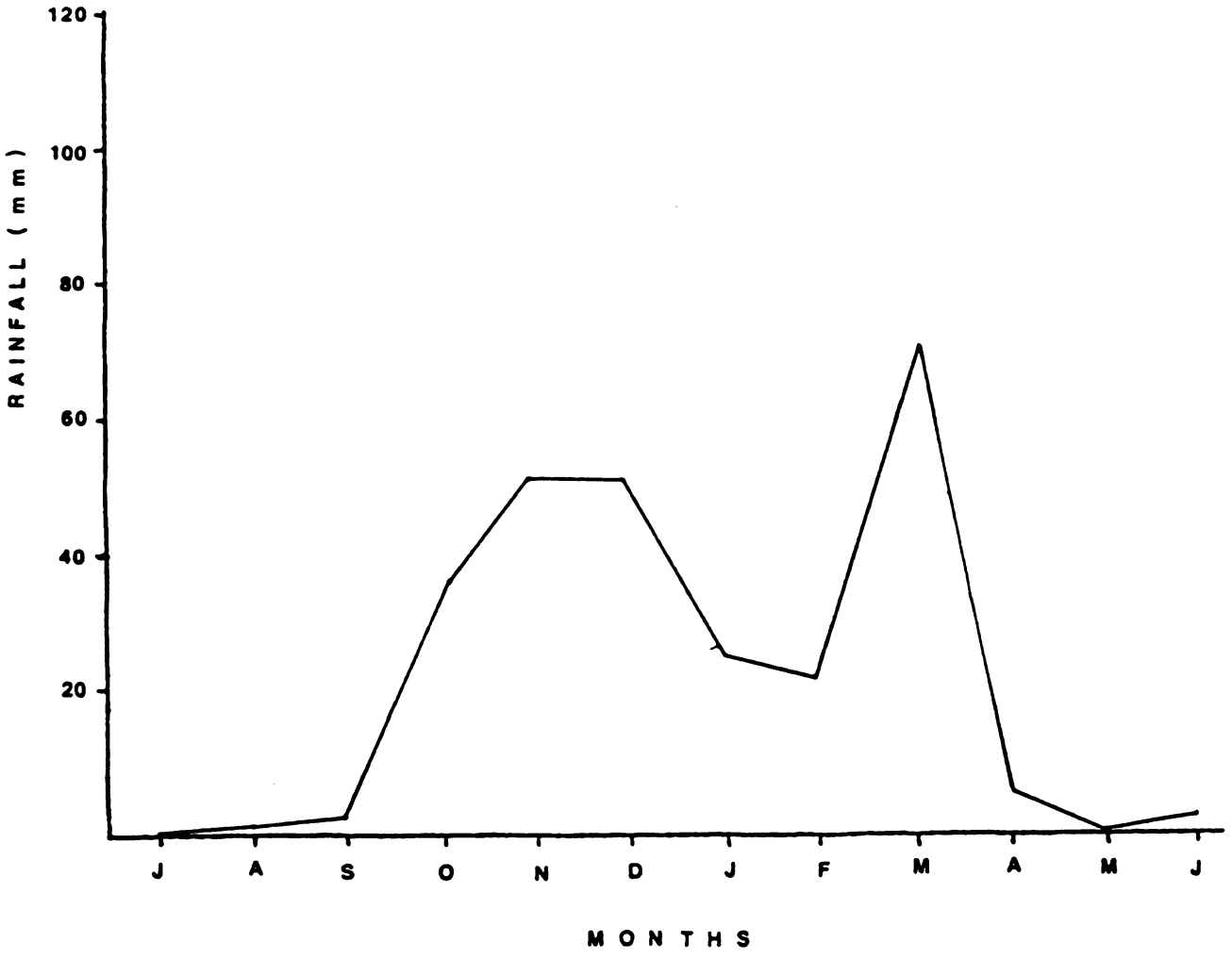

Figure D. Rainfall during the 1983-84 growing season (Sebele).

Table E. Climatological Information - Sebele.

Variable	1982-83	1983-84
Total rainfall (mm)	452.2	265.4
Mean maximum monthly air temperature (°C)		
low	20.1	23.0
high	36.0	34.9
Total hours of sunshine		
low	8.8	8.8
high	10.4	11.0
Mean monthly relative humidity at 1400 hrs.		
low	31%	19.0%
high	50%	35.0%
Solar radiation (MI m^{-2} day^{-1})		
low	13.8	15.6
high	24.9	27.4
Bare soil evaporation (mm)		
low	99.5	111.4
high	253.2	308.2

Table F. List of cowpea accessions used for nitrogen fixation screening.

Variety	Source	Variety	Source
IT81D-990	IITA	Vita 4-LS 21	IITA
IT81D-988	IITA	B143	Botswana
IT81D-984	IITA	B165	Botswana
IT81D-985	IITA	B144	Botswana
TVu3629	IITA	B149	Botswana
Vita 7	IITA	B178	Botswana
Vita 5	IITA	B154	Botswana
TVu3236	IITA	B160	Botswana
IT82E-47	IITA	B153	Botswana
IT82E-60	IITA	B157	Botswana
B175	Botswana	B142	Botswana
B137	Botswana	B174	Botswana
Vita 8	IITA	B150	Botswana
TVx2394-02F	IITA	B169	Botswana
TVx3380-01E	IITA	B166	Botswana
Vita 6	IITA	B170	Botswana
TVx1850-01E	IITA	B156	Botswana
TVx1948-01F	IITA	B167	Botswana
TVx2724-01F	IITA	TVx3404-012E	IITA
TVx1999-01F	IITA	B140	Botswana
TVx3381-02F	IITA	B148	Botswana
TVx2949-03J	IITA	B151	Botswana
ER7	IITA	B162	Botswana
Blackeye	(check)	B013-A	Botswana
Mounge	Borkina Faso	B019-A	Botswana
IT82E-38	IITA	B015-A	Botswana
IT82E-61	IITA	B017-A	Botswana
IT82E-13	IITA	B006-C	Botswana
IT82E-3	IITA	B007-A	Botswana
TVx3405-01E	IITA	IT82E-18	IITA
TVx3072-01E	IITA	IT82E-60	IITA
Kpockguegue	Borkina Faso	IT82E-39	IITA
TVx1999-02E	IITA	IT82E-52	IITA
IT81D-1137	IITA	IT82E-42	IITA
IT82E-58	IITA	IT82E-32	IITA
IT81D-1051	IITA	IT82E-54	IITA
IT82E-8	IITA	IT82E-10	IITA
IT82E-16	IITA	IT82E-9	IITA
IT82E-17	IITA	IT82E-49	IITA
B165	Botswana	IT82E-56	IITA
B159	Botswana	IT82E-50	IITA
B146	Botswana	B008-G	Botswana
B172	Botswana	M48/78	Botswana
B173	Botswana	B163	Botswana
B141	Botswana	B158	Botswana
B145	Botswana	B138	Botswana

Table F. (Continued).

Variety	Source
B176	Botswana
B147	Botswana
B007-B	Botswana
B139	Botswana
B155	Botswana
B164	Botswana
B161	Botswana
B168	Botswana

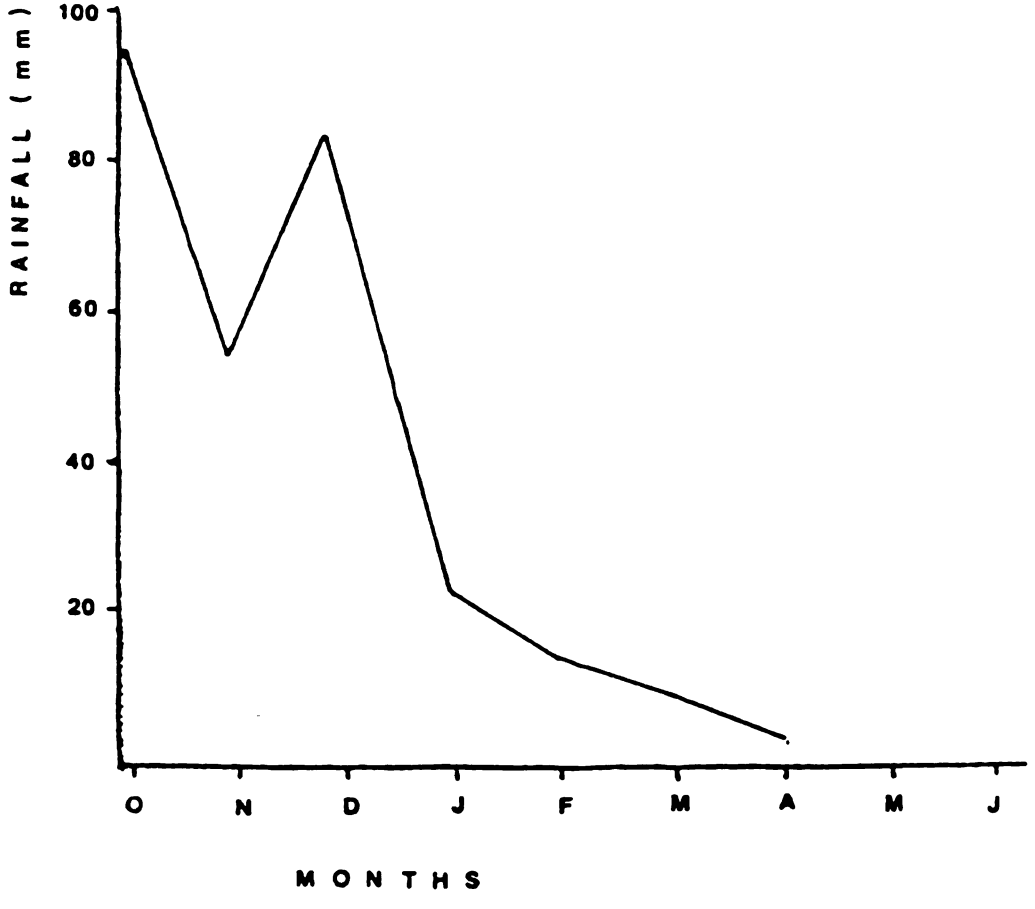

Figure G. Rainfall during 1982-83 growing season (Mahalapye).

Table H. Soil analysis results - Mahalapye and Sebele 1982-83.

Date[a]	Replication	Treatment	Mineral N ppm	Mineralizable N ppm	Soil pH
			Site 1 - Mahalapye		
3/29/83	1	-N	20.33	52.36	
	2	-N	8.33	77.14	
	1	+N	88.33	27.36	
	2	+N	51.33	0.09	
4/18/83	1	-N	56.33	33.39	
	2	-N	36.33	57.33	
	1	+N	40.33	66.42	
	2	+N	40.33	104.45	
			Site 2 - Sebele		
3/25/83	1	-N	56.33	0.34	5.1
	2	-N	36.33	32.76	4.8
	1	+N	128.33	168.00	4.1
	2	+N	4.33	64.76	5.4
4/23/83	1	-N	104.33	66.26	
	2	-N	100.33	109.74	
	1	+N	100.33	80.45	
	2	+N	228.33	100.57	

[a]100 kg N/ha applied to +N treatments at Site 1 on 11/10/82 and at Site 2 on 12/8/82.