GENOTYPIC VARIATION IN PHOSPHORUS REQUIREMENT AND UTILIZATION IN NODULATED COMMON BEANS (*PHASEOLUS VULGARIS* L.)

by

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ABSTRACT

A study to investigate the genotypic variations in phosphorus requirement and utilization by four common bean genotypes AA/2/5/6xK-2; 86EP 5091-B-2; EP 3-2 and Selian wonder, referred to as C_2, C_4, C_5 and C_8 respectively, inoculated with a locally produced Rhizobium strain, PV1, was carried out at the Sokoine University of Agriculture, Morogoro, Tanzania in a glasshouse pot experiment. The soil used as the growth medium was an Oxic Haplustult with low levels of total nitrogen and Bray-1 extractable phosphorus with a slight acid reaction. The levels of phosphorus applied were 0, 5, 10, 20, 40, 80, 120 and 160mgP/kg soil as KH_2PO_4 and these rates were each replicated six times for each common bean genotype.

The increases in the number of nodules, (hence nodulation), nodule weights, shoot dry weights, root dry weights, pod production, seed production, percent N in the shoots and percent P in the shoots with increasing rates of applied phosphorus were significant and these plant parameters were positively and significantly correlated to one another, suggesting the vital role of phosphorus in symbiotic nitrogen fixation.

The variations in phosphorus requirement and utilization based on the above plant parameters were attributed to the inherent genetic characteristics of the four common bean genotypes. The genotypes most tolerant to low levels of Bray-1 extractable phosphorus fixed more nitrogen symbiotically and accumulated more dry matter. The genotypes' tolerance to low levels of extractable phosphorus followed the order $C_5 > C_4 > C_2 > C_8$. The different phosphorus rates for optimum yields and symbiotic nitrogen fixation for each individual common bean genotype should be established in a similar investigation under field conditions. The ability of the genotypes to form nodules hence fix nitrogen, when inoculated with the Rhizobium strain PV1 indicate the ability of the Rhizobium strain PV1 to tolerate low levels of Bray-1 extractable phosphorus. Based on the results obtained in this study, the genotypes C_5 and C_4 inoculated with Rhizobium strain PV1 can be grown in soils deficient in available phosphorus without any effect on reducing seed yield.

DECLARATION

I, DOROTHY JOHN SIMON, OLE-MEILUDIE, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my original work and that it has neither been submitted nor concurrently being submitted for a degree in any other University.

Date: 31 July 1992

Signature: D.J. Meiludie

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CHAPTER ONE

INTRODUCTION

In most of the developing countries, the population growth rates are very high while food production is very low leading to food shortages and in some countries, starvation and famine. In order to satisfy the food requirements and needs of the ever increasing population and therefore ridding them of malnutrition, hunger and famine, there is need to chart out medium and long term agricultural strategies aimed at increasing food production. Among the strategies would include the adoption and application of modern and appropriate agricultural practices, like the use of fertilizers, growing seeds selected for high yields and resistant to disease and pest attacks, proper crop husbandry practices and the provision of good post-harvest crop storage facilities.

The diets of the majority of the population in the developing countries are composed of cereals like maize, rice, sorghum and wheat and pulses like beans, peas, cowpeas, green-gram and pigeon peas, just to mention a few. In Tanzania, pods or leaves of the common beans are included in many peoples' diet together with either rice, maize, cassava, bananas and other carbohydrate and starchy foods. Bean protein is rich in lysine and tryptophane which are deficient in the cereals. Therefore, inclusion of beans in the cereal diets improves the nutritional value of the diets.

In East Africa, Tanzania inclusive, beans are widely cultivated, but the yields are very low and as a consequence demand is far much higher than supply (CIAT, 1980; Grisley, 1990). In East Africa, for example, common bean yields range from 220-670 kg/ha compared to the expected yields of 1,100 kg/ha when the crop is well managed (Acland, 1971; Karel et al., 1980). The observed low yields of the common beans have been attributed to the variability among bean genotypes in nitrogen fixation (McFerson et al., 1981) genetic variability in ion absorption from soils, low levels of plant nutrients in the soils, disease and insect susceptibility, susceptibility to stress like drought and heat and poor crop management practices. Common bean yields could, therefore, be substantially increased if all the above are given due consideration.

In Tanzania beans are mainly cultivated by small scale farmers at subsistence level and most of the farmers intercrop the beans with other crops like maize, sorghum and other cereals, vegetables and even with perennial tree crops like coffee and fruit trees leading to very low yields per unit area. In some of the bean growing areas, the soils are highly weathered and therefore deficient in most of the essential plant nutrients (Sanchez, 1976) hence the observed low yields of beans. Bean yields in such areas could, therefore, be improved by applying the appropriate fertilizers.

Although common beans are able to fix nitrogen, it has been reported that they are generally poor nitrogen fixers as compared to, for example, soybeans and cowpeas (Graham, 1981; Graham and Temple, 1984; Pereira and Bliss, 1989). The poor nitrogen fixing capability by common bean is attributed to ineffective symbiotic association between the bean and the native *Rhizobium* strains, and limited availability of plant nutrients particularly phosphorus. Common bean yields could, therefore, be increased by growing common bean genotypes which have high nitrogen fixing capabilities. For example, high yields could be obtained by growing the climbing and late maturing types of the common bean which have demonstrated the abilities to fix more nitrogen as compared to the determinate types (McFerson *et al*., 1981). The symbiotic biological nitrogen fixation by the common bean genotypes could also be improved by inoculating them with effective and compatible *Rhizobium* strains.

Phosphorus has been reported to be one of the yield limiting factors in common bean production in most of the bean producing areas (Dale *et al*., 1977; Pereira and Bliss, 1987; 1989). Phosphorus is responsible for root growth stimulation in plants. However, some bean genotypes and strains of *Rhizobium* have shown the ability to fix nitrogen symbiotically at low levels of available phosphorus. Inoculating the above bean genotypes with the appropriate *Rhizobium* strains would result in improved common bean yields and also reduce the amounts of phosphate fertilizers to be used in bean production. In Tanzania, however, limited studies have been undertaken in this area (Mahatanya, 1977; Urio, 1984) with no conclusive results on the effects of phosphorus on nodulation, symbiotic nitrogen fixation and dry matter yields of the common bean.

The objectives of this study were:-

(a) to determine the variations in phosphorus requirement and utilization of four common bean genotypes inoculated with *Rhizobium*, hence response to phosphorus application;

(b) to determine the levels of available phosphorus at which each one of the four genotypes would give the optimum yields;

(c) to identify, among the four bean genotypes the one(s) which have the ability to fix nitrogen at low levels of available soil phosphorus; and

(d) to determine the overall effect of phosphorus on nitrogen fixation by the four bean genotypes.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction.

Common bean (Phaseolus vulgaris L.) is an annual plant which belongs to the family Leguminosae. The common bean is widely grown in Tanzania and other various countries in the world. The common bean is a very important crop in the areas where the major dietary energy sources are the cereals, starchy roots and tubers because of its high total protein content. Like all other legumes, the common bean protein contains relatively more of the essential amino acids, lysine, and tryptophane which are useful supplements of the amino acids supplied by cereals (Purseglove, 1969; Cobley and Steele, 1976). The proteins from beans further contain appreciable proportions of the sulphur-containing amino acids, methionine and cysteine. They also contain niacin and substantial amounts of calcium (Cobley and Steele, 1976). In Tanzania, apart from its direct contribution to the quality of the human diet, the beans constitute one of the export crops (Ministry of Agriculture, Tanzania, Report 1977/1978).

In East Africa, it has been observed that the yields of common beans per unit area are very low compared to the other countries in the world where the crop is grown. For example, Acland (1971) and Karel et al. (1980) observed that the bean yields in East Africa ranged from 220 to 1000 kg/ha while in other countries it ranged from 1000 to 4000 kg/ha. However, in some parts of East Africa where improved common bean varieties have been grown with good crop husbandry practices coupled with appropriate disease and pest control measures, yields of up to 1100 to 3000kg/ha have been recorded (Acland, 1971; Karel, 1985). In 1977, East African countries produced about 4.1% of the world's total bean production of 102milliom metric tons (Karanja and Wood, 1988). The low yields of beans recorded in Tanzania and in other developing countries could be attributed to several factors. Some of the factors include genetic variability in ion absorption from the soils, low levels of plant nutrients in the soils, susceptibility to plant diseases, pests, drought and heat and poor crop management practices. Higher bean yields could, therefore, be realized in the developing countries if the above constraints are given due consideration.

2.2 Symbiotic nitrogen fixation by the common bean.

Nitrogen fixation is the biological conversion of the atmospheric nitrogen gas (N_2) into various organic compounds (amino acids or amides), which can eventually turn into proteins or other nitrogen-containing macromolecules and be assimilated by higher plants as NH_4^+ or

NH_3. It is a reductive reaction which requires the input of biochemical energy and is catalysed by only a few members of prokaryotic organisms. Nitrogen fixation in common beans occurs as a result of association of the bacteria of the genus Rhizobium with the bean roots in most agricultural soils. The nitrogen fixing bacteria (also called microsymbiont) lives in the rhizosphere and at the surface of the plant roots. The growth and multiplication of the nitrogen fixing bacteria are favoured by the slime excreted by the cells of the root caps of the bean plants (also called macrosymbiont) while the bacteria supply fixed nitrogen to the plants (Grant and Long, 1981). This association is therefore, mutually beneficial, but it is very specific due to the fact that the bacterium strain responsible for nitrogen fixation in a given legume will not be equally effective in infecting other legumes (Grant and Long, 1981; Brady, 1984; Duque et al., 1985; Chowdhury, 1982; Bliss, 1987; Vencatasamy, 1985). The reason behind this is that the ability of a legume to nodulate and fix nitrogen is, among other things, genetically controlled (Vencatasamy, 1985). The appropriate strain of rhizobia may be present in the soil where a given legume has been growing regularly (Whiteaker et al., 1976).

Phaseolus vulgaris is infected by Rhizobium phaseoli (Mengel and Kirkby, 1987; Brady, 1984; Erdman, 1959). The bacterium infects the plant root hair and cortical cells of the host plant. The infection is achieved by reactions of the sugar binding proteins present in the plasmalemma of the root cell wall of the *Rhizobium* (Mengel and Kirkby, 1987; Rosenberg and Cohen, 1983). The infected cells grow and thereafter develop to bacteroides which contain nitrogenase, an enzyme responsible for atmospheric nitrogen fixation (Grant and Long, 1981). If the nodules formed are effective then nitrogen will be fixed (Grant and Long, 1981; Mengel and Kirkby, 1987). With the formation of effective nodules the common bean would have the potential to fix atmospheric nitrogen symbiotically to meet its requirements and sometimes those of cereal crops grown together in alternative cropping seasons (Foy *et al*., 1967) and in the same season through the excretion of the fixed nitrogen by the bean roots to the soil. Symbiotic nitrogen fixation efficiency is influenced by the factors which may affect the plant-nitrogen fixing *Rhizobium* association. Erdman (1959) and Bliss (1987) reported that the amount of nitrogen fixed by legumes symbiotically differs with *Rhizobia* strain, the host plant and the environmental conditions under which the two develop.

Although common beans are able to fix atmospheric nitrogen symbiotically, it has been observed that *P. vulgaris* is a poor nitrogen fixer (Graham and Temple, 1984; Piha and Munns, 1987). Piha and Munns (1987) observed that beans formed smaller and more numerous nodules than both soybean and cowpea, and hence lower nitrogen accumulation in beans compared to soybean and cowpea. This indicated lower nitrogen fixation in bean than in cowpea and soybean (Piha and Munns, 1987). The amount of nitrogen accumulated by a legume has been found (Piha and Munns, 1987) to be one of the factors which affect the amount of nitrogen fixed. For example, nitrogen accumulation in bean was found to be 56% to 78% while in the other legumes tested, soybean and cowpea, the average was found to be about 95% (Piha and Munns, 1987). The poor nitrogen fixation ability observed in beans in the field has been considered to be caused by the failure of the beans to establish effective nodulation due to inefficient symbiotic relationship between the common bean roots and the native Rhizobia strain in the soil (Piha and Munns, 1987). Further, Mengel and Kirkby (1987) and Brady (1984) argued that the poor nitrogen fixation characteristic in common bean was due to the low population of the natural rhizobia which then become inadequate to produce the adequate infection of the bean roots. In some cases it has been found that the strain of the Rhizobium species present in the soil, although in a reasonably high population was not effective in stimulating effective nodulation once the nodules have been formed (Urio, 1984, Piha and Munns, 1987; Mengel and Kirkby, 1987). This could also be attributed to undesirable soil and other environmental characteristics. Effective nodulation may be attained by treating the bean seeds with special cultures of rhizobia either by coating or inoculating them by direct application of the inoculant into the soil (Brady, 1984).

Piha and Munns (1987) found that one of the reasons for the modern bean cultivars failure to establish effective nodules in agricultural soil environment was a function of their genetic composition and high nitrogen evolution. For example, the nitrogen evolution in bean has been reported to range from 0.5 to 0.7 while in the other legumes tested, which were cowpea and soybean, it was found to be 0.95 for both of them (Piha and Munns, 1987). Cowpeas and beans sown on the same day were found to accumulate different amounts of nitrogen during their nitrogen fixation period (Piha and Munns, 1987). Hydrogen evolution is another factor that contributes to variations in the amount of nitrogen fixed by legumes. For example, evolution of hydrogen in cowpeas has been found to be less than in beans, hence faster accumulation of nitrogen and therefore higher fixation (Piha and Munns, 1987). Similar results were obtained by Cossman, et al.(1981a). The reasons for these observations were found to be due to competition for photosynthate between nodules and pods shortly after flowering in beans and the shorter period for nitrogen fixation in cowpeas hence more total nitrogen accumulation than in the beans (Piha and Munns, 1987). In addition, beans matured earlier than cowpea hence there was a shorter period during which photosynthate was available for nitrogen fixation without competition from nodule formation and pod filling (Piha and Munns, 1987).

Earlier work by Truog et al.(1947) revealed that phosphate nutrition in bean production was important especially during nodule formation period. Furthermore, Dale et al. (1977) and Pereira and Bliss (1987; 1989) observed that phosphorus is one of the factors which affect nitrogen fixation in beans. Apart from nitrogen, phosphorus is the principal bean yield limiting factor in most of the bean producing areas. The legume-rhizobia associations generally function best on soils that are not too acidic (below pH 5.0) and well supplied with phosphorus (Brady, 1984; Mengel and Kirkby, 1987). In acid soils, the toxicity resulting from Fe, Al and H affect both the growth and development of the microsymbiont and macrosymbiont populations (Alexander, 1961). Further, in acid soils Ca deficiency diminishes the development of nodules, resulting into reduced weight of nodules (Alexander, 1961). In soils deficient in phosphorus, bean growth is hampered (Dale et al., 1977; Brady, 1984; Saidou, 1985). The rate of symbiotic nitrogen fixation is further affected by climatic conditions and also the available nitrogen in the soil (Piha and Munns, 1987; Brady, 1984). Nodule weight and nodule numbers are diminished at relatively high nitrate or ammonium levels, but low concentrations of inorganic nitrogen salts often enhance nodulation (Alexander, 1961). Water stress has been found to be one of the major factors limiting nitrogen fixation in experimental bean fields with various bean cultivars. Franco et al. (1979) observed that nitrate reductase activity was lowest during peak nitrogen fixation but increased again following the decline in nitrogen fixation due to low percent of effective nodules and nitrogenase activity in relation to water stress. This means that due to differences in agronomic features of various bean genotypes there are genotypic variations in water absorption ability.

Bean genotype influences symbiotic nitrogen fixation due to various characteristics of a particular genotype (Duque et al., 1982; Whiteaker et al., 1976). Some of the factors include the differences existing in the activity of nitrate reductase and efficiency in nitrogen and phosphorus utilization, variation in nitrogenase activity and amount of carbohydrate accumulated in the plants (Franco et al., 1979; Pereira and Bliss, 1987;1989). Graham and Rosas (1977) observed that the determinate bush beans fix less nitrogen than the indeterminate prostrate lines, while the highest fixers are the climbing ones.

Likewise the late maturing bean genotypes have the ability to fix more nitrogen symbiotically than the early maturing ones (Graham and Rosas, 1977).

It has been observed that phosphorus is essential in the nitrogen fixation by common beans and by other legumes (Leif, 1990). In some parts of the tropics nitrogen fixation by common bean is limited by the very low concentration of phosphate in the soil solutions. Further the immobility of phosphate applied to soils in the form of fertilizers and the inherent limited ability of most common bean cultivars to absorb phosphate ions from the soil solution further limits the amounts of nitrogen fixed by the common beans. However, some genotypes have been reported to have the ability to fix nitrogen symbiotically at low levels of available phosphate in soils (Whiteaker _et al_., 1976). The above characteristic has partly attributed to the ability of some common bean genotypes' roots to grow extensively and spread to large volumes of soil from which they can absorb more of the available phosphorus and partly due to the genetic make-up and physiological and metabolic phosphate requirements of a given bean genotype (Whiteaker, _et al_., 1976; Leif, 1990). Also the differences existing in the efficiency of utilization of the absorbed phosphorus in different genotypes influence the amount of nitrogen fixed (Leif, 1990; Duque, _et al_., 1982; Shea _et al_., 1968). The density of roots and the volume of the soil that the roots occupy have been observed to be the main factors affecting phosphorus uptake (Leif, 1990). Furthermore, the large fine roots and mycorrhizae also increase P uptake in beans (Leif, 1990). It was established that the observed genotypic variations in phosphorus utilization are a result of differences in extent of three factors as they occur in each genotype (Leif, 1990). The factors include; translocation of the absorbed nutrients from the roots to the growing tissues, remobilization of nutrients out of senescencing tissues such as the leaves, and lack of balanced reproductive developments so that as much of the absorbed nutrient as possible is utilized for grain production.

In Tanzania the soil natural Rhizobia for nitrogen fixation in beans has been reported not to be very effective hence there is a need for inoculating the beans to enhance symbiotic nitrogen fixation and therefore increased bean yield (Urio, 1984). Inoculating beans with a Rhizobium strain has been observed to increase the rate of nitrogen fixation (CIAT, 1977). In a crop cycle of 100-130 days the rate of nitrogen fixation in P. vulgaris was observed to vary from 25 to 71 kg N fixed per hectare (CIAT, 1977).

2.3 Phosphate content in soils and its availability.

The total phosphorus content of a mineral soil is in the ranges of 0.02 to 0.5% P (Mengel and Kirkby, 1987; Sanchez, 1976; Lindsay and Vlek, 1977). Within the soil, the phosphorus can be considered to be in three fractions, namely the organic fraction, the inorganic fraction and a small, very variable part that is the soluble portion which can be absorbed by plants during their periods of growth. This is the fraction referred to as the plant available phosphorus (Barber, 1984). The relative contents of these fractions are variable in different soils. The concentration of phosphorus present in the soil solution even in soils with fairly high level of available phosphate is in the range of 0.3 to 3.0 kg P/ha (Mengel and Kirkby, 1987).

Apart from its low concentration in the soil solution phosphorus moves very slowly in the soil mostly by diffusion (Olsen et al., 1962). Phosphorus tends to accumulate close to the surface while a relatively small proportion penetrates to the subsoil where most of the soil water is stored (Olsen et al., 1962; Sanchez, 1976). The amount of P in the soil solution is low and hence its availability in various soils is often deficient for the growth of plants. Phosphorus is not recycled in rainfall nor readily released from organic residues hence the phosphate deficiency in soils can generally be corrected by applying phosphatic fertilizers.

The availability of phosphorus to plants is governed by the intensity, quantity, capacity rate and diffusion factors of the soil phosphorus (Hagin and Tucker, 1982; Brady, 1984; Ozanne, 1980). The concentration of phosphorus in the soil solution is measured by the intensity (I) of the soil phosphorus. Hagin and Tucker (1982) and Brady (1984) suggested that for maximum yield of most crops, P intensity has to be maintained at about 0.2 ppm or above. Plants utilize phosphorus from the soil solution, and when the amount contained in the soil solution is depleted it has to be replenished from the soluble solid phosphate fraction held on the soil particles surfaces to bring about an equilibrium with the soil solution phosphate. This source of solution replenishment is known as the quantity (Q) of phosphorus nutrition (Hagin and Tucker, 1982; Sanchez, 1976; Vincent, 1965; Brady, 1984). This is the slowly available form of phosphorus which is freshly precipitated as Fe, Al, Mn and Ca compounds and surface-absorbed phosphates that have not yet penetrated the particles on which they are held. Soils high in Fe and Al need relatively high (Q) level to maintain an intensity (I) level sufficient for normal plant growth. The quantity of this fraction in the top soil of most soils ranges from 150 to 500kg P/ha (Hagin and Tucker, 1982; Olsen and Watanabe, 1970). The rate of absorption of this fraction is governed by its phosphorus buffer capacity (Olsen and Watanabe, 1970; Hagin and Tucker, 1982). Olsen and Watanabe (1970) and Brady (1984) stated that the potential buffering capacity of a soil is a function of the change in (Q) to that of the change in (I) expressed as: phosphate buffer capacity (pbc) =

$$\Delta(Q)/\Delta(I)$$

where, $\Delta(Q)$ = the change in the quantity (Q),

$\Delta(I)$ = the change in the intensity (I)

Olsen and Watanabe (1970) found that the phosphate concentration of a soil and its phosphate buffering capacity are the factors which control the phosphate supply to plant roots.

2.4 Phosphate requirement by beans.

Phosphorus is important for leguminous plants because of its influence on the activity of nitrogen fixing bacteria. Sanchez (1976) observed that at low yields of about 1 ton/ha, beans require about 3.5kg of phosphorus. The critical levels of phosphorus in bean plant varies with the species, organ and age of the plant. Critical levels of phosphorus in the bean leaves were found to range from 0.31% to 0.4% (Qureshi, 1981; CIAT, 1977). Peck (1975) observed P content in mature trifoliate snap bean leaf to range between 0.3% to 0.50%.

Ozanne (1980) observed that if the concentration of phosphorus in the plant tissues approach the sufficiency level, there is very little chance that crops would respond to any additional P supply. It was generalized that in bean leaves or petioles, phosphorus concentration as low as 0.3% P approaches the critical level while 0.5% P is higher than the amount required for maximum yield. Amount of phosphorus that the whole plant can take up has been found to be 7.8kg P/ha while 4.8kg P/ha were found in the pods and seeds only (USDA, 1976). On the other hand total P in pods and seeds has been found to be 0.48% P by dry weight (USDA, 1976). A concentration of P in the soil solution is associated with a certain plant species maximum growth and yield. Fox et al. (1971 ; 1974) found that maximum growth of bean in oxisol and sandy soils could be attained when the soil solution in an oxisol was 0.07ppm P and 0.2ppm P in sandy soils.

Apart from the role of phosphorus in nitrogen fixation in bean plants, phosphorus is important to the plants physiological processes and other vital functions. Phosphorus is important in root development especially the laterals and fibrous rootlets, cell division, flowering and seed formation and energy transformation and transfers. It also hastens maturation thereby terminating excessive vegetative growth due to high nitrogen uptake (Vincent, 1965; Brady, 1984). Phosphorus is also important in improving the quality of the products especially that of the leaves and pods and seeds (Vincent, 1965; Greenwood et al., 1974). It has also been reported that phosphorus fertilization hastens disease resistance and seedling vigour in most plants (Draycott, 1972).

Bean genotypes differ in their ability to tolerate phosphorus stress levels (Whiteaker et al., 1976; Pereira and Bliss, 1987; Cossman et al., 1981b; Lynes, 1936, Graham and Rosas, 1979; Ssali and Keya, 1985). It has been established that if a mineral nutrient is a factor limiting in the soil, a plant or microbial population in the soil may become progressively adapted to it and become efficient in the biological use of it due to development of various adaptive mutants (Lyness, 1936; Bernard and Howell, 1964; Gerloff, 1963; Vose, 1963). The adapted nutrient use efficiency may furthermore result to a genetically diverse efficiency for that mineral nutrient within various species of that soil population and the diversity becomes heritable (Bernard and Howell, 1964; Shea et al., 1967; Smith, 1934). One of the factors which enable bean genotypes to tolerate low levels of available phosphorus could, therefore, be the development of adaptive mutants. Likewise the nitrogen fixing Rhizobium strain for such host plants may have developed adaptive mutants for low level of phosphorus tolerance. It has been found that, if bean genotypes which are able to tolerate low levels of soil available phosphorus are inoculated with the Rhizobium strain that is effective in nitrogen fixation and able to tolerate stress P levels, the need for adding nitrogenous and phosphatic fertilizers in bean will be reduced (Duque et al.,1985; Ssali and Keya, 1983; 1985). For example, the climbing cultivar of P. vulgaris when inoculated with Rhizobium strain CIAT 1057 was then able to fix 40kg N/ha giving 50% greater yield than when cultivated without any inoculation (CIAT, 1980).

2.5 Role of phosphorus in symbiotic nitrogen fixation by the common bean.

Phosphorus is responsible for root growth, stimulation and initiation of nodules in the process of nitrogen fixation by common bean (Mengel and Kirkby, 1987; Brady, 1984; Sanchez, 1976). The bean plant must therefore be well supplied with phosphates so that they can be able to fix nitrogen symbiotically with Rhizobium. Phosphate affects the number and mass of nodules per plant, the duration of efficiency for the rhizobium-legume symbiotic association and consequently the amount of nitrogen fixed by a particular variety at a given period (Brady, 1984).

Maximization of nitrogen fixation and yields in beans is greatly hampered by phosphorus deficiency (Dale et al., 1977; Saidou, 1985). After the infection of the bean plant roots by Rhizobium, phosphorus is required by the plant to continue with its growth as well as multiplication of the

Rhizobium. Thereafter more phosphorus is required for cell division, nodule initiation and development, flowering, pod filling, seed formation and maturation (Vincent, 1965; Brady, 1984). Phosphorus therefore, determines the amount of nitrogen fixed symbiotically by a particular bean genotype at a given period. The amount of nitrogen that can be fixed per year had been found to be 30-50kg N/ha (Brady, 1984; Burris, 1981). For each mole of atmospheric nitrogen reduced to NH_3 twelve (12) moles of ATP are required (Burris, 1981).

2.5.1 Effect of various phosphorus levels to bean yield.

Bean genotypes respond variably to different levels of phosphorus applied to them. Haag et al. (1977) observed significant differences in responses of 124 bean genotypes to two levels of phosphorus when evaluated in glass house conditions. There has been observed a significant increase in seed yield, number of pods per plant and number of seeds per pod and weight of a single seed at higher than at low p fertility level (Haag et al., 1977). The differences observed in the response of these genotypes were considered to be due to the nutrient supplying capacity of the soil and unique regulative properties contained by the genotypes in addition to the applied phosphorus.

Significant increases in common bean grain yield due to application of phosphorus at the rate of 30, 60 and 90kg P/ha were observed by Singh *et al*. (1981). Superior grain production was obtained between 60kg P/ha and 90kg P/ha than at 30kg P/ha (Singh *et al*., 1981). Similar results of grain yield increases with increase in phosphorus levels have been observed by Garg *et al*. (1971), Saidou (1985), Ssali and Keya (1985) and Mahatanya (1977).

Significant results in efficiency of phosphorus utilization in relation to bean yield have been observed (El-Leboundi *et al*., 1976) in terms of seed weight, pod weight and number of pods per plant. Seed yield ranged from 1243kg/ha to 1557kg/ha while pod yield was 14628.6kg/ha based on 19 pods per plant with a weight range of 18.33 to 28.08g/plant (El-Leboundi *et al*., 1976). The beans were inoculated with *Rhizobium* and treated with 0kg P/ha and 476kg P/ha. Increasing P rate from zero to 150kg P/ha increased seed yield by 390kg/ha (Ssali and Keya, 1983). The high nitrogen fixing cultivar which was Rose coco (a high yielding, early maturing, bush type cultivar) gave 1687kg/ha seeds at the lower level of phosphorus which was zero kg/ha while at the higher level it gave 2177kg/ha bean seeds (Ssali and Keya, 1985).

Different lines of bean grown in a growth culture medium at different levels of phosphorus showed extremes in response to low phosphorus level in terms of mean dry weight of the shoots and roots (Whiteaker et al., 1976). It was then established that the capacity to respond to low P was line-specific which resulted to variations in the relative growth of the lines with phosphorus concentration (Whiteaker et al., 1976). The most efficient lines gave the highest yields at the lowest level of added available phosphorus.

2.5.2 Effect of phosphorus on dry matter yield and nodulation.

Variations in dry matter yield and nodule mass have been observed in three different bean cultivars inoculated with a Rhizobium strain and treated with phosphorus at two levels (Ssali and Keya, 1985). Dry matter yield increased with increasing phosphorus level. This could be due to high amount of P absorbed by the high yielding cultivar which could be having a large root system that explored more phosphorus from the growth medium and therefore more nodulation which increased the leaf surface area and vegetative growth. The number of nodules and nodule mass of various bean cultivars have been observed to increase significantly as levels of phosphorus were increased (Ssali and Keya, 1983, 1985; Pereira and Bliss, 1989; Wolyn et al., 1988; Saidou, 1985). Increase of phosphorus from 100mMP to 400mMP increased the number of nodules from 215.8 to 228.6 and nodule weight from 204.2 to 486.2g/2 plants (Pereira and Bliss, 1989). This indicated the requirement of phosphorus for nodule initiation and formation which was increasing as more nodules were to be formed. The more the availability of phosphorus the higher the number of nodules formed. Similar results were observed by Rennie and Kemp (1983), Attwell and Bliss (1985) and Wolyn *et al*. (1988).

2.5.3 Effect of phosphorus on the amount of nitrogen fixed by common bean.

Nitrogen fixation by beans has been observed to increase with increasing phosphorus levels (Attwell and Bliss, 1985; Rennie and Kemp, 1983; Pereira, 1987; Pereira and Bliss, 1989; Graham and Rosas, 1977; Westermann and Kola 1978; Nash and Schulman, 1976). The increase in nitrogen fixation with increasing phosphorus levels have been observed to be due to the increase in the number of nodules and nodule mass with increasing P levels (Graham and Rosas, 1977). The high P utilization was for nodule initiation and nodule formation. However, at higher levels of P nitrogen fixation decreased with the increasing levels of P. The reason for the observed drop of N-fixation at higher levels of P could be due to reduced P requirement by the plants after the maximum amount of P required had been obtained at the lower levels of the applied P. Another reason for the fall in nitrogen fixation at the higher levels of P could be that the maximum amount of N that could be fixed by those cultivars was attained in the lower P levels. It can, therefore, be concluded that the cultivars used had ability to tolerate low levels of phosphorus to grow well and give high yields. It can also be concluded that the cultivars were efficient in extracting low soil P for their normal growth functions due to presence of large and extensive root mass. The amount of nitrogen fixed by nodulated bean cultivars has been observed to increase with increasing levels of phosphorus (Pereira and Bliss, 1989; Ssali and Keya, 1985).

It has been observed by Attwell and Bliss (1985) and Wolyn et al. (1988) that peak nitrogen fixation occurs at 50% bloom after which it falls as a result of decline in leghemoglobin concentration in the nodules due to nodule senescence and competition of carbohydrates during the pod filling stage. Leghemoglobin concentration is also affected by type of roots. For example lateral roots were found by Wolyn et al. (1988) to contain higher leghemoglobin than the crown roots and nodule mass.

It was found by Pereira and Bliss (1987) and Pereira (1987) that growth of nodules in beans is the primary factor limiting nitrogen fixation at low levels of phosphorus because the nodules were found to be rich in P.

This is because P in beans has a main function of initiating nodule formation and maintain their growth.

2.5.4 Effect of other mineral nutrients on nitrogen fixation in beans.

Leguminous plants grown in the presence of adequate nutrients usually accumulate greater concentrations of the basic elements in their tissues than do non-legumes (Burton et al., 1961). High contents of Ca, Mg and K in plant tissues are usually associated with a high nitrogen content (Burton et al., 1961; Stephens, 1966; Morris and Pierre, 1949).

Inoculated legumes are associated with high nitrogen content in the tissues. The high nitrogen content is a result of high level of mineral nutrient supply to the nitrogen fixing plants and stimulation in symbiotic nitrogen fixation (Burton et al., 1961). Increasing levels of mineral nutrients in effectively nodulated common beans has been observed by Burton et al. (1961) and Munns and Keyser (1979) to increase symbiotic nitrogen fixation. It has also been reported by Munns and Keyser (1979) and Vincent (1961) that Ca is not very much required as an essential nutrient for Rhizobium growth while Mg seems to be essential for all bacteria. The concentration of calcium at which normal growth of bacteria is attained has been found by Vincent (1961) and Fried and Peech (1946) to be 25μM at pH 4.5. Calcium absorption by plants is inhibited by high concentrations of manganese. In artificial media Rhizobia can tolerate very high levels of Mn (Materson, 1968; Holding and Lowe, 1971). It has been found by Kliever (1961) that 20ppm Mn in nutrient solution reduces the number of nodulated alfalfa plants to 50%. In acid condition (pH 4.4) nodule numbers are affected at 40ppm Mn in beans leading to reduced nitrogen fixation (Kliever, 1961).

Magnesium has been observed by Truog et al. (1947) to act as a carrier of the phosphorus used by the plants. Magnesium uptake and translocation is depressed by high levels of K^+, Ca^{2+} and NH_4^+ in beans and can lead to Mg deficiency (Schimansky, 1981). High concentration of Mg will therefore benefit nitrogen fixation especially in high available P containing soils. Magnesium has been found to activate all the reactions involving transfers of phosphates in plants (Jackson et al., 1962). It has been shown that P uptake is increased in the presence of Mg (Edwards, 1968; Franklin, 1969).

Thomas and Hungria (1988) established that potassium stimulates nitrogenase activity and accumulation of ureides in pod walls. Potassium is also responsible for stimulation of the transportation of nitrogenous compounds to developing fruits (Thomas and Hungria, 1988).

Nitrogenase activity and seed yield of beans can be increased by high rate of potassium (Thomas and Hungria, 1988).

2.6 Agronomic significance of the common bean-Rhizobium- phosphate interaction.

Generally nitrogen supply for common bean production especially in the developing countries depends on the soils natural fertility and symbiotic nitrogen fixation, as a result of the bean plant association with nitrogen fixing Rhizobium. Ability of the common bean to fix nitrogen symbiotically is low due to incompatibility between most bean genotypes and the nitrogen fixing rhizobia strain in most soils, small or ineffective population of the nitrogen fixing rhizobia and low levels of phosphorus in the soils. Phosphorus is a limiting factor in symbiotic nitrogen fixation in beans. However, some bean genotypes can tolerate low levels of phosphorus in soils due to their efficiency in phosphate absorption and utilization. If such genotypes are inoculated with an effective strain of nitrogen fixing Rhizobium their nitrogen fixation ability will be increased. This will result to genotypes with improved phosphorus utilization efficiency, high nitrogen fixing ability and therefore, improved bean yield. This will also be a biological solution of the phosphorus problems in symbiotic biological nitrogen fixation by common beans. The association between common-bean-Rhizobium will then be the source of N to most bean producing areas and will also decrease the problem of phosphorus inadequacy to the bean plants. Soils deficient in available phosphorus will be utilizable for bean production with less phosphatic fertilizer addition and finally the cost of producing beans will be reduced while bean yield will be increased. The N nutritional status of the soil will also be increased as a result of increased nitrogen fixation ability and therefore benefit the plants intercropped with the beans which are normally cereals.

2.7 Summary.

Bean production in various areas in the developing countries is characterized by low yield. In Tanzania, bean is cultivated in the cool and wet areas which receive high rainfall per year. The soils in these areas are highly weathered and some are acidic, hence deficient in most plant nutrients including phosphorus. Soil infertility and other factors like plant diseases, pests and drought susceptibility have contributed to the low bean yield in Tanzania and in the whole of East Africa. Fertilizer applications will improve the fertility of the soils but fertilization of the fields used for bean production is less practiced in Tanzania due to the high costs and therefore, unaffordable to farmers.

Nitrogen fixation in beans is greatly hampered by low levels of phosphorus and also ineffective and low soil nitrogen fixing Rhizobium populations in soils. Since some bean genotypes have been known to have the ability to tolerate low levels of available P in soils and therefore, lead to variations in the amount of nitrogen fixed and yield, bean yields can then be improved by inoculating the bean genotypes which can tolerate low levels of available phosphorus with the effective Rhizobium strains so as to improve nitrogen fixation and finally yield, which is the main objective of every farmer.

CHAPTER THREE

MATERIALS AND METHODS

THE GLASS-HOUSE POT EXPERIMENT

3.1 Introduction.

The study, to investigate the variations in phosphorus requirement and utilization by four common bean (Phaseolus vulgaris L.) genotypes, inoculated with a Rhizobium strain PVI was conducted at the Sokoine University of Agriculture, Morogoro. The four common bean genotypes used in the study were AA/2/5/6xK-2; 86EP 5091-B-2; EP 3-2 and Selian wonder and the genotypes were referred to as C_2, C_4, C_5 and C_8 respectively, in this study. The Rhizobium inoculum culture (PVI) used in this study is a mixture of strains produced locally at the Sokoine University of Agriculture, from bean nodules collected from various bean producing areas in Tanzania.

The soil used in the glass-house pot experiment had been classified by Kaaya (1989) as an Oxic Haplustult (USDA, 1975) or Dystric Nitosol (FAO, 1984). Some of properties and the description of the reference profile for the Oxic Haplustult are given in Table 1 and Appendix 1, respectively. The source of the phosphorus used in the study was a reagent grade KH_2PO_4.

3.2 Soil sampling and preparation.

The soil, Oxic Haplustult, used in the study was collected from the Sokoine University of Agriculture farm, Morogoro (Appendix 1). Soil samples to the depth of 15cm amounting to about 1500kg were collected from the periphery of the reference profile for the Oxic Haplustult. The 1500kg of soil were brought to the laboratory and air-dried by spreading the soil on a polythene paper. The air-dry soil was then ground to pass through a 2mm sieve. Four 400g soil sample portions were randomly sampled from the bulk 2mm sieved soil and used for the determination of relevant physical and chemical properties of the soil as presented in Table 2.

3.3 Application of phosphate treatments.

One hundred and ninety two plastic pots of five-litre capacity were filled with 5kg (based on oven-dry weight) of the air-dry 2mm sieved soil. The pots had three holes on the sides close to the bottom. The holes were plugged with cotton wool before the soil was put into the pots to prevent loss of soil. The purpose of the holes was to allow free drainage of excess soil solution above field capacity ($\geq$ 0.1 bar). The weighed soils in the individual pots were then thoroughly mixed with reagent grade KH_2PO_4 to give six replicates of 0, 5, 10, 20, 40, 80, 120 and 160 mg P/kg soil which were equivalent to 0, 0.110, 0.219, 0.439, 0.878, 1.756, 2.634 and 3.512g KH_2PO_4 per 5kg soil.

There were 192 observations.

Mixing of the soil with the appropriate amount of the KH_2PO_4 was achieved by transfering the weighed soil (5kg soil per pot) from each pot to a hard manilla sheet and sprinkling the appropriate weights of KH_2PO_4 on the surface of the soil. The soil and the added KH_2PO_4 were then thoroughly mixed and thereafter transferred to the appropriate plastic pots. The plastic pots were then labelled according to treatments (phosphorus rates). Tap water was added to all the pots to about field capacity ($\leq$ 0.1 bar) and left to equilibrate at this moisture level for seven days. The concentration of phosphorus in the tap water was found to be very small and therefore would not affect the phosphorus rates used in the experiment.

3.4 Seed sterilization.

At the end of the seven days of equilibration (Section 3.3), three hundred seeds of each genotype were sterilized by immersing them separately in 3% hydrogen peroxide for 3-5 minutes and thereafter thoroughly washed with sterilized water to completely and thoroughly get rid of the hydrogen peroxide. The seeds were then dried and stored in sterilized bottles and securely capped ready to be inoculated with the PVI <u>Rhizobium</u> strain.

3.5 Seed inoculation and planting.

At the end of the equilibration period and soon after the seeds had been sterilized the seeds were inoculated with PVI strain of Rhizobium. Each of the three hundred sterilized seeds for each genotype were stired in gum arabic solution and PVI inoculum cultures on yeast mannitol agar for two minutes. They were then left to dry for two hours in a sterile environment before they were planted in the appropriate pots.

Six replications of each phosphorus level were allocated to each of the four genotypes. The experiment was in a two factorial arrangement in completely randomized design. The pots were clearly labelled indicating the genotype and phosphorus rate. Five of the inoculated seeds were then planted in each pot according to the genotypes at a depth of about 1.5cm. Four days after germination the plants were thinned to three plants per pot. The moisture level of the soil in the pots was maintained at about field capacity throughout the active growing period of the plants.

3.6 Harvesting, plant material preparation and analysis.

At flowering, that was approximately four weeks from the planting date, three replications of each phosphorus rate, and for each genotype picked at random were harvested. The shoots were cut at soil level, washed with distilled water and dried at 70°C to constant weights. The dry plant materials were ground into fine powder. The ground plant materials were then used for the determination of percent phosphorus and nitrogen contents in the plants at the various levels of phosphorus applied to the soil using the procedures by Juo (1979).

Immediately after harvesting the shoots, the root systems of the plants for each treatment and genotype were carefully separated from the soil and thoroughly washed. The number of nodules on the roots were then counted and recorded as number of nodules per plant. Thereafter, the nodules were dried at 70°C to constant weights.

The plants in the remaining three replications for each treatment were maintained to grow up to maturity. The pods were then harvested, counted and weighed after which they were threshed, air dried for two weeks and the seeds were then weighed. The number of pods and weight of seeds for each phosphorus level and for each genotype were expressesd as number of pods per plant and weight of seeds per plant. The data collected were statistically analysed following the procedures by Montgomery (1976) and Snedecor and Cochran (1980).

Table 1. Some of the physical and chemical properties of the Oxic Haplustult profile (Kaaya, 1989).

Soil parameter/property	Horizon and horizon depth (cm)				
	Ap	B21t	B22t	B23t	B24t
	0-17	17-43	43-83	83-115	115-153
1. Bulk density (g/cm^3) (Blake, 1965)	1.24	1.29	1.30	1.38	1.41
2. Water holding capacity (Vol %) (USSCS, 1967)					
0.3 bar	29.9	27.5	24.9	29.6	31.1
15.0 bar	15.1	16.4	14.0	16.2	16.9
3. Available water capacity (Vol %) (USSCS, 1967)	14.8	11.1	10.9	13.4	14.4
4. Particle size distribution (Day, 1965)					
% sand	45.8	34.3	30.8	24.1	24.1
% silt	7.5	5.0	4.9	5.8	6.3
% clay	46.7	60.7	64.3	70.1	69.6
5. Soil pH: in water	6.6	6.1	6.0	7.0	6.3
in 0.01M $CaCl_2$	5.6	5.0	5.0	5.3	5.5
(Dewis and Freitas, 1970)					
6. Cation exchange capacity (me/100g soil) (Thomas, 1982)	20.09	16.17	16.17	19.11	16.66
7. Exchangeable bases (me/100g soil) (Thomas,1982) Ca^{2+}	5.03	2.23	1.29	1.33	2.04
Mg^{2+}	2.18	1.93	2.22	2.59	2.10
K^+	1.04	0.35	0.21	0.18	0.10
Na^+	0.18	0.21	0.24	0.53	0.73
8. Organic carbon (%) (Nelson and Sommers, 1982)	1.43	0.82	0.60	0.41	0.40
9. Organic matter (%) (Nelson and Sommers, 1982)	2.47	1.41	1.03	0.71	0.70
10. Total nitrogen (%) (Bremner and Mulvaney, 1982)	0.15	0.12	0.11	0.80	0.80
11. Bray-1 extractable P (mg/kg) (Watanabe and Olsen, 1965)	5.26	3.51	3.51	3.51	2.39

Table 2. Some of the physical and chemical properties of composite soil sample (Oxic Haplustult) used for the glass-house pot experiment.

Soil parameter/characteristic		Quantity/amount in the soil
1. Particle size distribution: (Day, 1965):	% sand	44.7
	% silt	7.3
	% clay	48.0
2. Available water capacity (%) (USSCS, 1967)		14.5
3. Soil pH:	in H_2O	6.2
(Dewis and Freitas, 1970)	in 0.01M $CaCl_2$	5.3
4. Cation exchange capacity (me/100g soil) (Thomas, 1982)		20.0
5. Exchangeable cation (me/100g soil) (Thomas, 1982)	Ca^{2+}	5.5
	Mg^{2+}	2.2
	K^+	1.02
6. Organic carbon (%) (Nelson and Sommers, 1982)		1.08
7. Total nitrogen (%) (Bremner and Mulvaney, 1982)		0.16
8. Bray-1 extractable P (mg/kg) (Watanabe and Olsen, 1965)		3.80

3.7 Data analysis.

Data obtained from this study were subjected to statistical analysis on IBM model 55 SX and Olivetti model M240 personal computers. Raw data were entered in a Microcomputer Program for the Design, Management, and Analysis of Agronomic Research Experiments (MSTAT, 1988) and later changed to ASC11 file using Lottus 1-2-3 spread sheet so as to be read by Statistical Package for Social Sciences (SPSS, 1989) program.

Means for the various variables of interest were calculated using MSTAT program while correlation coefficients for the same variables were calculated using SPSS. Duncan's Multiple Range Test (DMRT) was used to determine differences between means.

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Effect of phosphorus on nodulation.

The effect of increasing levels of phosphorus on number of nodules (that is the extent of nodulation) formed on the roots of the four bean genotypes under investigation were as presented in Fig.1 and Appendix 2. The number of nodules increased with increasing levels of added phosphorus up to a range between 80 to 100mgP/kg soil for genotypes C_2 and C_4, 120 to 140mgP/kg soil for genotype C_5 and kept on increasing beyond 160mgP/kg soil for genotype C_8 (Figure 1 and Appendix 2). The number of nodules for genotypes C_2 and C_4 and genotype C_5 decreased with increasing levels of phosphorus beyond the range 80 to 100mgP/kg soil and 120 to 140mgP/kg soil, respectively. At zero level of added phosphorus (control) the trend of nodulation observed in the four common bean genotypes followed the order, $C_5 \rangle C_4 \rangle C_8 \rangle C_2$, while at 80mgP/kg soil the trend was $C_8 \rangle C_5 \rangle C_4 \rangle C_2$ and at 160mgP/kg soil the trend was $C_8 \rangle C_5 \rangle C_2 \rangle C_4$. The differences between the mean number of nodules formed by the four genotypes at each level of added phosphorus were significant ($P \leq 0.05$). Based on the Duncan's Multiple Range Test (DMRT) the increase in the number of nodules between genotypes and within genotypes at the different levels of added phosphorus were also significant ($P \leq 0.05$).

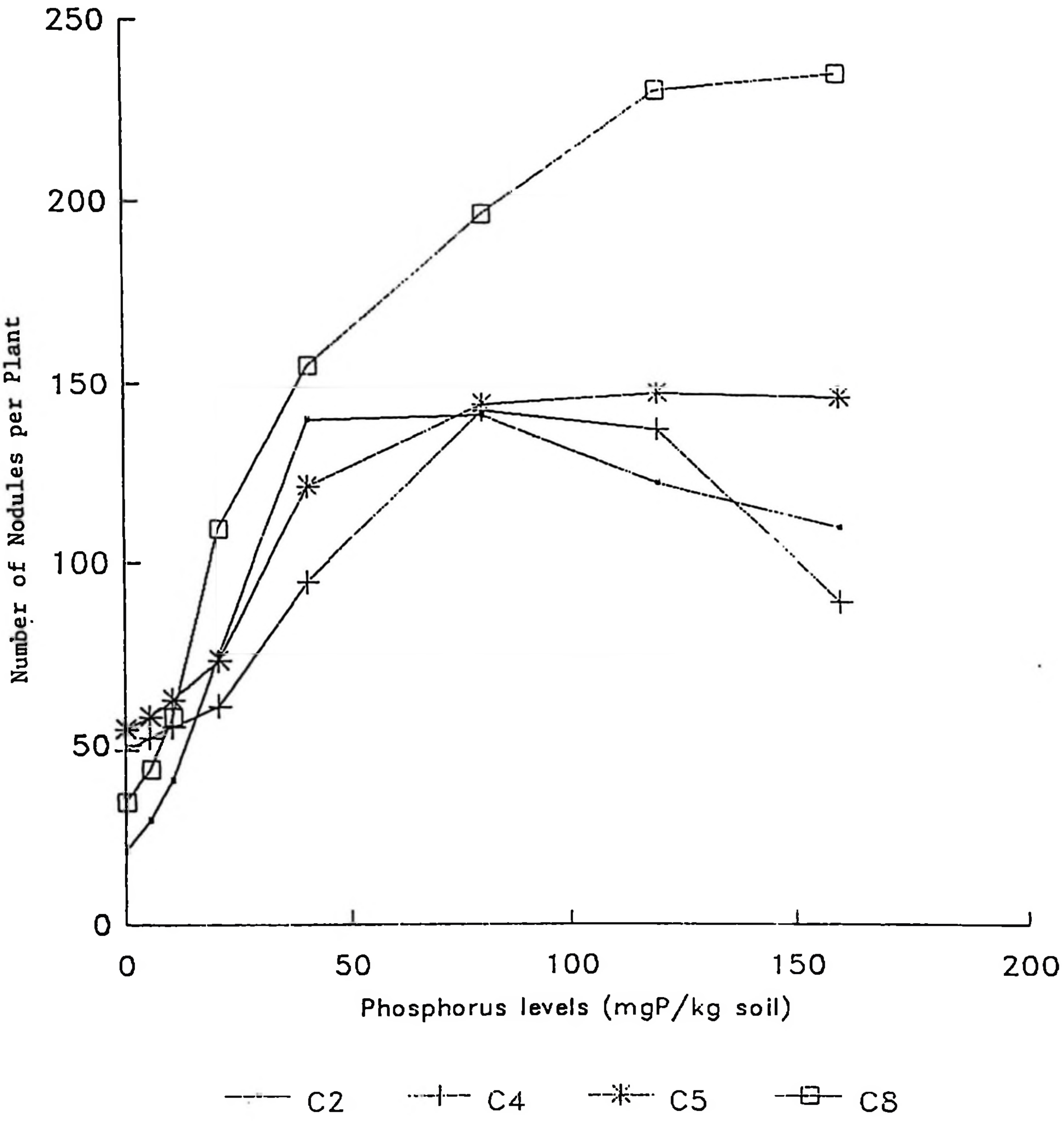

Figure 1. Effect of Phosphorus on Nodulation of Four Bean Genotypes.

The variations in the extent of nodulation between the four genotypes observed in this study could be attributed to the inherent genetical characteristics of the four genotypes to form nodules when inoculated with Rhizobium strain. Therefore, genotypes C_5 and C_4 are genetically superior to genotypes C_8 and C_2 with respect to nodulation. At the zero level of added phosphorus, the major factor controlling the number of nodules formed on the roots of the four common bean genotypes is the genetical variability between the genotypes because all other factors which could have contributed to the extent of nodulation were maintained constant (i.e. were the same for all the genotypes). Similar observations have been reported by Franco et al.(1979) and Graham and Rosas (1977) and Pereira and Bliss (1987; 1989).

The very pronounced increase in the number of nodules formed on the roots of the four common bean genotypes with increasing levels of added phosphorus up to 40mgP/kg soil, reflect the essentiality of phosphorus in nodulation in the common bean. Similar observations have been reported by Mengel and Kirkby (1987) and Sanchez (1976) and Brady (1984). The variations in the magnitude of nodulation between the four genotypes with increasing levels of phosphorus added to the soil up to 40mgP/kg soil reflect the variations in phosphorus requirements and utilization by the four bean genotypes with respect to nodulation. It can, therefore, be argued that the common bean genotypes C_5 and C_4 have the ability to form nodules at very low levels of soil available phosphorus, while for genotypes C_2 and C_8, low levels of soil available phosphorus limit the extent of nodulation. Similar observations have been reported by Whiteaker *et al*. (1976) and Leif (1990). The abilities of genotypes C_5 and C_4 to nodulate at the very low level of available phosphate (5mgP/kg soil) could be due to the genotypes' root ability to grow extensively and spread to large volumes of soil in the pots from which they could absorb more phosphate (Whiteaker *et al*., 1976; Leif, 1990) and to the genetic make-up and the physiological characteristic and metabolic phosphate requirements of genotypes C_5 and C_4 as compared to the other two genotypes, C_2 and C_8. However, the extensive root growth were not clearly reflected in the mass of roots, most probably due to the inability to recover all the roots from the soils in the pots.

The inherent limited ability of genotypes C_2 and C_8 to absorb phosphate from the soil at low level of added phosphorus could partly account for the small number of nodules formed. This is substantiated by the observed increase in the number of nodules mainly for the C_8 genotype (Fig. 1) with increase in the availability of phosphorus (i.e. increasing levels of added phosphorus). The increase in nodulation in the four common bean genotypes with increasing levels of added phosphorus up to 40mgP/kg soil observed in this study confirm the role of phosphorus in root growth and nodule initiation in common beans (Attwell and Bliss, 1985; Rennie and Kemp, 1984; Wolyn et al., 1988).

The attainment of maximum nodulation at 80-100mgP/kg soil for genotypes C_2 and C_4 and 120-140mgP/kg soil for genotype C_5, and beyond 160mgP/kg soil for genotype C_8 reflect the variations in the phosphate requirement and utilization by the four genotypes under study. The increase in nodulation up to the maximum or optimum levels could be attributed to the increased availability of phosphorus, increased root growth and increased uptake of other plant nutrients essential for plant growth (Haag et al., 1977). The increased root growth and vegetative growth with increasing levels of added phosphorus were noted in this study. The attainment of maximum nodulation beyond 160mgP/kg soil for genotype C_8 reflect the very high phosphorus requirement by this genotype. It could, therefore, be argued that genotype C_8 phosphorus requirement and consequently utilization is very high as compared to the other three genotypes and this characteristic might be genetically controlled.

The decrease in nodulation with increasing levels of added phosphorus above 80-100mgP/kg soil and 120-140mgP/kg soil for genotypes C_2 and C_4 and C_5, respectively, indicate the inability of these genotypes to tolerate high levels of phosphorus. For the three genotypes, C_2, C_4 and C_5, phosphorus levels beyond 80-100 and 120-140mgP/kg soil respectively could have resulted in reduced root growth, early maturation and reduced vegetative growth, hence reduced nodulation. Comparable observations have been reported by Piha and Munns (1987). Furthermore, the increased uptake of phosphorus could have distorted the ion equilibrium in the plants and consequently the metabolic processes in the plants hence the proportionate decrease in the number of nodules observed in this study among the four genotypes.

4.2 Effect of phosphorus on nodule weight.

The dry weights of the nodules formed on the roots of the four common bean genotypes under study increased with increasing levels of added phosphorus (Fig. 2 and Appendix 3). The increase in nodule weights were more pronounced in the 0-80mgP/kg soil treatments. For genotype C_4, C_5 and C_8 the highest nodule weight were attained in the phosphorus range 80-100mgP/kg soil, 40-60mgP/kg soil and beyond 160mgP/kg soil respectively. However, for genotypes C_4 and C_5 the nodule weights decreased with increasing level of added phosphorus above 100 and 60mgP/kg soil, respectively. The trends of the nodule weights with respect to the levels of applied phosphorus were in the

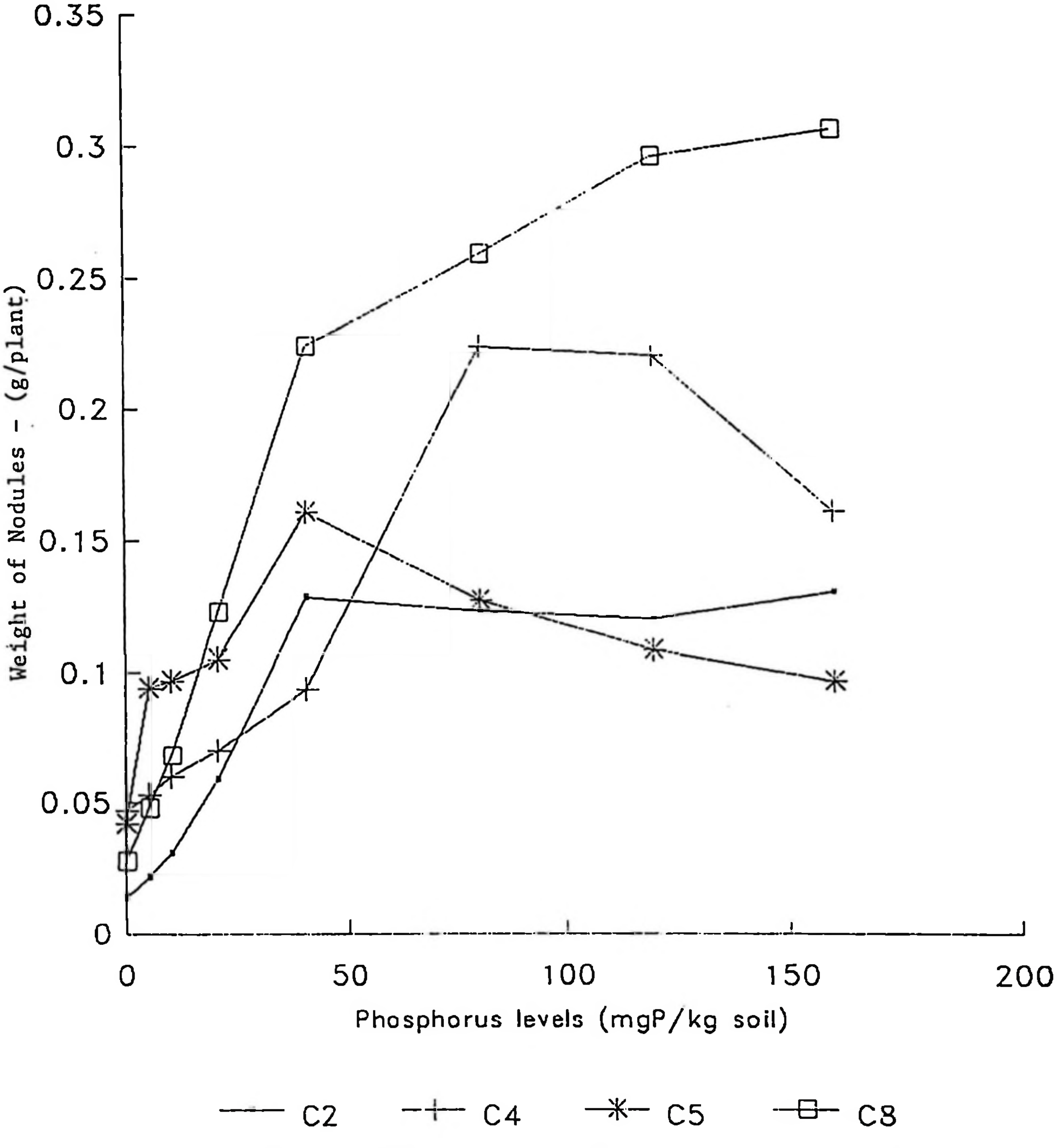

Figure 2. Effect of Phosphorus on the Weight of Nodules Formed on Four Common Bean Genotypes.

order $C_4 > C_5 > C_8 > C_2$, $C_8 > C_4 > C_5 > C_2$ and $C_8 > C_4 > C_2 > C_5$ at 0, 80 and 160mgP/kg soil of added phosphorus, respectively.

The response of the four common bean genotypes to the various levels of added phosphorus with respect to nodule weights were significant ($P \leq 0.05$) (Appendix 3). Further, based on the DMRT, it was observed that there were significant differences ($P \leq 0.05$) in nodule weights between the four genotypes and between the different levels of added phosphorus in each genotype (Appendix 3). The correlation coefficients between the number of nodules (Appendix 2) and the nodule weights (Appendix 3) were also very highly significant ($P \leq 0.01$).

The decrease in nodule weight beyond 100mgP/kg soil of added phosphorus observed for genotype C_4 (Fig. 2) conform with the decrease in the number of nodules (Fig. 1) for the same phosphorus range. However, the decrease in nodule weight for genotype C_5 at phosphorus level > 60mgP/kg soil and the increase and decrease in nodule weight for genotype C_2 in the phosphorus levels 40-160mgP/kg soil (Fig. 2) contradicts with the trends in the number of nodules formed by the respective genotypes.

The very pronounced increase in nodule weights for all four genotypes in the phosphorus range 0-80mgP/kg soil could be attributed to the low level of the available native soil phosphorus in the soil used in this study (Table 1 and 2) and the essentiality of phosphorus in nodule formation, hence nodule weights. The application of phosphorus to the soil, therefore, increased the level of phosphorus in the soil hence its availability to the plants. The decrease in the nodule weights observed for genotypes C_4 and C_5 at phosphorus levels > 120 and > 60mgP/kg soil, respectively, could be due to the assumption that at these levels, the optimum phosphorus requirements by the two genotypes had been exceeded. The excessive levels of available phosphorus with increased uptake of phosphorus by the two genotypes resulted into reduced vegetative growth, enhanced maturity and ion-imbalance in the soil and within the plants resulting in reduced nodule weights. The continuous increase in nodule weight with increasing levels of applied phosphorus > 160mgP/kg soil for genotype C_8 confirm the variations in phosphorus requirements by different bean genotypes. The decrease and increase in nodule weight for genotype C_2 at phosphorus levels > 60mgP/kg soil cannot be explained in the present study.

The observations made in this study with respect to the effect of phosphorus on nodule weight of the four genotypes conform with the observations by Ssali and Keya (1983; 1985) and Pereira and Bliss (1989) and Saidou requirement and utilization by the four common bean genotypes observed in the present study follow the order $C_8 > C_5 > C_4 > C_2$ (Fig. 2). The observed trends and variations in the rate of increase or decrease in nodule weights per unit increase of applied phosphorus (Fig. 2 and Appendix 3) further confirms the genetic variations in phosphorus requirement and utilization by the four bean genotypes and the role of phosphorus in symbiotic nitrogen fixation by common bean (Pereira and Bliss,1987; 1989; Dale *et al*.,1977; Franco *et al*., 1979; Haag *et al*., 1977; Leif, 1990). The positive correlation between nodule weights and nodule numbers obtained in this study conform to the results obtained by Pereira and Bliss (1989) and Haag *et al*. (1985) where increase in nodule numbers were also reflected in increase in nodule dry weights or nodule mass.

4.3 Effect of phosphorus on shoot weight.

The response of the four common bean genotypes with respect to shoot dry weights to increasing levels of added phosphorus was as presented in Fig. 3 and Appendix 4. The trends in the increase in shoot dry weights were in the order $C_4 \rangle C_5 \rangle C_8 \rangle C_2$, $C_4 \rangle C_8 \rangle C_2 \rangle C_5$ and $C_4 \rangle C_8 \rangle C_2 \rangle C_5$ at 0, 80 and 160mgP/kg soil, respectively. The increase in shoot dry weights between the four genotypes and within individual genotypes were significant ($P \leq 0.05$) (Appendix 4). The correlation coefficients between the weight of the shoots of genotypes C_2, C_4, and C_8 and the

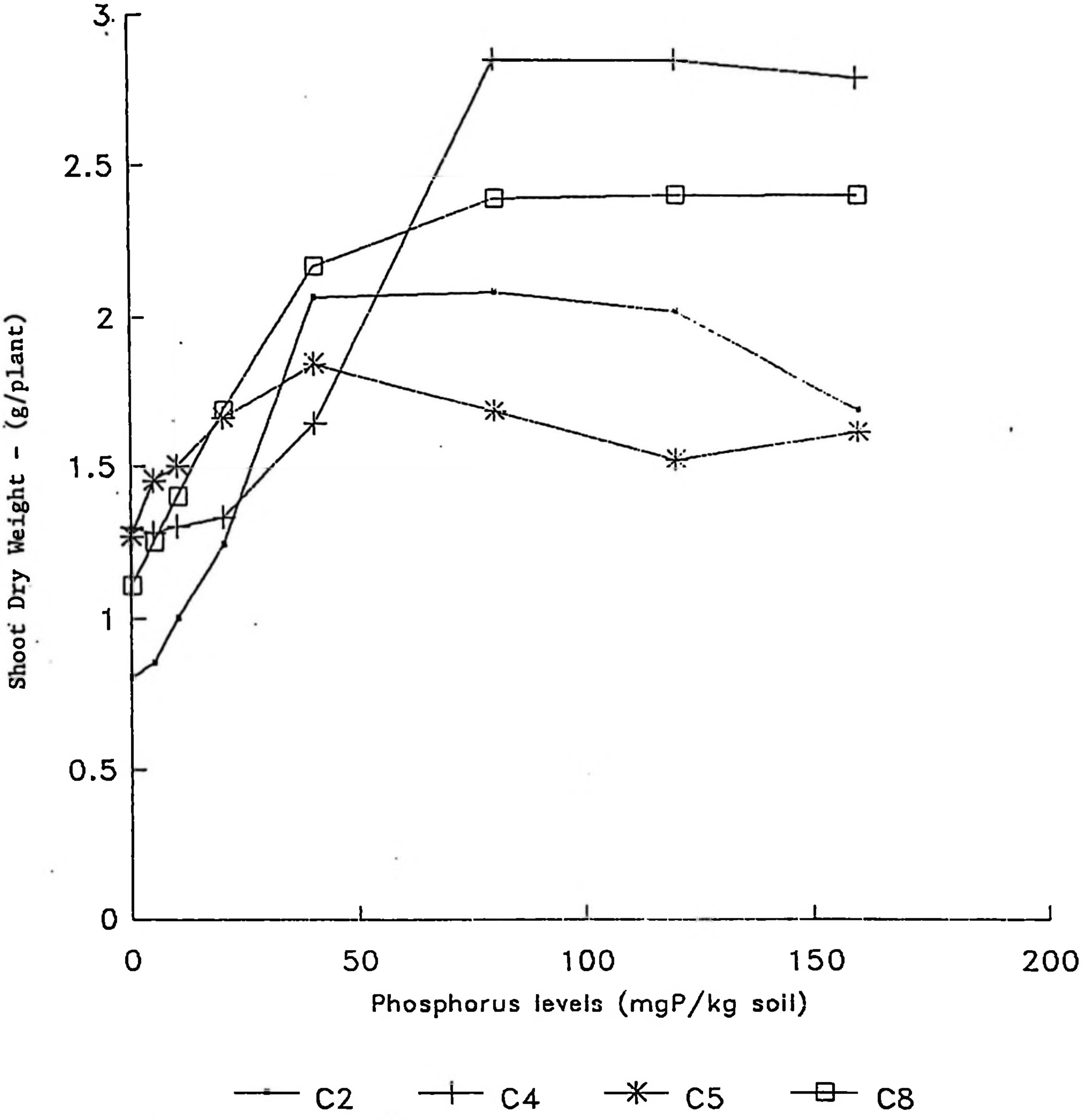

Figure 3. Effect of Phosphorus on Shoot Dry Weight of Four Bean Genotypes.

number of nodules formed on the roots of the same genotypes were highly significant ($P \leq 0.001$) (Tables 3(a)-(d)).

The variations in shoot dry weights without added phosphorus, express the differences in the yield potentials of the four genotypes and the response of the genotypes to <u>Rhizobium</u> inoculation, hence symbiotic nitrogen fixation. The rapid increase in shoot dry weight of the four bean genotypes with increasing levels of added P reflects the deficiency status of P in the soil used in this study and the essentiality of phosphorus in plant growth and development. The decrease in shoot dry weights at phosphorus rates ≥ 40, 80 and ≥ 120mg P/kg soil for genotypes C_4, C_2 and C_5 respectively indicates excessive uptake of P by the genotypes, hence enhanced maturity at the expense of vegetative growth. The continuous increase in shoot dry weight by genotype C_8 up to P rates ≥ 160mgP/kg soil reflect the high requirement of P by genotype C_8. However, the rates of increase in shoot dry weight per unit increase in added P were much lower, at P rates ≥ 80mgP/kg soil as compared to the rates between 0 and 80mgP/kg soil. This observation further explains essentiality of phosphorus in plant growth and development. The increase in shoot dry weight for genotype C_5 at P rate ≥ 120mgP/kg soil could be attributed to experimental error, otherwise a decrease in dry weight

Table 3(a). Correlation coefficients for pairs of some of the variables for genotype C_2.

Var[1]	NN	NW	SW	RW	PN	PW	SD	SP	SN
NW	0.835^{**}								
SW	0.912^{**}	0.744^{**}							
RW	0.07	0.245	-0.049						
PN	0.864^{**}	0.857^{**}	0.845^{**}	0.008					
PW	0.845^{**}	0.837^{**}	0.816^{*}	0.091	0.934^{**}				
SD	0.866^{*}	0.854^{**}	0.831^{**}	0.098	0.937^{**}	0.991^{**}			
SP	0.772^{**}	0.823^{**}	0.738^{**}	0.199	0.846^{*}	0.865^{**}	0.869^{**}		
SN	0.684^{**}	0.640^{**}	0.644^{**}	-0.066	0.819^{**}	0.810^{**}	0.784^{**}	0.602^{**}	

Table 3(b). Coefficients for pairs of some of the variables for genotype C_4.

Var[1]	NN	NW	SW	RW	PN	PW	SD	SP	SN
NW	0.381^{**}								
SW	0.813^{**}	0.808^{**}							
RW	0.535^{*}	0.547^{**}	0.581^{**}						
PN	0.588^{**}	0.626^{**}	0.742^{**}	0.613^{**}					
PW	0.778^{**}	0.755^{**}	0.855^{**}	0.659^{**}	0.915^{**}				
SD	0.755^{**}	0.715^{**}	0.833^{**}	0.651^{**}	0.892^{**}	0.973^{**}			
SP	0.729^{**}	0.680^{**}	0.807^{**}	0.601^{**}	0.849^{**}	0.905^{**}	0.902^{**}		
SN	0.670^{**}	0.433	0.472^{*}	0.322	0.377	0.484^{*}	0.485^{*}	0.607^{**}	

Table 3(c). Correlation coefficients for some of the variables for genotype C_5.

Var^1	NN	NW	SW	RW	PN	PW	SD	SP	SN
NW	0.455*								
SW	0.339	0.725**							
RW	-0.588*	-0.066	-0.101						
PN	0.668**	0.484*	0.379	-0.665**					
PW	0.777**	0.509*	0.369	-0.773**	0.877**				
SD	0.798**	0.545*	0.397*	-0.743**	0.877**	0.993**			
SP	0.884**	0.439*	0.277	-0.683**	0.682**	0.856**	0.862**		
SN	-0.013	0.319	0.203	0.122	0.219	0.199	0.244	0.190	

Table 3(d). Correlation coefficients for some of the variables for genotype C_8.

Var[1]	NN	NW	SW	RW	PN	PW	SD	SP	SN
NW	0.966**								
SW	0.825**	0.869**							
RW	0.828**	0.816**	0.629**						
PN	0.844**	0.788**	0.776**	0.685**					
PW	0.874**	0.852**	0.806**	0.742**	0.969**				
SD	0.757**	0.737**	0.749**	0.639**	0.909**	0.921**			
SP	0.942**	0.924**	0.769**	0.871**	0.876**	0.903**	0.812**		
SN	0.674**	0.616**	0.615**	0.573*	0.608**	0.573*	0.513*	0.696**	

[1] <u>Key for variables referred to in Tables 3(a-d):</u>

NN - nodule numbers per plant
NW - nodule weight per plant
SW - shoot dry weight
RW - root dry weight
PN - pod numbers
PW - pod dry weight
SD - seed dry weight
SP - shoot % phosphorus content
SN - shoot % nitrogen conten

* = 1-tailed significant at 0.01 level

** = 1-tailed significant at 0.001 level could have been observed. The phosphorus requirement and utilization by the four common bean genotypes could be assumed to follow the order $C_8 \rangle C_4 \rangle C_2 \rangle C_5$ with respect to shoot dry weight.

The optimum phosphorus requirements by the four genotypes were at the levels 80-100, 40-80, 40-50 and beyond 160mgP/kg soil for genotypes C_4, C_2, C_5 and C_8, respectively. The increase in shoot dry weights between P rates greater than zero to the optimum rates (the range at which highest shoot dry weights were attained) indicate the variations in phosphorus requirements and utilization by the genotypes and the yield potential-phosphorus requirement and utilization- interaction of the genotypes. The differences in shoot dry weights at zero level of added phosphorus further reflect the ability of the genotypes to tolerate phosphorus stress (Whiteaker et al., 1976; Lyness, 1936; Pereira and Bliss, 1987; Cossman et al., 1981(b); Graham and Rosas, 1979; Ssali and Keya, 1985; Leif, 1990). The ability of the four genotypes to tolerate low phosphorus levels in the soil was in the order $C_4 \rangle C_8 \rangle C_5 \rangle C_2$. The results obtained in this study conform with those reported by Ssali and Keya (1985) and Leif (1990).

4.4 Effect of phosphorus on root weight.

The response of the four common bean genotypes with

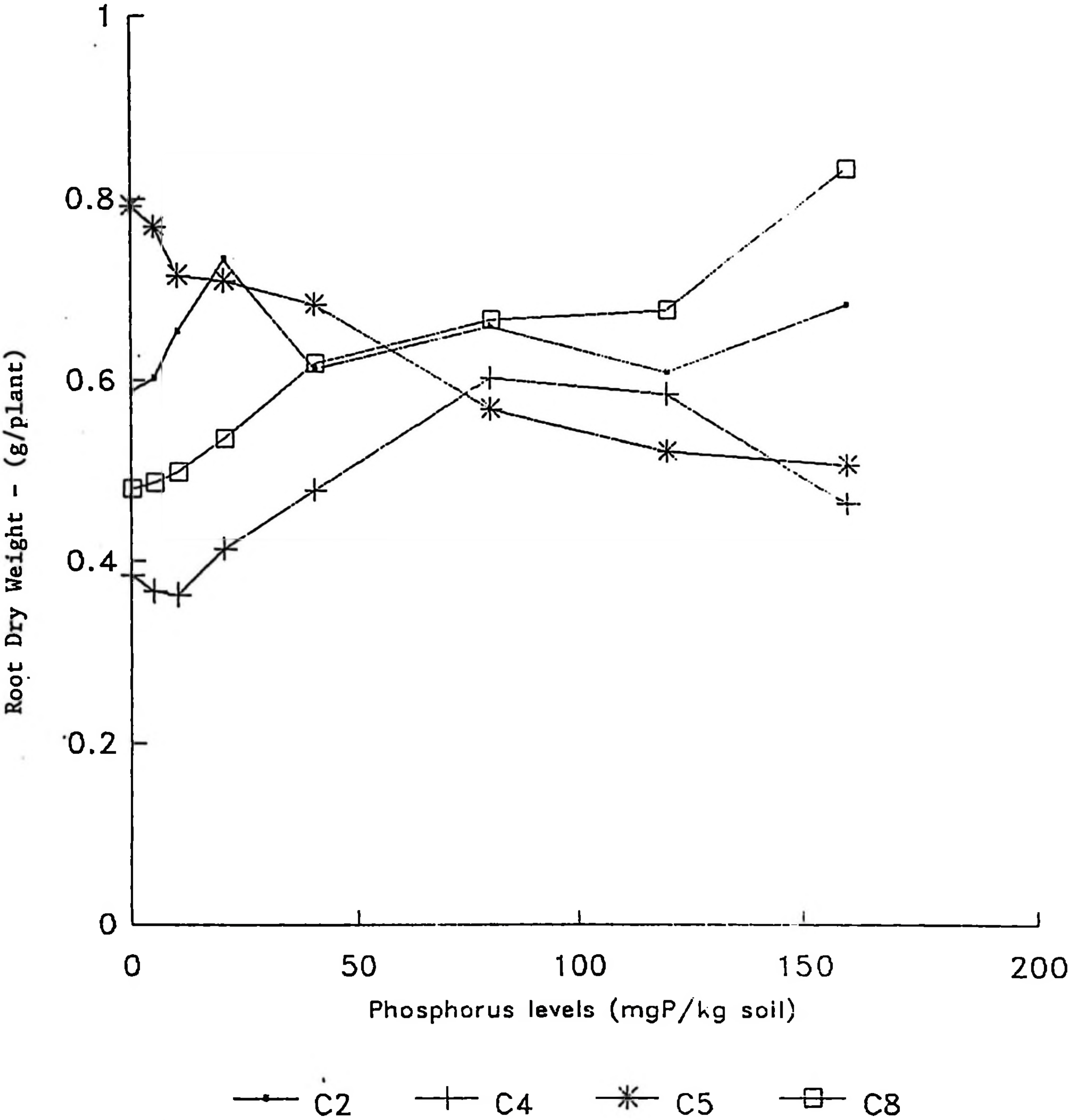

Figure 4. Effect of Phosphorus on the Weight of Roots of Four Bean Genotypes.

respect to root dry weight to applied phosphorus was as presented in Fig. 4 and Appendix 5. The increase or decrease in root dry weight at the various levels of applied phosphorus did not follow a well defined trend except for genotype C_8. The root dry weights at zero level of added phosphorus were in the order $C_5 \rangle C_2 \rangle C_8 \rangle C_4$ as compared to the shoot dry weights where the trend was $C_4 \rangle C_5 \rangle C_8 \rangle C_2$. The decrease or increase in root dry weights for the four common bean genotypes with increasing levels of added phosphorus were not significant ($P \leq 0.05$) (Appendix 5). Likewise, the correlation coefficients between the dry weight of the roots for genotype C_2 and all other variables for the same genotype studied were not significant ($P \leq 0.01$) but for C_8 it was significant ($P \leq 0.001$) (Tables 3(a)-(d)). For genotype C_4 the correlation coefficients between the roots dry weight and number of nodules, nodule weight, shoot dry weight, number of pods, pod weight, seed weight,and shoot P content were significant ($P \leq 0.001$) (Tables 3(a)-(d)). For genotype C_5 the only pairs of variables which were significantly ($P \leq 0.001$) correlated with the roots dry weight were number of nodules, pod weight, number of pods, and seed weight (Tables 3(a)-(d)). It was very difficult to collect all the root portions from the soil in the pots and hence could have somehow contributed to the observed trends in root dry weight data.

The high root dry weight at zero level of added phosphorus followed by a continuous decrease in root dry weight with increasing levels of added phosphorus for genotype C_5 could probably be due to the genetic characteristic of genotype C_5 to develop an extensive root system in soils with low plant available phosphorus. The extensive root system, would, therefore, explore more soil volume for available phosphorus, hence absorbing more phosphorus for plant growth and development. Additions of increasing levels of phosphorus to the soil, progressively increased the quantities of plant available phosphorus in the vicinity of the rooting system, hence stimulating the plant to progressively produce few roots. The above observation could be supported by the observations made by Whiteaker et al. (1976) and Pereira and Bliss (1987; 1989) that different lines (genotypes) showed extremes in response to low phosphorus in terms of root weight and other plant parameters. Further, the continuous decrease in root dry weight with increasing levels of phosphorus could be accounted for by good vegetative growth, increased photosynthates and the enhanced maturity with increased availability of phosphorus. However, more research has to be done and data has to be collected to confirm the above two postulations.

The trends in root dry weights with increasing levels of added phosphorus for genotypes C_2 and C_4 could not be explained, hence more investigations have to be undertaken in order to confirm as to whether the observed trends of the data were due to experimental errors or otherwise. The continuous increase in root dry weight with increasing levels of added phosphorus for genotype C_8 suggest that genotype C_8 has high phosphorus requirement. Contrary to genotype C_5, genotype C_8, increased levels of phosphorus availability resulted to increased root production and proliferation. The root weight data available in this study for the four common bean genotypes concur with the observations made by Whiteaker et al. (1976) that the different bean lines (or genotypes) respond differently to levels of available phosphorus in the soil. It can, therefore, be suggested that genotype C_5 was more efficient in phosphorus uptake and utilization as compared to the other genotypes, with respect to root dry weights. Based on the correlation coefficients between the various plant parameters (Tables 3(a)-(d)), it could be said that genotype C_8 was more efficient than the other genotypes in phosphorus uptake and utilization because addition of different levels of phosphorus to this genotype influenced the root weight of this genotype as well as all of the other plant variables.

4.5 Effect of phosphorus on pod production.

The response of the four common bean genotypes with respect to the number and dry weight of pods to increasing levels of applied phosphorus were as presented in Fig. 5 and 6 and Appendices 6 and 7, respectively. The number of pods per plant for each of the four genotypes increased with increasing levels of added phosphorus but the increase in pod numbers were not significant (Fig. 5 and Appendix 6). However, the increase in pod dry weights with increasing levels of added phosphorus were significant (Appendix 7). At zero, 80 and 160mgP/kg soil levels of added phosphorus, the trends of the number of pods and pod dry weights were in the order $C_5 > C_4 = C_8 > C_2$, $C_5 > C_2 > C_8 > C_4$, and $C_5 = C_2 > C_4 > C_8$ and $C_5 > C_8 > C_4 > C_2$, $C_4 > C_5 > C_8 > C_2$ and $C_5 > C_4 > C_8 > C_2$, respectively. There was a well defined relationship between number of pods and dry weight of pods in genotype C_5, while in the other genotypes the relationships were not that clear, as can be deduced from the correlation coefficient between the various plant parameters for the four common bean genotypes (Tables 3(a)-(d)).

The lack of clear cut relationship between the pod number and pod weights observed in this study for the three genotypes suggest that the pod weights is a function of the size of the pods, the size of the seeds in the pods and the number of seeds per pod. It could therefore be postulated that increasing levels of phosphorus did influence the above parameters equally, hence the observed variations.

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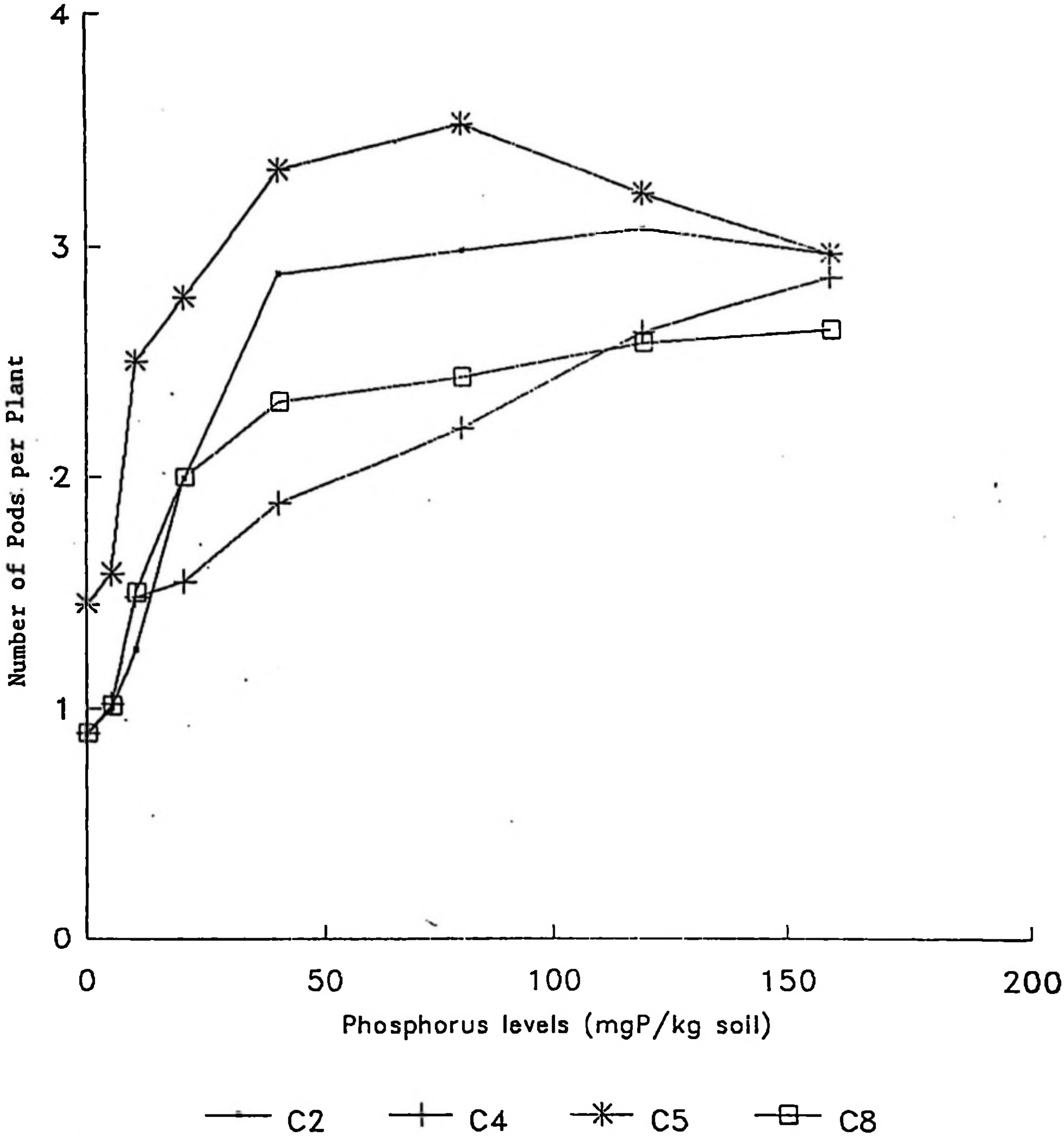

Figure 5. Effect of Phosphorus on the Number of Pods on Four Bean Genotypes.

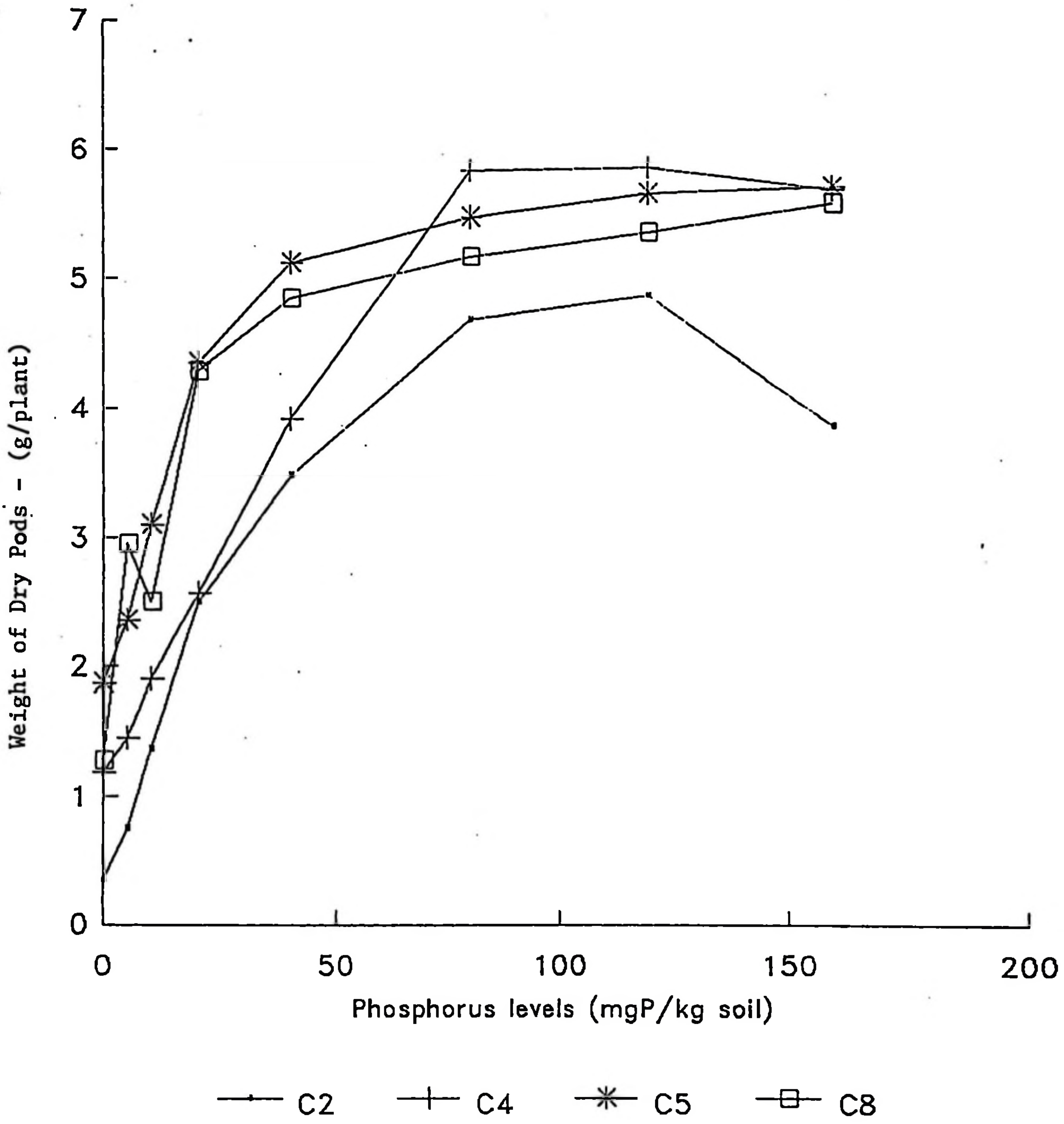

Figure 6. Effect of Phosphorus on the Weights of Pods of Four Bean Genotypes.

The number and dry weights of the pods for the four genotypes at zero level of added phosphorus (the control) express the yield potentials of the four genotypes when inoculated with the Rhizobium inoculum PV1 at very low levels of available phosphorus (Table 2). The number of pods and dry weights of the pods further express the ability of the individual common bean genotypes and the genotype-Rhizobium interaction to withstand low levels of plant available phosphorus (Dale et al., 1977; Pereira and Bliss, 1987; 1989; Whiteaker et al., 1976; Leif, 1990; Haag et al., 1977). In this study it could be suggested that genotype C_5 has the highest yield potential and the ability to maintain the high yield potential at low level of plant available phosphorus.

The rapid increases in the number and dry weight of pods in the phosphorus ranges 0-40 and 0-80mgP/kg soil added phosphorus for genotypes C_5 and C_8 and C_4 and C_2, respectively reflect the variations in phosphorus requirements and utilization by the four bean genotypes (McFerson et al., 1981). The decrease in the weights of the dry pods for genotypes C_4 and C_2 beyond 120mgP/kg soil could suggest that the available phosphorus was in excess of the optimum plant requirement for the genotypes. This, therefore, culminated into reduced vegetative growth and enhanced maturity hence reduced pod weights (pod production). Further, at the high levels of available phosphorus, symbiotic nitrogen fixation could have been depressed because of the enhanced maturity coupled with decreased vegetative growth and nutrient imbalances in the soil solution as well as in the plant tissues.

The very high rate of increase in the dry weight of pods (or pod weights) at 0-40mgP/kg soil in contrast to the low rate of increase in the dry weights in phosphorus ranges ≥ 40mgP/kg soil for genotypes C_5 and C_8 could suggest that the optimum phosphorus requirement and utilization for these two genotypes is within the 40-80mgP/kg soil, while for genotypes C_4 and C_2 is within 80-120mgP/kg soil. However, the above suggestion has to be verified by field trials.

Similar positive observations on the effect of phosphorus on number of pods and pod weights and variations in phosphorus requirements and utilizations have been reported (Haag et al., 1977; El-Leboundi et al., 1976; Ssali and Keya, 1983; 1985).

4.6 Effect of phosphorus on seed production.

The response of the four common bean genotypes in terms of seed production to increasing levels of added phosphorus was as presented in Fig. 7 and Appendix 8. The seed production increased with increasing levels of phosphorus and the increases were significant for all the

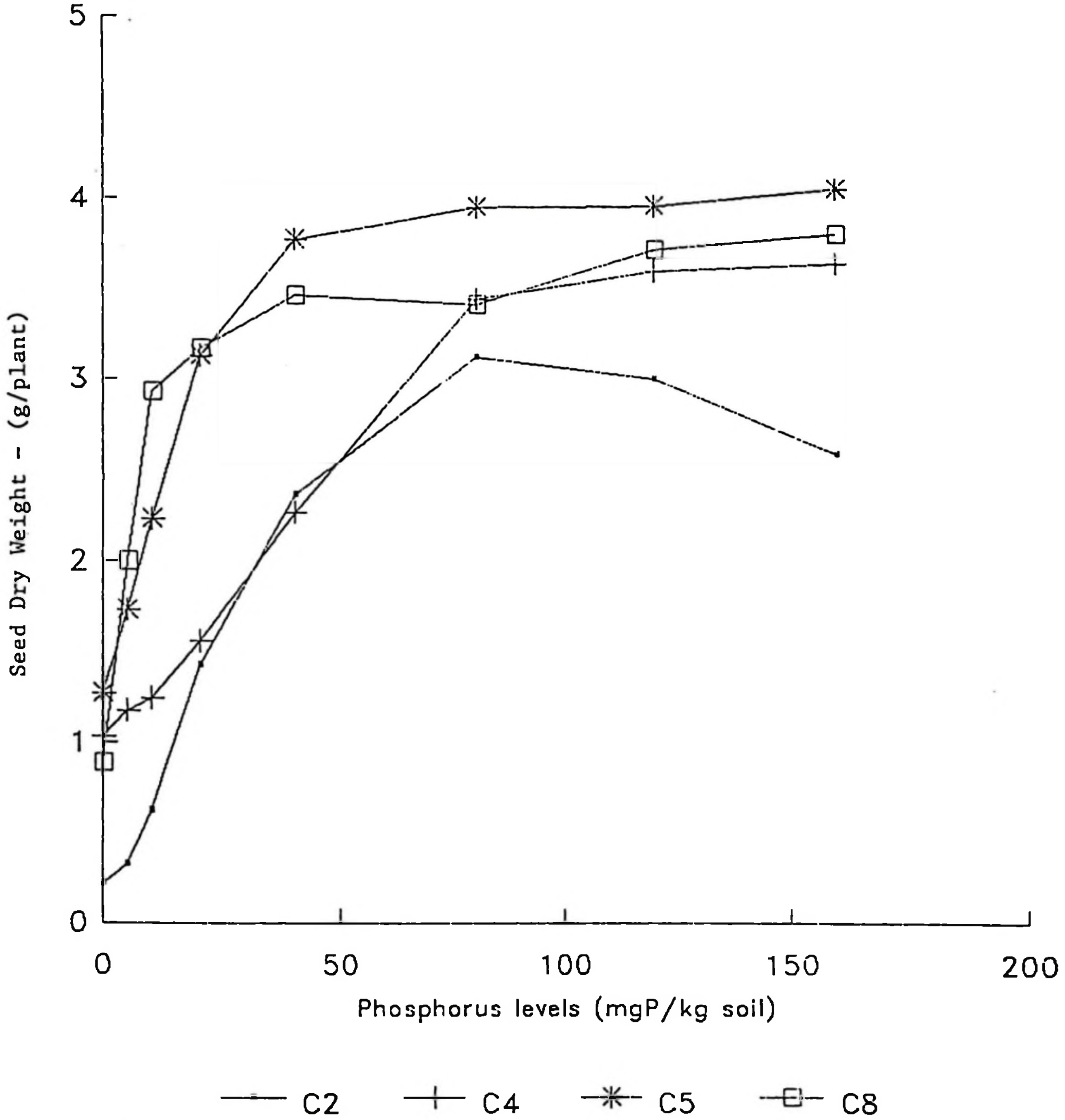

Figure 7. Effect of Phosphorus on Seed Weights of Four Bean Genotypes.

four genotypes (Appendix 8). The seed yields for the four genotypes followed the order $C_5 > C_4 > C_8 > C_2$, $C_5 > C_4 > C_8 > C_2$, and $C_5 > C_8 > C_4 > C_2$ at phosphorus levels 0, 80 and 160mgP/kg soil, respectively. The rates of increase in seed production per unit of added phosphorus were highest in the phosphorus levels > 0 to 40mgP/kg soil and > 0 to 80mgP/kg soil for genotypes C_5 and C_4 and genotypes C_8 and C_2, respectively. The rapid increase in the seed production suggest that phosphorus was the main factor limiting seed production (or generally growth) for the four genotypes. The variations in the rates of seed production with increases in the phosphorus levels > 0 to 40 and > 80mgP/kg soil is a reflection of the inherent genetical capacity of the four genotypes to withstand phosphorus stress, hence their levels of requirement and efficiency in phosphorus utilization.

The levels of seed production without added phosphorus reflect the yield potentials of the four bean genotypes when phosphorus is the major factor limiting growth (Dale *et al*., 1977; Pereira and Bliss, 1987; 1989). The differences in seed production at zero level phosphorus is an indication of the particular genotypes to extract and make maximum use of the low level of native phosphorus in the soil; in addition to their yield potentials. The observation that the rates of increase in seed yields per unit of phosphorus added were highest in the phosphorus levels 〉 0 to 80mgP/kg soil for all the four genotypes suggest that the optimum phosphorus requirement by the four genotypes lie within the phosphorus levels 20-80mgP/kg soil. This could be substantiated by the observation that the seed yields beyond 80mgP/kg soil levels of phosphorus remain constant and decline with increasing levels of phosphorus (Fig. 7 and Appendix 8). Application of phosphorus beyond 80mgP/kg soil would therefore prove to be uneconomic.

The decrease in seed production (seed yield/seed weight) for genotypes C_2 and C_5 at applied phosphorus rates $\geq$ 80 and $\geq$ 120mgP/kg soil, respectively could be an indication of excessive supply and uptake of phosphorus by the genotypes resulting to reduced vegetative growth, enhanced maturity and ion-imbalances in the plants; all these culminating to decline in seed production. The very small increases in seed production for genotypes C_4 and C_8 at phosphorus levels $\geq$ 80mgP/kg soil, suggest that, the critical levels of phosphorus for these genotypes is $\leq$ 80mgP/kg soil added phosphorus. Based on the data obtained in this study with respect to seed yield the common bean genotypes C_5 and C_4 should be selected and recommended to be grown in soils with low soil available phosphorus, or in areas where phosphate fertilizers are expensive, hence high application rates are unaffordable.

The four common bean genotype seed yields recorded at the various levels of applied phosphorus, hence the magnitude of plant available phosphorus are the interactions of the effective common bean genotype *Rhizobium* strain PV1, hence the amounts of nitrogen fixed symbiotically, the positive response by the genotypes and PV1 *Rhizobium* to phosphorus, hence more nitrogen fixation and the inherent genetical yield potentials of the common bean genotypes under investigation (McFerson *et al.*, 1981; Dale *et al.*, 1977; Pereira and Bliss, 1987; 1989; Haag *et al.*, 1977; Garg *et al.*, 1971; Saidou, 1985; Ssali and Keya, 1983; 1985; Whiteaker *et al.*, 1976; Leif, 1990).

4.7 Effect of phosphorus on nitrogen fixation.

The effects of phosphorus on the percentage nitrogen contents in the four bean genotypes, which reflect the extent of symbiotic nitrogen fixation by the *Rhizobium* PV1-common bean genotype associations were as presented in Fig. 8 and Appendix 9. The amounts of nitrogen accumulated by the four common bean genotypes increased with increasing levels of added phosphorus and the increases in the amounts of nitrogen fixed symbiotically were significant ($P \leq 0.05$) (Appendix 9). The trends in the amounts of nitrogen fixed followed the order $C_4 \rangle C_8 \rangle C_2 \rangle C_5$; $C_8 \rangle C_4 \rangle C_2 \rangle C_5$ and $C_8 \rangle C_4 \rangle C_2 \rangle C_5$ at zero, 80 and 160mgP/kg soil, respectively. The highest amounts of the % N contents were attained at 40, 40-60 and 120mgP/kg

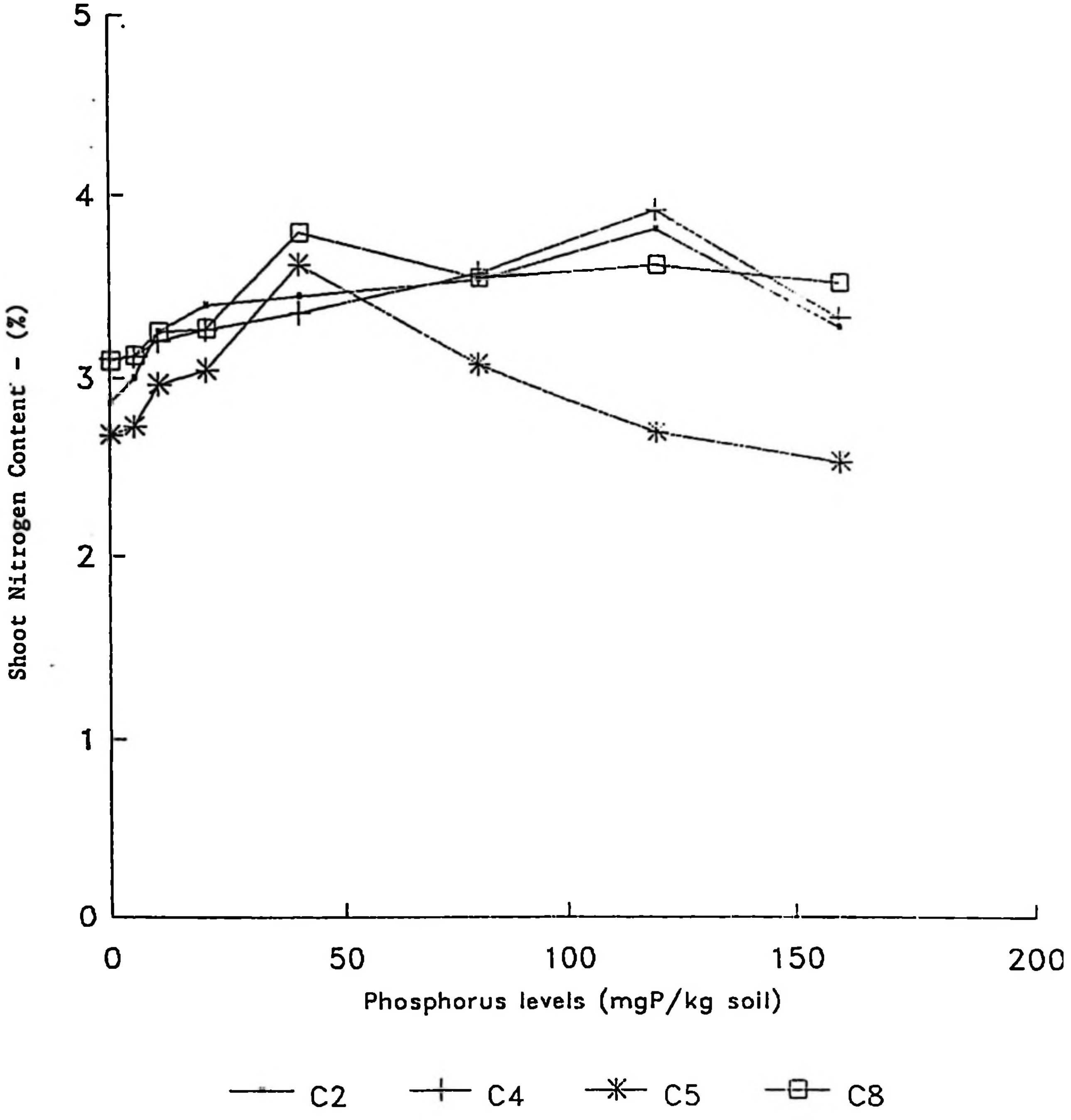

Figure 8. Effect of Phosphorus on the % N Contents in the Shoots of Four Bean Genotypes.

soil for genotypes C_5, C_8 and C_4 and C_2, respectively. The trends in % N contents in the shoots of the four common bean genotypes, particularly for genotypes C_5 and C_8 reflect the variations in the extent of the symbiotic association between the Rhizobium and the plants with increasing levels of added phosphorus hence plant available phosphorus. The trends in % N contents in genotypes C_4 and C_2 were almost similar, suggesting little variations among the two genotypes in symbiotic nitrogen fixation with the application of phosphorus.

The percentage N contents in the shoots of the four genotypes at zero level of phosphorus (control) is an indication of the capabilities of the individual genotypes to symbiotically fix nitrogen through the Rhizobium PV1-genotype associations at very low levels of available phosphorus (Table 2). The percentage N contents for the controls (zero level added phosphorus), is also an indication of the extent of the effectiveness of the Rhizobium PV1-genotype association to symbiotically fix nitrogen at very low levels of plant available phosphorus. This could be attributed to the genetic make up, physiological and metabolic phosphate requirement and utilization by the individual bean genotypes and Rhizobium (Whiteaker et al., 1976; Leif, 1990; Duque et al., 1985; Ssali and Keya, 1983; 1985). Further, the abilities of the genotypes to symbiotically fix nitrogen at very low levels of available native soil phosphorus could be attributed to the genotype's abilities to extract phosphorus from the soil, through the development of elaborative and extensive root system (Whiteaker et al., 1976; Leif, 1990; Duque et al., 1982; Ssali and Keya, 1985; Fox et al., 1974) which could explore considerable volume of the soil.

The rapid increase in the % N contents for genotypes C_5 and C_8 in the range of applied phosphorus between ≥ 0 to 40mgP/kg soil and the attainment of the highest % N at 40mgP/kg soil reflect the deficiency of the native soil phosphorus and the importance and essentiality of phosphorus in nitrogen fixation through its influence on the number and mass of nodules and the duration of the efficiency for the Rhizobium-legume symbiotic association (Duque et al., 1985; Mengel and Kirkby, 1976; Brady, 1984; Sanchez, 1976). It could, therefore, be argued that the phosphorus requirements and utilization by genotypes C_5 and C_8 in nitrogen fixation, could be considered as being low, hence they are more efficient in the utilization of phosphorus.

For genotypes C_2 and C_4 the increase in % N contents and attainment of the highest % N content at about 120mgP/kg soil reflect the higher requirement of phosphorus by these genotypes in relation to symbiotic nitrogen fixation. The very sharp decrease in % N contents for genotypes C_5 and C_4 and C_2 with increasing levels ≥ 40mgP/kg soil and ≥ 120mgP/kg soil, respectively, suggest the presence of excessive amounts of plant available phosphorus, hence reducing the ability of the Rhizobium-genotype association to symbiotically fix nitrogen. The high levels of phosphorus for individual genotypes could have, therefore, effectively influenced the efficiency and extent of duration of the Rhizobium-bean genotype symbiotic association (Duque et al., 1985). The observation that although maximum N accumulation for genotype C_8 was attained at 40mgP/kg soil, and thereafter decreased very slowly with increasing levels of phosphorus in contrast to genotypes C_5, C_4 and C_2 could be a reflection of the capacity of genotype C_8 to tolerate high levels of available (soil solution) phosphorus. For genotype C_5, the very high rate of decrease in the % N contents with increasing levels of phosphorus, ≥ 40mgP/kg soil until at 160mgP/kg soil and the % N content in the deficiency range suggest that the symbiotic Rhizobium-genotype C_5 association was rendered ineffective. The lack of clear cut trends between the % N accumulation (content) in the four common bean genotypes and the other plant parameters and the correlation coefficient (Tables 3(a)-(d)) indicate the intricacies, complexities and interactions involved in plant growth and development and hence the Rhizobium PV1-common bean genotype symbiotic associations. It could therefore, be argued that phosphorus is only one of the many factors influencing symbiotic nitrogen fixation.

Based on the fact that the percentage total nitrogen in the soil (Table 2) was low (Landon, 1984), it could, be considered that the % N in the shoots at zero level of added phosphorus for all the genotypes above the critical levels was an indication of effective Rhizobium-genotype symbiotic association. The observations made in this study where % N contents increased with increasing levels of phosphorus conform with those of Attwell and Bliss (1985), Rennie and Kemp (1983), Pereira (1987) and Pereira and Bliss (1989), and Graham and Rosas (1977). The decrease in percent nitrogen contents at various levels of phosphorus conform with those of Ssali and Keya (1985) and this could be attributed to excessive supply of phosphorus above the maximum levels required for maximum nitrogen accumulation, hence creating nutrient imbalances in the plants.

4.8 Effect of phosphorus on shoot % P contents.

The response of the four common bean genotypes with respect to % P contents in the four common bean genotypes with increasing levels of added phosphorus were as presented in Fig. 9 and Appendix 10. The increase in shoot % P contents in the four genotypes increased significantly with increasing levels of added phosphorus (Appendix 10). The trends in the percent P contents increasing continuously with increasing levels of phosphorus up to

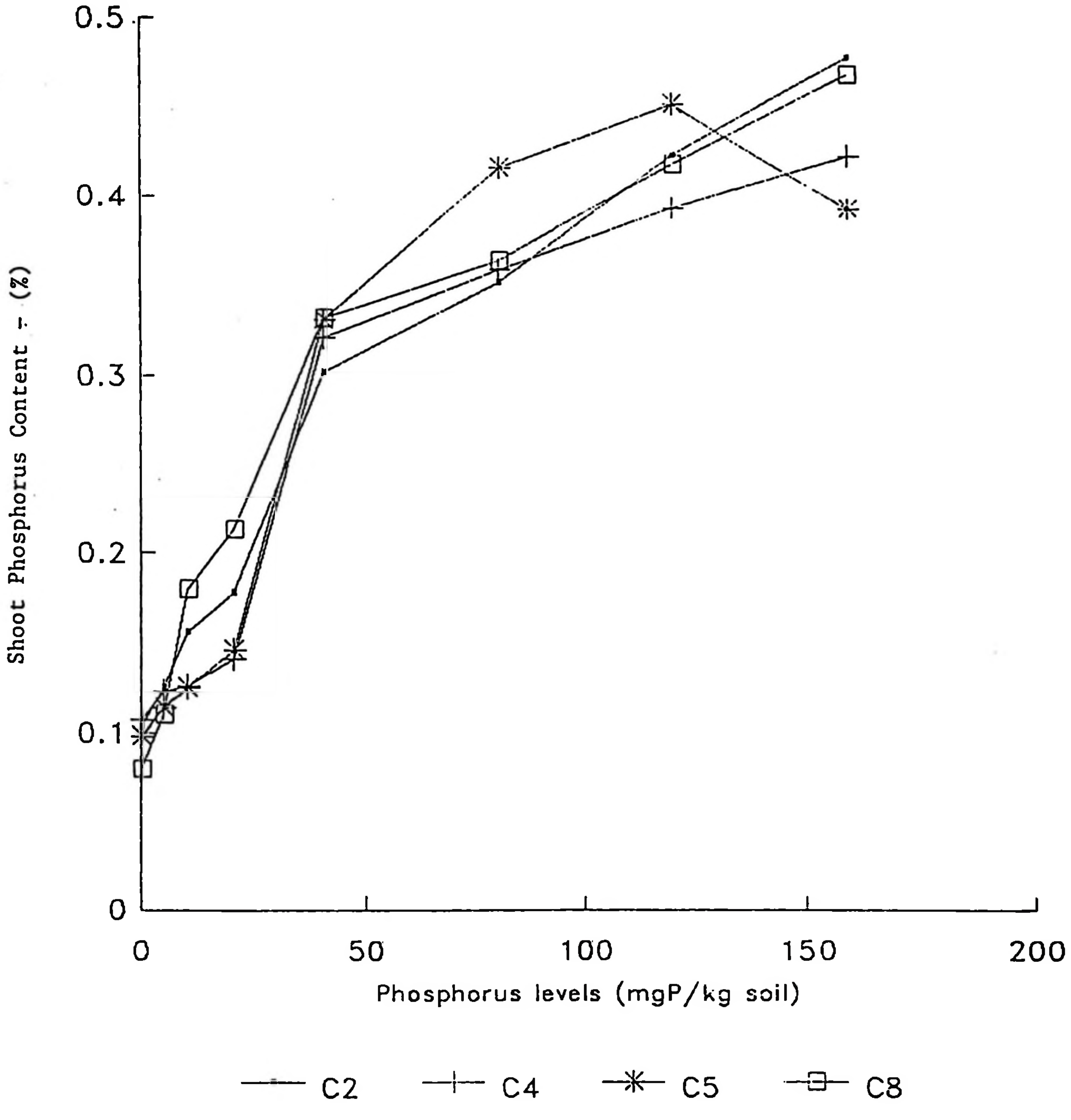

Figure 9. Effect of Phosphorus on the % P Contents in the Shoots of Four Bean Genotypes.

levels ≥ 160mg P/kg soil except for genotype C_5,indicate increased uptake with increasing level of phosphorus.

The very low % P contents (in the deficiency range) in the four common bean genotype shoots at zero level of phosphorus (the control) confirm the very low plant available soil phosphorus (Table 2). The observed percentage P contents (Fig. 9) suggest similar trends among the four genotypes in phosphorus requirement and utilization, except for genotype C_5 at higher levels of added phosphorus. The increase in phosphate availability with increasing levels of added phosphorus conform with the increase in % P contents in the genotype shoots equal and beyond the critical levels of 0.31 to 0.41% P (Qureshi, 1981; CIAT, 1977). The response to phosphorus beyond the critical % P in all the four bean genotypes reflect their variations in inherent genetical characteristics with respect to phosphorus requirement and utilization.

The significant ($P \leq 0.05$) correlation coefficient between the percentage P contents with other plant parameters (Tables 3(a)-(d)) confirm the importance and essentiality of phosphorus in the Rhizobium-genotype symbiotic association (Dale et al.,1977; Pereira and Bliss, 1987; 1989; Graham, 1981; Graham and Temple, 1984; Ssali and Keya, 1985).

CHAPTER FIVE

SUMMARY AND CONCLUSSIONS

The study of the genotypic variation in phosphorus requirement and utilization by four common bean genotypes carried in a glasshouse experiment at the Sokoine University of Agriculture demonstrated the differences in phosphorus requirement and utilization among the four genotypes. This was observed in different bean characteristics investigated during the study. The characteristics included nodulation of the roots, root and shoot dry weights, weight and number of pods, phosphorus and nitrogen contents in shoots and seeds and seed dry weight.

Addition of various levels of phosphorus into the soil in increasing order, increased nodulation in genotypes C_2, C_4 and C_5 up to 80-100mgP/kg soil above which there was a decrease in nodule weight for all the three genotypes. This observation could be due to reduced root growth, early maturation, and reduced vegetative growth and hence reduced nodulation. This indicated that each genotype had a certain means for controlling excessive uptake of phosphorus so as to prevent distortion of ion equillibrium and consequently the metabolic processes in the plants. It can therefore be concluded that genotypes

C_2, C_4 and C_5 were more tolerant to low levels of available phosphorus while genotype C_8 was not. This indicated that there were differences in the four genotypes with respect to their capability for absorbing and utilizing low available phosphorus. It also indicated that the four genotypes differed in the amount of available phosphorus that they required for their maximum nodulation. Research on the plants phosphorus absorption and utilization efficient rate of these four genotypes will probably confirm the above observations.

Genotype C_4 was furthermore capable in utilizing the absorbed phosphorus for growth and gave the highest shoot dry weight increase with the increasing levels of added phosphorus having its maximum shoot dry weight at 80-100mgP/kg soil. Genotype C_5 attained its maximum shoot dry weight at the lowest level (40-50mgP/kg) soil of added phosphorus compared to the other three. This is an indication of the genotype's high ability in utilizing the absorbed phosphorus for high dry matter accumulation or the genotypes ability to absorb more available phosphorus than the other genotypes for the observed dry matter accumulation. It can, therefore, be concluded that genotype C_5 had a better vegetative growth from its early stages of growth and, therefore, accumulated higher dry matter than C_4, C_8 and C_2. Since the genotype C_5 plants responded to phosphorus at the low levels of added phosphorus it means that the plants started to have firm functioning roots earlier than C_4, C_8 and C_2 and, therefore, started to fix nitrogen earlier than the rest. This lead to healthy plants which had more leaf area for photosynthesis than the rest meaning that more carbon compounds were transported to the rhizosphere to enable multiplication of the nitrogen fixers and therefore more ability to fix more nitrogen than for the other three genotypes. It can, therefore, be suggested that research on the amount of photosynthates being transported in these genotypes could help confirm the plants ability to respond or tolerate low phosphorus levels in relation to the amount of dry matter accumulated in each genotype. Knowledge on the initial soil <u>Rhizobium</u> population and strains will also help in confirming the above observation. However genotype C_5 had outstanding abilities for pod production, giving higher number of pods as well as higher pod dry weight and seed dry weight per plant compared to C_8 and C_2. Since genotype C_5 and C_4 had a better response to added phosphorus than C_8 and C_2 in nodulation and shoot dry weight and these variables were also correlatd to pod number, and pod dry weight as well as seed dry weight for the same genotype it suffices to conclude that the observed good response of the number and pod dry weight as well as seed dry weight was determined by these previously observed variables. Field experiment will further confirm this observation.

Highest percent shoot nitrogen contents at the lowest added phosphorus rate were attained at 40 and 40-60mgP/kg soil for genotypes C_4 C_5 and C_8, respectively. Furthermore, shoot nitrogen content of genotypes C_5 and C_8 increased highly with added phosphorus from 20mgP/kg soil to 40mgP/kg soil. This observation indicate their ranges of P requirements for maximum nitrogen fixation to meet the plants nitrogen requirements from their very initial stages of growth. Since the soil used was low in percent nitrogen content, the response of genotype C_5 and C_8 to low available phosphorus indicated the plants need for phosphorus in their early stages due to its essentiality in nitrogen fixation. Optimum percent shoot phosphorus accumulation for genotype C_5 which was attained at 120mgP/kg soil indicated that genotype C_5 had absorbed enough phosphorus for the plants growth and development at that level of added phosphorus. For the other three genotypes the optimum phosphorus absorbing capacity was higher than that level of available phosphorus. It can, therefore, be concluded that among the four genotypes used, genotype C_5 had the highest ability to tolerate and utilize low available phosphorus for its maximum growth, development and final yield. This was due to the genotype's tolerancy to low available phosphorus for its optimum yield as a result of its genetical and physiological characteristics as well as low metabolic phosphate requirement and utilization. Also genotype C_5 had a higher compatibility with the nitrogen fixing Rhizobium strain (PV1) which was used in inoculating the beans. The order of the four genotypes' ability to tolerate low available phosphorus can, therefore, be $C_5 > C_4 > C_2 > C_8$ based on all the yield variables observed. If genotype C_5 is inoculated with PV1 and used for seeds, bean yield in soils which are low in available phosphorus will be increased at low phosphatic fertilization.

For future research and further findings in this field the following studies can be suggested:

(1) Investigation on the size of the population of PV1 during different stages of the plants growth and other possible Rhizobium strains in the soil (which can as well contribute to nitrogen fixation in those bean genotypes) and their strength, i.e. to find out whether they can be more effective than PV1 in the experimental soil.

(2) Similar experiment be done in the field to find out the results under conditions similar to those of an ordinary subsistence farmer.

(3) Similar experiment be done using other sources of phosphorus both in the field and in controlled situations.

(4) Investigate effect of diseases, pests, heat and drought and management on the final yield of these PV1 inoculated four bean genotypes versus other R.

phaseoli strains so as to find out the extent at which bean production can be improved by inoculating any of the four genotypes.

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APPENDICES

Appendix 1. Profile description for the Oxic Haplustult profile.

(Kaaya, 1989)

1. Information on the site.

(a) Higher category classification:

- FAO: Dystric Nitosol
- USDA: Oxic Haplustult

(b) Date of examination: 6th April, 1987

(c) Location: UTM Zone 37m cc 489439

(d) Evaluation: 498m

(e) Land form:

- Physiographic position: concave slope
- Sorrounding land form: undulating
- Microtopography: nill

(f) Slope on which profile is sited: gently sloping, (4%)

(g) Land use: At the time of examination the land was under cultivation of good growing maize crop

(h) Climate: Sub-humid tropical type with bimodal rainfall distribution

2. General information on the soil.

(a) Parent material: Colluvial material from metasediments of the Uluguru mountains

(b) Drainage class: Class 4, well drained

(c) Moisture conditions in the profiles: dry throughout at examination time

(d) Depth of groundwater table: below 150cm at all times of the year

(e) Presence of surface stones and rock outcrops: none

(f) Evidence of erosion: none detected

(g) Presence of salts or alkaline: none

(h) Human influence: slight and confined to the plough layer.

3. Brief description of the profile.

- Deep, well-drained, red to dark red with clayey soil throughout
- It is characterized by presence of an ochric A horizon overlying an argillic B horizon

4. Profile description.

Ap 0-17. Dark red (10R 3/6) moist and red (10R 4/6) dry; clay; moderate medium crumb; sticky, slightly plastic (wet), friable (moist), hard (dry); many fine and few medium tubular pores; many very fine and fine roots; abrupt, smooth boundary.

B_{21t} 17-43cm. Red (10R 4/8) moist and red (10R 5/8) dry; clay; moderate medium sub-angular blocky; slightly sticky ,slightly plastic (wet)
friable (moist), slightly hard (dry); many very

fine to fine and few medium tubular pores; frequently very fine roots; clear, smooth boundary.

B_{22t} 43-83cm. Red (10R 4/8) moist and red (10R 5/8) dry; clay; weak very fine sub-angular blocky; slightly sticky, slightly plastic (wet), friable (moist), slightly hard (dry); common very fine and fine inped tubular pores; frequent very fine roots; clear, weavy boundary.

B_{23t} 83-115cm. Dark red (10R 3/6) moist and red (10R 4/8) dry; clay; weak very fine sub-angular blocky; slightly sticky, slightly plastic (wet), friable (moist), slightly hard (dry); common very fine and few fine inped tubular pores; very few fine roots; clear smooth boundary.

B_{24t} 115-153cm. Dark red (10R 3/6) moist and red (10R 4/8) dry; clay; moderate fine to medium angular blocky; slightly sticky, slightly plastic (wet), friable (moist), hard (dry); few very fine to fine tubular pores.

Appendix 2. Number of nodules per plant

Genotype(A)	Replication(R)	Treatment P mgP/g soil (B)							
		0	5	10	20	40	80	120	160
	1	12	22	40	52	139	158	135	130
	2	32	38	50	56	159	110	107	101
C_2	3	17	27	30	117	123	157	127	99
	Mean	20	29	40	75	140	142	123	110
	1	52	56	60	87	120	134	125	66
	2	55	59	62	54	71	126	120	110
C_4	3	39	41	43	42	91	169	169	93
	Mean	49	52	55	61	95	143	138	90
	1	42	53	61	80	135	157	160	139
	2	48	55	62	67	131	167	170	131
C_3	3	73	66	65	73	99	110	114	171
	Mean	54	58	63	73	122	145	148	147
	1	30	39	52	99	131	158	220	262
	2	29	35	49	93	142	223	235	193
$C_{[illegible]}$	3	43	55	73	136	190	211	241	256
	Mean	31	43	58	109	155	197	232	237

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	0.93			
A	28.30	4.266	12.060	20.66
B	74.23	6.033	17.056	
AB	4.76	2.067	34.1113	

NB: DMRT = Duncans's Multiple Range Test
LSD = Least Significance Difference CV = Coefficient of variation

Appendix 3. Nodules dry weight per plant (g)

Genotype(A)	Replication(R)	Treatment P mgP/g soil (B)							
		0	5	10	20	40	80	120	160
C_2	1	0.015	0.021	0.030	0.067	0.128	0.180	0.178	0.151
	2	0.117	0.023	0.029	0.054	0.119	0.118	0.125	0.132
	3	0.010	0.022	0.034	0.056	0.141	0.073	0.060	0.113
	Mean	0.014	0.022	0.031	0.059	0.129	0.124	0.121	0.0321
C_4	1	0.012	0.052	0.123	0.168	0.122	0.148	0.145	0.133
	2	0.032	0.048	0.048	0.017	0.018	0.058	0.262	0.260
	3	0.098	0.059	0.040	0.023	0.101	0.267	0.264	0.225
	Mean	0.048	0.053	0.060	0.070	0.094	0.226	0.223	0.163
C_5	1	0.022	0.074	0.089	0.093	0.143	0.137	0.115	0.104
	2	0.063	0.098	0.090	0.061	0.134	0.143	0.114	0.097
	3	0.041	0.110	0.112	0.162	0.206	0.105	0.098	0.094
	Mean	0.042	0.094	0.097	0.106	0.161	0.128	0.109	0.098
C_8	1	0.030	0.040	0.066	0.063	0.201	0.248	0.320	0.377
	2	0.011	0.045	0.072	0.163	0.155	0.273	0.285	0.226
	3	0.043	0.059	0.066	0.144	0.319	0.263	0.292	0.326
	Mean	0.028	0.048	0.068	0.123	0.225	0.261	0.299	0.310

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	1.43			
A	21.01	0.001287	0.0258	34.96
B	29.30	0.012909	0.03649	
AB	3.95	0.02581	0.07299	

Appendix 4. Shoot dry weight per plant (g)

Genotype(A)	Replication(R)	Treatment P mgP/g soil(B)							
		0	5	10	20	40	80	120	160
	1	0.911	0.954	1.025	1.207	1.763	2.053	2.025	1.624
	2	0.883	0.890	0.952	1.039	2.034	1.742	1.625	1.487
C_2	3	0.610	0.706	1.023	1.471	2.400	2.454	2.410	1.985
	Mean	0.801	0.850	1.000	1.239	2.066	2.083	2.020	1.699
	1	1.06	1.057	1.452	1.861	1.150	2.856	2.751	2.124
	2	1.711	1.675	1.344	1.560	1.765	2.487	2.812	2.941
C_4	3	1.117	1.108	1.104	0.570	2.021	3.207	2.987	3.231
	Mean	1.296	1.280	1.300	1.300	1.645	2.850	2.850	2.765
	1	0.743	1.482	1.490	1.656	1.925	1.599	1.530	1.526
	2	1.650	1.500	1.520	1.144	1.737	1.583	1.521	1.534
C_5	3	1.404	1.368	1.490	2.186	1.863	1.876	1.509	1.786
	Mean	1.258	1.450	1.500	1.663	1.847	1.685	1.520	1.615
	1	1.062	1.230	1.392	1.369	1.911	2.158	2.200	1.781
	2	1.010	1.260	1.412	1.617	1.679	2.243	2.300	2.485
C_3	3	1.251	1.260	1.396	2.028	2.914	2.760	2.700	2.944
	Mean	1.108	1.250	1.400	1.672	2.168	2.387	2.400	2.403

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	5.75			
A	11.75	0.063	0.1787	18.21
B	28.17	0.089	0.2528	
AB	3.47	0.1788	0.5057	

Appendix 5. Dry weight of roots per plant (g)

Genotype(A)	Replication(R)	Treatment P mgP/g soil(B) 0	5	10	20	40	80	120	160
C_2	1	0.699	0.700	0.705	0.880	0.675	0.792	0.600	0.879
	2	0.757	0.800	0.735	0.594	0.583	0.802	0.756	0.660
	3	0.305	0.306	0.528	0.724	0.584	0.395	0.483	0.529
	Mean	0.587	0.602	0.656	0.730	0.614	0.663	0.613	0.689
C_4	1	0.442	0.400	0.362	0.442	0.369	0.535	0.621	0.650
	2	0.440	0.426	0.358	0.440	0.639	0.700	0.625	0.284
	3	0.271	0.275	0.369	0.360	0.434	0.582	0.521	0.473
	Mean	0.384	0.367	0.363	0.414	0.480	0.605	0.589	0.469
C_5	1	0.760	0.770	0.701	0.725	0.811	0.544	0.521	0.511
	2	0.664	0.720	0.712	0.603	0.458	0.710	0.580	0.567
	3	0.953	0.817	0.735	0.805	0.785	0.4637	0.477	0.458
	Mean	0.793	0.769	0.716	0.711	0.685	0.573	0.526	0.522
C_9	1	0.424	0.452	0.490	0.520	0.578	0.651	0.690	0.993
	2	0.546	0.515	0.520	0.531	0.650	0.607	0.670	0.843
	3	0.472	0.497	0.490	0.559	0.622	0.753	0.686	0.683
	Mean	0.481	0.488	0.500	0.537	0.617	0.670	0.682	0.839

<u>Statistic variables:</u>

Variable	F-value	DMRT	LSD	CV
R	1.74			
A	1.83			82.55
B	1.07			
AB	1.13			

Appendix 6. Number of pods per plant

Genotype(A)	Replication(R)	Treatment P mgP/g soil(B) 0	5	10	20	40	80	120	160
C_2	1	0.67	0.70	1.15	2.30	3.33	3.00	3.00	3.00
	2	0.67	0.80	1.00	1.67	2.33	3.00	3.15	3.00
	3	1.30	1.50	1.60	2.00	3.00	3.00	3.15	3.00
	Mean	0.88	1.00	1.25	1.99	2.89	3.00	3.10	3.00
C_4	1	0.67	1.00	1.35	1.33	1.33	1.67	2.40	3.00
	2	1.00	1.05	1.55	1.65	3.00	2.33	2.75	3.00
	3	1.00	1.01	1.54	1.67	1.33	2.67	2.80	2.67
	Mean	0.89	1.02	1.48	1.55	1.89	2.22	2.65	2.89
C_5	1	1.67	1.75	2.70	3.33	3.00	3.33	3.28	3.00
	2	1.67	1.80	2.90	3.00	3.33	3.00	2.95	2.33
	3	1.00	1.19	1.90	2.00	3.67	3.00	3.52	3.67
	Mean	1.45	1.58	2.50	2.78	3.33	3.11	3.25	3.00
C_3	1	0.67	1.00	1.40	2.67	2.67	2.33	2.33	2.33
	2	1.00	1.00	1.50	2.00	2.33	2.33	2.60	2.67
	3	1.00	1.03	1.60	1.33	2.00	2.67	2.87	3.00
	Mean	0.89	1.01	1.50	2.00	2.33	2.44	2.60	2.67

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	0.25			
A	23.10	0.079	0.2234	18.10
B	48.18	0.1118	0.3160	
AB	1.29	--	--	

Appendix 7. Weight of pods per plant (g)

Genotype(A)	Replication(R)	Treatment P mgP/g soil(B) 0	5	10	20	40	80	120	160
C_2	1	0.47	0.80	1.55	2.54	3.80	4.60	4.70	3.17
	2	0.11	0.55	1.35	2.50	3.47	5.24	5.40	4.90
	3	0.47	0.90	1.60	2.46	3.20	4.25	4.60	3.63
	Mean	0.35	0.75	1.50	2.50	2.49	4.70	4.90	3.90
C_4	1	0.93	1.30	1.85	1.70	3.21	4.89	5.75	5.41
	2	0.97	1.40	1.80	3.54	5.39	5.08	5.95	5.50
	3	1.66	1.65	2.05	2.48	3.17	7.61	6.00	6.31
	Mean	1.19	1.45	1.90	2.57	3.92	5.86	5.90	5.74
C_3	1	2.48	2.50	3.20	5.21	4.49	5.77	5.90	5.74
	2	2.66	2.20	2.80	4.28	5.34	4.95	5.20	5.66
	3	0.47	2.35	3.30	3.56	5.57	5.79	6.00	5.88
	mean	1.87	2.35	3.10	4.35	5.13	5.50	5.70	5.76
C_1	1	0.93	1.60	2.30	5.45	5.09	5.41	5.60	5.29
	2	1.46	2.20	2.70	4.38	4.68	4.71	5.00	5.70
	3	1.43	2.05	2.50	3.05	4.79	5.47	5.60	5.92
	Mean	1.27	1.95	2.50	4.29	4.85	5.20	5.40	4.64

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	1.15			
A	3.76	0.44	1.25	56.68
B	6.37	0.6239	1.7639	
AB	1.01	--	--	

Appendix 8. Dry weight of seeds per plant (g)

Genotype(A)	Replication(R)	Treatment P mgP/g soil(B)							
		0	5	10	20	40	80	120	160
C_2	1	0.31	0.35	1.65	1.47	2.56	3.03	2.95	2.03
	2	0.05	0.30	1.50	1.18	2.41	3.55	3.40	3.34
	3	0.30	0.34	0.74	1.65	2.15	2.84	2.74	2.49
	Mean	0.22	0.33	0.63	1.43	2.37	3.14	3.03	2.62
C_4	1	1.48	1.19	1.30	0.93	2.12	3.12	3.60	3.63
	2	0.53	1.17	1.40	2.24	3.19	3.00	3.25	3.32
	3	1.08	1.18	1.05	1.52	1.50	4.26	4.04	4.08
	Mean	1.03	1.18	1.25	1.56	2.27	3.46	3.63	3.68
C_3	1	1.73	1.70	2.30	3.69	3.61	4.26	4.15	4.03
	2	1.79	1.75	2.15	3.06	3.82	3.59	3.80	4.13
	3	0.30	1.74	2.24	2.65	3.91	4.05	4.02	4.13
	Mean	1.28	1.73	2.23	3.13	3.78	3.97	3.99	4.10
C_5	1	0.64	1.85	2.95	3.86	3.52	3.68	3.70	3.46
	2	0.98	2.30	3.00	3.05	3.39	2.79	3.70	3.94
	3	1.04	1.85	2.84	2.61	3.51	3.83	3.85	4.13
	Mean	0.89	2.00	2.93	3.17	3.17	3.43	3.75	3.84

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	0.11			
A	54.31	0.08	0.235	16.41
B	85.18	0.117	0.332	
AB	2.54	--	--	

Appendix 9. Shoot % N-content

Genotype(A)	Replication(R)	Treatment P mgP/g soil(B) 0	5	10	20	40	80	120	160
	1	2.695	2.852	3.125	3.395	3.675	3.395	3.975	3.325
	2	2.695	2.963	3.200	3.185	3.185	3.710	3.860	3.395
C_2	3	3.185	3.185	3.425	3.605	3.500	3.570	3.715	3.220
	Mean	2.858	3.000	3.250	3.395	3.453	3.558	3.850	3.313
	1	3.150	3.200	3.205	3.360	3.710	4.130	4.250	3.430
	2	3.101	3.106	3.200	2.905	3.080	3.220	3.800	3.640
C_4	3	3.045	3.039	3.195	3.535	3.290	3.430	3.806	3.010
	Mean	3.099	3.115	3.200	3.267	3.360	3.593	3.952	3.360
	1	2.695	2.800	3.925	3.745	1.025	3.220	2.900	2.730
	2	2.660	2.700	2.800	2.835	3.430	2.730	2.625	3.395
C_5	3	2.660	2.675	2.870	2.555	3.430	3.325	2.620	1.540
	Mean	2.672	2.725	2.965	3.045	3.628	3.092	2.715	2.555
	1	3.045	3.115	3.200	2.975	3.780	3.010	3.350	3.570
	2	3.150	3.155	3.250	3.340	4.025	3.920	3.850	3.780
C[illegible]	3	3.080	3.099	3.303	3.500	3.605	3.780	3.750	3.325
	Mean	3.092	3.123	3.251	3.272	3.803	3.570	3.650	3.558

Statistic variables:

Variable	F-value	DMRT	LSD	CV
R	1.02			
A	13.31	0.062	0.18	9.32
B	7.45	0.087	0.25	
AB	1.47	--	--	

Appendix 10. Phosphorus content in shoots (%)

Genotype(A)	Replication(R)	Treatement P mgP/g soil(B)							
		0	5	10	20	40	80	120	160
C_2	1	0.060	0.100	0.140	0.160	0.342	0.360	0.420	0.480
	2	0.190	0.195	0.195	0.20	0.292	0.390	0.435	0.480
	3	0.060	0.080	0.133	0.173	0.275	0.310	0.420	0.480
	Mean	0.103	0.125	0.156	0.178	0.303	0.353	0.425	0.480
C_4	1	0.102	0.120	0.130	0.130	0.310	0.428	0.400	0.420
	2	0.090	0.115	0.140	0.147	0.378	0.342	0.390	0.435
	3	0.130	0.134	0.135	0.147	0.275	0.310	0.395	0.420
	Mean	0.107	0.123	0.135	0.141	0.321	0.360	0.395	0.425
C_5	1	0.102	0.110	0.115	0.160	0.342	0.445	0.460	0.405
	2	0.090	0.100	0.120	0.147	0.342	0.378	0.450	0.480
	3	0.102	0.132	0.140	0.130	0.310	0.428	0.449	0.300
	Mean	0.098	0.114	0.125	0.146	0.331	0.417	0.530	0.395
C_8	1	0.090	0.100	0.175	0.218	0.342	0.325	0.450	0.525
	2	0.075	0.115	0.215	0.230	0.360	0.410	0.420	0.450
	3	0.075	0.115	0.150	0.190	0.295	0.360	0.390	0.435
	Mean	0.080	0.110	0.180	0.213	0.332	0.365	0.420	0.470

<u>Statistic variable:</u>

Variable	F-value	DMRT	LSD	CV
R	6.03			
A	1.86	--	--	12.17
B	232.49	0.0091	0.026	
AB	1.82	0.0182	0.0516	