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# ADAPTIVE VARIATION OF MALAWIAN BEAN LANDRACES TO NITROGEN AND PHOSPHOROUS

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# ABSTRACT

## ADAPTIVE VARIATION OF MALAWIAN BEAN LANDRACES TO NITROGEN AND PHOSPHOROUS

By

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Twelve Malawian bean landrace cultivars from high (145-201 lb P/ac) and low (11-15 lb P/ac) phosphorous sites were evaluated for response to Rhizobium phaseoli inoculation and nitrogen (N) and phosphorous (P) fertilization. Added P significantly improved the N use efficiency of plants reliant on soil and symbiotic N. In the same plants a higher but insignificant increase in seed and biological yield was observed. Stem and leaf weights and nutrient use efficiency appeared to be related to the P status of the cultivars' habitats.

The response of eight components of a bean landrace seed mixture to Rhizobium inoculation was also investigated. Nodule dry weight and seed yield were higher but not significant in inoculated plants.

It is suggested that resource utilization and habitats of the landrace cultivars can be related. A thorough investigation in their natural habitats is recommended.

## DEDICATION

To my parents
who have enabled me
to grow up in wisdom and age
before God and mankind.

To the Malawian farmers
who recognize the vagaries of nature
and still take the risk.

And to all of us . . .especially,
who sit in the backrow.

Henry Mloza-Banda
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# CHAPTER I

# NITROGEN AND PHOSPHOROUS IN BEAN PRODUCTION: A REVIEW

## 1.1 Introduction

Indigenous subsistence agriculture depends on a reliable and annual yield of crops more than maximum yields of genetically uniform select cultivars that respond to definable water and nutrient levels. It is axiomatic that the nitrogen (N) and phosphorous (P) nutrition of most soils under cultivation declines progressively in a manner characteristic of the climate, cultural practices, and soil type. Invariably, most of the N required for plant growth must come from external sources, namely, through biological fixation and fertilizer application.

Current research is aimed at reducing the application of N fertilizers because of their high cost, and, to promote the use and to increase the efficiency of the legume--*Rhizobium* symbiosis. Without substantial fertility inputs, symbiotic $N_2$ fixation is one of the most equitable technical alternatives available to small farmers.

The amount of N fixed by a given system is related to the potential of this system, which is determined by the crop grown and the effectiveness of strains of *Rhizobium* in

fixing N with the host. This potential is often limited by environmental conditions (temperature, moisture, day length, high light intensity), general soil fertility, and the extent to which soil N is assimilated by plants. It is these factors whose influence should be determined to increase actual $N_2$ fixation. However, some of the causal factors have been shown to be of overriding importance in limiting grain yields than biological $N_2$ fixation per se. Thus it is pertinent to view biological $N_2$ fixation only as a component of a larger research strategy for meeting crop demand for N.

A prominent feature of many tropical soils is micro-variability associated with biological activity of various kinds. Among the many genetic variations in crop plants, certain individuals, and even groups, appear to possess survival value in certain environments, such as those borne of the diversity of soil.

By experience farmers know the difference in productivity of the various soils within their holdings. They are known to select crops, cropping sequences, and management practices that will adjust best to the soils. It has therefore been said that ". . . there is nothing unique about the type of soil fertility problems encountered in the tropics. What is unique is the magnitude of soil fertility problems in relation to quantities of resources which are being committed to alleviate those problems" (42).

In re-examining the principles and practices that make up Malawi's bean production, a more fundamental change is represented by increasing evidence that plant germplasm can be collected and evaluated and superior cultivars developed on the basis of adaptation to the subsistence farming system. While many questions remain to be answered, the principles are available for immediate investigation and use. An attempt, therefore, is made here to convey the essential ideas of adaptation to mineral nutrition of some of the Malawi bean landrace cultivars, albeit, using a minuscle fragment of the available genotypic wealth.

## 1.2 Literature Review

### Bean Production

Dry beans (Phaseolus vulgaris, L.) are an important grain legume in the traditional farming systems of Malawi. This is reflected in their wide range of adaptability to various environmental conditions, and provision of subsistence needs to indigenous farmers who produce over 90% of the crop (32).

Surveys of bean production trends in Malawi, as in other tropical and sub-tropical countries (27, 90), reveal that production has been characterized by poor seed yield per unit of land (32). According to FAO (1982) yearbook (38), average grain yield (kg/ha) was 565 in Latin America, 655 in Africa, and 230 in India, against 1,328 in

the Near East and about 1,500 in the U.S.A. In Malawi, an average yield of 662 kg/ha has been quoted (38), although under experimental conditions, 3,000 kg/ha of seed yield has been achieved (32).

Among the primary constraints to increase bean production in Malawi, is the limited use of fertilizer and/or inoculation of seed with the nitrogen (N) fixing bacteria, Rhizobium phaseoli, L. (32). Other factors include diseases, pests, and the lack of suitable varieties where most of what is grown is mixed seed (32). Fertilizer use on monocropped beans or an intercrop of beans and other crops, particularly maize (Zea mays, L.), has been recommended but not widely practiced (31). An average of 20-60 kg/ha of supplemental N is recommended (31). It can be expected that this rate may result in a sizable investment for the growers, increase the potential for N loss by leaching and run-off, and according to other studies (23), such a rate can supplant the $N_2$-fixing capability of plants.

Farmers apply little or no fertilizer for a number of reasons (31). First, bean production occurs on 95,000 hectares of land compared to 250,000 and 1,100,000 hectares of groundnuts (Arachis hypogea, L.) and maize, respectively. Thus the crop may be considered minor. Secondly, the traditional method of growing beans is to

interplant them with other crops and this accounts for over 90% of total bean production (32). It is expected that where beans are grown in association with maize, for example, they may benefit from fertilizer applied to the maize crop. Finally, under the premise that beans are a leguminous crop, it is likely that beans are considered self-sufficient in their N nutrition.

Nitrogen and Phosphorous as Limiting Factors

Mineral nutrition deficiency, particularly of N and phosphorous (P), remains a major problem in the bean production regions of Malawi (4). An inference can be made that bean production occurs in a farming system and/or ecosystem which cannot allow self-sustenance under overall N-limited conditions. Fertilizer addition to a plant community may therefore represent the simplest method of demonstrating the importance of N and P as limiting factors (64).

In the tropical and subtropical environments, the rapid mineralization of inorganic N from organic material has been attributed to year round high temperatures (23, 27, 6). The period of most rapid nitrate ($NO_3$) increase is at the start of the rainy season. At this time, crops are as yet unestablished to take advantage of peak N release (6, 27). A large part of N is moved rapidly down in the soil beyond easy reach of arable crops and

this may account for losses of between 20-50% of N fertilizer applied (6, 88). Variation in N losses would be expected in different rainfall, soil, and crop regimes (6, 88). For instance, a lower soil N content as rain- fall decreases has been observed in east Africa (7). Invariably, the nutrient which is nearly always in short supply is N. Most crops including beans (30), will give increased yield if N is added. And it is not only the absolute amount of N supply, but also its timing that is crucial (64).

The situation with P is different. Phosphorous does not occur as abundantly in soil as N, nor is it recycled in rainfall or readily released from organic residues (34, 68, 71). Soluble P fertilizers are very rapidly changed to less soluble compounds which with time become less and less available to plants (34, 68, 71). The contention that fixation of applied P is high in the tropics has been argued (34). Soils with high P fixation capacity occur in both the tropical and temperate latitudes (83) and the soils have been shown to hold P in much the same way (34). However, the initial P status of tropical soils is much lower than found for temperate soils with the consequence that it may take a number of small applications for an appreciable residual effect to be shown (34).

In Malawi, extensive areas in the northern region have been shown to have inadequate levels of P (4, 20). But P deficiencies in the central region appear mainly on worn-out land after prolonged cultivation (4). In the southern region, soils are reported to be generally better supplied with P although local variation has been discerned (4).

Because P can be replenished only from external sources, P removed from the soil in crop products and climatic factors, over a few decades, can be a significant portion of that contained in the pedon (34). Thus under a predominantly subsistence farming system, P remains a limiting nutrient.

Inferences From Soil N and P Status

Two scenarios have been established: that climatic and soil factors intensify the transitory nature of N and P availability in the soil resulting in high soil N and P demand; and, that indigenous growers do not have a specific technology to support both the soil and crop demand. These considerations, in part, explain the preponderance of low yields of beans. The development and application of innovative procedures aimed at alleviating N and P deficiencies should be of paramount importance. The high bean yields obtained under experimental conditions (32), may reflect both the superiority of the

environment and/or the quality of the genotype (60, 75). Thus, plants themselves may be a ready-made source for correcting the soil nutrition problem. For instance, most of the dramatic improvements in crop yield of soybeans (Glycine max, L.) and groundnuts, are reported to have originated from exceptional varieties--varieties that respond consistently to superior management, including Rhizobium inoculation (60).

It has been established that the physiological performance of plants, particularly their response to nutrients, will be a function of their innate genetic constitution and of the environment in which they are adapted (10, 34, 55, 75). And owing to the great diversity of soil, plants may also be adapted, genetically, to this diversity (34, 75). This paper is in part an argument for the potential use of genetic diversity, such as is prevalent among Malawi bean landraces (1) in an attempt to meet crop and soil demand for N and P.

## Malawi Bean Landraces

Initially, a corpus of descriptive notes (1) revealed that there exists a rich array of bean germplasm differentiated into a vast number of landraces so that beans produced in Malawi were heterogenous mixtures of different plant types and different seed size, shape, and color. These landraces had been widely grown, over many

years without conscious selection and were well adapted to the areas where they are grown (1).

The contemporary variability of these mixtures and the probable mechanism by which this variability arises and persists has been investigated (67). Evidence for a broad regional adaptation and site-specific adaptation was obtained. For instance, several phenological traits, such as days to flowering or duration of flowering, were associated with latitude. Seed yield and some morphological characters, such as leaf size or seed size, were associated with soil P levels and soil potassium (K) levels, respectively. Furthermore, a north-south clinal pattern, attributed to differences in flowering and maturity times of beans, was discerned.

These findings are supportive of the theory of evolutionary diversification within species of plants, particularly the concept of "ecotypes" (35, 47, 75, 102). Plants termed ecotypes, are those that belong to the same annual species, growing in geographically separated areas and showing characteristic features related to their habitats which may be retained when these plants are grown together (35, 47, 102). They are also known to grade into one another in a succession, or cline in response to some variables of the environment, such as altitude, climate, or soil (47, 102).

The major corollary in the present paper is that N and P are plant nutrients known to be widely deficient in many soils in Malawi. Yet a vast array of bean landraces are peculiarly thriving in varying indigenous environments. Typical effects of an abundance or deficiency of N and P should be of great ecological importance whereby the responses of plants may suggest their adaptation to the local soil environments involved. It is envisaged that growing a landrace seed mixture and bean lines from different locations in Malawi in two field experiments containing different levels of Rhizobium inoculum and N and P, respectively, may provide additional evidence to the adaptive variation of Malawian bean landraces to environmental soil conditions.

# CHAPTER II

# RESPONSE OF BEAN LANDRACES TO NITROGEN AND PHOSPHOROUS FERTILIZATION AND *RHIZOBIUM* INOCULATION

## 2.1 Introduction

Numerous reports have shown that cultivars of some crops differ widely in their capacity to extract and utilize plant nutrients from the soil. This capacity is especially important when crops are grown on soils with low or marginal levels of available nutrients. Problem soils also often exhibit more than one nutritional constraint for the economical production of field crops.

These phenomena may often be manifested by adaptation of certain plants or groups of plants to certain environments, and the predominance of some plants over others. The present experiment hypothesized that there exists an explicit relation between crop ontogeny and resource utilization, and the phosphorous status of the natural habitat in which bean landrace cultivars have been grown.

The primary objective was to evaluate available evidence indicating that differences in efficiency of

nitrogen and phosphorous utilization by landrace cultivars do exist and have been recognized. A second emphasis was to discuss the possible morphological and physiological mechanisms responsible for these efficiency differences. For practical reasons, only a number of possible mechanisms for efficiency differences have been considered with supporting evidence included when available.

## 2.2 Literature Review

### Mechanisms For Nutritional Adaptation

Different soils represent a broad range of mineral nutrition environments (35, 57). The significance of the diversity of soil environments is three-fold (57). Firstly, the presence or absence of particular nutrients or elements in the soil can affect the growth of plants thereby profoundly influencing their distribution in indigenous habitats. Secondly, the apparently wide distribution of a species may also reflect nothing more than the persistence of similar microenvironments in different regions. Finally, in many instances, the widespread distribution of a species may be achieved as a consequence of the existence of genetically distinct populations each of which is adapted to a relatively narrow range of conditions. Climatic factors are known to determine largely whether a given plant (or crop) is potentially suitable for

an area, but soil factors may actually determine whether it actually is found there and in what abundance (102).

The pre-eminence of the chemical composition of the soil as a decisive factor has been reviewed (103). It has been presumed that when a seedling germinates in a particular soil, at the root surface there will be a direct interaction of structural and catalytic proteins with the chemical constituents of the soil solution. Thus, a direct and often severe selective 'sieve' would be established resulting in a rapid selection of closely adapted individuals (103). Further, the exposed proteins may have important enzyme properties, such as those of carriers in ion transport systems. The interaction of the root surface and soil solution would likely evoke a rapid response in overall metabolism of plants. This response could impinge on a large number of interlinked enzyme systems that would determine the expression of physiological or morphological traits of adaptation (103).

Recently, the cation exchange capacity (C.E.C.) of roots has been shown to be adaptive to the growth medium and the differing ability of varieties to respond to certain nutritional conditions has been attributed to varying ability for C.E.C. adaptation (35, 94). The 'carrier' concept and active uptake of different ions has been demonstrated (46, 94). For example, the uptake's system's

varying affinity for $NO_3^-$ has been suggested to be of ecological significance (46).

Potential mechanisms of nutritional variation are immense and this paper will concentrate on certain mechanisms governed by the principles of absorption, translocation and metabolism.

Root Growth and Nutrient Uptake

The importance of phosphorous (P) and potassium in relation to adaptive physiological (seed yield) performance and morphological characteristics of Malawi bean landraces has been indicated (67). Beans share with other legumes a larger than average P requirement. Phosphorous is needed not only for plant growth but also for nodulation and biological nitrogen fixation (43, 70). Further, compared to Graminaceous plants, legumes are known to have a restricted root system and are therefore poor foragers for P (70). Thus, considering the immobility of P in the soil (45), and the evidence of N movement in soils (41) the importance of root density, and exploitable soil volume has been emphasized on the rate of N and P uptake (41, 45). Exploitable soil volume is known to depend on soil physical and chemical properties which in turn limit root density and root length (41). Therefore, for a given gain in root dry weight, subsequent N and P uptake may be maximized by the optimum allocation of this dry matter between root

density and length (41, 45, 71). Consequently, nutrient efficiencies of different cultivars of beans and other crops (45, 94) have been directly related to a high ratio of total plant growth present as the root system, that is, root:shoot ratio (45).

The ability of beans to improve their P uptake by means of mycorrhizal association between their roots and soil fungi has been documented (21). Although the particular significance of mycorrhizal infection is still under investigation (70), it has been shown that nodulated beans may fix more N (17, 21), and may have large N content in shoots and seeds (3, 22). Mycorrhizae and added P have been reported to show similar effects on plant growth, nodulation, and $N_2$ fixation (70). The absence of a response to mycorrhizal infection in some cultivars has been found to be closely related to the absence of response in the same cultivars to P fertilization (28). As in the legume-Rhizobium system, in a typically P-deficient soil, a certain amount of soluble P fertilizer has been found to be necessary to obtain a significant response to endomycorrhizal infection (28). It was therefore suggested that mycorrhizae cannot replace P fertilizer but increase fertilizer efficiency allowing a reduction of the rate of application through improved P uptake by the host plant.

Nutrient Redistribution

Differences in re-export of mobile elements, particularly N, from deficient or senescing plant parts has been shown to be another basis for variation in N and P efficiency (45, 72, 84, 85).

The N nutrition of the plant is known to influence seed yields indirectly by affecting leaf area and the duration for which leaves remain functional during grain filling (45, 72, 73). The timing of N redistribution from leaves in species with a high N demand, such as beans, may be important as loss of protein could reduce their capacity to supply photosynthate late in the life of crops (42, 45, 73). Thus the concomitant decline in N uptake and in leaf N concentration is reported to have led to the proposal that the rate of senescence in "self destructive" crops, such as soy or dry beans, is controlled largely by remobilization of N from leaves to grain (84, 85). However, sink strength has emerged as the characteristic which expresses genotypic differences in efficiency of N redistribution because high N uptake has not necessarily resulted in extensive redistribution to grain since some cultivars lack the sink strength to promote this movement of N (56, 96).

Metabolism and Nutrient Efficiency

The view that the primary basis for the differences in N and P response are due to differences in efficiency of element utilization in metabolic processes has been advanced (45, 100). The argument is that if access to soil nutrients is a limiting factor, morphological features of the root system may be critical and efficiency of ion uptake mechanism may then be of secondary importance (45). But when the arrival at the root surface is non-limiting, the uptake mechanism may be a key factor in the overall uptake (45).

Intraspecific differences in growth of beans at stress levels of P support this theory (100). Bean lines from diverse sources were grown in nutrient culture under P stress to isolate phenotypes which differ in efficiency of P utilization. Physiological basis for differences in efficiency of P utilization were shown to be strain specific and involved participation of P in metabolic processes other than absorption from the environment.

Another biochemical change which has been cited (45) is the increase in phosphatase activity when plant tissues become P deficient. It has been suggested that the adaptation of plants to P stress might be a result of high phosphatase activity.

In terms of N nutrition, differences in efficiency of metabolic functions may result from the fact that biological $N_2$ fixation and $NO_3$ assimilation represent the major sources of reduced N for plant growth and seed yield in beans, as a legume crop (44). Beans have been shown to fix more N per hectare (measured as $C_2H_2$ reduction per hectare), in low-N soils (77).

Nonetheless, beans are considered weak in symbiotic $N_2$ fixation and attempts to supplement $N_2$ fixation with fertilizer N have given inconsistent yield responses (44). Low levels of combined N during initial stages of plant development have been reported to enhance nodulation and $N_2$ fixation (44, 99). Several environmental and plant factors are known to control the amount of N incorporated through $N_2$ fixation (49). Cultivar differences in symbiotic--nonsymbiotic N relationship, and plant type differences in relative rates of N uptake from the soil, have been reported (49, 51, 98, 99). Relatively more rapid absorption of soil N has been shown in bush bean cultivars than in climbing cultivars (51). However, this has been reported to be associated with lowered fixation presumed to arise from decreased carbohydrate supply to nodules (51). In field studies, nodulation and $N_2$ fixation of climbing and strongly indeterminate cultivars of beans has been reported to be superior to that of most bush cultivars (50, 51).

Nutrient Efficiency of Unimproved Cultivars

Metabolic differences in nutrient efficiency may represent a complex of interactions between plants and their natural habitat enabling them to adapt to differing ecological situations.

Differential responses of improved and unimproved cultivars of dry beans to N and P fertilization has been studied (53). The rationale was that improved genotypes probably had been grown under conditions of high soil fertility and that those types capable of utilizing a large quantity of nutrients were higher yielding. The results showed that neither positive nor negative response capacity was specific to the improved or unimproved lines in relation to seed yield and yield components. Further, it was suggested that "the response of a genotype and the nutrient supply capacity of its native soil may not be unrelated."

In Kenya, the response of beans to N fertilization has been shown even in landrace cultivars (59). In another study in France, some tropical landrace cultivars and temperate cultivars were compared for nitrate reductase activity and nitrogenase activity (39). When the two enzymatic activities, in plants of equivalent vegetative cycles were compared, on average, tropical cultivars showed a higher level of $C_2H_2$ reduction and a lower nitrate reductase activity than temperate ones. The important nitrogenase activity was associated with a higher amount of

nodules. It was inferred that there probably exists in Phaseolus vulgaris enough genetic variability for $NO_3^-$ reductase and nitrogenase activities for a possible improvement of $N_2$ fixation ability.

Differences in the efficiency of N utilization have also been ascribed to an inherent capacity to develop adequate nitrate reductase (94). In legumes, this has been shown to be related to differences between amide-producing and ureide-forming cultivars (74, 86).

## Plasticity and Plant Nutrition

Whatever the exact cause, one part of the armoury of a successful plant is the ability to cope with variation in nutrient levels in time and space (10, 11). The capacity of the individual plant to endure such variation may be manifested in two ways.

Firstly, the plant may need to have the necessary phenotypic plasticity to adjust its metabolic processes to take maximum advantage of changes in environment, or it must be so well buffered with storage systems that it can maintain its internal characteristics no matter what the external environment is (9, 10, 24).

Nutrient use in legume/non-legume intercropping studies may be an illustration of the presence of such morphological or physiological mechanisms. The rationale behind gains in nutrient use in intercropping was that $N_2$

fixation or the eventual use of fixed N by other crops should be more efficient than when the crops are grown separately but in some suitable sequence (8, 101). It was reported that pigeonpeas were shown to nodulate better when their roots intermingled with those of intercropped sorghum (101). In another study, an increase in nodule number and weight on soybeans growing with maize was observed although not statistically significant. The explanation for these results was that the cereals depleted soil N and consequently stimulated $N_2$ fixation (101). Thus a plant that is capable of adjusting its N or P niche through enhanced nodulation and $N_2$ fixation or mere uptake would presumably be more successful than one that cannot.

Secondly, the cosmopolitan distribution of the species Phaseolus vulgaris L., accompanying different architectural types is suggestive of the immense genetic diversity inherent in beans (2). But along with this diversity, genetic flexibility may be a necessary component to counter nutrient variations. It is most essential though to show that the ability to change genetically is under the influence of nutrients (10).

## Method of Study

One of several approaches to the identification, specification, and measurement of differential behavior in respect of the uptake, transport and utilization of

nutrients has been described (52, 102). Groups of plants drawn from particular habitats are examined under a variety of standardized conditions, some involving a particular environmental stress. A list of attributes may be compiled for each ecotype and, in certain cases, these attributes may provide an indication of the plant components which are (or have been) the subject of selection in the habitats concerned. For example, a measure which seems worthy of wider application in nutritional ecology is the use of nutrient contents of plants as a clue to the nutritional status of plants collected from various environments (45).

## 2.3 Materials and Methods

### Plot Preparation

The experiment was conducted during summer of 1985 at the Crop Science research facility in Montcalm County, Michigan. The soil contained 175 kg P/ha before planting. A preplant herbicide combination of Dual (metolachlor) and Amiben (chloramben) was incorporated at a rate of 1.8 kg/ha active ingredients. After seedling emergence, weeding was done by hand.

### Experimental Design

A 2 x 2 factorial in a split plot design with two replications and with cultivars as subplots was used. Inadequate seed material curtailed additional replication of treatments. The design consisted of 4 x 3-metre plots,

0.5-metre row width, and 0.2-metre within-row plant spacing.

In Table 2.1 below, nitrogen (N) and phosphorous were applied as 46-0-0 (urea) and 0-46-0, respectively. Both fertilizers were applied at 20 kg/ha. All plots received 20 kg/ha of potassium as 0-0-60.

Table 2.1 Treatment arrangement.

| | | | |
|---|---|---|---|
| Treatment 1 | $N_0$ $P_0$ | | $N_0$ = no fertilizer N |
| Treatment 2 | $N_0$ $P_1$ | where | $N_1$ = 20 kg N/ha |
| Treatment 3 | $N_1$ $P_0$ | | $P_0$ = no fertilizer P |
| Treatment 4 | $N_1$ $P_1$ | | $P_1$ = 20 kg P/ha |

A granular inoculum of R. phaseoli from Nitragin Co., Milwaukee, Wisconsin, was applied during seeding at a rate of 1 g/m-row to all plots.

Cultivars

Twelve dry bean landrace cultivars originating from five locations in Malawi were randomly picked from the Malawi bean collection. The five locations differ in soil P levels. Site and soil characteristics of the source of seed material are given in Table 2.2 and Table 2.3, respectively.

Table 2.2 Site characteristics* of source of seed.

| Accession # of Cultivar | Treatment Code of Cvr. | Source area /Altitude (m) | Latitude /Temperature | Rainfall (mm) Day Length |
|---|---|---|---|---|
| 6506 | 7 | Misuku Hills | 9° 42'S | 2005 |
| 6512 | 12 | 1609 | 21.9°C | 12h.37' |
| 3509 | 2 | Misuku Hills | 9° 44'S | 2698 |
| 3517 | 8 | 1578 | 21.9°C | 12h.38' |
| 3521 | 3 | | | |
| 4107 | 11 | Livingstonia | 10° 35'S | 2368 |
| 4111 | 1 | 1152 | 20.8°C | 12h.42' |
| 5512 | 10 | South Rukuru | 11°52'S | 2132 |
| 5518 | 4 | 1210 | 21.0°C | 12h.45' |
| 3407 | 9 | Mabulabo T.A. | 12° 36'S | 2287 |
| 3417 | 6 | 1207 | 21.0°C | 12h.47' |
| 3424 | 5 | | | |

*Source of site characteristics: G. B. Martin, 1984. Genetic diversity of bean landraces in Northern Malawi. M. S. Thesis. Michigan State University. U.S.A.

Table 2.3 Soil characteristics of source of seed.

| Source area /Cultivar | $NO_3^-$ ppm | P lb/ac | K lb/ac | OM % | pH |
|---|---|---|---|---|---|
| Misuku Hills 7, 12 | 0.174 | 201 | 729 | 5.40 | 7.0 |
| Misuku Hills 2, 3, 8 | 0.375 | 15 | 362 | 5.52 | 5.9 |
| Livingstonia 1, 11 | 0.454 | 11 | 488 | 3.67 | 5.6 |
| South Rukuru 4, 10 | 0.309 | 145 | 168 | 2.26 | 6.4 |
| Mabulabo T.A. 5, 6, 9 | 0.454 | 172 | 111 | 3.83 | 7.7 |

Source: G. B. Martin. 1984. Genetic diversity of bean landraces in Northern Malawi. M.S. Thesis. Michigan State University. U.S.A.

General

Seedling emergence was severely affected by seed corn maggots owing to the use of untreated seed. The number of plants was reduced from 30 plants per 2-row net plots to 5 plants per 2-row net plots in some plots (Table B.2 in Appendix B). This precluded acquisition of phenological and agronomic data that was vital to this study. Due to lack of seed, the plots were not replanted but they were thinned to single (non-competitive) plant stand. All data are reported on a per plant basis.

Plants were sampled for nutrient analysis at approximately the same stage of maturity starting 45 days after planting (DAP), just prior to the first signs of physiological maturity (yellowing of pods) and enhanced leaf senescence. Two plants per plot were harvested for dry weight determination and nutrient analyses of plant tops. A shovel was used to dig up roots from a depth of about 30 cm. Duplicate samples of plants were separated into roots, stems, leaves, and pods; this material was placed in a forced-air oven at 60°C for 72 hours. After dry weights were recorded, the plant components were bulked and ground in a Wiley mill to pass through a 40-mesh screen for N and P analyses. The method for total plant N analysis was that described by Bremner (1965) (12) while as the method described by Watanabe and Olsen (1965) (95), was used for total plant P analysis.

During the growing season all plots were irrigated once a week. Insect infestation was evident and adequate control was effected by two applications of Sevin (Carbaryl). At harvest, seed yield was adjusted to 10% moisture.

Statistical Analysis

All data were subject to analysis of variance. Only significant mean squares were presented and they are given in Table B.3 through Table B.6 in Appendix B.

Correlation coefficients ($r^2$), were calculated where stated and the results are shown in Table B.2 in Appendix B.

Comparisons of cultivar means are given in Table 2.5 through Table 2.8 in the Results section. Comparison of treatment means was done only when treatment means were found to be significantly different in the analysis of variance. The Duncan's Multiple Range (DMR) test was quoted at an alpha level of 0,05. It should be admitted that the significance of the magnitude of differences in ranked means was less critical in the presentation of results given a backlog of different vegetative cycles of plants, inadequate plots and replications.

Table B.1 in Appendix B, is a summary of statistical notations and abbreviations used in the procedures.

## 2.4 Results

### 2.4.1 Field Observations

Plant Type

Cultivars grown were of unknown plant habit. At the time of flowering, growth habit was assessed according to the system used at CIAT. In Table 2.4, it is apparent that cultivars randomly picked for this study represented the range of growth habit. Cultivars drawn from different sites were either of the same or of different growth habits. However, growth habit of many cultivars of *P. vulgaris* is known to be very variable and dependent on the environment (CIAT, 1981). Classification of growth habit thus refers particularly to the growth habit observed under the growing conditions the landrace cultivars were subjected to.

It was anticipated that different growth habits would be relevant to responses of plants to N and P fertilization and *Rhizobium* inoculation. In fact current legume breeders have shown interest in variability in plant form and architectural display in their attempts to achieve the best possible adaptation between progeny and intended environment (2).

Table 2.4 Plant type and crop maturity.

| Type | Cultivar | Approx. duration | Cultivars |
|---|---|---|---|
| I | 1, 5 | 9 weeks | 2, 5, 6, 9 |
| II | 2, 3, 8, 11 | 9-1/2 weeks | 3, 7, 8, 12 |
| III | 4, 7, 10 | 11 weeks | 1, 11 |
| IV | 6, 9, 12 | -- | 4, 10 |

Crop Maturity

Cultivars grown were also of unknown growth duration. Location of study area precluded determination of precise number of days to physiological maturity. Table 2.4 gives an approximate measure of crop maturity. Cultivars #4 and 10 were still at the mid-pod filling stage at the time of harvest. It appears that maturity was associated with the site or source of seed than plant type. Cultivars #6 and 9 were both Type IV's; yet they matured earlier than Cultivar #1 a Type I or Cultivar #11 a Type II.

### 2.4.2 Morphological Components[1]

Root Weight

Nonetheless, the AOV of root weight revealed significant P and cultivar effects. The range test for P effect showed no significant effect of P fertilizer on mean root weight. However, four of the cultivar means #6, 5, 9, and 7, from high P sites were ranked least compared to a corresponding four, #11, 1, 3, and 8, from low P sites, that had high mean root weight. When root weight was expressed as a percent of total plant weight, the above observation remained true only for cultivar means #9 and 7, and 11, 1, and 3.

Stem Weight and Leaf Weight

The AOV showed significant cultivar effect on means of stem and leaf weight, respectively. The range showed a striking pattern whereby the top five means, for both stem and leaf weights, were of cultivars from high P sites. These means were followed by those of cultivars from low P sites. However, means of cultivars #5 and 6, both from one high P site, were ranked least. When stem and leaf weights were expressed as percent of total plant weight, these observations were not apparent. Cultivars #4 and 10, with longer maturity periods had higher ratios of stem or leaf to total weight.

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[1]The range tests of cultivar means of morphological components are displayed in Table 2.5 and 2.6.

Table 2.5 Duncan's range test of morphological components at 45 DAP.

| RANKED ORDER OF MEANS FOR | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| root wt. | | stem wt. | | leaf wt. | | pod wt. | | total wt. | | leaf:steam ratio | | shoot:root ratio | |
| cvr. | order | cvr. | order | cvr. | order | cvr. | order | cvr. | order | cvr. | order | cvr. | order |
| 10 | $2.53^{a}$ | 12 | $38.72^{a}$ | 12 | $47.39^{a}$ | 12 | $87.26^{a}$ | 12 | $175.41^{a}$ | 5 | $1.87^{a}$ | 9 | $117.64^{a}$ |
| 11 | $2.33^{ab}$ | 4 | $34.79^{ab}$ | 10 | $40.01^{ab}$ | 8 | $76.30^{ab}$ | 7 | $137.10^{b}$ | 8 | $1.79^{ab}$ | 7 | $115.21^{a}$ |
| 1 | $2.24^{ab}$ | 10 | $33.53^{abc}$ | 4 | $39.05^{abc}$ | 7 | $69.71^{abc}$ | 8 | $130.87^{bc}$ | 6 | $1.74^{abc}$ | 12 | $90.36^{b}$ |
| 3 | $2.18^{abc}$ | 7 | $27.59^{bcd}$ | 7 | $38.02^{abc}$ | 9 | $61.47^{bcd}$ | 9 | $128.24^{bc}$ | 3 | $1.57^{a-d}$ | 4 | $75.16^{bc}$ |
| 12 | $2.03^{a-d}$ | 9 | $26.41^{cd}$ | 9 | $36.79^{abc}$ | 2 | $57.16^{bcd}$ | 3 | $119.27^{bcd}$ | 1 | $1.50^{bcd}$ | 8 | $74.00^{bc}$ |
| 8 | $1.89^{a-e}$ | 1 | $24.28^{de}$ | 3 | $36.19^{abc}$ | 3 | $55.55^{b-e}$ | 4 | $116.86^{bcd}$ | 11 | $1.89^{bcd}$ | 2 | $72.35^{bcd}$ |
| 4 | $1.68^{b-f}$ | 3 | $23.74^{de}$ | 1 | $35.64^{abc}$ | 5 | $49.96^{cde}$ | 10 | $115.14^{bcd}$ | 9 | $1.47^{b-e}$ | 5 | $69.32^{bcd}$ |
| 2 | $1.54^{c-f}$ | 11 | $23.41^{de}$ | 11 | $34.57^{bc}$ | 6 | $47.29^{de}$ | 2 | $109.72^{bcd}$ | 2 | $1.39^{cde}$ | 6 | $69.23^{bcd}$ |
| 6 | $1.50^{c-f}$ | 2 | $19.73^{de}$ | 8 | $33.04^{bc}$ | 11 | $44.56^{de}$ | 11 | $107.60^{bcd}$ | 7 | $1.37^{de}$ | 3 | $54.26^{cd}$ |
| 5 | $1.47^{def}$ | 8 | $19.64^{de}$ | 6 | $29.94^{bcd}$ | 1 | $41.56^{de}$ | 1 | $106.71^{bcd}$ | 12 | $1.30^{de}$ | 1 | $51.88^{cd}$ |
| 9 | $1.33^{ef}$ | 6 | $17.08^{ef}$ | 2 | $26.98^{cd}$ | 4 | $41.34^{e}$ | 6 | $98.49^{cd}$ | 10 | $1.23^{de}$ | 11 | $44.97^{d}$ |
| 7 | $1.19^{f}$ | 5 | $11.02^{f}$ | 5 | $19.59^{d}$ | 10 | $39.07^{e}$ | 5 | $87.63^{d}$ | 4 | $1.11^{e}$ | 10 | $44.78^{d}$ |
| $s_{\bar{x}}$ | 0.21 | | 2.68 | | 3.72 | | 6.11 | | 10.59 | | 0.11 | | 8.49 |

Table 2.6 Duncan's range test of morphological components as percent of total plant dry weight at 45 DAP.

| RANKED ORDER OF MEANS FOR | | | | | | | |
|---|---|---|---|---|---|---|---|
| root wt. | | stem wt. | | leaf wt. | | pod wt. | |
| cvr. | order | cvr. | order | cvr. | order | cvr. | order |
| 10 | 2.29$^{a}$ | 4 | 30.47$^{a}$ | 10 | 34.90$^{a}$ | 5 | 63.60$^{a}$ |
| 11 | 2.29$^{a}$ | 10 | 28.77$^{a}$ | 1 | 33.68$^{ab}$ | 8 | 50.40$^{ab}$ |
| 1 | 2.11$^{ab}$ | 1 | 22.58$^{b}$ | 4 | 33.48$^{abc}$ | 2 | 56.45$^{bc}$ |
| 3 | 1.86$^{abc}$ | 12 | 22.13$^{b}$ | 11 | 31.69$^{abc}$ | 7 | 51.41$^{cd}$ |
| 5 | 1.65$^{b-d}$ | 11 | 21.59$^{b}$ | 6 | 30.84$^{a-d}$ | 9 | 50.40$^{cd}$ |
| 6 | 1.52$^{cde}$ | 9 | 20.15$^{bc}$ | 3 | 30.59$^{a-d}$ | 6 | 49.84$^{cd}$ |
| 4 | 1.48$^{cde}$ | 7 | 20.11$^{bc}$ | 0 | 28.75$^{b-e}$ | 12 | 48.87$^{d}$ |
| 8 | 1.48$^{cde}$ | 3 | 19.75$^{bc}$ | 12 | 27.80$^{b-f}$ | 3 | 47.81$^{de}$ |
| 2 | 1.45$^{c-f}$ | 2 | 17.88$^{c}$ | 8 | 27.54$^{cf}$ | 11 | 44.52$^{de}$ |
| 12 | 1.29$^{def}$ | 6 | 17.80$^{c}$ | 8 | 25.35$^{def}$ | 1 | 41.63$^{e}$ |
| 9 | 1.01$^{ef}$ | 8 | 14.77$^{d}$ | 2 | 24.52$^{ef}$ | 4 | 34.86$^{f}$ |
| 7 | 0.93$^{f}$ | 5 | 12.37$^{d}$ | 5 | 22.39$^{f}$ | 10 | 34.04$^{f}$ |
| $s_x$ | 0.17 | | 0.99 | | 1.83 | | 2.22 |

Root weight was significantly correlated with both stem weight (0.40) and leaf weight (0.44).

Pod Weight

The variation in pod weight was significantly due to cultivars as revealed by the AOV. Cultivars #4 and 10, with longer maturity period, had the least mean pod weight. The range test also showed the ranking of adjacent means of cultivars from the same site eg. 4, 10; 1, and 11. Means of pod weight expressed as percent of total plant weight departed slightly from the observed trend.

There was significant correlation between pod weight and both stem (0.37) and leaf weight (0.37) but none with root weight.

Leaf:Stem Ratio and Shoot:Root Ratio

The AOV indicated a significant cultivar effect on both ratios. Mean separation was not informative. There was no correlation between either of the ratios and leaf weight. Shoot:root ratio was negatively but highly (-0.66) correlated with root weight while as leaf:stem ratio was negatively but highly (-0.55) correlated with stem weight.

Total Plant Weight

Total plant weight was significantly a cultivar effect. The range test was supportive of the trend observed for pod weight, ie., means of cultivars from

similar sites were ranked adjacently in several cases. Total plant weight was highly correlated with all plant components but negatively correlated with either leaf: stem or shoot:root ratio.

Simple ratios of the dry weights of the different parts of total planting weight have served as adequate summaries of partition data (40). In Table 2.6, it is evident that at this stage of growth, ie., prior to the beginning of physiological maturity, 34-64% of total dry weight was made up of pod weight; 22-35% of leaf weight, 12-30% of stem weight, and 1-2.3% of total dry weight was made up of root weight. Thus in some plants, more than 50% of total dry weight had accumulated in the growing fruit. Although nutrient concentration accumulation and translocation lags 10% behind that of dry weight, (93), it would be expected that a higher proportion of nutrients was being invested in fruit formation.

### 2.4.3 Nutrient Analysis[2]

Nutrient Concentration

The AOV for %N, %P, and N:P ratio revealed significant cultivar effects on nutrient concentrations. The range test showed that some cultivars that were late maturing (see Table 2.4), had high %N and %P. There was no

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[2]The range test of cultivar means of nutrient analysis are given in Table 2.7.

Table 2.7 Duncan's range test of nutrient analyses at 45 DAP.

| RANKED ORDER OF MEANS FOR | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| %N cvr. | order | %P cvr. | order | N:P ratio cvr. | order | total wt/gN cvr. | order | total wt/gP cvr. | order | seed w/gN cvr. | order | seed wt./gP cvr. | order |
| 1 | $2.53^{a}$ | 10 | $0.28^{a}$ | 5 | $10.30^{a}$ | 12 | $3.59^{a}$ | 12 | $0.45^{a}$ | 12 | $1.27^{a}$ | 12 | 0.16 |
| 10 | $2.48^{a}$ | 11 | $0.28^{a}$ | 1 | $9.17^{b}$ | 10 | $2.88^{ab}$ | 7 | $0.34^{b}$ | 8 | $1.15^{ab}$ | 8 | 0.14 |
| 4 | $2.40^{ab}$ | 4 | $0.28^{a}$ | 3 | $9.00^{b}$ | 7 | $2.84^{abc}$ | 10 | $0.32^{b}$ | 9 | $1.04^{bc}$ | 9 | 0.12 |
| 11 | $2.36^{abc}$ | 1 | $0.280^{a}$ | 10 | $9.00^{b}$ | 4 | $2.78^{bc}$ | 4 | $0.32^{b}$ | 6 | $0.94^{cd}$ | 6 | 0.11 |
| 2 | $2.32^{abc}$ | 2 | $0.27^{ab}$ | 4 | $8.82^{3}$ | 9 | $2.72^{bc}$ | 9 | $0.32^{b}$ | 3 | $0.86^{de}$ | 2 | 0.10 |
| 3 | $2.16^{bcd}$ | 12 | $0.26^{abc}$ | 2 | $8.72^{b}$ | 1 | $2.70^{bc}$ | 2 | $0.30^{b}$ | 2 | $0.84^{de}$ | 3 | 0.09 |
| 9 | $2.12^{cde}$ | 7 | $0.25^{abc}$ | 6 | $8.71^{b}$ | 3 | $2.56^{bcd}$ | 1 | $0.29^{b}$ | 1 | $0.79^{def}$ | 7 | 0.09 |
| 5 | $2.11^{cde}$ | 9 | $0.25^{abc}$ | 8 | $8.69^{b}$ | 2 | $2.55^{bcd}$ | 11 | $0.29^{b}$ | 7 | $0.78^{def}$ | 1 | 0.09 |
| 7 | $2.07^{de}$ | 3 | $0.24^{abc}$ | 9 | $8.63^{b}$ | 11 | $2.51^{bcd}$ | 3 | $0.29^{b}$ | 5 | $0.74^{efg}$ | 11 | 0.08 |
| 6 | $2.03^{de}$ | 6 | $0.24^{bcd}$ | 11 | $8.52^{b}$ | 8 | $2.45^{bcd}$ | 8 | $0.29^{b}$ | 11 | $0.65^{fgh}$ | 5 | 0.07 |
| 12 | $2.02^{de}$ | 8 | $0.22^{cd}$ | 7 | $8.43^{b}$ | 6 | $2.01^{cd}$ | 6 | $0.23^{bc}$ | 4 | $0.61^{gh}$ | 4 | 0.07 |
| 8 | $1.89^{e}$ | 5 | $0.21^{d}$ | 12 | $8.06^{b}$ | 5 | $1.86^{d}$ | 5 | $0.18^{c}$ | 10 | $0.51^{h}$ | 10 | 0.06 |
| $s_x$ | 8.06 | | 1.12 | | 0.37 | | 0.25 | | 3.16 | | 5.24 | | 0 |

correlation between %N, %P and root or total weight. There was a negative correlation between N:P ratio and most of the morphological components. However both %N and %P were significantly correlated with leaf:stem ratio and shoot: root ratio. Percent P was highly correlated with %N (0.64).

Percent P ranged from 0.21 to 0.28. This range approached the critical level considered sufficient for plant growth (71, 93). Generally, if %P (expressed on dry weight basis) exceeds 0.25% the P concentration of the plant is considered sufficient. If the P concentration is less than 0.20%, the plant is considered to be low in P, and if less than 0.15%, the plant is considered to be very deficient. In leaves and petioles of beans, concentrations as low as 0.3% total P approaches the critical level, while 0.5% is regarded to be higher than required for maximum yields (71).

Percent N ranged from 1.89 to 2.53. This was far below the critical level of 4.25 to 5.50% considered sufficient in dry beans (76, 93). In another study, it was found that total N represented 2.5 to 3.1% of the dry weight at the pod stage. But since analysis of both N and P was carried out in the present experiment, the ratio of N to P should have indicated whether N or P was likely the limiting element. In many healthy tissues, the ratio of

N:P has been found to be roughly 10:1 (71). A range of 8:1 to 10:1 obtained in this experiment did not represent a significant departure indicative of a nutrient imbalance.

Nutrient Use Efficiency (NUE)

a) NUE as total plant weight/unit nutrient

When %N and %P were expressed as a fraction of total plant weight, the AOV showed significant P and cultivar effects. The range test for means separation showed that means for fertilizer P treatment were not significantly different from means of no P treatments. However, in Table 2.7, a trend similar to that of stem and leaf weights (Table 2.5) was evident. The means of N use efficiency and P use efficiency of cultivars #12, 10, 7, 4, and 9, from high P sites were ranked higher than means of cultivars #1, 3, 2, 11, and 8, from low P sites. However, means of cultivars 5 and 6, from one high P site were ranked least.

Apart from correlation with total plant weight, the highest correlation of both NUE's were positive correlations with leaf and stem weight and a negative correlation with leaf:stem ratio. This observation was surprising since pod weight made up 34-64% of plant dry weight (Table 2.6). But if high stem and leaf weights in cultivars #10, 1, 4, 11, were associated with low pod weight then the situation may be explainable. Moreover since nutrient

accumulation in a given plant part lags behind dry weight accumulation, higher dry weight contributed by pod weight may not have necessarily been accompanied by nutrient contribution.

b) NUE as seed weight per unit of nutrient

When %P was expressed as a fraction of seed yield per plant, the AOV showed significant cultivar effects and N x cultivar interaction effects. The range test did not reveal any informative trend.

On the other hand, the AOV of N use efficiency indicated significant P, N x P interaction, and cultivar effects. The range test revealed that adding fertilizer P significantly improved the N use efficiency of plants. Separation of means of the N x P interaction showed that P-fertilized plants reliant on soil and symbiotic N had the highest N use efficiency although not significantly different from N-fertilized or NP-fertilized plants. Separation of cultivar means did not reveal any important trend.

### 2.4.4 Yield and Harvest Index[3]

Seed Yield

The AOV for seed yield per plant showed that seed yield was significantly influenced by an N x P interaction effect, and cultivar effects. The range test showed that

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[3]The range test cultivar means of yield and harvest index are given in Table 2.8.

Table 2.8 Duncan's range test of yield and harvest index (HI)

| RANKED ORDER OF MEANS FOR | | | | | | | |
|---|---|---|---|---|---|---|---|
| seed cvr. | yld/plant order | 100-seed cvr. | wt. order | biological cvr. | yld. order | HI cvr. | order |
| 12 | 62.98$^{a}$ | 7 | 49.88$^{a}$ | 4 | 139.34$^{a}$ | 9 | 0.61$^{a}$ |
| 8 | 60.98$^{a}$ | 2 | 44.41$^{b}$ | 12 | 134.28$^{a}$ | 6 | 0.58$^{a}$ |
| 9 | 48.16$^{b}$ | 8 | 43.31$^{bc}$ | 7 | 117.11$^{ab}$ | 8 | 9,54$^{a}$ |
| 6 | 45.77$^{b}$ | 5 | 40.40$^{cd}$ | 8 | 115.23$^{abc}$ | 5 | 0.53$^{a}$ |
| 3 | 39.46$^{c}$ | 3 | 39.71$^{d}$ | 1 | 97.40$^{bcd}$ | 11 | 0.47$^{ab}$ |
| 7 | 37.52$^{c}$ | 12 | 36.69$^{c}$ | 10 | 96.00$^{bcd}$ | 3 | 0.47$^{ab}$ |
| 2 | 36.25$^{cd}$ | 11 | 33.32$^{f}$ | 3 | 84.83$^{cd}$ | 2 | 0.47$^{ab}$ |
| 5 | 34.18$^{cd}$ | 1 | 32.28$^{f}$ | 9 | 80.51$^{d}$ | 12 | 0.47$^{ab}$ |
| 1 | 31.04$^{de}$ | 4 | 31.42$^{f}$ | 2 | 78.08$^{d}$ | 1 | 0.37$^{bc}$ |
| 11 | 27.42$^{e}$ | 10 | 25.87$^{g}$ | 6 | 76.91$^{d}$ | 7 | 0.32$^{c}$ |
| 4 | 25.61$^{ef}$ | 9 | 22.56$^{h}$ | 11 | 71.67$^{d}$ | 10 | 0.24$^{c}$ |
| 10 | 20.38$^{f}$ | 6 | 21.45$^{h}$ | 5 | 67.72$^{d}$ | 4 | 0.24$^{c}$ |
| $s_x$ | 1.88 | | 1.05 | | 10.03 | | 4.47 |

P-fertilized plants reliant on soil and symbiotic N had the highest seed yield although not significantly different from N-fertilized or NP-fertilized plants. Plants reliant on soil and symbiotic N had the lowest yield although not significantly different from that of NP-fertilized or N-fertilized plants.

Cultivars #4 and 10, and to a lesser extent #1 and 11, which were still at the late pod-filling stage at harvest, had the least mean yields. Yield was not correlated with any morphological components except total plant weight and shoot:root ratio. This was intriguing because correlation between seed yield and stem and leaf weights would implicate the known phenomenon of assimilate remobilization as a contributor to yield.

100-Seed Weight

Nitrogen, N x P interaction, and cultivars had a significant effect on 100-seed weight as revealed by the AOV. When the means for N effects were tested, there were no significant differences between them. Other tests revealed that P-fertilized plants reliant on soil and symbiotic N had higher seed weight although not significantly different from that of plants that were N-fertilized had the least 100-seed weight though not significantly different from plants that were only N-fertilized or not fertilized at all. The range test for cultivar means

suggested that even though cultivars 4 and 10 were still at the pod-filling stage, they had higher seed weight than some early maturing cultivars such as 6 or 9. On account of this, the 100-seed weight of cultivars prior to planting would have been informative. Lack of adequate seed precluded such a preemptive measure.

## Biological Yield

The AOV for biological yield indicated significant cultivar effect. Because some cultivars had not yet reached physiological maturity at the time of harvest, it was expected that data of biological yield would have been more revealing. The range test did not show any particular trend. There was a significant correlation between biological yield and %P and none with %N. It was also correlated with seed yield (0.37), N use efficiency (0.38) and P use efficiency (0.44). All values are relatively small.

## Harvest Index

Evidence for significant cultivar effect on HI was portrayed by the AOV. The range test did not reveal any trend. The HI was negatively but highly correlated with biological yield (-0.50) and positively correlated with seed yield (0.49).

## 2.5 Discussion

### 2.5.1 Morphological Components

Root and Shoot Growth

It was convenient simply to record root weight, as the indicator of root growth. Surface area or volume, and root length, are known to be acceptable criteria for quantifying the absorbing power of root systems (41). Adhering soil particles of oven-dried roots that might increase root weight or thickened roots that would contribute to weight do not contribute to the absorptive function of the root. Nevertheless, in this experiment, root weight provided a reasonable compromise between ease of measurement and physiological validity.

Root weights were correlated with nutrient use efficiency. Comparatively, some cultivars from low P sites had higher mean root weights but lower nutrient use efficiencies. The converse was true for cultivars from high P sites. It has been reported that if access to soil nutrients is a limiting factor, morphological features of root systems will be critical, and the efficiency of uptake mechanisms will be secondary (45). If the results of the experiment represent an adaptation, then the low nutrient use efficiency of cultivars from low P sites agrees with findings (5), which suggested that the more deficient the soil is in P, the less efficient crops are in responding to added P.

If the root is restricted by a poor supply of nutrients, the shoot will be relatively more restricted since it is further from the limited supply (41, 78). Thus a lowering of the shoot:root ratio is considered a general characteristic common to both N and P deficiency ascribed to the lowering of auxin level in the whole plant (78). Phosphorous deficient soybean plants have shown a marked disturbance of N metabolism as manifested by an accumulation of total soluble N and of amides and carbohydrates in leaves, resulting in a high leaf:steam ratio (78).

These observations merit more than a simple analysis because they may be of great ecological importance. For example, the adaptive features of crop plants that evolved under domestication with marginal supplies of mineral N have shown that the N-nutrition controls the rate of root/shoot growth (14).

### 2.5.2 Nutrient Analysis

Nutrient uptake as measured by chemical analyses of root or shoot fractions is known to provide two valuable pieces of information: (a) what the plant has managed to extract from its growth medium in a given time, and (b) how much net translocation there has been from root to shoot (81). For plants grown in different environments nutrient analysis would indicate nutrient availability regardless of soil type. In terms of nutrient variation, they

represent a departure from basing differences in response on morphological characteristics since physiological variations that account for nutrient variations are not as readily detected as are structural modifications.

Nitrogen Concentration

The concentration of both N and P is known to vary between plant organs and to change within the same organ according to the stage of development (56). Accumulation and distribution of either N or P in crops has been shown to depend on their supply, plant genotype, and environmental factors.

The results of this experiment show that variation in %P, %N, and the N:P ratio was significantly influenced by cultivars and not N or P effects. This is worth noting for two reasons. Firstly, large differences in physiological maturity may have confounded some of the results. Secondly, nutrient distribution is often increased in nutrient stressed plants. This has been shown in P-deficient soybeans (98) and N-deficient sorghum (56). It appears then that plants were not stressed in this trial. But one disadvantage of tests based on total N (eg., Kjeldhal method (12), is the relatively small change in tissue concentration of reduced N as the supply of N increases from deficit to sufficient (56).

It has been established that P concentration is related to chronological age of the plant (71, 93). In beans, the part and time most commonly selected for is a leaf near the top of the plant at full bloom. In the present trial, total P was obtained from whole plant and at near mid-pod fill. It may be inarguable that the P, and even N concentration, were out of range. When the N:P ratio, that would have indicated the limiting nutrient, was calculated, the relative concentrations did not depart from the range observed in many healthy tissues.

Examining the effect of nutrient supply on N concentration in the plant has been one way of assessing nutrient involvement in symbiotic $N_2$ fixation (80). Under some conditions, it has been shown that correcting deficiencies of P in legumes also increased N concentration in shoots, suggesting an involvement of P in symbiotic $N_2$ fixation. Nutrient concentrations of cultivars do not relate to this observation in the current paper.

## Nutrient Use Efficiency

Efficiency of a nutrient element to produce the organ to be harvested has been expressed by the amount of the harvested organ produced by a unit amount of the element absorbed by a crop (45, 89, 97). In this experiment, nutrient use efficiency was expressed as total plant weight (at 45 DAP) per unit of nutrient and seed weight (at harvest) per unit of nutrient.

At 45 DAP, means of cultivars from high P sites represented higher nutrient use efficiencies than means of cultivars from low P sites. The ranking of means was similar to that obtained for leaf and stem weight and nutrient efficiencies were correlated with leaf and stem weight.

On the other hand, when nutrient efficiencies were expressed as seed weight per unit of nutrient, added P fertilizer had a significant influence on N use efficiency. This was also apparent in P-fertilized plants reliant on soil and symbiotic N alone which had the highest but not significant N use efficiency. Since seed yield is an integration of many factors, it appears that seed-weight based nutrient efficiencies did not relate to the P status of the natural habitats of the cultivars.

If these results represent an adaptation phenomena, then the high N use efficiency of plants from high P sites may give them an adaptive advantage, particularly if the same sites are low in N. It has been reported that plants with a superior N use efficiency also have better P utilization efficiency (18). Without detailed knowledge of the composition and spatial localization in various tissues of pools of N or P, the underlying basis of these findings might be inexplainable, particularly the correlation between the nutrient use efficiencies and leaf and stem weights. For the latter case, however, a close association

between the translocation of N and P and that of photosynthate has been reported in wheat (37, 45). The direction of movement was shown to be governed by the supply and demand for carbohydrates and not specifically by supply or demand of phosphate. The study suggested that only in situations where shoot growth was reduced would there be less movement to the deficient shoot. There were no indications that shoot growth was limited in this experiment by either N or P. Thus, both nutrients might have been preferentially transported to the shoots.

Finally, it might be argued that cultivars from high P sites which showed high nutrient use efficiencies were of Type III or Type IV plant habits. However, higher efficiency was obtained in cultivar #1, a Type 1 than cultivar #6, a Type IV. In a study on the influence of plant genotype on some parameters of $N_2$ fixation in Phaseolus vulgaris L. (29), it was demonstrated that certain cultivars of Type II plant habit were superior to Type I and Type III cultivars in N use efficiency.

Combined N

One question of significance for understanding $N_2$ fixation in plant communities concerns the use of soil or fertilizer N as opposed to symbiotic N. Beans are facultative for amount and source of N (49). Availability of N is important for seedling development before root nodules

begin forming although a large N application diminishes $N_2$ fixation usually without reducing productivity.

A soil test was conducted prior to the establishment of the experiment and the amount of N quoted was too high (0.06%). The lack of response to fertilizer N, however, might imply that there was sufficient residual N from previous fertilizer or mineralization to support development of a vigorous and effective symbiotic association. The effect of an excess of N should have been revealed by a high biological yield or low harvest index in N-fertilized treatments. There were no significant fertilizer N effects in either parameter.

### 2.5.3 Yield and Harvest Index (HI)

A food crop is genetically improved through the improvement of either total dry matter production, harvest index, or both (58). Total biological yield represents the crops photosynthetic efficiency while HI represents the efficiency of the crop to convert photosynthesized products into an economically valuable form. Harvest index is reported to vary with the rate of N applied, spacing, and season.

The results of this experiment suggest that neither fertilizer N nor fertilizer P had a significant influence on biological yield or HI. However, significant N x cultivar interactions obtained were expected because variety and

plant density have been shown to interact with fertilizer N (96). It should be reiterated that all measurements were on single plant plots since seedling emergence was severely affected by corn root maggots. In this respect it can be surmised that an individual subjected to the pressures of a plant community may respond very differently from an otherwise identical individual growing alone.

With respect to the influence of season on yield and HI, a number of cultivars grew into the cool temperatures that mark the end of the growing season. Differences in temperature, daylength, and water supply and in the plants responses to them could have been the determinants of many morpho-physiological responses including nutritional ones.

Useful derivatives of seed yield data were the seed yield-nutrient concentration relationships. Seed yield also responded to added fertilizer P. It has been reported that if a yield increase to applied P is obtained then corresponding or even a larger increase in the amount of atmospheric $N_2$ fixed may result (71). However increase in fertilizer P requirements observed in soybeans dependent on symbiotic $N_2$ fixation has been ascribed to nodulation which is known to inhibit root extension (69).

## 2.6. Summary

In view of the diversity in structure and physiology among the Malawian bean landrace cultivars, it may be hardly surprising that nutritional responses which have been discussed exist. Such physiological differences may be great enough to necessitate profound modification in agronomic practices if contrasting cultivars are cultivated. The application of nutrients on a rational basis will not only require knowledge of cultivar response to certain nutrients or Rhizobium inoculation, but also consideration to space and time for making these nutrients available given the ecology of nitrogen and phosphorous in the bean producing areas of Malawi.

It appears that among the factors which contributed to variation among cultivars to nitrogen and phosphorous is the phosphorous status of their natural habitats and growth duration which was also associated with source of seed. In fact two of the cultivars grew into the cool temperature season.

These differences may in turn be related to variation in capacity for uptake, translocation, or accumulation of nutrients. If uptake and translocation of nitrogen and phosphorous were, to a large extent, through association with morphological characteristics, such as stem weight and leaf weight, then the nutrient use efficiencies of either

nitrogen or phosphorous could have been associated with varying capacities of cultivars from different phosphorous sites and their respective growth duration to utilize phosphorous and nitrogen (including symbiotic nitrogen).

While it is true that ecotypes varying in their response to mineral nutrients can be found on soils differing in fertility, it is pertinent to recognize that even within a habitat large changes of nutrient conditions may occur. Perhaps this might explain why some cultivars of similar growth habit and from the same habitat eg., #8 versus 2 and 3, did not perform similarly. In addition, there was no guarantee that the factors of the comparisons ie., the experimental conditions, cultivars used, the plant features chosen for examination or even the nutrients nitrogen and phosphorous, would be the ones that would expose the difference of importance in the field. The identification of the nutrients or nutrient responsible for selection in soils of varying fertility is not easy. For instance, nutrients are known to interact strongly both in the native soil and experimental conditions.

These aspects of nutritional variations in plants imply that crop yield improvement will always include a conscious attempt to evaluate and develop lines that are able to make more productive use of the soil environment for which they are intended. While this paper is by no

means sterile of results, the collective nature of the action of the environmental influence during the investigation deny hasty application of principles to precise problems. The adaptive response to Malawian bean landrace cultivars to nitrogen and phosphorous can only genuinely be evaluated in the environment in which they are grown.

# CHAPTER III

# RESPONSE OF A LANDRACE SEED MIXTURE TO *RHIZOBIUM* INOCULATION

## 3.1 Introduction

The contribution of symbiotic N fixation in supplying cultivated legumes with part of their N requirement depends on the efficiency of the association, and on the available soil fertility when due attention is paid to environmental factors.

It has normally been assumed that seed or soil inoculation is necessary for full benefit from symbiotically fixed N. This is the case when inefficient rhizobia, or no rhizobia are present in the soil. In naturally poor soils, or soils impoverished by cropping, and where little or no fertilizer is applied, $N_2$ fixation by indigenous rhizobia may contribute significantly to N demand by plants.

Natural nodulation has commonly been observed in dry beans. The present experiment attempted to evaluate the need for inoculation of a landrace natural seed mixture. The hypothesis was that because of the diversity of soil environment, components of a seed mixture, in a manner characteristic of their natural habitat, have the same

agro-physiologic response to the soil-plant environment and, in particular, to *Rhizobium* inoculation.

## 3.2 Literature Review

### Potential of $N_2$ Fixation

Grain legume production is dependent on an adequate supply of N that can be obtained from nitrate ($NO_3$) assimilation or from di-nitrogen fixation in the presence of effective indigenous rhizobia or through inoculation (49, 62).

The potential of $N_2$ fixation is known to depend on the crop grown, the effectiveness of the strains of rhizobia in fixing N with the host, the environmental conditions of temperature, moisture, daylength, light intensity, general soil fertility, and the extent to which soil N is assimilated (46). Integral to these factors is that for the desired inoculum strain to have an impact on crop yield, it must be able to survive in the soil and compete with indigenous strains for nodulation of the host (15, 33, 49, 62). Inoculum strains superior in $N_2$ fixation have been shown to fail to compete successfully with indigenous rhizobia (16, 33). Thus the integration of the legume--*Rhizobium* system into agricultural practice will require the close examination of many facets of production far removed from the direct study of $N_2$ fixation.

## Indigenous Rhizobia

The need for adequate attention or indigenous soil populations of rhizobia, compared to efforts on improving and monitoring the survival of introduced strains has been expressed (54, 79). The need has been ascribed to the complexity of native populations of rhizobia that may contain many strains that are difficult to distinguish and an apparent emphasis on adding inoculum rhizobia at each annual planting of the legume crop (54, 79). Yet a prominent feature of many tropical soils is microvariability associated with biological activity of various kinds (42). By experience, indigenous farmers are known to distinguish the productivity of various soils within their holdings and they select crops, cropping sequences and management practices that adjust best to their land (42). It can be anticipated that one source of microvariability which can lead to a wide range of production within small areas is the occurrence of naturalized populations of rhizobia and that these populations contribute to nutritional adaptation of beans grown in varying habitats.

## Adaptation

Adaptation of the legume--*Rhizobium* system to the environment may be manifested at three levels (75, 86). Firstly, differences between genera and species of host are known to be the largest and most easily detected. They

have been shown to underlay many established practices of legume selection for climate, soil type, or agricultural management. Secondly, variation at the level of host cultivar and Rhizobium strain have been reported to be sufficient to justify selection for improved symbiotic yield, for example, in acid soils, in soils rich in combined N, or in exacting temperature conditions. Finally, the survival of the free-living Rhizobium in soil has been shown to confer advantages to Rhizobium of different host groupings. The present paper is concerned with the second type of variation, namely, adaptive variation of host cultivar and Rhizobium strain.

It has been stated that a symbiotic association may be exposed to a continually changing pattern of environmental stresses, each potentially limiting (75). In consequence, it may not be easy to ascertain which factor may be responsible for permitting one particular association to perform better than another in a given set of environment (75). For example, the effectiveness of isolates of Rhizobium trifolii was shown not to be related to soil pH or management practices (13). On a range of Iraqi soils (13), no relationship was found between soil population levels of Rhizobium meliloti and pH, conductivity, lime content or geographic location. Yet it is axiomatic that in indigenous habitats, the effective symbiotic relationship between the legume and Rhizobium represents an

integration, for that soil, of many factors, such as pH, soil fertility, temperature, water availability, and biological factors (13, 75, 86). In such situations, qualities of agronomic importance may take second place (75). For example, rate and efficiency of $N_2$ fixation may be much less important than the ability of nodules to withstand extremes of soil temperature and moisture (75).

Whatever the basis for superior performance, whether of symbiotic origin or not, it has been noted that the long term survival of species within particular ecological situations has undoubtedly encouraged evolution of associations superlatively equipped for operating in their home environment (75). Host variation in ecotypes and Rhizobium strain adaptation has, thus, been well documented in a variety of situations (17, 75). However, in annual arable crops, in which factors, such as sowing time, inoculation practice, soil texture and nutrient and water status are likely to be pre-arranged close to optimum, adaptive ability, if any at all, may confer little or no advantage (75).

## Natural Seed Mixture and Symbiotic $N_2$ Fixation

When land is cultivated, the N content of most soils is known to gradually decline to a status characteristic of the climate, cultural practices, and soil type (28). Thus the N required for plant growth may have to

come from external sources, namely, through symbiotic fixation and N fertilizer application (66). But in the absence of large applications of N fertilizers, as is the usual case with subsistence farming, declining soil N levels and an increase in the proportion of N derived from fixation in legumes can be anticipated (66). Symbiotic $N_2$ fixation, may then be envisaged as a process of adaptation to a disequilibrium in N balance (65, 86). And the supply of N to the ecosystem via biological fixation would represent the ecological niche of the legume--Rhizobium association (65, 86).

A landrace seed mixture of beans may be composed of components varying in nodulation and $N_2$ fixation conferred upon them by the level of effectiveness of indigenous rhizobia strains that they were exposed to.

Members of an ecologic race are known not to be homogeneous (102). Certain biotypes within the race may be better adapted than others. The fact that when crossed, contrasting ecotypes produce many new forms (67, 92), emphasizes the presence of a backlog of unused adaptive potential which can be of agronomic significance.

The present trial assessed the nodulation response of a natural seed mixture of beans using two Rhizobium strains. Both the seed and strains were from Malawi.

## 3.3 Materials and Methods

### Plot Preparation

The experiment was conducted during summer of 1955 and it was located at the Crop Science Research Farm in East Lansing. The soil contained 68 kgN/ha and 155 kgP/ha before planting. A tank mixture of Dual (metolachlor) and Amiben (chloramben) was incorporated pre-plant at a rate of 1.8 kg/ha active ingredient. After seedling emergence, all weeding was done by hand.

### Experimental Design

The experimental layout was a randomized complete block design with two replications. Rows were 2 meters long and spaced 0.5 meters apart. Each cultivar was sown in two adjacent rows at a seeding rate to give 10 plants per metre-row.

An agar-based Rhizobium inoculum, prepared following the method of Thompson (91), was applied during seeding, to two treatments including one that also received 20 kgN/ha as urea. This approach has been described by Date (25). The inoculum consisted of a mixture of two strains of Rhizobium phaseoli, MG 336 and MG 300, that are indigenous to Malawi. All plots received 20 kgP/ha as 0-46-0.

In order to reduce the risk of contamination and subsequent inoculation of the control, uninoculated

treatments were handled before inoculated treatments. A test for the presence of naturalized rhizobia in the soil (92), was done prior to seeding. Test plants were inadequately nodulated suggesting that naturalized rhizobia were not promiscous in modulation of test plants.

Cultivars

Components of a landrace seed mixture of 47 seeds from Malawi were separated into different sizes and seed color. Twenty-four classes, each of three seeds, were multiplied in the Plant and Soil Science greenhouse facility during Fall, 1984. Only eight components of the seed mixture had sufficient seed material to meet the minimum requirements of this study. This limitation curtailed replication of treatments, the number of plants sampled for growth analysis, and the number of times plants were sampled.

General

At the end of the vegetative growth of plants ie. prior to flowering (about 45 DAP) and 2 weeks later (about 60 DAP) ie. prior to the end of flowering, 2 plants/plot were harvested for plant growth analysis. A shovel was used to dig up roots from a depth of about 30 cm. Table 3.1 is a synopsis of symbols and formulae for growth analysis.

Table 3.1 Synopsis of growth analysis symbols and formulae.

| Dervied Quantity | Instant Value | Mean Value |
|---|---|---|
| Crop growth rate | $\frac{1}{P}\frac{dw}{dT}$ | $\bar{C}_{1-2} = \frac{1}{P} \cdot \frac{W_2 - W_1}{T_2 - T_1}$ |
| Leaf area duration | none | $\bar{D}_{1-2} = \frac{(L_{A1} + L_{A2})(T_2 - T_1)}{2}$ |
| Leaf area index | $\frac{LA}{P}$ | $I_{1-2} = \frac{L_{A2} - L_{A1}}{2}$ |
| Leaf area ratio | $\frac{LA}{W}$ | $F_{1-2} = \frac{(LA_1 / W_1) + (LA_2/W_2)}{2}$ |
| Leaf weight ratio | $\frac{LW}{W}$ | $LWR_{1-2} = \frac{(L_{W1}/W_1) + (L_{W2}/W_2)}{2}$ |
| Relative growth rate | $\frac{1}{W} \cdot \frac{dW}{dT}$ | $R_{1-2} = \frac{\ln W_2 - \ln W_1}{T_2 - T_1}$ |
| Specific leaf area | $\frac{LA}{LW}$ | $\overline{SLA}_{1-2} = \frac{(LA_1/LW_1) + LA_2/LW_2)}{2}$ |
| Unit leaf rate (Net assimilation rate) | | $E_{1-2} = \frac{W_2 - W_1}{T_2 - T_1} \cdot \frac{\ln LA_2 \ln LA_1}{LA_2 - LA_1}$ |

Source: Wayne J. C. et al. (97).

The only characteristic related to nodulation and $N_2$ fixation was nodule dry weight at 50 DAP. Plant samples were separated into components, namely, nodules, roots, stems, and leaves. This material was over-dried at 60°C for 72 hours before dry weight measurements were taken. All weight quoted in the paper refer to dry weight. Seed yield was adjusted to 10% moisture.

During the course of the experiment, there was evidence of mild forms of common blight and a severe insect infestation. An insecticide, Sevin (Carbaryl) was applied at weekly intervals till crop maturity to reduce insect damage.

## Statistical Analysis

All data were subject to analysis of variance. Only variances with significant mean squares were tabulated. Comparison of treatment means were done only when treatment means were found to be significantly different in the analysis of variance. Duncan's Multiple Range (DMR) test values are quoted at the 0.05 alpha level. Table B.1, in Appendix, is a summary of statistical notations and abbreviations used in the procedures.

## 3.4 Results[4]

### Morphological Components and Growth Analysis Variables

Inoculation and cultivars were not significant sources of variation in morphological components, such as leaf weight and stem weight, nor in all of the growth analysis variables (Table 3.2).

### Days to Flower

The AOV of number of days to flowering showed significant cultivar effect. The range test of mean separation showed that the number of days to flower varied significantly from 47 to 61 days after planting.

### Nodule Weight (50 DAP)

Because of the difficulty of harvesting roots and nodules, data of nodule dry weight was dealt with moderation. Nonetheless, inoculation effect was the main source of variation in nodule weight as indicated by the AOV. Nodule weight was highest when plants were inoculated than when uninoculated. However, the means were not significantly different from each other. Inoculated and N-fertilized plants had the least mean nodule weight which did not significantly depart from that of uninoculated treatment. There was no correlation between nodule dry weight and days to flowering.

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[4]The AOV and range tests mentioned in context refer to Tables 3.2 and 3.3.

Table 3.2 Analysis of variance of same plant characteristics of landrace seed mixture.

| Source of variation | d.o.f. | MEAN SQUARES FOR: days to flower | nodule wt. 50 DAP | root wt. 67 DAP | leaf wt. 67 DAP | seed yld. /plot | 100-seed wt. |
|---|---|---|---|---|---|---|---|
| Replicate | 1 | 2.521 | .004 | .020 | 183.770 | 713.812 | 27.847 |
| Inoculation | 2 | 9.083 | .020* | .088 | 60.648 | 13979.344*** | 11.561 |
| Cultivar | 7 | 128.592*** | .008 | .696* | 443.939* | 5824.555*** | 702.595*** |
| Inoc. x cvr. | 14 | 9.226 | .008 | .800* | 318.930* | 5603.617*** | 24.445 |
| Error | 23 | 10.043 | .007 | .333 | 168.394 | 633.492 | 32.470 |
| % C.V. | | 6.05 | 94.5 | 25.32 | 25.69 | 13.87 | 17.24 |

Table 3.3 Duncan's range test of some plant characteristics of a landrace seed mixture.

| RANKED ORDER OF MEANS FOR | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| days to flower | | root wt. (67 DAP) | | leaf wt. /plot | | seed yld. wt. | | 100-seed wt. | |
| cvr. | order | cvr. | order | cvr. | order | cvr. | order | cvr. | order |
| 7 | 61.33$^{a}$ | 3 | 2.75$^{a}$ | 3 | 69.50$^{a}$ | 1 | 214.67$^{a}$ | 4 | 53.00$^{a}$ |
| 8 | 55.83$^{b}$ | 5 | 2.68$^{a}$ | 2 | 53.47$^{b}$ | 4 | 212.74$^{a}$ | 8 | 44.40$^{a}$ |
| 5 | 52.67$^{bc}$ | 2 | 2.45$^{ab}$ | 1 | 51.73$^{b}$ | 6 | 204.57$^{ab}$ | 3 | 31.44$^{c}$ |
| 2 | 52.17$^{bc}$ | 6 | 2.22$^{ab}$ | 7 | 48.68$^{b}$ | 8 | 201.00$^{ab}$ | 1 | 30.13$^{c}$ |
| 4 | 51.33$^{cd}$ | 7 | 2.18$^{ab}$ | 5 | 48.29$^{b}$ | 3 | 174.11$^{bc}$ | 6 | 30.09$^{c}$ |
| 3 | 51.17$^{cd}$ | 8 | 2.15$^{ab}$ | 8 | 46.48$^{b}$ | 7 | 164.30$^{cd}$ | 2 | 29.87$^{c}$ |
| 1 | 47.83$^{de}$ | 1 | 2.10$^{ab}$ | 4 | 44.98$^{b}$ | 5 | 145.79$^{cd}$ | 5 | 27.67$^{c}$ |
| 6 | 46.50$^{e}$ | 4 | 1.70$^{b}$ | 6 | 40.92$^{b}$ | 2 | 134.80$^{c}$ | 7 | 17.75$^{d}$ |
| $s_x$ | 10.29 | | 0.24 | | 5.30 | | 10.28 | | 4.17 |

Root Dry Weight (67 DAP)

The AOV of root weight at 67 DAP indicated significant cultivar effects and inoculation x cultivar interaction effect. The range test showed that the mean root weight was not significantly different among cultivars. There was no correlation between root weight and nodule dry weight.

Leaf Weight (67 DAP)

The AOV for leaf weight also showed significant cultivar and inoculation c cultivar interaction effects. The mean leaf weight among the cultivars did not vary significantly. There was no correlations between leaf weight and days to flowering, nodule weight, nor root weight.

Seed Yield/Plot ($0.3m^2$)

Seed yield was strongly influenced by inoculation, cultivars, and inoculation x cultivar interaction effects. The range test revealed that the means for the three inoculation levels were significantly different from one another. Inoculated plants produced the highest seed yield followed by the inoculated N-fertilized plants. There was no correlation between seed yield and any of the morphological components nor with days to flower.

100-Seed Weight

Apart from the extremes of 17.75 g/100 seeds and 53.0 g/100 seeds, 100-seed weight varied very little among the remaining six components of the seed mixture. The range test showed no significant differences in the ranked means. The AOV ascribed variation in 100-seed weight to cultivar effect only. It is evident that the size of seeds of the components was essentially identical.

## 3.5 Discussion

### Growth Analysis and Crop Growth

The use of growth analysis was to understand and explain crop growth and yield of the components of the seed mixture with respect to an anticipated response to *Rhizobium* inoculation. Most of the factors affecting crop yield and some of the genetic ones have been traced back to the influence of the factors on growth components, such as leaf area, and thus on photosythetic performance and dry weight yield. For example, N nutrition is known to affect leaf expansion and thus affecting leaf area index (40).

To measure crop growth of the seed mixture which had an unknown agro-physiologic response, growth analysis, ensuing from the dry weight of whole plants or its parts, was seen as the accepted measurement. Since more than 90% of the dry material of plant has been shown to be the product of photosynthesis (40), plant growth analysis

defined in dry weight terms was intended to measure the performance of plants as producers of photosynthate.

## Growth Analysis, $N_2$ Fixation and Crop Productivity

Another argument for the use of growth analysis was borne of recent evidence which relates more to the existence of an interdependence between $N_2$ fixation and photosynthetic capacities rather than the dependence of the former on the latter (36). Elaboration of this concept is out of scope in the present discussion. However, the lesson is that an increase in either $N_2$ fixation or photosynthetic capacity without a concomitant increase in the other cannot be expected to influence growth and productivity very much (63). Factors influencing photosynthate production, such as leaf area duration, have been shown also to affect nodulation and $N_2$ fixation. For example, the lower canopy leaves of beans supplied over 85% of the $^{14}CO_2$ absorbed to roots and nodules (48). Yet it is these leaves that senesce first affecting both dry weight production for investment in seed yield and photosynthate required by the $N_2$ fixation process or nutrient uptake.

## Growth Response of Seed Mixture

The results of plant growth analysis indicated that there were no significant differences due to inoculation or cultivar effects. The explanation for these results may be two-fold aside from the main effects of inoculation.

Firstly, since plant samples cannot be destructively harvested on more than one occasion, the classical approach to growth analysis requires very large samples to be taken so that errors are kept within aceptable limits. Such errors could not have been reduced to manageable proportion with two replications and 2-metre row plots. The coefficients of variation for the AOV's of growth analysis variables, like that of nodule weight (94.5%), were unacceptable.

Secondly, the plant type display of the components of the seed mixture suggested that all the eight cultivars were of Type IV plant habit. However, the aggressive vegetative growth that produced a leaf area index of 9 in some plots showed that the plants were sensitive to environmental variables, such as photoperiod temperature or even soil moisture.

Inoculation Response

The assessment of bean cultivars for $N_2$ fixation on the basis of nodule number and mass is laborious and time consuming. One would prefer a more readily determinable plant trait such as yield. But yield is influenced by so many factors of the environment other than the symbiotic process. Nodule weight, which is an inferior measure compared to nodule mass, has traditionally been used to indicate or quantify $N_2$ fixation (26). From a technical

feasibility standpoint, nodule weight was chosen as one measure of cultivar response to Rhizobium inoculation.

The lack of significant differences in cultivar response to inoculation leaves room for argument given a high coefficient of variation (94.5%) in the analysis of variance (Table 3.2). Inoculated and uninoculated plants were not significantly different in their response. It is possible though that the inoculation treatment indicated a highly competitive but ineffective strain. Such an outcome has been suggested for similar three-treatment inoculation trials (25). The low nodule weight in inoculated and N-fertilized plants, may be an effect on nodulation suppression by combined N. This has been demonstrated in numerous studies (49).

One of the most important aspects of the ecology of rhizobia is interstrain competition within the rhizosphere, for nodule formation (49). Due to the complex nature of the problem very few unequivocal statements can be made given the simple nature of this study. Characteristically, if a host is sown into soils containing low numbers of indigenous rhizobia capable of nodulating the host, the inoculum strain will form a larger proportion of the nodules (49). A recent study on the effect of delayed inoculation on nodule occupancy of Rhizobium japonicum strains has suggested that the critical stage for competition among R. japonicum strains whether the inoculum

strain or the naturalized strain becomes the primary strain in the root zone and not numbers, per se (61).

The agar-based inoculum has been used in other studies with variable success (87). This form of inoculum has been shown to lack the protective effect afforded by peat to the rhizobia on seed following inoculation. In this study, the form of inoculum, lack of adaptiveness of strains, inadequate cell numbers in the mixed inoculum, and faulty preparation of inoculum, can be cited as possible causes of poor inoculation response.

## Yield Response

Although inoculation and cultivar effects did not significantly influence crop growth or nodulation response, it is tempting to view seed yield with less circumspection. It is evident that seed yield responded more positively to inoculation than to inoculation plus N. No case can be made that fertilizer N in the N-fertilized inoculated plants promoted vegetative growth. But the yield inhibiting effect of the treatment may be due to a transitory supply of N. The aggressive growth of plants could have exhausted the N supply and the repressed symbiotic apparatus may not have been able to satisfy the N demand created.

When cultivar means of seed yield were ranked, 50% of the cultivars had similar mean yields. This suggested that the cultivars were essentially similar in seed yield.

## 3.6 Summary

Rhizobium inoculation was marginally able to meet nodulation requirements of the seed mixture. Significant variation among cultivars was shown for number of days to flowering but other growth components including nodulation, and seed yield elicited non-significant responses. There were no significant treatment effects in all the variables measured for growth analysis.

The extent to which sensitivity to photoperiod and variation of day and night temperature determined the symbiotic process and crop productivity is undoubtedly significant given the aggressive growth noted. It is reiterated, therefore, that an effective symbiotic relationship between a legume and its associated Rhizobium represent an integration for a particular soil, of many factors. The maintenance of genetic characteristics of bean landraces, as represented by the natural seed mixture, has been ensured by the inherent stability and permanence of the traditional system of farming which itself does not undergo abrupt changes. Even though the hypothesis in the paper was tested against a background of uncontrolled

variables, it is pertinent to view the natural seed mixture and corresponding Rhizobium in an ecological perspective.

# CHAPTER IV

# VARIATION IN PLANT NUTRITION OF MALAWIAN BEAN LANDRACES: A RESEARCH PROPOSAL

## 4.1 Summary

The Malawi bean germplasm collection represents a unique opportunity for evaluation of landrace cultivars better adapted to present or potential production areas and farming systems. Among the current constraints to bean production is that dry beans are normally grown without N fertilization and no inoculation is practiced. This suggests that the symbiotic relationship with indigenous Rhizobium and/or soil N sources provide most of the N needed by this legume.

There is strong evidence of host variety and strain of Rhizobium differences in $N_2$ fixing ability and differences in assimilation of inorganic N. The potential for exploitation of $N_2$ fixation and nutrient use may be further influenced by additional adaptive variation of these landrace cultivars to low soil fertility. For dry beans to continue as a major supplier of protein in Malawi, cultivars with improved ability to fix $N_2$ and which assimilate inorganic N more efficiently will be required.

This paper proses to use current cultivars with widespread production and acceptable culinary characteristics, and landrace cultivars of high yield potential, to identify promising cultivars for $N_2$ fixation and nutrient use efficiency. Multilocal field trials combining both attributes and including Rhizobium strain selection will be conducted. The ultimate objective is to incorporate nutrient use efficiency, including $N_2$ fixation, in seed and plant types preferred by indigenous growers and consumers.

## 4.2 Description of Proposal

### 4.2.1 Review of Literature

#### Exploiting Dry Bean - Rhizobium symbiosis

The integration of Rhizobium strain selection procedures with varietal development programs has been viewed in terms of exploiting the variability in both host plant and strains of Rhizobium (25). It has been stated that most researchers are aware of the benefits of inoculating legume seed with suitable strains of nodulating bacteria. Yet in many situations, the selection of effective strains of Rhizobium has begun before knowing whether there will be a response to inoculation or without knowing which criteria needed be applied in strain selection programs. The need for inoculation will depend on the legume to be grown, the presence or absence of native Rhizobium and their effectiveness in $N_2$ fixation with the

introduced host, and on the previous history of the husbandry of the area (25).

With respect to nodulation and $N_2$ fixation of beans, inconsistent results have been reported. For example, Keya et al (59) citing data of Mughogho (1979), reported that efforts to increase yield through inoculation have not been particularly successful in Malawi, like elsewhere in East Africa. Graham (49) cited two studies in Kenya; a study by Keya (1977) found limited natural nodulation in Kenyan soils, but another study by Souza (1969) reported natural nodulation to be both abundant and effective. Recently (59), a survey of 68 bean producing regions in Kenya showed that most soils contained effective Rhizobium phaseoli L. and yield responses to inoculation ranged from 7-47%. However, among inoculation treatments, best results were usually obtained using commercial imported inoculant culture.

A study on $NO_3^-$ reductase and nitrogenase activities of common beans from different geographic locations gave evidence that comparisons of both enzymatic activities upon eight tropical and temperate cultivars of equivalent vegetative cycles indicated on the average, that tropical cultivars have a higher level of $C_2H_2$ reduction and a lower $NO_3^-$ reductase activity than temperate cultivars (39). Thus, the rationale inherent in the study, that tropical

varieties of common beans which have not been bred under conditions favoring assimilation of soil $NO_3$ may exhibit higher efficiency of $N_2$ fixation, was demonstrated.

These observations suggest that there probably exists in cultivars of beans enough genetic variability and potential for $N_2$ fixation owing to the diversity of soils of generally low N status in Malawi, like elsewhere in the tropics. Specifically, the fundamental hypotheses inherent in this research proposal may confirm, modify, or even disprove generally held views of inoculation and $N_2$ fixation status in Malawi. While it is inarguable that the potential for $N_2$ fixation is dependent on the host legume-*Rhizobium* interaction and other environmental factors including general soil fertility, survival and competitiveness of inoculum rhizobia is integral to the effectiveness of the symbiosis (46, 49, 62). Therefore, the need for inoculation must be determined as a prerequisite to *Rhizobium* strain-selection programs which in turn should complement $N_2$ fixation varietal development programs.

Cultivar Differences in Nutrient Use

The potential of $N_2$ fixation may be limited by environmental conditions (temperature, moisture, day length, high light intensity), general soil fertility, and the extent to which soil N is assimilated by plants (46, 49, 62). Soil fertility and assimilation of N have been

shown to be of overriding importance than $N_2$ fixation per se. For example, recently germinated bush bean cultivars have been reported to absorb soil N more rapidly than do climbing cultivars (51). A lowered carbohydrate supply to nodules with consequent lowering of $N_2$ fixation was suggested.

Under some conditions, it has been shown that correcting a mineral nutrition deficiency has resulted in enhanced $N_2$ fixation. Correcting deficiencies of phosphorous in most legume crops has been shown to increase N concentration in shoots suggesting an involvement of P in symbiotic $N_2$ fixation (80).

Recent studies have related differential response of bean cultivars to the mineral nutrition status of the habitats in which cultivars have been grown (53, 67). Among the Malawian bean landrace cultivars, seed yield and some morphological characters were related to soil phosphorous and potassium levels, respectively (67).

Plants may therefore differ markedly in their ability to take up and use nutrients. In situations where mineral nutrition efficiency represents a response to productivity of various soils (53, 65, 67, 75, 86), these differences may often be manifested by adaptation of the cultivars to the corresponding soils. Differential efficiency of plants for uptake and use of mineral elements

may be better understood if mineral requirements for plants are better understood and defined (Clark and Loneragan, 1976).[5]

A mineral-efficient plant has been defined as one that yields the most dry matter for the least amount of mineral applied or taken up. Clark (1976) argued that this may not be a plant that yields the highest total output. A modified definition was that a mineral efficient plant is one that grows better, produces more dry matter, and develops fewer deficiency symptoms than another plant when grown at low levels of a mineral element. At higher nutrient levels it may have a greater ability to take up and make mineral elements more available but have a lower requirement.

Loneragan (1976) further contended that the concept of efficiency cannot be divorced from the soils and climate in which the plant is to be grown and from the use to which the products are put. Thus, the plant efficiency in use of a nutrient was defined as the relationship between the nutrient present in the soil below a unit area of land and the rate of production of a plant growing on it.

Differences in efficiency of nutrient use have also been defined as the ability of a genotype to produce above

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[5]In Plant Adaptation to Mineral Stress in Problem Soils. Proceedings of a Workshop held at the National Agricultural Library, Beltsville, Maryland, Nov. 22-23, 1976.

average yield under low soil fertility conditions and to possess the capacity to produce higher yield when additional nutrients are supplied (53). This may be one practical method of assessing variability in nutrient use efficiency among cultivars, particularly in situations where facilities preclude plant nutrient analysis.

In summary, given evidence of some success in inoculation and $N_2$ fixation, and evidence of differential capability for nutrient use, there would appear sufficient justification for undertaking a bean varietal improvement program that combines $N_2$ fixation and mineral nutrition to produce varieties capable of yielding well under predominantly low fertility soils.

### 4.2.2 Goals and Hypotheses

Research Goals

1. Identify through screening and selection, landrace cultivars having:
   a. high and low N and P use efficiency, and
   b. high and low levels of $N_2$ fixation
2. To determine differential yield response and $N_2$ fixation capacity in selected cultivars:
   a. landrace cultivars with a high yield potential
   b. current cultivars of widespread production and known culinary characteristics grown in representative agro-ecologic zones of Malawi.
3. To determine whether soil or crop management changes would or could lead to greater similarity in response of cultivars grown in different locations.

4. To assess the importance of certain plant growth and phenological characteristics upon parameters of $N_2$ fixation and cultivar differences in nutrient use.

5. To determine whether it is feasible to select for both $N_2$ fixation and nutrient use concurrently in the same population, and whether cultivars superior in both characteristics simultaneously could be produced.

6. To evaluate Rhizobium strains from different agro-ecologic zones under glasshouse and field conditions, and to select strains of Rhizobium efficient in $N_2$ fixation which are competitive with resident strains and persistent over time.

7. To use the information and genetic materials obtained in the experimental phases as sources of improved germplasm in varietal development programs.

8. To develop commercial classes of beans for estate production, small holder producers (tea, coffee, rice, sugar and schemes) and canning industry.

Research Hypotheses

1. That differences in $N_2$ fixation and nutrient use efficiency can be shown in:
   a. landrace cultivars,
   b. current varieties of widespread production and known culinary qualities.

2. That $N_2$ fixation and nutrient use efficiency can be shown to be either adaptive or neutral upon 'fitness' which can be measured as differential yield response or differential nutrient use efficiency.

3. That various morphological (growth analysis) and physiological measurements can be used to evaluate favorable expression of both $N_2$ fixation capacity and nutrient use efficiency.

4. That $N_2$ fixation and nutrient use efficiency are heritable and can be transferred to plants

of varying seed and architectural characteristics by standard breeding methods.

### 4.2.3 Rationale

The improvement of the legume-*Rhizobium* association has received more attention in the past decade than any other field of agricultural research (49). This is in view of the rapid expansion of world population which has and will further exacerbate the already poor state of human protein nutrition, and the tremendous increase in cost of fertilizer N as a consequence of increased petroleum prices.

Attempts to improve biological nitrogen fixation in dry beans have produced variable results (44). Varietal comparisons in the field have demonstrated a cultivar influence upon $N_2$ fixation in dry bean-*Rhizobium* association (49, 51, 98, 99). Taking account of this cultivar influence, the Malawi bean germplasm collection with material from different agro-ecological zones represent a ready made source for attempts at correcting the soil nutrition problem.

In some cases, the integration of dry bean-*Rhizobium* system into agriculture practice will require the close examination of other facets of production far removed from the direct study of $N_2$ fixation (46). For instance, adaptation of plants to unusually low fertility soils may

confer significant differential yield responses (53, 67). Incorporation of nutrient use efficiency and $N_2$ fixation into development of varieties would be complementary to advancement of crop yield. Therefore, it is imperative to determine the extent of variability in dry bean-*Rhizobium* association and nutrient use efficiency as founding objectives of bean varietal improvement in Malawi.

## 4.3 Research Methodology

### 4.3.1 Studies on *Rhizobium* Strain-Selection

1. Soils will be collected from different agro-ecologic zones. *Rhizobium* population will be determined by MPN counting and the soil will also be tested for macro-and micro nutrients.

2. *Rhizobium* isolated from nodules of plants inoculated with soil suspensions in (1) will be evaluated for $N_2$ fixation in Leonard jar assemblies and relative efficiencies in fixation determined.

3. N fertilization - $N_2$ fixation interactions will be studied, particularly in relation to the effect of N on *Rhizobium* since beans are usually the associated crop in N-fertilized maize stands.

4. Small inoculation trials with inoculum shown to be superior in $N_2$ fixation will be conducted under field and greenhouse conditions. In the former case, *Rhizobium* strain(s) will be evaluated in different locations.

### 4.3.2 Screening and Selection of Landrace Cultivars for Differential Inoculation, Nutrient or Yield Response

1. Cultivars selected from the germplasm collection at Bunda will be used for screening trials. The experiment will include

representative plant and seed types from distinct agro-ecological zones in Malawi. It will also include cultivars with acceptable size, shape, color and culinary factors.

2. Cultivars will be raised in sand culture or pot soil culture in polyethylene-lined clay pots.

3. The culture medium, including nutrient solution, will be purified and will simulate general growing conditions. Cultivars will be screened at low (stress), medium, and normal levels of nutrient supply.

4. A split-plot design with factorial components of nitrogen and phosphorous and cultivars in six replications will be employed in growing plants and in analysis of data. The first three replications will be for nodulation and plant growth analysis data.

5. Nutrient use efficiency will be expressed as differential yield response ie. ability of a cultivar to produce yield above average yield under low soil fertility conditions and to possess the capacity to produce higher yield when additional nutrients are supplied.

### 4.3.3 Multilocale Field Experiments

a) Need for inoculation trials
b) Cultivar differences in nutrition

1. Test plants will be grown in representative agro-ecologic zones of Malawi preferably on low and high fertility sites and for a number of seasons.

2. The choice of test plants will be a matter for decision by experimenter on the basis of local conditions. However, it is suggested that existing cultivars of wide spread production and acceptable culinary preferences, and landrace cultivars of high yield potential will be used in most locations where beans grow satisfactorily but in some circumstances, other legume crops that are locally grown may be evaluated together with beans.

3. A suitable Rhizobium strain in a suitable inoculant preparation will be necessary. Existing Rhizobium strains developed at Chitedze, Lilongwe (Malawi) may be adequate for preliminary trials.

4. The choice of a non-legume control, particularly maize, will be an added advantage because beans are sometimes grown with N-fertilized maize. Intercropping would also show whether its advantages are affected by changes in the level of productivity arising from fertilizer treatments in low and high fertility sites.

5. The choice of experimental design will also be a matter for decision by the experimenter. While the inclusion of plus/minus inoculation, plus/minus (stress) N or P may complicate a primary objective of simplicity, the fertilizer treatments would show whether nutrient deficiency is a problem. If nutrient deficiency interferes with nodulation and nodule function, this could be resolved by inspection of plant nodulation and recourse to the soil dilution experiment for estimation of Rhizobium numbers which would also indicate whether ineffective strains are present. Since inoculant establishment failure is not an uncommon condition, consideration will be given to making serological facilities available.

6. Morphological (growth analysis), phenological, and agronomic data to contribute as measures of adaptive fitness, nodulation, and nutrient use efficiency will be taken.

# LITERATURE CITED

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1. Adams, M. W. 1982. Genetic, agronomic, and socio-cultural analysis of diversity among bean landraces in Malawi. Bean/cowpea (CRSP) Title XII Project. Michigan State University, East Lansing, Michigan. U.S.A.

2. Adams, M. W. and J. J. Pipolly. 1980. In Advances in Legume Science. R. J. Summerfield and A. H. Bunting (Eds.). Royal Botanic Gardens. England. 1-16.

3. Andrew, C. S. 1977. In Exploiting the Legume-Rhizobium Symbiosis. J. M. Vincent et al (Eds.). University of Hawaii, College of Tropical Agriculture, Misc. Publ. 145, 253-274.

4. Anonymous. 1969. Agriculture in Malawi. D. A. Jones (Ed.). Ministry of Agriculture. Zomba, Malawi.

5. Baker, D. E. 1976. In Plant Adaptation to Mineral Stress in Problem Soils. M. J. Wright (Ed.). Cornell University, New York. 127-149.

6. Barthlomew, W. V. 1977. In Biological Nitrogen Fixation in Farming Systems of the Tropics. A. Ayanaba and P. J. Dart (Eds.). John Wiley and Sons, Ltd., New York. 3, 27-42.

7. Birch, H. F. and M. T. Friend. 1956. The organic matter and nitrogen status of East African soils. J. Soil Sci. 7, 156-167.

8. Boucher, D. H. and J. Espinoza. 1982. Cropping system and growth and nodulation responses of beans to nitrogen in Tobasco, Mexico. Trop. Agric. (Trinidad) Vol. 59. No. 4.

9. Bradshaw, A. D. 1965. Evolutionary significance of phenotypic plasticity in plants. Adv. Genet. 13, 115-151.

10. Bradshaw, A.D. 1969. In Ecological Aspects of the Mineral Nutrition of Plants. I. H. Rorison (Ed.) Blackwell Scientific Publications. V, 415, 428.

11. Bradshaw, A. D. 1984. In Evolutionary Ecology. B. Shorrocks (Ed.) Blackwell Scientific Publications. 1, 1-26.

12. Bremner, J. M. 1965. In Methods of Soil Analysis. C. A. Black et al (Ed.). American Society of Agronomy, Madison, WI. Agronomy #9, pt. 2. 83, 1149-1178.

13. Bushby, H. V. A. 1982. In Nitrogen Fixation, Vol. 2: Rhizobium. W. J. Broughton (Ed.). Oxford University Press. 2, 35-75.

14. Bulow, J. F. W. 1977. Plant influences in symbiotic fixation. Limitations and Potentials for Biological Nitrogen Fixation in the Tropics. Vol. 10. 75.

15. Caldwell, B. E. 1969. Initial competition of root-nodule bacteria on soybeans in a field environment. Agron. J. 61, 813-815.

16. Chowdhury, M. S. 1980. Potentials and Limitations of Biological Nitrogen Fixation in Eastern Africa. Dar Es Salaam University Press. Dar es Salaam. 16.

17. CIAT (Centro Internacional de Agricultura Tropical), 1978. Annual report of the Bean Production Program.

18. CIAT. (1980). Centro Internacional de Agricultura Tropical (CIAT). Bean Program Annual Report.

19. CIAT. (1981). Centro Internacional de Agricultura Tropical (CIAT). Bean Program Annual Report.

20. Cutting, C. V. 1954. In Proceedings, Second Inter-African Soils Conference. Leopodville. 30, 431-440.

21. Daft, M. J. and A. A. El Giahmi. 1974. Effect of endogenous mycorrhizae on plant growth. VII. Influence of infection on the growth and nodulation in french bean (Phaseolus vulgaris). New Phytol. 73, 1139-1147.

22. Daft, M. J. and A. A. El Giahmi. 1976. Studies on nodulated and mycorrhizal peanuts. Ann. Appl. Biol. 83, 273.

23. Dart, P. J. and B. A. Krantz. 1977. In Exploiting the Legume - Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent et al (Eds.). University of Hawaii, College of Tropical Agriculture. Misc. Publ. 145, 120-154.

24. Devine, T. E. 1976. In Plant Adaptation to Mineral Stress in Problem Soils. M. J. Wright (Ed.). Cornell University, Ithaca, N.Y. 65-72.

25. Date, R. A. 1977. In Biological Nitrogen Fixation in Farming Systems of the Tropics. A. Ayanaba and P. J. Dart (Eds.). John Wiley & Sons. 14, 169-180.

26. Dobereiner, J. 1966. Evaluation of nitrogen fixation in legumes by the regression of total plant nitrogen with nodule weight. Nature. 210, 850-852.

27. Dobereiner, J. and A. B. Campello. 1977. In A Treatise on Dinitrogen Fixation. R. W. F. Hardy and A. H. Gibson (Eds.). Section IV: Agronomy and Ecology. Wiley - Interscience. 191-220.

28. Dommergues, Y. R. 1982. Scarcely explored means of increasing the soil N pool through biological $N_2$ fixation. Whither Soil Research, Panel Discussion Papers. 12th International Congress of Soil Science. New Delhi, India. 8-16 February 1982. 138-149.

29. Duque, F. F., L. T. G. Salles, J. C. Pereira, and J. Dobereiner. 1982. In Biological Nitrogen Fixation Technology for Tropical Agriculture. P. H. Graham and S. C. Harris (Ed.). Centro Internacional de Agricultura Tropical (CIAT). Colombia. 63-66.

30. Edje, O. T., L. K. Mughogho, and U. W. U. Ayonoadu. 1975. Responses of dry beans to varying nitrogen levels. Agron. J. 67, 251-255.

31. Edje, O. T., L. K. Mughogho, and Y. P. Rao. 1980. Increasing bean production in Malawi. Paper

presented at the Sixteenth session of parliament, 20 March 1980. Zomba, Malawi.

32. Edje, O. T., L. K. Mughogho, Y. P. Rao, and W. A. B. Msuku. Regional Workshop on Potential for Field Beans in Eastern Africa, Lilongwe, Malawi, 1980. Proceedings Cali, Colombia, Centro Internacional de Agricultura Tropical, 1981. 226 p.

33. Ellis, W. R., G. E. Ham, and E. L. Schmidt. 1984. Persistence and recovery of Rhizobium japonicum in a field soil. Agron. J. 76, 573-576.

34. Engelstad, O. P., and G. L. Terman. 1980. In The Role of Phosphorous in Agriculture. F. E. Kamprath et al (Eds.). American Society of Agronomy, and others. Madison, Wisconsin. USA 12, 311-329.

35. Epstein, E. 1972. Mineral nutrition of plants: principles and perspectives. John Wiley & Sons, Inc. New York, NY. 12-13, 323-334.

36. Erikson, F. I. and A. S. Whitney. 1984. Effects of solar radiation regimes on growth and $N_2$ fixation of soybean, cowpea, and bushbean. Agron. J. 76, 529-535.

37. Evans, L. T., and I. F. Wardlaw. 1976. Aspects of the comparative physiology of grain yield in cereals. Adv. Agron. 28, 301-350.

38. FAO. 1982. Statistical yearbook.

39. Felix, J. F., M. Obaton, C. M. Messiaen, and L. Saljac. 1981. Nitrate reductase and nitrogenase activities of common beans (Phaseolus vulgaris, L.) from different locations. Plant and Soil 63, 427-438.

40. Fisher, N. M. 1984. In The Physiology of Tropical Field Crops. P. R. Goldsworthy and N. M. Fisher (Eds.). John Wiley and Sons. 4, 119-162.

41. Fisher, N. M., and R. J. Dunham. 1984. In The Physiology of Tropical Field Crops. P. R. Goldsworthy and N. M. Fisher (Eds.). John Wiley and Sons Ltd. 3, 85-118.

42. Fox, R. L. and B. T. Kang. 1977. In Exploiting the Legume - Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent (Ed.). University of Hawaii, Coll. of Trop. Agric. Miscl. Publ. 145, 183-210.

43. Franco, A. A. 1977. In Exploiting the Legume - Rhizobium Symbiosis in Tropical Agriculture J. M. Vincent et al (Ed.). University of Hawaii. Coll. of Trop. Agric. Misc. Publ. 145, 237-252.

44. Franco, A. A., J. C. Pereira, and C. A. Neyra. 1979. Seasonal patterns of nitrate reductase and nitrogenase activities in Phaseolus vulgaris L. Plant Physiol. 63, 421-424.

45. Gerloff, G. C. 1976. In Plant Adaptation to Mineral Stress in Problem Soils. M. J. Wright (Ed.). Cornell University, Ithaca, NY. 161-174.

46. Gibson, A. H. and D. C. Jordan. 1980. Ecophysiology of nitrogen-fixing systems. Encyl. of Plant Physiol. 12C(8), 301-390.

47. Goodman, P. J. 1969. In Ecological Aspects of the Mineral Nutrition of Plants. I. H. Rorison (Ed.). Blackwell Scientific Publications. Oxford. 237-253.

48. Graham, P. H. 1980. In Biological Nitrogen Fixation Technology for Tropical Agriculture. P. H. Graham and S. C. Harris (Ed.). Centro Internacional de Agricultura Tropical (CIAT) Colombia. 27-38.

49. Graham, P. H. 1981. Some problems of nodulation and symbiotic nitrogen fixation in Phaseolus vulgaris L.: A review. Field Crops Research. 4, 93-112.

50. Graham, P. H., and J. Halliday. 1977. In Exploiting the Legume - Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent et al (Eds.). University of Hawaii, Coll. of Trop. Agric. Misc. Publ. 145, 313-334.

51. Graham, P. H. and J. C. Rosas. 1977. Growth and developoment of indeterminate bush and climbing cultivars of Phaseolus vulgaris, L. inoculated with Rhizobium. J. Agric. Sci. Camb., 88, 503-508.

52. Grime, J. P. and J. G. Hodgson. 1969. In Ecological Aspects of the Mineral Nutrition of Plants. I. H. Rorison (Ed.). Blackwell Scientific Publications. Oxford. I, 67-100.

53. Haag, W. L., M. W. Adams, and J. V. Wiersma. 1978. Differential responses of dry bean genotypes to N and P fertilization of a Central American Soil. Agron. J. 70, 565-568.

54. Hagedorn, C., A. H. Ardahl, and L. A. Materon. 1983. Characteristics of Rhizobium trifolii populations associated with subclover in Mississippi soils. Soil Sci. Soc. Am. J. 47, 1148-1522.

55. Harlan, J. R. 1975. Geographical patterns of variation in some cultivated plants. J. of Heredity. 66, 184-191.

56. Hocking, P. J., B. T. Steer, and C. J. Pearson. 1984. Nitrogen nutrition of non-legumnous crops: A review. Field Crop Abstracts. 37, 625-741.

57. Jefferies, R. L. and D. Laycock. 1969. In Ecological Aspects of the Mineral Nutrition of Plants. I. H. Rorison (Ed.). Blackwell Scientific Publication IV, 281-308.

58. Kawano, K. and P. R. Jennings. 1980. In Potential Productivity of Field Crops Under Different Environments--Symposium. International Rice Research Institute. Phillipines. 81.

59. Keya, S. O., V. R. Balasundaram, H. Ssali, and C. Mugane. 1982. In Biological Nitrogen Fixation Technology for Tropical Agriculture. P. H. Graham and S. C. Harris (Eds.). Papers presented at a Workshop Held at CIAT, March 9-13, 1981. Cali, Colombia, Centro Internacional de Agricultura Tropical. 231-234.

60. Khan, T. N. 1977. In Exploiting the Legume - Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent et al (Eds.). University of Hawaii, College of Tropical Agriculture. Misc. Publ. 145, 313-334.

61. Kosslak, R. M., B. Bon Bohlool, S. Dowdle, and M. Sadowsky. 1983. Competition of Rhizobium

japonicum strains in early stages of soybean nodulation. Appl. Environ. Microbiol. 46, 870-873.

62. Kremer, R. J. and H. L. Peterson. 1983. Field evaluation of selected Rhizobium in an improved legume inoculant. Agron. J. 75, 139-143.

63. Lawn, R. J. and W. A. Brun. 1974. Symbiotic nitrogen fixation in soybeans. I. Effect of photosynthetic source-sink manipulations. Crop Sci. 14, 11-16.

64. Lee, J. A., R. Harmer, and R. Ignaciuk. 1981. In Nitrogen as an Ecological Factor. J. A. Lee et al (Eds.). Blackwell Scientific Publications. Oxford. 5, 95-112.

65. Lopes, E. S. 1977. In Limitations and Potentials for Biological Nitrogen Fixation in the Tropics. J. Dobereiner et al (Eds.). Plenum Press. New York. 173-187.

66. Mahon, J. D. 1983. In Nitrogen Fixation, Vol. 3: Legumes. W. J. Broughton (Ed.). Oxford University Press. 8, 299-326.

67. Martin, G. B. 1984. Genetic diversity of bean landraces in Northern Malawi. M. S. Thesis. Michigan State University, East Lansing, Michigan U.S.A.

68. Mengel, K. and E. A. Kirkby. 1982. Principles of Plant Nutrition. International Potash Institute. Switzerland.

69. Munns, D. N. and A. A. Franco. 1982. In Biological Nitrogen Fixation Technology for Tropical Agriculture. P. H. Graham and S. C. Harris (Eds.). Centro Internacional de Agricultura Tropical (CIAT). Colombia. 133-152.

70. Munns, D. N. and B. Mosse. 1980. In Advances in Legume Science. R. S. Summerfield and A. H. Bunting (Eds.). University of Reading, England II, 115-125.

71. Ozanne, P. G. 1980. In The Role of Phosphorous in Agriculture. F. E. Kamprath et al (Eds.). American Society of Agronomy, and others. Madison, Wisconsin, U.S.A. 20, 559-589.

72. Pate, J. S., and C. A. Atkins. 1983. In Nitrogen Fixation Vol. 3: Legumes W. J. Broughton (Ed.). Oxford University Press. 7, 245-298.

73. Pate, J. S. 1980. Transport and partitioning of nitrogenous solutes. Ann. Rev. of Plant Physiol. 31, 313-340.

74. Pate, J. S. 1983. In Nitrogen as an Ecological Factor. J. A. Lee et al (Eds.). Blackwell Scientific Publication. 11, 225-256.

75. Pate, J. S. 1976. In Symbiotic Nitrogen Fixation in plants. P. S. Nutman (Ed.). Cambridge University Press, Cambridge. 27, 335-360.

76. Peck, N. H. and G. E. McDonald. 1984. Snap bean plant responses to nitrogen fertilization. Agron. J. 76, 247-253.

77. Phillips, D. A. 1980. Efficiency of symbiotic nitrogen fixation in legumes. Ann. Rev. Plant Physiol. 31, 29-49.

78. Pirson, A. 1955. Functional aspects in mineral nutrition of green plants. Ann. Rev. of Plant Physiol. 6, 71-114.

79. Robert, F. M., and E. L. Schmidt. 1983. Population changes and persistence of Rhizobium phaseoli in soil and rhizospheres. Appl. Environ. Microbiol. 45, 550-556.

80. Robson, A. D. 1983. In Nitrogen Fixation Volume 3: Legumes. W. J. Broughton (Ed.). Oxford University Press. NY. 2, 36.

81. Rorison, I. H. 1969. In Ecological Aspects of the Mineral Nutrition of Plants. I. H. Rorison (Ed.) Blackwell Scientific Publications. 155-176.

82. Ruschel, A. P., P. B. Vose, E. Matsui, R. L. Victoria, and S. M. Tsai Saito. 1982. Field evaluation of nitrogen fixation and nitrogen utilization by Phaseolus bean varieties determined by $N^{15}$ isotope dilution. Plant and Soil. 65, 397-407.

83. Sanchez, P. A. and G. Uehara. 1980. In Role of Phosophorous in Agriculture. F. E. Khasawneh et al (Eds.). American Society of Agronomy and others. Wisconsin. 17, 471-509.

84. Sinclair, T. R., and C. T. DeWit. 1975. Photosynthate and nitrogen requirements for seed production by various crops. Science 189, 565-567.

85. Sinclair, T. R. and C. T. DeWit. 1976. Analysis of the carbon and nitrogen limitations of soybean yield. Agron. J. 68, 319-324.

86. Sprent, J. I. 1981. In Nitrogen as an Ecological Factor. J. A. Lee et al (Eds.). Blackwell Scientific Publications. Oxford. 2, 29-42.

87. Strijdom, B. W. and C. C. Deschodt. 1976. In Symbiotic Nitrogen Fixation in Plants. P. S. Nutman (Ed.). Cambridge University Press. 151-168.

88. Subba Rao, N. S. 1977. In A Treatise on Dinitrogen Fixation. R. W. F. Hardy and A. H. Gibson (Eds.). Section IV: Agronomy and Ecology. Wiley-Interscience. 1, 3-30.

89. Tanaka, A. 1980. In Potential Productivity of Field Crops Under Different Environments - Symposium International Rice Research Institute. Phillipines. 61.

90. Temple, S. R., and L. Song. 1980. In Advances in Legume Science. R. J. Summerfield and A. H. Bunting (Eds.). Royal Botanical Gardens. England. 365-374.

91. Thompson, J. A. 1980. In Methods for Evaluating Biological Nitrogen Fixation. F. J. Bergersen (Ed.). John Wiley and Sons. N.Y. 2, 489-534.

92. Vincent, J. M. 1970. A manual for the practical study of the root-nodule bacteria. IBP Handbook No. 15. Blackwell Scientific Publications.

93. Vitosh, M. L. 1978. In Dry Bean Production - Principles and Practices. L. S. Robertson and R. D. Fraser (Eds.). Extension Bulletin E-1251. Michigan State University, Michigan. U.S.A.

94. Vose, P. B. 1963. Varietal differences in plant nutrition. Herbage Abstracts. 33, 1-13.

95. Watanabe, F. S. and S. R. Olsen. (1965). Test of an ascorbic acid method for determining P in water and $NaHCO_3$ extracts from soil. Soil Sc. Society, Proc. 677-678.

96. Watson, D. J. 1965. The physiological basis of variation in yield. Adv. in Agron. 4, 101-144.

97. Wayne, J. C., S. T. Ball, G. H. Elkon, T. G. Isleib, and T. J. Schneemeis. 1982. In Biological Nitrogen Fixation Technology for Tropical Agriculture. P. H. Graham and S. C. Harris (Ed.). Centro Internacional de Agricultura Tropical (CIAT). 67-76.

98. Westermann, D. T. and J. J. Kolar. 1978. Symbiotic $N_2$ ($C_2H_2$) fixation by bean. Crop Sci., 18, 986-990.

99. Westermann, D. T., G. E. Kleinkopf, L. K. Porter, and G. E. Leggett. 1981. Nitrogen sources for bean seed production. Agron. J. 73, 660-664.

100. Whiteaker, G., G. G. Gerloff, W. H. Gabelman, and D. Lindgren. 1976. Intraspecific differences in growth of beans at stress levels of phosphorous. J. Am. Soc. Hortic. Sci. 101, 472-475.

101. Willey, R. W. 1979. Intercropping - its importance and research needs. Field Crop Abstracts. 32, 1-10, 72-81.

102. Wilsie, C. P. 1962. Crop Adaptation and Distribution. W. H. Freeman and Company. 4, 66-86.

103. Woolhouse, H. W. 1969. In Ecological Aspects of the Mineral Nutrition of Plants. I. H. Rorison (Ed.). Blackwell Scientific Publication. IV, 357-380.

# APPENDICES

# APPENDIX A

## CHARACTERISTICS OF BEAN-*RHIZOBIUM* FROM TWO SOILS OF CENTRAL MALAWI[1]

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[1]Contribution from Botany 803, Special Problem: Plant Physiology. Winter, Term, 1985.

# ABSTRACT

Identification and characterization of field isolates is a prerequisite to laboratory and field *Rhizobium* strain-selection programs. Like other micro-organisms, bean-*Rhizobium* exhibit tremendous diversity in the substrates that they can use for growth and in their sensitivity to antibiotics. The two characteristics, and including nodulating and $N_2$ fixation ability, were used to identify and characterize bean-*Rhizobium* field isolates from two soils of Central Malawi.

Different media formulations of carbon and nitrogen and different antibiotics were able to support, retard, or prevent growth of the isolates. The methods were not definitive for identification of a single strain. When the isolates were re-inoculated on test plants, they were able to nodulate and fix N with the host plants.

# INTRODUCTION

Dry beans (*Phaseolus vulgaris* L.) grown under field conditions in Malawi are usually nodulated (4). The cultivation of this crop for more than three centuries (5) probably accounts for the general occurrence of bean rhizobia in soils. However, as is true in other legume-*Rhizobium* symbiotic associations, the mere presence of nodules on the roots of bean plants has been no assurance that these plants benefit through their association with rhizobia (7). In fact there has been concern that indigenous soil populations of *Rhizobium* have received little attention compared with efforts on monitoring the survival of introduced strains (10). This situation is ascribed to both the complexity of native populations that may contain many strains that are difficult to distinguish and an emphasis on legume row crops where inoculum rhizobia may be added at each annual planting (10).

Sometimes inoculation of beans has resulted in increased seed yield, but frequently plants have been poorly nodulated and yield not increased (7, 8). Variability in field responses to nodulation has been ascribed to unsatisfactory host-microsymbiont interactions (8) or to the environment (7). A major problem is the competition

between inoculant strains and native soil populatios of R. phaseoli for nodule sites (7, 10). Soils may contain rhizobia that are highly competitive against those applied in inoculants. Studies in Kenya reported natural nodulation to be both abundant and effective (7). However, indigenous rhizobia may be ineffective in $N_2$ fixation. Many field isolates of R. phaseoli from bean soils of Colombia proved ineffective when tested for $N_2$ fixation under controlled conditions (7). Thus more disturbing than the effect of the host variety is the possibility that the success of a given strain is a major function of the microflora of the soil, particularly the presence of an indigenous population of R. phaseoli.

Many factors are known to affect the microflora of the soil (1, 17), but little published information on basic ecological relationships governing strain growth, survival or competition of the rhizobia in its native environment is available. If inoculant rhizobia are to be managed effectively, knowledge of the spatial structure of indigenous populations will provide a better understanding of the population variability and may ultimately lead to identification of inoculant strains which are both efficient and competitive.

The following experiments were planned to study: (a) the prevalence of effective and ineffective isolates of

*R. phaseoli* in two soils from Malawi, and (b) whether homogeneity in cultural characteristics and $N_2$ fixation (measured as $C_2H_2$ reduction) occurred among the isolates from selected nodules.

## MATERIALS AND METHODS

### Isolation of Rhizobia

Soils collected from Bunda and Mvera in Malawi were used as sources of rhizobia. From each soil sample, 10 g of soil was suspended in 90 ml of sterile distilled water. Five ten-fold dilutions were made from the initial suspension to give a dilution series of six for each soil sample.

Seeds of *P. vulgaris* L. cvs. Wisconsin 2158 were surface-sterilized by immersion with agitation for 10 minutes in 0.1% mercuric chloride (acidified with 5 ml/l concentrated HCl). They were rinsed with three rinses with sterile distilled water and subsequently immersed in a 3% calcium hypochlorite solution for 10 minutes. The disinfectant was removed by five rinses with sterile distilled water. Seeds were pre-germinated on moistened filter paper in sterile Petri dishes. A pair of two day-old seedlings (radicles of 2 to 3 cm) were aseptically transferred to 250-ml Erlenmeyer flasks containing sterile 1:2 mixture of perlite and vermiculite moistened with modified Fahraneus N-free solution (6). One (1) ml of soil suspension was

carefully dribbled onto the seedling. The N-free base medium was composed of $Na_2HPO_4$, 150; $KH_2PO_4$, 100; $CaCl_2$, 100; $MgSO_4 \cdot H_2O$, 120; Fe citrate, 5 and a trace element solution as described by Gibson (6). A thin film of sterile distilled water was maintained throughout the growing period. The flasks were covered with cotton wool which was worked around the seedlings after they grew up to the top of the flasks. Plants were grown in growth chambers maintained at 24°C and 20°C day and night temperatures and 14 hours of light. A large flask of water was placed in the chamber to maintain adequate humidity.

All plants were harvested after 4 weeks. The rhizobia were isolated by conventional techniques (14) from five, medium to large nodules from each pair of plants. For ease of handling, nodules were cut so as to leave a small amount of root. Nodules were exposed momentarily to 95% ethanol and immersed in 0.1% acidified mercuric chloride for 3-4 minutes and subsequently rinsed with six changes of sterile distilled water. Nodules were crushed and the milky fluid from within the nodule spread over the surface of yeast extract-mannitol (YEM) agar (12), in petri plates. The YEM agar base medium was of the following composition (in grams per litre): mannitol, 10; yeast extract (Difco Laboratories), 1.0; $K_2HPO_4$, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; NaCl, 0.1; agar, 15. The pH was adjusted to 6.8

with 0.1 N HCl. Cycloheximide, 25 mg/ml water, was added to retard fungal growth.

Plates were incubated at 29°C and after 2 days, they were checked for isolated colonies of spreading that are conformable with the growth expected of Rhizobium phaseoli (14). From a typical isolated colony, a loopful of cells was picked for restreaking to ensure purity of the isolates and the make permanent cultures which were maintained in yeast minimal media. The isolates were labelled and recorded and the culture numbers were related to the source of soil. For instance, isolates from Bunda were designated B and those from Mvera, M.

Carbon and Nitrogen Sources

Various carbon and nitrogen sources in Table A.1 were incorporated in yeast agar (YA) medium (14). The base medium was of the following composition (in grams per litre): $MgSO_4 \cdot 7H_2O$, 100; $CaCl_2 \cdot H_2O$, 220; $K_2HPO_4$, 220; $FeCl_3$, 20. Two (2) mls of each stock were added to 2 litres of distilled water. The pH was adjusted to 6.8 and 38 g of agar was added. About 200 mls of the medium were dispensed in 250 ml Erlenmeyer flasks and autoclaved for 15 minutes at 35 p.s.i. To each Petri plate 15 mls of the YA medium, 0.5 ml of N source, 0.1 ml of the carbon sources were added. With a 30-point grid underneath the Petri plate, all the isolates were individually inoculated on the

Table A.1 Index to carbon and nitrogen sources.

| Carbon Sources | Nitrogen Sources |
|---|---|
| 1. Na-citrate | 1. $KNO_3$ |
| 2. Succimic acid | 2. $NH_4Cl$ |
| 3. Inositol | 3. Na-glutamate |
| 4. Sucrose | |
| 5. Mannitol | |
| 6. Galactose | |
| 7. Fructose | |
| 8. Lactose | |
| 9. Glucuronic acid | |
| 10. d-xylose | |
| 11. Na-gluconic acid | |
| 12. Glucose | |
| 13. Rhamnose | |
| 14. Maltose | |
| 15. Glycerol | |
| 16. Cellobiose | |
| 17. Sorbitol | |
| 18. Raffinose | |
| 19. Soluble starch | |
| 20. Inulin | |
| 21. Trehalose | |
| 22. Mannose | |
| 23. Dulcitol | |
| 24. Arabinose | |

surface of the agar with a sterile toothpick for each isolate. Growth was scored by measuring the diameter of the colonies. For a diameter of less than 1 mm growth was nil. The number of isolates that had colonies above 1 mm in diameter are shown in Table A.4.

Antibiotic Sensitivity Test

The basic agar diffusion procedure (16) was used to test antibiotic sensitivity of the field isolates as an aid to characterization. Each Petri plate containing 15 ml of YEM agar (12) was inoculated with field isolates using conventional techniques (14). Commercially available filter paper discs, each containing defined concentration of specific antibiotic Table A.2 were released onto the agar surface using an automatic dispenser. The inoculated plates were incubated at 29°C for 2-3 days during which the antibiotics diffused from the discs to the agar. The minimal inhibitory concentration (MIC) for the antibiotic was recognized by the presence of growth inhibition (clear) zone at a particular distance from the discs (18). The diameters of the zones were measured with a ruler and compared with a standard table, Table A.3.

Re-inoculation and $C_2H_2$ Reduction Tests

The isolates were subject to further testing to verify (Koch's postulates) the initial presumptive identity

Table A.2 Antibiotic sensitivity discs*

| Antibiotic | | Concentration (mcg, units) |
|---|---|---|
| SSS | Triple sulfa | 0.25 |
| S | Streptomycin | 10 |
| P | Penicillin | 10 units |
| F/M | Nitrofurantoin | 300 |
| SXT | Sulfamethoxazole + Trimethroprim | 23.75 + 1.25 |
| CB | Carbenicillin | 100 |
| E | Erythromycin | 15 |
| PB | Polymyxin B | 300 units |
| K | Kanamycin | 30 |
| C | Chloramphenicol | 30 |
| N | Neomycin | 2 |
| GM | Gentamicin | 10 |
| NA | Nalidixic acid | 30 |
| AM | Ampicillin | 10 |
| OX | Oxacillin | 1 |
| CL | Colistin | 10 |
| Te | Tetracycline | 30 |

*Expiry of stock ranged from 1981-1989.

Table A.3 Zone-size interpretive standards and approximate MIC breakpoints for the disc diffusion technique.

| Antimicrobial Agent | Disc Potency | Inhibitory Zone Diameter (to nearest mm) Resistant | Intermediate | Susceptible |
|---|---|---|---|---|
| Penicillin G and ampicillin | 10 units<br>10 μg | | | |
| Staphylococci | | 20 or less | 21-28 | 29 or more |
| Enterobacteriacene and enterococci | | 11 or less | 12-13 | 14 or more |
| Other organisms | | 11 or less | 12-21 | 22 or more |
| Methicillin | 5 μg | 9 or less | 10-13 | 14 or more |
| Nafcillin or oxacilin | 1 μg | 10 or less | 11-12 | 13 or more |
| Vancomycin | 30 μg | 9 or less | 10-11 | 12 or more |
| Cephalothin | 30 μg | 14 or less | 15-17 | 18 or more |
| Cephalonidine | 30 μg | 11 or less | 12-15 | 16 or more |
| Carbenicillin | | | | |
| Pseudomonas sp. | | 12 or less | 13-14 | 15 or more |
| Proteus and E. coli | | 17 or less | 18-22 | 23 or more |
| Polymyxin B | 300 units | 8 or less | 9-11 | 12 or more |
| Chloramphenicol | 30 μg | 12 or less | 13-17 | 18 or more |
| Tetracycline | 30 μg | 14 or less | 15-18 | 19 or more |
| Erythromycin | 15 μg | 13 or less | 14-17 | 18 or more |
| Lincomycin | 2 μg | 9 or less | 10-14 | 15 or more |
| Clindamycin | 2 μg | 11 or less | 12-15 | 16 or more |
| Kanamycin | 30 μg | 13 or less | 14-17 | 18 or more |
| Neomycin | 30 μg | 12 or less | 13-16 | 17 or more |
| Streptomycin | 10 μg | 11 or less | 12-14 | 15 or more |
| Gentamicin | 10 μg | 12 or less | 13-14 | 15 or more |
| Sulfonamides | 300 μg | 12 or less | 13-16 | 17 or more |
| Nitrofurantoin | 300 μg | 14 or less | 15-18 | 19 or more |
| Nalidixic acid | 30 μg | 13 or less | 14-18 | 19 or more |

Source: E. R. Wright (18).

that they were bean-Rhizobium because they were isolated from nodules of bean test plants. Using procedures already described (14), test plants were inoculated with all the isolates and were grown under growth chamber condition. After 4 weeks the plants were assayed for $N_2$ fixation using the acetylene ($C_2H_2$) reduction method (6).

Freshly obtained nodulated roots were placed and sealed in 15 ml serum bottles. Prior to addition of 1.5 ml of purified acetylene (15%, v/v), an equivalent amount of air was removed using a syringe. The bottles were inoculated for 60 minutes at the appropriate growth temperature. A sample of gas ($C_2H_2$), 50 $\mu$liters, was withdrawn from each bottle and assayed on a gas chromatograph equipped with a flame ionization detector and a Porapak N column. The $N_2$ fixed was represented as a ratio of acetylene and ethylene peaks displayed on the recorder taking into account amplification or attenuation. For example, the ratio of acetylene to ethylene for isolate B-9 was calculated as follows:

peak of ethylene 7.3 attenuation 16 x $10^{-4}$

peak of acetylene 3.45 attenuation 16 x $10^{-9}$

$$\frac{7.3 \times (16 \times 10^{-11})}{3.45 \times (16 \times 10^{-9})} = 0.304 \text{ or } 3.04\%$$

## RESULTS AND DISCUSSION

### Carbon and Nitrogen Sources

Table A.4 shows that growth is stimulated by a particular carbon source given the variation of growth response of various isolates. Secondly, that a great variety of carbon compounds supported growth of rhizobia isolates. In addition to the carbohydrates, the higher alcohols such as mannitol, dulcitol, and glycerol are utilized by some of these organisms. Comparatively the monohexoses were almost as readily utilized as were some of the di- and tri-hexoses. In the case of the salts of the organic acids, neither the succinate nor the citrate were judiciously utilized by the isolates. The nature of the N compound was a very important factor and to a large extent, determined eventual growth. When $KNO_3$, $NH_4Cl$, and Na-glutamate replaced undefined (complex) organic nitrogenous compounds that constitute yeast extract, establishment of growth was affected much more with $NH_4Cl$ than the others.

The inherent hypothesis was that because micro-organisms exhibit a great diversity in their nutritional requirements due to differences in uptake, transport, and utilization pathways (9), isolates could be characterized by differences in carbon and nitrogen nutrition.

Table A.4 Number of isolates which grow on carbon/nitrogen combinations.

| | Isolate Sources: | $KNO_3$ B | $KNO_3$ M | $NH_4Cl$ B | $NH_4Cl$ M | NA-glutanate B | NA-glutanate M | Mean B | Mean M | Overall Mean |
|---|---|---|---|---|---|---|---|---|---|---|
| MONOSACCHRIDES | | | | | | | | | | |
| -Hexoses | -glucose | 19 | 0 | 0 | 5 | 22 | 26 | 13.66 | 10.33 | 12.00 |
| | -mannose | 12 | 26 | 1 | 11 | 15 | 26 | 9.33 | 21.00 | 15.16 |
| | -galactose | 21 | 27 | 0 | 3 | 22 | 27 | 14.30 | 19.00 | 16.65 |
| | -fructose | 0 | 2 | 2 | 8 | 22 | 27 | 8.00 | 12.33 | 10.16 |
| | -rhamnose | 18 | 20 | 0 | 2 | 22 | 27 | 13.33 | 16.33 | 14.83 |
| -Pentoses | -xylose | 20 | 27 | 1 | 0 | 20 | 26 | 13.66 | 17.66 | 15.66 |
| | -arabinose | 11 | 27 | 0 | 2 | 18 | 27 | 9.66 | 18.66 | 14.16 |
| -oxidation products | -glucuronic acid | 1 | 1 | 2 | 0 | 11 | 1 | 4.66 | 0.66 | 2.66 |
| | -gluconic acid | 2 | 0 | 2 | 0 | 10 | 0 | 4.66 | 0.00 | 2.33 |
| -Reduction products | -mannitol | 20 | 27 | 0 | 1 | 22 | 27 | 14.0 | 18.33 | 16.16 |
| | -sorbitol | 2 | 21 | 8 | 1 | 22 | 24 | 10.66 | 15.33 | 12.99 |
| | -inositol | 0 | 3 | 0 | 2 | 9 | 27 | 3.0 | 10.66 | 6.83 |
| | -glycerol | 10 | 7 | 1 | 0 | 22 | 25 | 11.0 | 10.66 | 10.83 |
| | -dulcitol | 27 | 0 | 0 | 11 | - | 27 | 11.33 | 9.33 | 10.66 |
| DISACCHRIDES | | | | | | | | | | |
| -Reducing | -maltose | 0 | 0 | 0 | 0 | 0 | 0 | 0 | - | - |
| | -lactose | 18 | 22 | 0 | 0 | 19 | 24 | 12.33 | 15.33 | 13.83 |
| | -cellobiose | 9 | 22 | 2 | 3 | 22 | 25 | 11.0 | 16.66 | 13.83 |
| -Non-reducing | -sucrose | 17 | 27 | 0 | 4 | 22 | 23 | 13.0 | 18.0 | 15.5 |
| | -trehalose | 20 | 26 | 11 | 21 | 22 | 27 | 17.66 | 24.66 | 21.16 |
| TRISACCHRIDES | -raffinose | 22 | 27 | 4 | 4 | 14 | 25 | 13.33 | 18.66 | 15.99 |
| POLYSACCHRIDES | -soluble starch | 19 | 0 | 21 | 0 | 0 | 5 | 7.33 | 4.66 | 9.66 |
| | -inulin | 22 | 0 | 0 | 11 | 0 | 3 | 7.33 | 4.66 | 9.66 |
| ORGANIC ACID SALTS | -succinate | 14 | 0 | 0 | 0 | 16 | 1 | 10.0 | 0.33 | 5.16 |
| | -Na-citrate | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0.33 | 0.16 |
| | Mean | 3.08 | 13.0 | 2.29 | 3.7 | 14.93 | 18.79 | | | |
| | Overall Mean | 13.4 | | 2.99 | | 16.87 | | | | |

To draw conclusions that would characterize a particular kind of growth to a group of isolates which would in turn constitute a single strain would be far-fetched because of the tremendous overlap of growth, given various sources of carbohydrates in combination with several sources of N. However, rhizobia, like other microorganisms, have the ability to split complex macro-molecules into their constituent molecules which can be transported into the cell and used in the biosynthetic energy-yielding reactions (18). Therefore, if B-isolates are able to utilize polysacchrides such as soluble starch or inulin (Table A.4) as sources of carbohydrates to release component monosacchrides for utilization, then the organism must be able to produce a polysacchride-splitting enzyme. This would make B-isolates unique from M-isolates which very sparingly utilized inulin or soluble starch when grown on nitrate as N source and conversely when glutamate was the N source. This finding would be true for differences within either B- or M-isolates and it would be amenable to the establishment of a selective medium for a particular isolate or for differentiating B-isolates from M-isolates. Finally, it has been shown that with many rhizobial strains used in inoculants, a mixture of carbohydrates, usually mannitol and succinate, give an increased concentration of cells compared to that obtained with

one carbohydrate (15). Compatible combinations of carbohydrates can be drawn from this kind of study.

Antibiotic Sensitivity Test

From the results shown in Table A.5, it is evident that different isolates responded differently to various antibiotics giving a range of inhibitory zone diameters. The antibiotics with the strongest effect on rhizobia were tetracycline, erythromycin, and carbenicillin. A weaker effect was exerted by ampicillin and streptomycin while other antibiotics such as polymyxin B, nitrofurantoin and nalidixic acid did not inhibit growth. Where triple sulfa and sulfa methoxazde were used, the preparations exhibited little effect with predominance of many scattered resistant colonies.

Factors that might have affected the results were changes in the cells themselves and age of antibiotic discs. The first problem can be overcome by maintenance of a permanent culture whereas the latter problem was due to inavailability of own stock of antibiotics which necessitated use of different stocks of antibiotics.

The method of interpretation that uses the standard table, Table A.3, was too stringent to be of any use in growing the isolates towards naming a new strain. This was evident when the approximate MIC breakpoints of Table A.3 are compared to results shown in Table A.5. The author

Table A.5 Range of inhibitory zone diameter (to nearest mm) of antibiotics on all isolates.

| Antibiotic | B-isolates | | M-isolates | |
|---|---|---|---|---|
| | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| Triple sulfa | R | NA | R | NA |
| Streptomycin | 6- 8 | 5- 8 | 4-10 | 3-10 |
| Penicillin | 3- 9 | R | R | R |
| Nitrofurantoin | R | R | R | R |
| Sulfamethoxazole + Trimethoprin | R | R | R | NA |
| Carbemicillin | 8-16 | 3-11 | 4-16 | 3-10 |
| Erythromycin | 6-14 | 4-10 | 8-12 | 4-10 |
| Polymyxin B | R | R | R | R |
| Kanamycin | 5- 8 | 1- 3 | 4- 9 | 1- 5 |
| Chloramphemicol | 2-12 | 1- 7 | 2- 5 | 1- 6 |
| Neomycin | 1- 3 | 1- 3 | 1- 3 | 1- 5 |
| Clindamycin | R | R | R | R |
| Gentamycin | 2- 4 | 2- 4 | 2- 4 | 1- 9 |
| Nalidixic acid | R | NA | R | NA |
| Ampicillin | 7-10 | 2- 7 | 4-12 | 3- 8 |
| Oxacillin | R | R | R | R |
| Colistin | R | R | R | R |
| Tetracycline | 10-21 | 14-21 | 10-24 | 12-24 |
| Rifampicin | NA | 2- 6 | NA | 1- 8 |

R* = resistance (no growth inhibition zone)
NA** = not available

suggested identifying a grouping of strains based on proximity given a particular zone diameter. If such a grouping is identified over a number of antibiotics under test, one would assume that the grouping may constitute one strain. For example, looking at raw data (data not shown) of B-isolates, isolate numbers 23, 6, 10, 3, 22, 20, 16 were almost always adjacent to one another and their MIC's were higher than other isolates over a number of antibiotics. The next step would have been to test the isolates for growth at different concentrations of a number of antibiotics to ascertain that growth of the isolates was similar. If there would appear to be homogeneity in growth response the isolates may perhaps be one strain. But even prior to this procedure, the dilemma would be in distinguishing a group of isolates as discussed above from another that could also be conceived by proximity. Thus it became clear that the use of sensitivity to antibacterial agents as natural markers in characterization of rhizobia strains was far from being definitive. Such results have been suggested (13). In fact, the ecological significance of antibacterial properties is doubtful in view of the ease with which microorganisms in the same environment decompose antibiotics (13). Greater reliability of identification has sometimes been achieved by typing strains on the basis of both bacteriocin production and antibiotic sensitivity (13).

Since it appears that strains within a species tend to be similar in most properties including sensitivity to antibiotics, mutants, which can be spontaneous or induced may provide one of the most reliable means of differentiation (13). For example the finding that all the isolates were resistant to nalidixic acid is significant in the sense that nalidixic acid has been used in selective media at varying concentrations to produce spontaneous mutants (13). Many studies have also used streptomycin (16). Antibiotic-resistant mutants are therefore valuable for laboratory and field experiments. The antibiotic aids in isolation and enrichment of the resistant strains (ie. most bacterial contaminants are eliminated during isolation), and in identification.

Plant Infection Test: Re-inoculation

Having obtained pure rhizobia isolates and characterized them, it was necessary to verify (Koch's postulates) that the isolates were indeed bean-*Rhizobium* and could be evaluated for nodulation and $N_2$ fixation activity.

The results of this exercise are shown in Table A.6. All the isolates inoculated on test plants exhibited $N_2$ fixing potential measured as percent acetylene ($C_2H_2$) reducted. B-isolate numbers 4, 8, 21, 9, and 18 were among the best in $C_2H_2$ reduction whereas M-isolate numbers 21, 4,

Table A.6 Ratio of acetylene reduced to ethylene produced expressed as percent.

| B-isolates | % $C_2H_2$ converted | M-isolates | % $C_2H_2$ converted |
|---|---|---|---|
| 1 | 0.05 | 9 | 0.10 |
| 11 | 0.05 | 18 | 0.10 |
| 5 | 0.08 | 25 | 0.10 |
| 14 | 0.10 | 11 | 0.15 |
| 16 | 0.20 | 6 | 0.16 |
| 20 | 0.31 | 1 | 0.20 |
| 23 | 0.38 | 3 | 0.20 |
| 7 | 0.40 | 5 | 0.20 |
| 17 | 0.57 | 16 | 0.20 |
| 19 | 0.67 | 20 | 0.20 |
| 2 | 0.65 | 14 | 0.30 |
| 10 | 0.65 | 28 | 0.38 |
| 6 | 0.90 | 22 | 0.47 |
| 24 | 1.00 | 17 | 0.56 |
| 18 | 1.35 | 13 | 0.60 |
| 3 | 1.90 | 19 | 0.66 |
| 9 | 2.10 | 2 | 0.68 |
| 21 | 3.04 | 26 | 0.76 |
| 8 | 3.33 | 29 | 0.80 |
| 4 | 3.50 | 7 | 0.90 |
| | | 10 | 1.03 |
| | | 24 | 1.05 |
| | | 12 | 1.28 |
| | | 27 | 1.57 |
| | | 4 | 2.20 |
| | | 21 | 3.00 |

27, and 10 were superior in $C_2H_2$ reduction. Among isolates there was no relationship between $C_2H_2$ reduction and either antibiotic sensitivity or carbon and nitrogen use.

While the decision as to whether a culture is a member of the genus Rhizobium does depend primarily on its nodulating and $N_2$ fixing ability neither distinguishes different isolates since nodulation and $N_2$ fixation represent an integration of host cultivar-Rhizobium compatibility, a characteristic that is also profoundly influenced by variation in host cultivar, Rhizobium strain, and environment.

In summary, it has been suggested that when rhizobia isolates that come from a related situation (same nodule, plant and immediate locality) are indistinguishable, for practical purposes, they can be regarded as one strain. A convenient characteristic that can include carbon and nitrogen sources or antibiotic sensitivity can foster strain identification of a large collection of rhizobia isolates since the characteristics used in this experiment by themselves or in combination have been inconclusive. Bacteriophage sensitivity, bacteriogenicity, lysogeny, gel electrophorersis, although unsuitable for screening large population of cells, have been used in strain identification. It is suggested in this paper that

nodulation and $N_2$ fixation in controlled environments (greenhouse, growth chamber) provide adequate presumptive characteristics for strain differences.

Finally, in the legume-Rhizobium system, the plant and the strain are two sources of variation (2). Frequently the legume bacteriologist is confronted with a plant that has already been selected and for which he must find a suitable strain thus leaving strain variability for exploitation (2). It has been suggested that the host may exercise a selective preference for effective strain(s) in a field containing a mixed population of effective and ineffective strains (15). Need for inoculation trials (3) may expose this observation and may provide an indication of the criteria to be used for Rhizobium strain-selection trials.

## LITERATURE CITED


1. Bushby, H. V. A. 1982. In Nitgrogen Fixation, Vol. 2: Rhizobium. W. J. Broughton (Ed.). Oxford University Press 2, 35-75.

2. Chowdhury, M. J. 1977. In Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent et al (Eds.). Univ. of Hawaii, Coll. of Trop. Agric. Misc. Publ. 145.

3. Date, R. A. 1977. In Biological Nitrogen Fixation in Farming Systems of the Tropics. A. Ayanaba and P. J. Dart (Eds.). John Wiley and Sons. 14, 169-180.

4. Davis, P. E. 1982. Legume microbiology research in Malawi. Overseas Development Agency. Lilongwe Malawi.

5. Edje, O. T., L. K. Mughogho, Y. P. Rao, and W. A. B. Msuku. Regional Workshop on Potential for Field Beans in Eastern Africa, Lilongwe, Malawi, 1980. Proceedings Cali, Cotombia, Centro Internacional de Agricultura Tropical, 1981.

6. Gibson, A. H. 1980. In Methods for Evaluating Biological Nitrogen Fixation. I. J. Bergersen (Ed.). John Wiley & Sons. N.Y. 4, 139-184.

7. Graham, P. H. 1981. Some problems of nodulation and symbiotic nitrogen fixation in Phaseolus vulgaris L.: A review. Field Crops Research. 4, 93-112.

8. Graham, P. H., and J. Halliday. 1977. In Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent et al (Eds.). University of Hawaii, Coll. of Trop. Agric. Misc. Publ. 145, 313-334.

9. Guirard, B. M., and E. E. Snell. 1981. In Manual of Methods for General Bacteriology. American Society of Microbiology. Washington, D.C.

10. Hagedorn, C., A. H. Ardahl, and L. A. Materon. 1983. Characteristics of Rhizobium trifolii populations associated with subclover in Mississippi soils. Soil Sci. Soc. Am. J. 47, 1148-1522.

11. Kremer, R. J., and H. L. Peterson. 1983. Field evaluation of selected Rhizobium in an improved legume inoculant. Agron. J. 75, 139-143.

12. Robert, F. M., and E. L. Schmidt. 1983. Population changes and persistence of Rhizobium phaseoli in soil and rhizospheres. Appl. Environ. Microbiol. 45, 550-556.

13. Schwinghamer, E. A., and W. F. Dudman. 1980. In Methods for Evaluating Biological Nitrogen Fixation. F. J. Bergersen (Ed.). John Wiley and Sons, NY. 9, 337-366.

14. Vincent, J. M. 1970. A manual for the practical study of the root-nodule bacteria. IBP Handbook No. 15. Blackwell Scientific Publications.

15. Vincent, J. M., A. S. Whitney and J. Bose. 1977. In Exploiting the Legume-Rhizobium Symbiosis in Tropical Agriculture. J. M. Vincent et al (Eds.). University of Hawaii. Coll. of Trop. Agric. Misc. Publ. 145.

16. Weistreich, G. A. and M. D. Lechtman. 1984. Laboratory Exercises in Microbiology. Macmillan Publishing Company. New York.

17. Wollum, A. G., II, and D. K. Cassel. 1984. Spatial variability of Rhizobium japonicum in two North Carolina soils. Soil Sci. Soc. Am. J. 48, 1082-1086.

18. Wright, E. R. 1984. General Microbiology, Laboratory Manual. Times Mirror/Mosby College Publishing. St. Louis.

# APPENDIX B

Figure B.1 Field map showing number of plants that survived corn seed maggott damage in 2 x 3 metre net plots at Montcalm, Summer 1985.

| Replicate I | | | | Replicate II | | | |
|---|---|---|---|---|---|---|---|
| $N_oP_1$ | $N_oP_o$ | $N_1P_1$ | $N_1P_o$ | $N_1P_o$ | $N_1P_1$ | $N_oP_1$ | $N_oP_o$ |
| 5 (112)* | 14 (124) | 17 (136) | 10 (148) | 17 (212) | 14 (224) | 6 (236) | 10 (248) |
| 22 | 17 | 17 | 10 | 20 | 19 | 8 | 14 |
| 19 | 10 | 14 | 13 | 4 | 6 | 13 | 12 |
| 7 | 5 | 7 | 14 | 8 | 11 | 6 | 7 |
| 7 | 5 | 5 | 12 | 5 | 9 | 10 | 9 |
| 7 | 9 | 7 | 10 | 6 | 6 | 12 | 6 |
| 10 | 16 | 13 | 6 | 6 | 11 | 12 | 7 |
| 11 | 4 | 9 | 8 | 6 | 5 | 10 | 13 |
| 9 | 8 | 6 | 16 | 5 | 6 | 5 | 11 |
| 5 | 7 | 6 | 12 | 6 | 9 | 5 | 5 |
| 9 | 9 | 13 | 16 | 8 | 6 | 7 | 6 |
| 7 (101) | 9 (113) | 15 (125) | 6 (137) | 14 (201) | 8 (213) | 13 (225) | 10 (237) |

*Plot numbers are given only for the beginning and end of the main plots.

Table B.1 Notations and Abbreviations

Statistical

* = significance at 0.10 probability level
** = significance at 0.01 probability level
*** = significance at 0.001 probability level

AOV = analysis of variance
% CV = coefficient of variation, percent
d.o.f. = degree of freedom
DMR = Duncan's Multiple Range test quoted at alpha level = 0.05
n.s. = not significant

Others

C or cvr = cultivar
I = inoculation
N = nitrogen
P = phosphorous
yld = yield
wt = dry weight (g)
DAP = days after planting
# = number(s)

Table B.2 Correlation coefficient ($r^2$) of some plant characteristics of 12 bean landraces planted at Montcalm, Summer, 1985.

| | root | steam | leaf | pod wt. | total wt. | leaf: stem ratio | shoot: root ratio | % N | % P | N:P ratio | total wt./gN | total wt./gP | seed yld. | biological yld. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| stem wt. | .40*** | | | | | | | | | | | | | |
| leaf wt. | .44*** | .82*** | | | | | | | | | | | | |
| pod wt. | n.s. | .37*** | .37*** | | | n.s. | | | | | | | | |
| total wt. | .32*** | .80*** | .81*** | | | -.25* | .39** | | | | | | | |
| leaf:stem ratio | n.s. | -.55*** | n.s. | | | | | | | | | | | |
| shoot:root ratio | -.66*** | .18* | n.s. | .50*** | | | | | | | | | | |
| % N | n.s. | .20* | n.s. | -.39*** | n.s. | -.37*** | -.37*** | | .64*** | | | | | |
| % P | n.s. | .34** | .20* | n.s. | n.s. | -.37** | -.18* | | | | | | | |
| N:P ratio | n.s. | -.22* | -.19* | | -.30** | n.s. | n.s. | .21* | -.60*** | | -.50*** | -.20* | | |
| total wt./gN | .45*** | .87*** | .79*** | .63*** | .90*** | -.35*** | .21* | .28** | .38*** | -.20* | | | | |
| total wt./gP | .38*** | .82*** | .77*** | .67*** | ..90*** | 0.35*** | .26* | n.s. | .52*** | -.50*** | | | | |
| seed wt./gN | n.s. | n.s. | n.s. | .38*** | .32*** | n.s. | .19* | n.s. | n.s. | n.s. | .27** | .27** | | |
| seed wt./gP | n.s. | .19* | .24* | .45*** | .40*** | n.s. | .22* | n.s. | n.s. | -.33*** | .31** | .40** | | |
| seed yld. | n.s. | n.s. | n.s. | n.s. | .37*** | n.s. | .28** | -.34** | -.19* | n.s. | .18* | .22* | | .37** |
| biological yld | n.s. | .41*** | .31** | | .40*** | .23* | .27** | n.s. | .20* | -.20* | .38*** | .44*** | | |
| harvest index | n.s. | -.40*** | -.27** | n.s. | n.s. | .33** | n.s. | | | | .26** | -.25** | .49*** | -.50*** |

Table B.3 Analysis of variance of morphological components at 45 DAP.

| Source of variation | d.o.f. | MEAN SQUARES FOR | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | root wt. | leaf wt. | stem wt. | pod wt. | total wt. | leaf:stem ratio | shoot:root ratio |
| Rep | 1 | 2.214 | 202.885 | 61.440 | 813.170 | 2712.909 | 0.030 | 1867.762 |
| N | 1 | 0.004 | 407.303 | 13.128 | 8.882 | 714.277 | 0.575 | 267.286 |
| P | 1 | 1.080* | 247.491 | 232.815 | 426.727 | 2775.866 | 0.114 | 32.465 |
| NP | 1 | 0.313 | 15.649 | 0.350 | 432.226 | 556.710 | 0.008 | 908.935 |
| Error (a) | 3 | 0.144 | 134.700 | 93.772 | 628.656 | 1727.921 | 0.029 | 353.929 |
| C | 11 | 1.513*** | 395.847*** | 489.230*** | 1727.104*** | 4018.429** | 0.420*** | 4719.914*** |
| NC | 11 | 0.512 | 114.822 | 75.211 | 217.656 | 604.202 | 0.169 | 402.569 |
| PC | 11 | 0.563 | 39.302 | 78.286 | 194.761 | 676.581 | 0.159 | 1300.409 |
| NPC | 11 | 0.121 | 174.046 | 126.248* | 399.720 | 1717.454* | 0.086 | 784.310 |
| Error (b) | 44 | 0.366 | 110.782 | 57.619 | 298.696 | 896.453 | 0.098 | 576.908 |
| %CV | | 33.19 | 30.23 | 30.37 | 29.910 | 25.07 | 21.02 | 32.78 |

Table B.4 Analysis of variance of morphological components expressed as % of total plant dry weight at 45 DAP

| Source of variation | d.o.f. | MEAN SQUARES FOR root wt. | stem wt. | leaf wt. | pod wt. |
|---|---|---|---|---|---|
| Rep | | 2.214 | 61.440 | 202.885 | 44.514 |
| N | | 0.236 | 4.287 | 120.822 | 71.157 |
| P | | 0.019 | 23.903 | 5.565 | 54.548 |
| NP | | 0.118 | 4.999 | 13.241 | 38.668 |
| Error (a) | | 0.199 | 4.005 | 25.301 | 50.916 |
| C | | 1.588*** | 206.424** | 123.430*** | 622.718*** |
| NC | | 0.215 | 21.391** | 41.161 | 96.164 |
| PC | | 0.300 | 14.473* | 14.771 | 19.498 |
| NPC | | 0.267 | 5.897 | 24.217 | 32.334 |
| Error (b) | | 0.223 | 7.870 | 26.820 | 39.631 |
| % CV | | 29.54 | 13.57 | 17.68 | 13.00 |

Table B.5 Analysis of variance of nutrient analyses at 45 DAP.

| | | MEAN SQUARES FOR | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Source variation | d.o.f. | %N | %P | N:P ratio | total wt. /gP | total wt. /gN | seed wt. /gP | seed wt. /gN |
| Rep | 1 | 0.00 | .007 | 8.746 | 0.066 | 1.745 | 0.002 | 0.003 |
| N | 1 | .040 | .002 | 0.400 | 0.019 | 0.638 | 0.000 | 0.000 |
| P | 1 | .073 | .003 | 0.970 | 0.047* | 2.702* | 0.003 | 0.149* |
| NP | 1 | .025 | .001 | 0.473 | 0.000 | 0.036 | 0.004 | 0.199* |
| Error (a) | 3 | .053 | .001 | 0.943 | 0.008 | 0.423 | 0.001 | 0.012 |
| C | 11 | .335*** | .004*** | 2.384*** | 0.033*** | 1.533** | 0.007*** | 0.395*** |
| NC | 11 | .061 | .001 | 0.505 | 0.006 | 0.477 | 0.003*** | 0.214*** |
| PC | 11 | .048 | .001* | 1.217 | 0.006 | 0.453 | 0.001*** | 0.063** |
| NPC | 11 | .036 | .001 | 0.403 | 0.014** | 0.928 | 0.004*** | 0.270*** |
| Error (b) | 44 | .052 | .001 | 1.092 | 0.008 | 0.507 | 0.000 | 0.022 |
| %CV | | 10.32 | 12.73 | 11.83 | 29.64 | 27.16 | 17.11 | 17.41 |

Table B.6 Analysis of Variance of yield and harvest index.

| Source of variation | d.o.f. | MEAN SQUARES FOR seed yld. /plant | 100-seed wt. | biological yld. | harvest index. |
|---|---|---|---|---|---|
| Rep | 1 | 0.893 | 9.856 | 104.855 | 0.014 |
| N | 1 | 5.731 | 40.560* | 95.44 | 0.000 |
| P | 1 | 91.857 | 8.556 | 764.841 | 0.002 |
| NP | 1 | 275.384* | 58.004* | 1859.000 | 0.004 |
| Error(a) | 3 | 32.772 | 5.500 | 1768.589 | 0.024 |
| C | 11 | 1413.952*** | 683.326** | 4799.190*** | 0.125*** |
| NC | 11 | 399.554*** | 8.541 | 1379.937* | 0.010 |
| PC | 11 | 132.512*** | 10.914 | 1052.117 | 0.011 |
| NPC | 11 | 565.190*** | 16.960* | 3801.434*** | 0.026 |
| Error (B) | 44 | 28.258 | 8.829 | 805.600 | 0.016 |
| %CV | | 13.58 | 8.46 | 29.39 | 28.56 |