SCREENING COMMON BEAN (*PHASEOLUS VULGARIS* L. Savi) GENOTYPES ADAPTED TO LOW SOIL PHOSPHORUS

BY

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ABSTRACT

Two screen house pot experiments were conducted to identify bean genotypes, diverse in their places of origin and seed size, based on vegetative growth and P uptake and final grain yield for adaptation to low phosphorus. Three phosphorus levels; low P (0 added P), medium P (40 mg P/kg soil) and high P (160 mg P/kg soil) were main plots whereas 27 genotypes were subplots, in a split-plot laid out in a completely randomised design. The test soil was very fine, kaolinitic Kanhaplic Haplustult. Out of 27, seven bean genotypes were further evaluated for yield components and final grain yield at low P in the same soil. Shoot biomass, root biomass, shoot P concentration and P uptake increased with increased P supply and genotypic variability in these parameters was significant ($P < 0.05$). Genotypes G92, PRETO 143, MILENIO, VEF 88(40), BAT 477, A785, ANT 22, DOR 714 and AFR 708 performed better than other genotypes at low P level; also their response to P addition was significant. It was revealed that P was a major limiting factor to bean growth in the soil used for the experiment. Number of pods/plant increased significantly with increase in P levels and was correlated with grain yield at low P and adequate P. P treatments did not affect the seed size of genotypes. Genotypes BAT 477, MILENIO, DOR 714 and A785 had significantly higher grain yield than other genotypes at low P and may be favoured in the selection process. Although genotypes produced some grain yield at low P, soil fertility improvement by applying medium P or appreciable amounts of organic manure may improve yields and increase productivity per unit area.

DECLARATION

I, SIXBERT KAJUMULA MOURICE, do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor concurrently being submitted for a degree award in any other University.

Signature.....

Date.....20/11/2006.....

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DEDICATION

To my Mom, Helmengilda Bangaoburungi and Dad, Mourice Muhanga for their painstaking responsibility in educating the family.

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LIST OF ABBREVIATIONS

#	Number
ANOVA	Analysis of variance
ATP	Adenosine triphosphate
CABI	Commonwealth Agricultural Bureau International
CEC	Cation exchange capacity
CIAT	Centro Internacional de Agricultura Tropical (International Centre for Tropical Agriculture)
CV	Coefficient of variation
DAP	Days after planting
DAS	Days after sowing
DMRT	Duncan's Multiple Range Test
DNA	Deoxyribose nucleic acid
DM	Dry mass
FAO	Food and Agriculture Organisation
GY	Grain yield
HI	Harvest index
m.e	milliequivalents
NA	not available
NPP	Number of pods/plant
NS	not significant
NSP	Number of seeds per pod
OC	Organic carbon
p.p.m.	parts per million

RNA	Ribonucleic acid
SA	Sulphate of ammonia
SE	Standard error of the mean
TSP	Triple super-phosphate
USDA	United States Department of Agriculture

CHAPTER ONE

1.0 INTRODUCTION

The field or common bean (*Phaseolus vulgaris* L. Savi) is the most important grain legume in Tanzania as it is a basic staple and an important source of protein in both rural and urban diets (Barton and Ashock, 1984). Common bean is mainly grown in the rural parts in the higher and wetter areas where it is chiefly for local consumption although a small portion of the produce finds its way to the urban markets (Wortmann *et al.*, 1998). *Per capita* bean consumption in Tanzania in 2004 was estimated at 21 kg (FAO, 2006); however, it exceeds 50 kg in eastern and southern Africa (Wortmann *et al.*, 1998; FAO, 2006). Bean yields are still low in Tanzania with an average of 729 kg/ha (FAO, 2005). This can be attributed to a number of factors namely pests occurrence, poor weather conditions, poor agronomic practices and lack of bean varieties adapted to soil related constraints (Wortmann *et al.*, 1998). Most important soil related constraint is the phosphorus deficiency (Thung, 1991), which limits 50% of bean production in tropical soils (CIAT 1992, cited by Beebe *et al.*, 1997).

Phosphorus is second only to nitrogen as the most limiting element for plant growth (Vance *et al.*, 2000, cited by Vance, 2001) and its deficiency limits crop yield on 40% of the world's arable land (Vance, 2001). In Africa, Wortmann *et al.* (1998) found phosphorus levels in the soil to be limiting for plant growth in all but 11 out of 95 bean sites evaluated. Like other crop plants, common bean (*Phaseolus vulgaris* L. Savi) requires phosphorus for proper growth as it is involved in energy metabolism, amino acids and protein synthesis and building plant tissues and cell organelles (Marschner, 1997). Phosphorus deficiency in common bean leads to slow growth, stunting, leaf yellowing,

early seedling death, reduced overall dry matter and yield production (Singh *et al.*, 2003). Phosphorus deficiency also results in flower and pod abortion (Marschner, 1997).

Phosphorus deficiency can be overcome by using corrective soil fertility amendments, for instance by liming, particularly in acid soils to raise soil pH and by application of phosphatic fertiliser (Thung, 1991). In Tanzania the use of fertilizer is very low, such that there is an overall negative nutrient balance for N, P and K of -27, -4 and -18 kg/ha/year respectively (URT, 2000, cited by Majule, 2004). It is reported that phosphorus is applied at an average rate of 1.9 kg P/ha of cultivated land (Agricultural Input Study, 1997; cited by ICRA, 2002). This is due to high costs of the fertiliser materials. ICRA (2002) reported that in the 1995/96 cropping season, a farmer needed to sell three 90 kg-bags of maize in order to buy one 50 kg-bag of urea. This ratio may be extended to phosphate fertilisers, as the price difference between the two fertiliser materials is not high. Even when phosphorus fertiliser is applied, it succumbs to fixation by aluminium and iron oxides in acidic soils (Brady, 1990).

Since it is not easy to undertake soil fertility amendments by farmers in developing countries, an alternative must be sought by developing crops that either acquire or use phosphorus more efficiently (Hammond *et al.*, 2004). This will not only enhance yield for a given bean genotypes but also reduce production costs and dependence of farmers on soil amendment inputs (Singh *et al.*, 2003).

Common bean genotypes have varying abilities and/or efficiencies with which phosphorus is acquired from low phosphorus substrates (Fawole *et al.*, 1982; Vadez and Drevon, 2001). Phosphorus efficiency in common bean is very important as it can enable bean

production with little or no P fertiliser inputs especially in the case of resource-poor farmers. Also with P-efficient genotypes, common beans have the ability to enhance soil fertility by symbiotic nitrogen fixation. Thus, genotypes that are efficient in acquisition from the soil and utilisation of P under deficient situation need to be screened, identified, and availed to the farmers in Tanzania and elsewhere so that food security and nutritional situation can be improved. Equally important is to help the farmers who cannot afford the costs of phosphatic fertilizers and thus improving productivity, halt shifting cultivation and consequent environmental degradation.

The general objective of this study was to screen for low phosphorus adaptation in 27 common bean genotypes

The specific objectives were to:

i. Identify superior common bean genotypes under phosphorus deficiency based on shoot and root biomass, shoot P content and P uptake, and
ii. Evaluate common bean genotypes with respect to economic yield under P deficiency conditions

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Common bean (*Phaseolus vulgaris* L. Savi)

Common bean (*Phaseolus vulgaris* L. Savi) is a leguminous crop, which belongs to the subfamily Papilionoideae of the family Leguminosae (Polhill, 1981 cited by Debouck, 1991). The plant is an annual herb, erect and bushy, 20-60 cm tall or twining with stems 2-3 m long (Duke, 1983).

2.1.1 Morphology and anatomy

The bean plant has a root system comprised of a taproot, basal roots emerging from the basipetal end of the taproot and adventitious roots emerging from the subterranean portion of the hypoctyl (Duke, 1983). The root system is fibrous and may contain nitrogen-fixing nodules. The main stem originates from the axis of seed embryo and starts at the insertion of the root system (Debouck, 1991). The stem has nodes and internodes. For instance, the first node is found at the insertion of cotyledons whereas the first internode is found between the cotyledons and primary leaves. The leaves are alternate (except the first leaves), green or purple, trifoliate and possess stipules, petiole and marked pulvinus at the base. Leaflets are ovate, entire, acuminate, 6-15 cm long and 3-11 cm wide in the middle (Duke, 1983). Flowers are in lax, axillary and zygomorphic i.e. bilaterally symmetrical. The colours of the flowers are diverse, ranging from variegated, white, pink or purplish and are about 1 cm long (Duke, 1983). Pods are slender, cylindrical or flat, green, yellow, purple or black in colour depending on the cultivar. The pods are 8-20 cm long and 1-1.5 cm wide, which also depends on the cultivar. The pods are usually grabulous i.e. have a smooth surface but sometimes, puberulent pods exist and the beak of the pod is prominent.

The seeds can be white, purple red, grey, tan or black, often variegated. They are reniform, oblong or globose up to 1.5 cm long (Duke 1983). One hundred seeds weigh between 17 and 100 g depending on the cultivar (Voysest and Dessert, 1991). Surface texture of the seed may be shiny (brilliant), opaque or intermediate.

2.1.2 Growth habits

Common bean has two general growth habits namely, determinate and indeterminate growth habits (CIAT, 1987). Strong and erect stem branches, short internodes and terminal inflorescence are attributes typical to determinate cultivars (Debouck, 1991) while indeterminate cultivars possess such aspects as long internodes, weaker stems and others with long guides for climbing (CIAT, 1987).

2.1.3 Origin

The common bean (*Phaseolus vulgaris* L. Savi) originated and was domesticated in two primary centres, Central America and the southern Andes with a minor centre in northern Andes (Gepts and Debouck, 1991). Multiple domestications in the two primary centres have resulted in the formation of two gene pools, one Mesoamerican (Central America primary centre) and another Andean (southern Andes primary centre). Evolutionary forces have resulted into significant changes in morphological, physiological and genetic characteristics including growth habits, seed size, dormancy, photoperiod sensitivity, aspects related to leaf photosynthesis and phenology (Gepts and Debouck, 1991). Bean became established as a food crop in Africa even long before the colonial era and was probably introduced to the eastern Africa coasts by Portuguese traders in the sixteenth century (Greenway 1945, cited by Wortmann *et al.*, 1998). Then it was probably carried to the interior by Arab slave traders and by Swahili merchants.

2.1.4 Uses

Beans are highly nutritive, relatively low cost protein food. Green snap bean contains 6.2% protein, 0.2% fat and 63% carbohydrates whereas dried bean has 22.9% protein and 1.3% minerals (Duke, 1983) and 69.4% carbohydrate (Shellie-Dessert *et al.*, 1991). Leaves are also eaten when tender in parts of Tanzania, Malawi, Rwanda, Uganda and Zambia (Shellie-Dessert and Bliss, 1991). The green immature pods are cooked and eaten as vegetables while others are marketed fresh, frozen or canned. Mature, ripe beans are cooked. Beans generate income to the growers after harvest as it is transported to urban centres where it fetches good prices.

2.1.5 Bean production in Tanzania

In Tanzania, common bean is grown in the high altitude and wet areas especially in the northern and southern highlands and also in the northwest part in Kagera region (Wortmann *et al.*, 1998). Comparing the data by FAO (2005) (Table 1) and the yield potential of 1000 kg/ha estimated by Barton and Ashock (1984), the actual yields are still very low, despite the importance of bean as a leader of all grain legumes in supplying dietary protein in Tanzania. The rate of annual production increase is very low whilst the area under production is more or less stable (Table 1).

2.2 Phosphorus in common bean

2.2.1 General overview

Compared to other major nutrient elements, phosphorus is by far, the least mobile (Ragothama, 1999) and the most unavailable and inaccessible macronutrient in the soils and frequently limits plant growth (Vance *et al.*, 2003). It is also the most deficient macronutrient in all acidic soils of the world on which most bean production is undertaken

(Thung, 1991). When phosphorus is supplied in sufficient amounts, bean responds to P more than to other nutrients (Malavolta, 1972, cited by Thung, 1991). Bean yield increases proportionately with an increase in P fertilisation (Guzman 1980, cited by Thung, 1991) although a maximum economic fertiliser level for bean production was found to be 47 kg P/ ha in Brazil (Filho and Silva, 1994, cited by Araujo *et al.* 2000). Production increases can yet be found with the application of up to 947.6 kg P/ha (Stolberg-Wernigerode 1997, cited by Thung 1991). When phosphorus is low, proper plant growth is not warranted and can lead to up to 100% yield loss in severe deficiency situation (Singh *et al.*, 2003).

Table 1: Dry beans production statistics in Tanzania 1991-2004

Year	Area harvested x 1000 ha	Yield (kg/ha)	Production x 1000 tons
1991	420	643	270
1992	305	639	195
1993	320	614	205
1994	300	633	190
1995	340	676	230
1996	400	700	280
1997	340	676	230
1998	360	694	250
1999	360	708	255
2000	365	712	260
2001	365	712	260
2002	370	729	270
2003	370	729	270
2004	370	729	270

Source: FAO (2005)

2.2.2 Phosphorus content and availability in tropical soils

In most soils, inorganic phosphorus occurs at low concentrations in the soil solution while a large proportion of it is more or less strongly held by diverse soil minerals (Hinsinger, 2001). Phosphate ions can be adsorbed onto positively charged minerals such as Fe, and Al oxides (Brady, 1990). It can also form a range of minerals in combination with such metals as Ca, Fe and Al. Within the soil, phosphorus can be considered to be in three fractions namely the organic fraction, the inorganic fraction and a small, variable part that is soluble and can be absorbed by plants, known as the plant available P (Barber, 1995; Tan, 1995). The concentration of P present in the soil solution, even in soils with high level of available P ranges between 0.3 and 3.0 kg P/ha (Mengel and Kirkby, 1987) or rarely exceeds 10 μM in soil solutions (Bieleski 1973, cited by Schachtman *et al.*, 1998). Availability of phosphorus in soil solution is affected by a number of factors namely: -

(a) Soil pH: Phosphorus in the soil solution changes with prevalent soil pH such that the pKs for the dissociation of H_3PO_4 into $H_2PO_4^-$ and then into HPO_4^{2-} are 2.1and 7.2, respectively (Schachtman *et al.*, 1998). Therefore, below pH 6.0, most inorganic phosphorus will be present as the monovalent H2PO4– species, whereas H_3PO_4 and HPO_4^{2-} will be present only in minor proportions (Hinsinger, 2001). Most studies on the pH dependence of P uptake in higher plants have found that uptake rates are highest between pH 5.0 and 6.0, where $H_2PO_4^-$ dominates (Hinsinger, 2001). It is this monovalent phosphate that is taken up by the plant. In acidic conditions, soluble iron (Fe) and aluminium (Al) oxides react with phosphate ions to form Al and Fe phosphates, which are insoluble and thus making P unavailable to plants (Brady, 1990).

(b) Adsorption and desorption of P ions: The concentration of P ions is also controlled by processes of adsorption onto and desorption from various soil constituents. The major ones being those bearing positive charges (Hinsinger, 2001) as these comprise of variable charge compounds that contain hydroxyl (Al and Fe oxides), carboxyl (organic matter) or silanol (clays) groups. Besides these being of high point of zero charge, they occur mostly as small crystals and poorly ordered minerals that have a considerable specific surface area and hence strong reactivity as sorbents (Hinsinger, 2001). They thus play a prominent role in the adsorption of P ions in most soils including ferralsols of the tropics with high concentration of Al and Fe and in soils in alkaline pH range such as calcareous soils (Strauss *et al.*, 1997, cited by Hinsinger 2001).

2.2.3 Phosphorus in Tanzania's soils

There is a growing concern over the decline in the productivity of most agricultural soils in Tanzania. This is a result of unsustainable land use practices that have led to soil erosion and consequent changes in chemical and physical properties of the soil (Majule, 2004). For example, P content has been reported to decline by 53% in cultivated areas of semi-arid northern Tanzania due to vegetation clearance and low input agriculture there (Solomon and Lehman, 2000). In bean growing areas of Tanzania there is declining soil phosphorus due to continued nutrient mining without replenishment. For example, it is estimated that beans remove 12.5 kg P/ha (Kaihura, *et al.*, 2001), which is very high compared to addition in terms of P fertiliser by resource-poor farmers.

2.2.4 Role of P in common bean growth and nitrogen fixation

Phosphorus is the main component in nucleic acids, which, as units of DNA molecules are the carriers of genetic information and as units of RNA are the structures responsible for

translation of genetic information (Marshner, 1997). Phosphorus is also known for its structural role in biomembranes in the cell systems as it acts as a bridge between the glycerides and other molecules such as amino acid, alcohol and amines (Marshner, 1997). It is also involved in energy metabolism in the cell by forming an energy rich intermediate or coenzyme, principally ATP. This intermediate is used in biochemical processes such as photosynthesis and starch synthesis.

The involvement of phosphorus in nitrogen fixation in common bean has been under intensive study by many workers. Vadez and Drevon (2001) reported an increase in nitrogen fixed per plant (up to 360 mg N/plant) in genotype BAT 271 when high P (540 µmol P/plant/week) was supplied. Olivera *et al.* (2004) found a four-fold increase in nodule biomass when bean plants were grown on high P availability (2 mM). It was then suggested that nitrogen fixation requires more P than does plant growth (Olivera *et al.*, 2004).

The exact role of P and mechanism of its involvement in symbiotic nitrogen fixation in legumes is not yet clear (Høgh-Jensen *et al.*, 2002). Sa and Israel (1998) reported that low phosphorus reduced shoot growth, and as a result; there was lower rate of photosynthesis, which limited the supply of carbon to the nodule, thereby limiting nitrogen (N_2) reduction. Almeida *et al.* (2000) reported similar observation that, as the concentration of P in the nutrient solution decreased, the plant mass decreased by a factor of four, leading to a very low apparent demand for nitrogen. But, when plants grow faster under sufficient P, the gap between the mineral N uptake from growth substrate and the plant's N demand increases, resulting in an increase in percentage symbiotically fixed N (Almeida *et al.*, 2000). Therefore, at high P, N demand surpasses the supply, thus legumes use symbiotic fixation

as an alternative source to meet the demand. This fact conforms to the work by Sanginga *et al.* (2000) in cowpea (*Vigna unguiculata* L.) and Vincent and Drevon (2001) in common bean, where nodules number and dry mass increased with an increase in P supply. These findings led Almeida *et al.* (2000) to suggest that nodulation and nitrogen fixation were regulated by an N feedback mechanism induced by low P.

2.2.5 Effects of mineral N on symbiotic nitrogen fixation

When common bean is cultured under sufficient amounts of mineral nitrogen, nitrogen fixation process is halted because bean plants would shift to an energy-saving N absorption from the soil (Lindemann and Glover, 2004). The sensitivity of legumes to higher concentrations of mineral nitrogen in the soil can vary significantly and nitrogen fertilization suppresses effective nodulation and N-fixation in soybean (*Glycine max* L.) and *P. vulgaris* (Giller and Wilson, 1991). It was reported that common bean was even more sensitive to nitrogen fertilisation than soybean (Abaidoo *et al.*, 1989, cited by Giller and Wilson 1991). Almeida *et al.* (2000) pointed out that high plant N content leads to high concentration of amino acids in vascular tissue, reducing nodule formation and nitrogen fixation. Vadez and Drevon (2001) working with common bean *(Phaseolus vulgaris* L.Savi) did not find any nodules when 10 mM potassium nitrate (KNO_3) (approximately 13.9 mg N/litre) was added weekly (97.3 mg N/litre for seven weeks) into treatments as nitrogen source.

2.2.6 Effects of low P on growth and development of common bean

Common bean [*Phaseolus vulgaris* L. Savi] crop exhibits pronounced response to phosphorus fertilisation (Thung, 1991). Critical levels of phosphorus in the soil for common bean production vary between 8 and 15 ppm (Bray II method) (Thung, 1991),

below which the deficiency is serious, resulting in reduced yield and perhaps poor quality grain. Thung (1991) pointed out that plants which suffered from P deficiency had a tissue phosphorus content of less than 0.2% in the upper leaves during flowering. The critical level of P in tissues of bean plant varies between 0.2 and 0.4% although it is considered as 0.2% in the shoot (Bingham, 1965).

In partitioning the photosynthates, between the roots and shoots, more is taken to the roots when nutrients or water is limiting, whereas more biomass is partitioned to shoots when irradiance or CO_2 is limiting (Poorter and Nagel, 2000). Common bean plants also respond in similar manner in partitioning their biomass between below ground and above ground when cultured under extreme P regimes. This allocation or partitioning depends on the genetic ability among the genotypes (Hunt, 1981), ontogeny and on the environment experienced by the plant (Poorter and Nagel, 2000). The work by Yan *et al.* (1995a) was consistent with the functional equilibrium theory (Brouwer 1963, cited by Poorter and Nagel, 2000), where root/shoot ratio decreased with increasing P levels. This could suggest that in common bean more biomass is allocated to the shoot at adequate P and less to the roots and the opposite at low P. According to Yan *et al.* (1995a) there was a significant variation in root/shoot ratio among bean genotypes under deficient P, suggesting that possession of superior root growth may be a strategy for P acquisition when it is deficient.

2.4 Plant adaptations to P deficiency

The ability of common beans to acquire and utilise soil P at low levels varies between the genotypes (Whiteaker *et al.*, 1976; Yan *et al.*, 1995a, b, Araujo *et al.*, 1997) and this ability is heritable (Fawole *et al.*, 1982). Under deficient P conditions, common bean genotypes and other legumes show differences in the manner they react to the P stress. Responses

such as increased root/shoot ratio (Yan *et al.*, 1995a), decreased shoot (Nielsen *et al.*, 2001), altering the growth angle (Ho *et al.*, 2003) and production of adventitious roots (Miller *et al.*, 2003) confer the bean plants an ability to acquire P under deficiency conditions.

Research on plants responding to P deficiency shows that plants have developed many physiological strategies to cope with the situations (Hammond *et al.*, 2004). Plants have evolved two broad strategies for P acquisition and use in P limiting environments (Vance *et al.*, 2003). These strategies are:

a) Processes aimed at conservation of P use include: Decreased growth rate (Schachtman *et al.*, 1998), increased growth per unit uptake, remobilisation of internal P (Hammond *et al*, 2004), modifications in carbon metabolism that bypass P requiring steps (Plaxton and Carswell 1999, cited by Hammond *et al.* 2004) and possession of alternative respiratory pathways; and

b) Processes that lead to enhanced P uptake. These include: Increased production and secretion of phosphatases (Miller *et al.*, 2001), organic acids exudation (Hinsinger, 2001), increased root growth and modified architecture (Lynch, 1995) and enhanced expression of P transporters (Schachtman *et al.*, 1998).

Generally, adaptation to deficient P situations in common bean may appear not to be related to the chemical modification of the rhizhosphere (Yan *et al.*, 1995 a, b) but does appear to be related to root architecture and morphology, which are highly variable among genotypes (Miller *et al.*, 2003).

2.5 Phosphorus uptake and utilisation efficiency in common bean

Föhse *et al.* (1988) defined phosphorus efficiency as the ability of plant to produce certain percentage of its maximum yield at a certain level of soil P. It can also be referred to as external P requirement, i.e. the P content in soil required to produce 80% of the maximum yield (Yan *et al.*, 1995a). Phosphorus efficiency in common bean is described by a number of parameters. Vadez *et al.* (1999) used the ratio of plant N concentration per plant P concentration as an estimate of the plant's P use efficiency for symbiotic nitrogen fixation. P uptake efficiency was defined as the mg of P taken up in the shoot biomass per dry mass of roots (Sanginga *et al.*, 2000). This is a measure of plants' below ground investment in absorbing a particular amount of nutrient. Dechassa *et al.* (2003) showed that cabbage (*Brassica oleraceae* L.) had higher P efficiency than carrot *(Daucus carrota* L) and potato (*Solanum tuberosum* L) by attaining 80% of its maximum yield at zero applied P. The cabbage had also the least root/shoot ratio. Therefore, P uptake efficiency implies that plants that invest less in below ground parts but absorb sufficient nutrient level to produce high biomass have high uptake efficiency.

Phosphorus utilization efficiency on the other hand is defined as the relative ability to produce biomass for each unit of P accumulated (Elliot and Lauchli 1985, cited by Araujo *et al.* 1997). Sanginga *et al.* (2000) working with cowpea lines, defined P use efficiency as the dry mass of shoot (g) per P content (mg) in shoots. Plants that have higher shoot P concentration at low P supply means a greater internal P requirement for growth (Fohse *et al.*, 1988), thus inefficient and vice versa. Israel and Rufty (1988) reported that P utilization efficiency increased with increased P supply up to external concentration of 0.25-0.5 mM, beyond which the utilisation efficiency declined because the tissue P concentration increased without additional biomass. Araujo *et al.* (1997) found that wild

bean genotypes had lower P utilisation efficiency than the cultivated ones since the latter had higher shoot P concentration and less shoot biomass in low P treatment. P utilisation efficiency is important as it refers to the ability of the plants to produce biomass at a given nutrient level. It can be used to screen for plant adaptation to low or deficient nutrient availability.

A number of P efficient common bean genotypes has been revealed. For example, genotypes ICA *Pijao*, BAT 271 and *San Cristobal* 83 have been reported to be tolerant to P deficient conditions due to their ability to exhibit high ratio of plant N per plant P concentration (Vadez *et al.*, 1999). Based on grain yield production, genotypes *Rio Tibaji* and *Carioca* were termed as P efficient genotypes at 5 mg/ kg available soil P.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Experiment 1

3.1.1 Experimental site

Two pot experiments were conducted at Sokoine University of Agriculture (SUA) main campus, at latitude of 6°45' S, longitude of 37°40'E and an altitude of 547 m above sea level (masl), Morogoro, Tanzania. The soil for the experiments was randomly sampled and collected from the University Farm in Magadu area at the depth of 0-15 cm. The soil has been classified as isohyperthermic, very fine, kaolinitic Kanhaplic Haplustult (according to USDA) or Chromic Acrisol (according to FAO) (Szilas, 2002). The soil at the site is well drained, characterized by very low available P, and derived from intermediate metamorphic rocks (Szilas, 2002). The site was under cowpea (*Vigna unguiculata* L.), after which it was left to fallow for one season (about one year) prior to taking the soil for the experiment. Before cowpea crop, the site was being planted with maize and pastures, years back.

3.1.2 Soil preparation

The soil from the site was bulked on a plastic sheet and thoroughly mixed while breaking down large lumps, using a spade. It was then sieved through a 6.3 mm sieve to remove over size clods, some stones and other debris including plant roots, and dead leaves.

3.1.3 Soil analysis

A sub-sample of the sieved soil was taken for determination of appropriate chemical characteristics. Soil pH was determined in 1:2.5 soil: water suspension (pH_w).

Exchangeable calcium (Ca) and magnesium (Mg) were determined by atomic absorption spectrophotometry whereas K and Na were extracted using ammonium acetate and analysed by flame photometry. Cation exchange capacity (CEC $_{pH\ 7}$) was determined with ammonium acetate saturation method at pH 7.0. Available P was extracted using the Bray 1 method and determined by ascorbic acid-molybdate blue colour method. Organic carbon (OC) was determined with the Walkley-Black wet combustion method (Tan, 1995) and total N was determined using the Kjeldahl method.

3.1.4 Experimental procedures

3.1.4.1 Soil and nutrient mixing

The soil weighing 972 kg was mixed thoroughly with the following basic nutrients (rates in mg/kg soil); nitrogen 40 (as SA), potassium 10 (as KCl), zinc 10 (as zinc sulphate) and molybdenum 1 (as ammonium molybdate). From this uniform soil-nutrients mixture, 324 kg was filled into 81-four kg pots each containing four kg of soil. Another 324 kg of soil nutrients mixture was thoroughly mixed with 40 mg P/kg soil and filled into 81 pots. The remaining soil was mixed thoroughly with 160 mg P/kg soil and filled into plastic pots. The pots used were yellow in colour perforated at the bottom to allow drainage. The perforations were plugged with cotton wool. The pots were later arranged in the screen-house on top of meshed steel benches, one metre from the ground.

3.1.4.2 Phosphorus treatments and experimental layout

Triple super-phosphate (TSP, 46% P_2O_5) was used as a source of phosphorus. There were two phosphorus treatments and one control (no P was added), medium level of P 40 mg P/kg soil (80 kg P/ha) and a high level of P (160 mg P/kg ≈320 kg P/ha). There were a total of 243 pots organized in a split-plot completely randomised design, with three

replications for each treatment. The use of CRD was based on assumptions that the environmental conditions would be uniform across all locations in the screen-house. The main plots were the P rates and subplots were the common bean genotypes.

3.1.5 Planting materials

Common bean genotypes, diverse in their seed size, seed coat colour, seed shape, growth habits and even their places of origin were used, out of which 27 were selected for use as planting materials (Table 2). There were no specific and strict criteria in choosing the genotypes although aspects like places of origin and size were considered. This was because P efficiency is suggested as being related to geographical origin (Beebe *et al.*, 1997) and seed size (Yan *et al.*, 1995b) among bean genotypes. Of the selected genotypes, 14.8% were large (40-45g/100 seeds), another 14.8% medium (25-40 g/100 seeds) and 70.4% were small seeded (less than 25g/100 seeds) (CIAT 1987).

3.1.6 Sowing and general management

After arranging the pots into their respective positions with regard to P treatments, the pots were watered to 90% of field capacity (FC), equivalent to 800 ml of distilled water. Four seeds were sown but no rhizobia inoculation was done because of lack of the resource. Moisture in the pots was monitored through weighing moisture control pots, which indicated the amount of water (g) lost, so that the difference was replenished by adding similar volume.

At 7-10 days after sowing (DAS), the plants were thinned to two uniform seedlings per pot. All management practices were carried out accordingly; watering was done to maintain the moisture at approximately 90% ±10% of FC throughout the experiment

duration. Thirty days after sowing, just when the first flower raceme had appeared [corresponding to growth stage R5: (CIAT, 1987)] two plants were harvested, shoots were cut just above the soil level, thereafter put into paper bags and oven dried. Harvesting of the plants at R5 aimed at determining the vegetative response of the bean genotypes to varying P levels. Root system from each pot was carefully separated from the soil by soaking the mass in a bucket full of water. Roots were done out with hands. The clinging soil particles were washed off on low-pressure tap water. The roots were left for a while for water to drain away, thereafter put into paper bags and oven dried at 60°C for 60 hours. The shoots were oven dried at 70°C for 48 hours.

3.1.7 Weighing and analysis of plant materials

After shoots and roots had attained constant mass at 70°C and 60°C, respectively (established by weighing the samples until no changes in mass at the interval of two hours), dry mass was taken and recorded. Shoots were then ground and analysed for phosphorus by dry ashing and ascorbic acid-molybdate blue method. The concentration of P in the shoots was determined using spectrophotometer. P uptake (mg/pot) was calculated as the product of shoot biomass (g/pot) and shoot P content (in mg/g).

3.1.9 Data analysis for experiment 1

All data were subjected to analysis of variance (ANOVA) using the MSTAT-C software based on the following split plot model and ANOVA table (Appendix 2) (Gomez and Gomez, (1984).

$$Yij = \Pi + \alpha i + \varepsilon i + \beta j + \alpha\beta ij + \varepsilon ij$$

Where,

Y=observation due to i^{th} and j^{th} treatment

Π= General effect

α = Main factor effect

β = Sub factor effect

$\alpha\beta$ = Interaction

εi= error due to α

εij =error due to β and $\alpha\beta$

Separation of means was done using Duncan's multiple range test (DMRT) and differences between treatment means were declared significant at $P \leq 0.05$.

3.2 Experiment 2

Experiment two was carried out to evaluate grain yield performance of selected genotypes that had superior performance in terms of P uptake from experiment one. Materials and methods were similar to those of experiment one, section 3.1.1 through 3.1.4; except that 0.5 mg B/kg soil (as sodium pentaborate) was added at 18 DAP in this experiment.

3.2.1 Planting materials

Seven bean genotypes were used as planting materials (Table 3).

Table 2:Common bean genotypes used as planting materials in experiment 1

Sn	Variety	Origin (Country)	Seed Coat Colour	Seed Size
1	G92	Colombia	Brown Black Stripes	Small
2	AMADEUS	Honduras	Red	Small
3	HHL 30-75	CIAT	Dark Red	Small
4	PRETO 143	Brazil	Black	Small
5	CIM 9314-36	Congo	Red Rose Coco	Large
6	FEB 192	Colombia	Cream/Beige	Small
7	A 785	Colombia	Black	Small
8	EG 44	Na[1]	Red	Medium
9	DORADO	Honduras	Dark/Red/Black	Small
10	VEF 88 (40)	Congo	Deep Red	Small
11	IMC 4A TYPE 3	Na	Brown Striped	Small
12	HHL MD 30-75	Na	Dark Red	Small
13	EG 10 TYPE 1	Na	Purple	Medium
14	PTC-955-10	Honduras	Red	Small
15	BILFA 12	Na	Red	Large
16	BAT 477	Colombia	Cream/Beige	Small
17	EG 40	Na	Red	Medium
18	ANT 22	Colombia	Red	Large
19	IMC 4A	Na	Brown	Small
20	NR 4B	Na	Red	Medium
21	A 80	Colombia	Cream/Beige	Small
22	MILENIO	Honduras	Red	Small
23	AFR 708	Congo	Black	Small
24	LISA (BILFA)	Na	Black	Large
25	BICO DE OURO	Brazil	Cream/Beige	Small
26	KC 10	Na	Purple/Striped	Large
27	DOR 714	Colombia	Black	Small

[1]Na= Not available

Table 3: Common bean genotypes used as planting materials in experiment 2

Sn	Variety	Origin (Country)	Seed colour	Growth habit[1]	Seed size
1	BAT 477	Colombia	Cream/Beige	IV	Small
2	MILENIO	Honduras	Red	IV	Small
3	DOR 714	Colombia	Blackish Red	IV	Small
4	VEF 88 (40)	Congo	Deep Red	III	Small
5	AFR 708	Congo	Black	IV	Small
6	A 785	Colombia	Black	IV	Small
7	ANT 22	Colombia	Red Calima	I	Large

[1] (CIAT 1987) I - determinate bush; III - Indeterminate Prostrate; IV - indeterminate climbing

During late flowering and early pod filling (37-43 DAP; corresponding to stages R7-R8, (CIAT, 1987) most plants in the adequate P treatment and some in medium P treatment began to show N deficiency-like symptoms by changing leaf colour from green to yellow. Two leaves (excluding petiole) from low P treatment (without deficiency symptoms) and from adequate P treatment (with deficiency symptoms) were sampled per pot and analysed for N using Kjeldahl method. Between 75 and 90 DAP, the bean plants were harvested and the following parameters were determined: number of pods/plant, number of seed/pod, 100-seed mass and grain yield (g).

3.2.2 Yield components and grain yield

When the plants attained maturity, indicated by drying of pods between 75 and 90 DAP, pods were counted and their numbers recorded for each plant, shelled and grains sun-dried for three days. The seeds were counted and weighed to give grain yield in g/plant. Ten seeds from each pot were picked randomly and weighed to determine the weight of 100 seeds or seed size. Harvest index (HI) (the weight of seeds per total mass of biological

yield) was not determined because at the time of harvest, almost all leaves had senesced and dropped.

3.2.3 Data analysis for experiment 2

Data were analysed as in the first experiment as described in section 3.1.9.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Experiment 1

This experiment aimed at identifying bean genotypes with superior vegetative growth and P uptake at low P supply.

4.1.1 Soil properties

The soil from Magadu area within the University Farm was analysed for pH, total nitrogen, organic carbon, available phosphorus, CEC and exchangeable Ca, Mg, Na, and K. The results were obtained as indicated (Table 4).

According to soil pH rating by Landon (1991) the soil used was acidic as it had low pH. It had also low exchangeable Ca, K and Na; low total N, and available P. Organic Carbon (OC), which reflects the organic matter content of the soil, was low, implying that the soil had low organic matter content. It had medium CEC and medium exchangeable Mg. Due to the acidic nature of this soil; it seems that low P availability was a result of fixation by Al and Fe oxides, which are abundant in soils of such pH (Hinsinger, 2001). Low total N is attributed to low organic carbon, implying small additions of organic matter and relatively high decomposition rate.

This soil was generally poor in fertility because almost all properties were below the critical level. Its use for agriculture may be possible given the use of fertilizers. For optimum common bean production, fertility amendment is necessary particularly P and N supplying fertilizers, although bean genotypes adapted to low P soils such as this are

needed if the fertility amendments are not possible. The aim of the present work was to screen common bean genotypes, which are better adapted to low P soils as the one used for the experiment, for instance, with very low available phosphorus of 7.3mg/kg as this is the typical soil common to bean growing areas.

Table 4: Selected properties of the soil used for the experiment

Soil character	Unit	Value	Rating/remarks[1]
pH	-	5.4	Low
Organic Carbon	%	1.0	Low
Total Nitrogen	%	0.1	Low
Bray-1-P	mg/kg	7.3	Low
CEC	me/100 g soil	15.8	Medium
Exchangeable Ca	me/100 g soil	2.2	Low
Exchangeable Mg	me/100 g soil	2.2	Medium
Exchangeable K	me/100 g soil	1.1	Low
Exchangeable Na	me/100 g soil	1.2	Low

[1]Landon (1991)

4.1.2 Shoot growth

The plants responded significantly to phosphorus treatments such that plants in the high P treatment were normal with respect to size and leaf expansion, as one would expect from a well-managed field without nutrient stress (Plates 1 and 2). Shoot biomass of all genotypes increased significantly with increasing P levels thus confirming severe deficiency in the unfertilised treatment or control. Variations due to genotypic influences were profound and the differences between them were highly significant at $P < 0.05$ (Table. 5). The maximum

shoot biomass was 5.195 g/plant while the lowest was 1.656 g/plant for genotypes A785 and BILFA 12, respectively, irrespective of the P treatment.

Table 5: Statistical significance levels derived from ANOVA for variables determined on 27 bean genotypes in three P treatments

Trait	P levels (P)	Genotypes (G)	P x G
Shoot biomass	***	***	*
Root biomass	**	***	*
Shoot P content	*	***	NS
P uptake	***	***	*

NS- not significant, *, **, *** Significant F values at 0.05, 0.01, 0.001, respectively

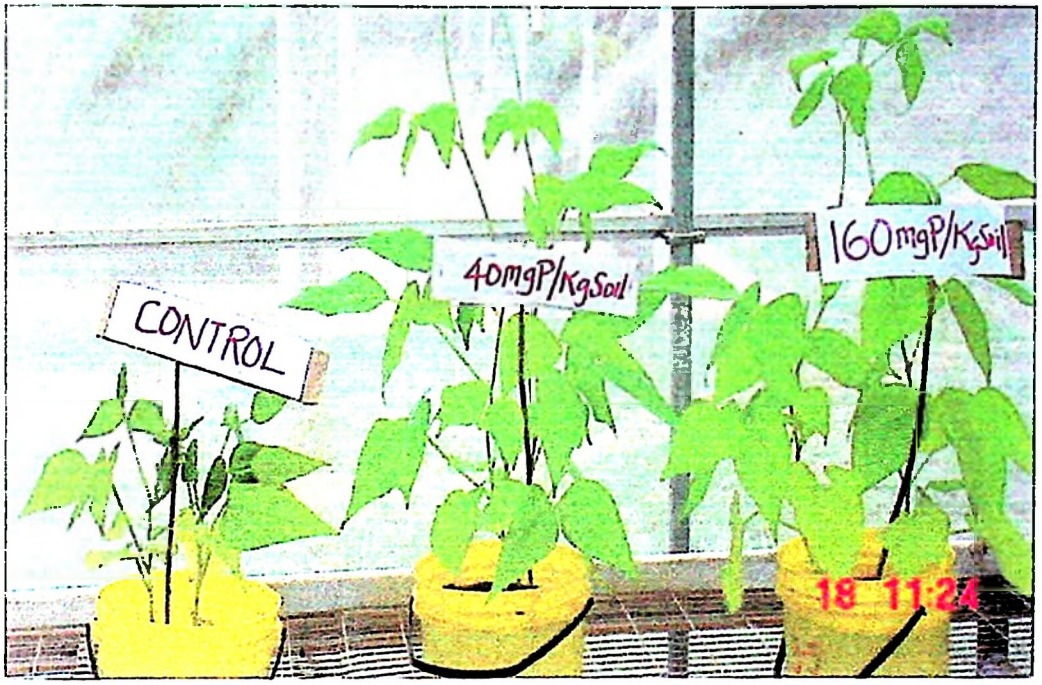

Plate 1: Effects of three P levels on plant vigour of genotype CIM 9314-36 at 30 DAP.

Plate 2: Effects of three levels of P on plant size of genotype NR 4B at 30 DAP.

Table 6: Shoot biomass (g/plant) for 27 bean genotypes in response to three P levels at 30 DAP.

Sn	Genotype	P=0	P=40mgP/kg	P=160mgP/k
1	G92	4.19 a	3.97 abcdef	4.53 c-f
2	AMADEUS	3.03 abcdefg	5.16 abc	5.67 bcde
3	HHL 30-75	1.96 cdefgh	3.04 fgh	4.49 ef
4	PRETO 143	2.88 abcdefg	3.39 defg	5.35 abcdef
5	CIM 9314-36	2.39 bcdefgh	3.95 abcdef	5.77 abcde
6	FEB 192	3.11 abcdefg	4.99 abcde	5.79 abcde
7	A 785	3.61 abcd	5.45 a	6.53 a
8	EG 44	1.70 fgh	3.35 efgh	3.78 f
9	DORADO	3.09 abcdefg	5.08 cd	6.16 abcd
10	VEF 88	3.83 ab	4.64 cdef	4.94 abcdef
11	IMC 4A	2.64 abcdefgh	3.76 bcdefg	5.62 abcde
12	HHL MD	1.90 defgh	3.47 cdefg	4.36 ef
13	EG 10	2.66 abcdefgh	3.73 bcdefg	5.25 bcdef
14	PTC 9557-10	2.99 abcdefg	5.36 b	6.27 b
15	BILFA 12	1.09 h	2.24 gh	1.64 g
16	BAT 477	2.97 abcdefg	4.98 abcde	5.68 abcde
17	EG 40	1.50 gh	3.35 gh	1.37 g
18	ANT 22	3.47 abcde	1.76 h	4.48 def
19	IMC 4A	2.79 abcdefg	3.13 fgh	5.58 abcde
20	NR 4B	2.70 abcdefgh	2.96 fgh	4.68 bcdef
21	A80	2.36 bcdefgh	4.18 abcdef	5.09 abcdef
22	MILENIO	3.66 abc	4.94 abcde	5.73 abcde
23	AFR 708	3.09 cdefg	4.28 abcdef	6.07 abcde
24	LISA	2.28 bcdefgh	4.00 abcdef	5.61 bcde
25	B. DE OURO	2.74 bcdefgh	3.88 abcdefg	5.94 abcde
26	KC 10	1.77 efgh	3.32 efgh	4.76 bcdef
27	DOR 714	3.36 abcdef	4.28 abcdef	6.23 abc

Means followed by the same letter are not significantly different according to mean separation by DMRT ($P < 0.05$)

There was a significant genotype x phosphorus interaction at $P < 0.05$ (Table 4). In P deficient treatment, few genotypes were able to produce shoot biomass of 3.0 g/plant or more and the highest (4.2 g/plant) was for G92 and the lowest (2.2 g/pant) for BILFA12. Genotypes that produced relatively high biomass (≥3 g/plant) in phosphorus deficient treatment are (in descending order) G92, VEF 88(40), MILLENIO, A785, ANT 22, DOR 714, AFR 708, DORADO and MADEUS.

At medium P (i.e. 40 mg P/kg soil), shoot biomass generally increased although one genotypes (ANT 22) (Table 6) showed a decline in this parameter. At higher or adequate P treatment there was a significant response in general with regard to shoot biomass but with exceptions. The genotype BILFA, showed a decline in shoot biomass at adequate phosphorus level. This response was abnormal but there might be stresses other than phosphorus, which interacted with high P availability and caused a decrease in biomass production. Shoot biomass was very responsive to added phosphorus and at adequate P level all genotypes responded positively except EG 40, which had even lower shoot biomass than at low P level.

The results showed an increase in shoot biomass production as phosphorus level was increased, agreeing with the results by Araujo *et al.* (2000). Genotypic variation in shoot biomass production at all P levels was highly significant ($P < 0.05$), agreeing with the results by Yan *et al.* (1995a). The genotypes with high dry matter yield at low P treatment might signify that they use low P more efficiently to produce relatively large biomass while others with low shoot biomass lack such ability.

For genotypes BILFA 12 and EG 40, the maximum yield was obtained when 40 mg P/kg soil was added. These genotypes perhaps are not able to thrive under very low P availability but the range between deficiency and adequacy may be very narrow. However, the genotypes KC10, HHL-MD, EG 44, BICO-DE-OURO and HHL-30-75 needed high doses of P to attain maximum yield. According to CIAT 1987) these genotypes which may yield less under P stress but yields the same or more than the efficient genotypes (such as A785, VEF 88 and DOR 714) at adequate P are termed as inefficient responsive genotypes. They are, therefore, not suitable for low P soils like the one used in the experiment. Genotypes G92, and ANT 22, showed relatively low response to added P, as there were 8.3% and 29% shoot biomass increase respectively, at adequate P treatment. May be these genotypes had superior P acquisition mechanism or utilize little P available in their tissues more efficiently to produce large dry matter. They can also be classified as efficient genotypes according to CIAT (1987) because their biomass production was not appreciably affected by increased phosphorus availability nor were they significantly affected at very deficient P level.

The data indicated that the P requirement for the common bean production was very crucial for some genotypes if commercial production is to be undertaken, because if plants at deficient P levels had as low shoot biomass as 1.7 g/plant (e.g. EG 44) it is likely that such a genotype will only produce just a few pods per plant thus lowering productivity per unit area. The differences in response to P levels among genotypes as far as shoot biomass is concerned may mean different physiological mechanisms that are involved in phosphorus use efficiency or tolerance to P stress.

At low nutrient availability, plants partition large fraction of resources to the root system and as a result, leaf growth and expansion become restricted such that there is a decline in above ground biomass and eventually decline in yield (Poorter and Nagel, 2000). Genotypes with low shoot biomass under low P may mean that leaf area development was restricted by P deficiency, which in turn hindered shoot growth. This was because P was an essential component in carbon assimilation (Thung, 1991). For screening purposes, the genotypes, which were able to produce fairly high shoot biomass at low soil P would be favoured against ones with comparatively low shoot biomass at the same rate because this parameter may reflect the grain yield potential of a plant. Genotypes with high biomass than others account for the difference in their adaptation to low P soils and such adaptation may be a larger root system (Yan *et al.*, 1995a), high density of root hairs and the intensity of ramification through the soil (Russell, 1973) or arbuscular mycorrhiza establishment.

At low P, the plants might have responded to this situation by diverting large fraction of their net carbon assimilation to the production of heterotrophic rather than photosynthetic tissues, which in turn might have resulted in an increase in root: shoot ratio (Nielsen *et al.*, 2001). Therefore, the genotypes that had high mean shoot biomass at deficient phosphorus may be termed as efficient, probably because, soil P was not a problem or they had invested more to the roots for enhanced soil exploration to support shoot biomass production. For this case, genotypes G92, VEF 88(40), MILLENIO, A785, ANT 22, DOR 714, FEB 192, DORADO, AFR708 and AMADEUS were relatively superior in shoot biomass production at low soil phosphorus level.

4.1.3 Root growth and nodule formation

At 30 days after planting (DAP), roots were extracted from the soil for examination of presence of symbiotic nodules and for determination of root biomass. There was no symbiotic nodule on the plant roots at all phosphorus levels, contrary to what was anticipated.

Analysis of variance for root biomass revealed highly significant ($P < 0.05$) effects of phosphorus level, and genotypes (Table 4) on this parameter. The interaction between phosphorus and genotypes was significant at $P \leq 0.05$. Root biomass increased significantly ($P < 0.05$) as the phosphorus levels increased among the genotypes, being low at deficient and high at adequate phosphorus. Genotypes A785 had the highest average root biomass of 2.15 g/plant while genotype BILFA 12 had the lowest, with a mean of 0.56 g/plant irrespective of phosphorus levels. Only 41% of the bean genotypes under study had root biomass less than 1.6 g/plant while the remaining 59% had root biomass ranging between 1.6 and 2.15 g/plant and there was no significant difference within this range.

Genotype FEB 192 had higher root biomass at low P treatment (1.56 g/plant) whereas genotype EG 40 had the least root biomass of 0.5 g/plant (Fig. 2). At high P treatment, most genotypes responded significantly ($P < 0.05$) by increasing their root biomass, but with exceptions. For example, genotypes EG 44, VEF 88(40), BILFA 12, BAT 477 and EG 40 had less root biomass at high P treatment whereas genotypes EG 10, ANT 22, NR 4B had low biomass at P= 40 mg P/kg soil compared to their biomass at low P level. Others had consistent root biomass increase as the level of P increased. Genotypes FEB 192, DORADO, VEF 88 (40), BAT 477, ANT 22, DOR 714, G92, MILLENIO, PTC

9557-10 and PRETO 143 had relatively higher root biomass at low P level than other genotypes.

4.1.3.1 Symbiotic nodules

Inability of these common bean genotypes to form symbiotic nodules may be due to individual factors or an interaction of several factors, some of which may be edaphic and/or environmental. Primarily, the seeds were not inoculated with any rhizobia due to lack of the inoculant and the area where the soil was taken from had no record of common beans being planted there. From Table 3, it is evident that the experimental soil was very acidic (pH 5.4), therefore may be this acidity was not suitable for effective establishment of rhizobia-bean root symbiosis. According to Subba-Rao's classification of *rhizobium* (Subba-Rao, 1989), the acidic nature of the soil might not have restricted the growth of fast growing rhizobium like *Rhizobium phaseoli* and *R. leguminosarum*. However, slow growing non-acid forming *rhizobia* like *R. japonicum*, and *R. lupin* would probably be there, but were unable to infect the bean root hairs due to their specificity. Although high doses of mineral N fertiliser hinder symbiotic N fixation (Lindemann and Glover, 2004), nitrogen fertilizer (40 mg N/kg soil) that was added at planting cannot be associated with the lack of symbiotic nodules because this amount was low.

Table 7: Effects of P levels on root biomass (g/plant) of 27 bean genotypes at 30 DAP.

SN	Genotype	P=0mg P/kg soil	P= 40 mg P/kg soil	P= 160mg P/kg soil
1	G92	1.42 ab	1.76 abcd	1.96 bcd
2	AMADEUS	1.20 ab	1.88 abc	2.43 abc
3	HHL 30-75	0.82 ab	1.25 cde	1.76 cd
4	PRETO 143	1.23 ab	1.43 abcde	2.42 abc
5	CIM 9314-36	1.09 ab	1.84 abcd	2.57 abc
6	FEB 192	1.56 a	1.91 abc	2.37 abc
7	A 785	1.14 ab	2.30 a	3.00 a
8	EG 44	0.79 ab	1.19 cde	1.17 de
9	DORADO	1.55 a	1.69 abcd	2.72 ab
10	VEF 88(40)	1.54 a	2.07 abc	1.98 bcd
11	IMC 4A	1.03 ab	1.38 bcde	2.29 abc
12	HHL MD	1.04 ab	1.15 cde	1.69 cd
13	EG 10	1.18 ab	1.17 cde	2.05 bc
14	PTC 9557-10	1.26 ab	2.25 ab	2.45 abc
15	BILFA 12	0.51 b	0.74 e	0.43 ef
16	BAT 477	1.51 a	2.33 a	1.92 bcd
17	EG 40	0.49 b	1.35 cde	0.37 f
18	ANT 22	1.49 a	0.72 e	1.87 bcd
19	IMC 4A	1.11 ab	1.15 cde	2.25 abc
20	NR 4B	1.12 ab	0.95 de	1.95 bcd
21	A80	1.04 ab	1.73 abcd	2.15 abc
22	MILENIO	1.40 ab	2.29 a	2.51 abc
23	AFR 708	1.24 ab	2.04 abc	2.24 abc
24	LISA (BILFA)	0.88 ab	1.99 abc	2.44 abc
25	B. D'OURO	1.16 ab	1.88 abc	2.40 abc
26	KC 10	1.10 ab	1.30 cde	1.82 bcd
27	DOR 714	1.52 a	1.89 abc	2.99 a

Means followed by the same letter are not significantly different according to Mean separation by DMRT ($P < 0.05$)

Vadez and Drevon (2001) did not find any symbiotic nodules when high dosage of mineral N (97.3 mg N/litre over seven weeks) was added as potassium nitrate into treatments when working with common bean *(Phaseolus vulgaris* L.) genotypes.

Therefore, it is likely that the failure of nodulation was due to lack of specific and effective natural rhizobia, or other factors.

4.1.3.2 Root biomass

Phosphorus deficiencies in plants cause a drop in new tissue biosynthesis, for example leaf expansion, which in turn, decreases aerial part growth (Le Bot *et al.*, 1998). Likewise, under deficient P conditions, plants respond by allocating large fraction of their net carbon assimilation to the production of root rather than photosynthetic tissues (Poorter and Nagel, 2000), resulting in increase in root/shoot ratio (Nielsen *et al.*, 2001).

The results showed significant ($P < 0.05$) genotypic variations among bean genotypes for root biomass, thus agreeing with the work by Fawole *et al.* (1982). The results also show that root biomass was significantly enhanced by P addition (Table 7), agreeing with the results from work by Yan *et al.* (1995a). It is obviously clear that phosphorus is relatively immobile in soil (Ragothama, 1999; Hinsinger, 2001), thus extensive root system would be an important trait for adapted genotypes in exploring large soil volume. Because root growth is heritable in common bean, it is possible for the selection of plants with larger root system (Fawole *et al.*, 1982) at deficient phosphorus. Some superior genotypes (indicated by high shoot biomass) had also higher root biomass in the P deficient treatment and these included FEB 192, VEF 88 (40), BAT 477, ANT 22, DOR 714, G92 and MILLENIO) than inferior genotypes e.g. BILFA 12 and EG 40. Therefore, superior genotypes may be efficient in acquisition of P from the soil by having large root system manifested by large root biomass. Especially important are the genotypes ANT 22, VEF 88 (40), G92, MILENIO and DOR 714, which had relatively higher shoot biomass as well as relatively higher root biomass at low P level. It suffices here to say that root biomass

cannot be used as a direct screening criterion for adaptability to low P soils because due to subterranean nature and fineness of the root system, plants grown on soil medium succumb to incomplete recovery of the whole root system.

4.1.4 Shoot P concentration

Analysis of variance showed significant effects of phosphorus treatments and genotypes on the shoot P concentration of the 27 bean genotypes (Table 5). The shoot P concentration varied significantly ($P < 0.05$) between the high P and medium P treatments but there was no significant difference between low P and medium P treatments. The shoot P concentration varied significantly ($P < 0.05$) between genotypes and EG40 had the highest mean shoot P concentration (0.27%) while genotype VEF 88(40) had the lowest (0.18%) across P levels. About 20 of all genotypes had shoot P content of more than or equal to 0.2% P, the level indicated by Bingham, (1965) and Wilcox and Fageria (1976), cited by Thung (1991) as the critical P content in common bean. The remaining 7 genotypes had P content well below the critical level, in the deficient P treatment.

From analysis of variance (Table 5), there was no significant effect of phosphorus x genotype interaction on the shoot P content, probably because, at deficient P, plants exhibited high P content due to low shoot biomass but again, plants at medium and high P levels had high shoot P content because they absorbed much of it as it was available. Therefore, lack of genotype x phosphorus interaction effect may be due to high tissue P accumulation and/or dilution effects. Mean separation could reveal the performance of genotypes for each P treatment. At deficient P level ($P = 0$ mg P/kg soil) there were ten genotypes which had shoot P content of more than or equal to 0.22% P, well above the critical level of 0.2% (Bingham, 1965). These were EG 40, HHL-30-75, PRETO 143, and

IMC 4A, A785, HHL-MD, BICO DE OURO, DOR 714, ANT 22 and BAT 477 in descending order. Genotype EG 40 had the highest shoot P content at deficient P treatment with 0.28% P whereas BAT 477 had the least, 0.22% P in that order. The possession of high shoot P content in such genotypes as HHL-30-75, PRETO 143, IMC 4A, HHL-MD and BICO-DE-OURO may not be a sign of P efficiency but rather P-accumulation effects (Thung, 1991), since all these genotypes had relatively low shoot biomass at deficient P level.

At medium P treatment, genotypes AMADEUS, CIM 9314-36, A785, VEF 88(40) PTC 9557-10, BILFA 12, BAT 477, MILENIO, AFR 708, KC 10 and DOR 714 had P concentration of less than 0.2% (Table 8). These genotypes showed abnormal behaviour, opposite to what was anticipated.

Between deficient and medium P treatments, shoot P content did not differ significantly. This was contrary to what could be anticipated, as the shoot P concentration was expected to increase at high P level. Because there was a general increase in shoot biomass at P = 40 mg P/kg soil among the genotypes the decline in P concentration might have been due to dilution effect (Thung, 1991; Machado and Furlani, 2004) because absorbed P is distributed within a large mass than when the biomass is smaller. Another reason might have been that Al, Mn and Fe oxides fixed much of the P at 40 mg P/kg soil, thus there was less P to be taken up by plants. Sanginga *et al.* (2000) obtained different results with cowpea breeding lines, where there was significant differences in shoot P content between 0 kg P/ha and 60 kg P/ha. This might have been due to the fact that the soil used by authors had pH of 6, which is slightly acidic (Landon 1991) therefore there might be little fixation by Al, Mn and Fe.

The genotypes grown in the high phosphorus treatment had significantly ($P < 0.05$) higher shoot P concentration than at both low and medium P treatments, because it is likely that 160 mg P/kg soil was a sufficient level leading to high rate of uptake (Hinsinger, 2001).

Genotype PRETO 143 had significantly ($P < 0.05$) higher P concentration at low P than at both medium and high P treatment (Table 8). When plants were grown in media containing very high P tissue P concentration was regulated by either reducing uptake or P is lost from tissues by efflux (Bieleski and Ferguson 1983, cited by Schachtman *et al.*, 1998). Probably genotype PRETO 143 required low P in its shoot tissues such that when P is high, uptake is restricted, or excess P is removed by efflux. Another explanation could be that this genotype had a constant P uptake irrespective of P level in the soil. Therefore, the low shoot P concentration at adequate P might have been the result of increasing biomass with increase in P levels while P uptake remained constant.

Shoot P concentration cannot be used as a screening criterion without considering shoot biomass production because of dilution effect described earlier. For that matter, only genotypes that had above critical level P content and relatively high shoot biomass stood the chance of being selected, rather than using P content alone because genotypes like EG 44, EG 40 and BILFA 12 would be selected if it was the only criterion. Therefore genotypes, DOR 714, BAT 477, ANT 22, and FEB 192, were eligible for being selected with respect to this particular parameter. Genotypes G92, A785, VEF 88 (40), MILENIO, AFR 708, IMC 4A, DORADO and AMADEUS seemed to have tissue P deficiency as far as shoot P concentration was concerned although they were outstanding in the case of shoot biomass production.

Table 8: Effects of P levels on shoot P concentration of 27 bean genotypes at 30 DAP

Sn	Genotype	P=0 mg P/kg soil	P=40mgP/kg	P=160mgP/kg
1	G92	0.19 cdef	0.21bcd	0.25 abcdefgh
2	AMADEUS	0.17 def	0.19 bcd	0.24 bcdefgh
3	HHL 30-75	0.26 ab	0.20 bcd	0.29 abc
4	PRETO 143	0.26 abc	0.22 abcd	0.23 bcdefgh
5	CIM 9314-36	0.15 f	0.19 bcd	0.19 h
6	FEB 1192	0.21 bcde	0.20 bcd	0.25 abcdefgh
7	A 785	0.17 def	0.17 cd	0.23 bcdefgh
8	EG 44	0.21 bcde	0.25 ab	0.28 abcd
9	DORADO	0.19 def	0.19 bcd	0.24 bcdefgh
10	VEF 88(40)	0.17 ef	0.16 d	0.19 h
11	IMC 4A	0.18 def	0.21 abcd	0.26 abcdefg
12	HHL MD	0.22 bcde	0.20 bcd	0.24 bcdefgh
13	EG 10	0.19 def	0.22 abcd	0.26 abcdefg
14	PTC 9557-10	0.20 bcdef	0.17 cd	0.23 defgh
15	BILFA 12	0.21 bcdef	0.17 cd	0.29 ab
16	BAT 477	0.21 bcde	0.16 d	0.24 bcdefgh
17	EG 40	0.28 a	0.24 ab	0.28 abcd
18	ANT 22	0.22 bcde	0.25 ab	0.20 gh
19	IMC 4A	0.24 abcd	0.24 ab	0.23 defgh
20	NR 4B	0.19 cdef	0.27 a	0.28 abcde
21	A80	0.17 def	0.21 bcd	0.22 efg
22	MILENIO	0.18 def	0.19 bcd	0.23 cdefgh
23	AFR 708	0.19 def	0.17 cd	0.21 fgh
24	LISA (BILFA)	0.21 bcdef	0.23 abc	0.27 abcdef
25	B. D'OURO	0.22 bcde	0.20 bcd	0.31 a
26	KC 10	0.21 bcde	0.19 bcd	0.31 a
27	DOR 714	0.22 bcde	0.18 bcd	0.25 abcdefgh

Means followed by the same letter are not significantly different according to Mean separation by DMRT ($P < 0.05$)

This corresponded to the results by Dechassa *et al.* (2003) where it was found that cabbage (*Brassica oleraceae* L.) attained 80% of its maximum yield at low soil P. Dechassa *et al.* (2003) concluded that cabbage plants had high P efficiency than carrot or potato plants. This may be due to dilution effects and it is possible that these genotypes had high internal P utilization efficiency.

4.1.5 Phosphorus uptake

Phosphorus uptake was defined as the total amount of P in the shoot (mg /plant) and was calculated as the product shoot biomass (g/plant) and shoot P content (mg P/g dry matter). Phosphorus uptake was influenced by P level and genotype x P interaction (Table 5). There was a highly significant ($P < 0.05$) genotypic variability with respect to phosphorus uptake at 30 DAP. Phosphorus uptake of most genotypes increased with increase in the rates of P application to the soil except for genotype EG 40 for which P uptake declined at high P treatment (Table 9). For genotype ANT 22, P uptake was high at low P treatment but this declined at 40mgP/kg soil but increased again at high P level. Genotype A785 had the highest P uptake among all genotypes at low P treatment whereas BILFA 12 had the lowest P uptake at low P treatment. Phosphorus uptake can be regarded as the best criterion for judging the genotypes, for efficiency in acquiring P from soils low in available P. Genotypes A785, G92, DOR 714, ANT 22, PRETO 143, IMC 4A, FEB 192, MILLENIO, VEF 88 and BAT 477 had P uptake of between 16.56 and 12.54 mg P/pot at low P treatment (Table 9). These were the best ten genotypes in terms of P uptake in the low available P treatment.

From the ten genotypes above, six of them appeared to have had high P uptake but had either low shoot biomass or P concentration. For instance, genotypes G92, A785, MILLENIO VEF 88(40), IMC 4A, and AFR 708, had relatively higher shoot biomass but had low shoot P concentration at low P treatment, which was below the critical level. They are thus efficient in utilising internal P (Sanginga *et al.*, 2000). The low P concentration might have been due to dilution effect (Thung, 1991). On the other hand, genotypes IMC 4A, PRETO 143 and BAT 477 had significantly high shoot P content but all these did not feature in shoot biomass i.e. had low shoot biomass. Out of the ten, three genotypes

featured consistently for all three traits (namely shoot biomass, shoot P concentration and P uptake) and these are A785, DOR 714 and ANT 22. These genotypes might have been efficient in acquisition and utilisation of P from low substrates since, besides producing higher biomass, they were able to acquire and maintain high P in their shoots.

Phosphorus uptake in most genotypes increased with an increase in P availability. The difference among the genotypes at deficient and medium P availability was not significant whereas the uptake rose significantly from 40 to 160mgP/kg soil treatment (Table 9). Genotype BILFA 12 had narrow response between low and high P treatments, while EG 40 had higher uptake at medium P, uptake at lower and adequate P availability remaining nearly equal (Table 9). Genotype ANT 22 had lower P uptake at 40 mgP/kg soil than at both lower and high P levels. This trend and the fact that the increase in P uptake at adequate P was not significant made ANT 22 questionable for P efficiency.

Plant P uptake depends not only on P available in the soil but also on plant adaptation and properties (Föhse *et al.*, 1988) such as root architecture (Yan *et al.*, 1995a), possession of adventitious roots (Miller *et al.*, 2003) and exudation of anions in the rhizosphere (Hinsinger, 2001).

P uptake is equal to amount of P that was taken into shoots, expressed as mg P/plant The differences in P uptake among the genotypes across P treatments show the efficiency with which bean plants were able to absorb phosphorus from the deficient soil and this ability differed among the genotypes. P uptake was the best indicator as it combines shoot biomass and shoot P content such that genotypes, which performed better or poorly in either of the two, are easily identified and therefore not favoured in the selection.

The increase in P uptake with increased P availability among genotypes agrees with the work by Valizadeh, *et al.* (2002) working with phosphorus responsive wheat genotypes. At low P, the genotypes that had both high shoot biomass and phosphorus content had also high P uptake while those, which had low P content and low biomass had low P uptake. The genotypes that had high shoot P concentration but with low shoot biomass e.g. HHL-MD, EG 40, EG 44, and BILFA 12 had low P uptake. These exhibited low P efficiency because they could not utilize efficiently the absorbed P as evidenced by high shoot P concentration and low shoot biomass, but also they have less root biomass, an important organ in P acquisition. This is evident in the work by Dechassa *et al.* (2003).

The increased P uptake at adequate P availability may be due to increased root and shoot growth resulting from increased carbon assimilation rates per unit leaf area (de Willigen and van Noordwijk, 1987). Response to P addition among bean genotypes is important, as it is possible to identify the genotype's ability when P is not limiting. All genotypes except BILFA 12, EG 40 and ANT 22 did respond vigorously to P addition in terms of P uptake. It seems that genotypes BILFA 12, EG 40 and ANT 22 have narrow range between deficient and adequate P levels or there was some other environment-P interactions, which hindered their high response to P additions. Such genotypes are not worthy adopting for a farmer who aims at increasing yield by P addition because the rate of returns to the investment can be negatively affected.

Also such genotypes as HHL-MD, BILFA 12 and EG 40 are not suitable to be grown in P deficient soils because of their poor P efficiency. For a resource-poor farmer who would not afford fertility amendment strategy these genotypes are not likely to give reasonable grain yield, on the basis of their P efficiency in terms of uptake.

Table 9: Effects of P levels on P uptake in 27 bean genotypes at 30 DAP.

Sn	Genotype	P=0mgP/kg		P=40mgP/kg		P=160mgP/kg	
1	G92	16.25	a	16.1	ab	21.92	bcdc
2	AMADEUS	11.27	abc	20.15	a	26.83	abcde
3	HHL 30-75	10.24	abc	12.29	ab	25.92	abcde
4	PRETO 143	13.6	abc	14.23	ab	24.42	bcde
5	CIM 9314-36	6.64	bc	15.06	ab	22.23	bcde
6	FEB 1192	12.96	abc	15.75	ab	28.85	abc
7	A 785	16.56	a	19.15	a	30.38	ab
8	EG 44	11.58	abc	15.85	ab	28.5	abc
9	DORADO	11.8	abc	20.01	a	29.82	abc
10	VEF 88(40)	12.57	abc	15.11	ab	19.46	de
11	IMC 4A TYPE 3	10.03	abc	15.77	ab	29.41	abc
12	HHL MD	8.18	abc	14.3	ab	21.09	cde
13	EG 10	10.34	abc	15.77	ab	27.9	abcd
14	PTC 9557-10	12.02	abc	18.32	a	28.56	abc
15	BILFA 12	4.56	c	7.66	b	9.7	f
16	BAT 477	12.54	abc	15.69	ab	26.48	abcde
17	EG 40	7.97	abc	14.93	ab	8.16	f
18	ANT 22	14.32	ab	8.29	b	18.3	e
19	IMC 4A	13.23	abc	15.11	ab	25.47	bcde
20	NR 4B	10.48	abc	16.06	ab	26.1	abcde
21	A80	9.27	abc	17.37	a	22.36	bcde
22	MILENIO	12.93	abc	18.53	a	26.27	abcde
23	AFR 708	12.13	abc	14.82	ab	26.	abcde
24	LISA (BILFA)	9.89	abc	19.02	a	30.64	ab
25	B. D'OURO	11.69	abc	17.72	a	34.79	a
26	KC 10	8.55	abc	13.24	ab	28.6	abc
27	DOR 714	14.46	ab	15.92	ab	30.89	ab

Means followed by the same letter are not significantly different according to Mean separation by DMRT ($P < 0.05$)

Genotypes DOR 714, G92, VEF 88(40), BAT477, AFR 708, MILENIO, PRETO 143 and A785 had high P uptake at medium and high P availability, meaning that better yields can be obtained when grown on P rich soils. Also these genotypes exhibited high P efficiency in terms of P uptake at low available P.

4.2 Experiment 2

This experiment aimed at examining if performance of bean genotypes in terms of vegetative growth and phosphorus uptake can be translated into grain yield, which is the ultimate goal of crop production. In addition, it aimed at evaluating the effect of high P application on tissue nitrogen status of the bean genotypes.

4.2.1 Leaf N content

Leaf nitrogen contents for different genotypes at low P and adequate P treatment were compared (Table 10).

Table 10: Leaf nitrogen content for different genotypes at low and high P levels at 40 DAP

Sn	Genotype	N (%) at P0 (mgP/kg)	N (%) at P160 (mg P/kg)
1	BAT 477	2.94	2.66
2	MILENIO	3.05	2.05
3	DOR 714	3.46	2.00
4	VEF 88(40)	3.18	2.35
5	AFR 708	2.85	1.96
6	A 785	3.15	2.46
7	ANT 22	2.70	1.83

At low P level, leaf N concentration ranged between 2.70% (ANT 22) and 3.46% (DOR 714), whereas at high P, leaf N concentration ranged between 1.83% (ANT 22) and 2.66% (BAT 477). According to Thung (1991), plants at low P level exhibited no N deficiency, but at the adequate P level genotypes ANT 22, AFR 708, DOR 714 and MILENIO were deficient in N. Genotypes BAT 477, VEF 88(40) and A785 had nearly sufficient or sufficient leaf N content at adequate P level.

At high P supply there was increased plant growth rate, making the plants' nitrogen demand high (Almeida *et al.*, 2000). This high N demand can be satisfied by either more N acquisition from the soil by plant roots or increased symbiotic N fixation by rhizobia, hence high nodule mass at sufficient P supply (Vadez and Drevon, 2001). Therefore, plants at low P treatment did not exhibit N deficiency probably because of low N demand due to reduced plant growth at low P level. At adequate P levels, genotypes, which had nearly sufficient or sufficient leaf P concentration, may possess mechanisms of N economy, although much is not known about this.

4.2.1 Number of pods/plant (NPP)

From analysis of variance (ANOVA) (Table 11) there was highly significant difference ($P \leq 0.05$) between the P levels with respect to the number of pods per pot. Genotypes at adequate P had large mean number of pods per pot followed by genotypes at medium P level, with low P level having the least mean NPP (Fig. 1)

Table 11: ANOVA of yield components determined for 7 common bean genotypes at three P levels

Parameter	P levels (P)	Genotypes (G)	P x G
Number of pods/pot	***	NS	NS
Number of seeds/pod	NS	***	NS
Grain yield (g/pot)	***	*	NS
100 seed weight	NS	***	NS

NS- not significant, *, **, *** Level of significance at 0.05, 0.01, 0.001 respectively

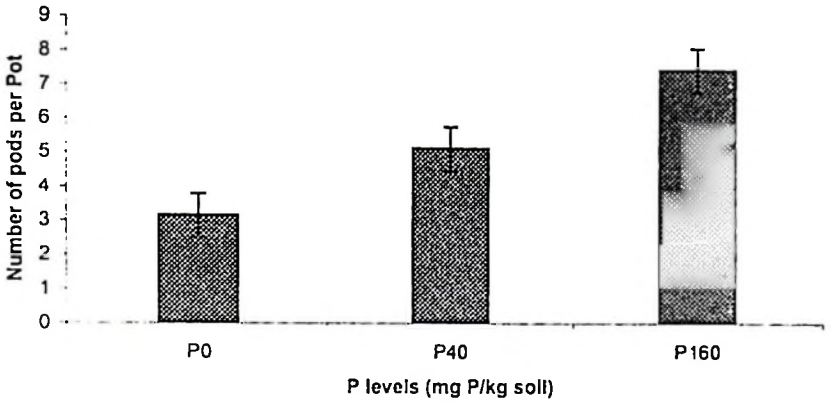

Figure 1: Effects of P levels on number of pods/plant (NPP)

There was no significant difference among genotypes with respect to this yield component irrespective of P levels, although NPP ranged from 6 for genotype BAT 477 to 4 for genotype ANT 22. There was no significant effect of P x G interaction on NPP indicating that there was increased genotype response with increase in P levels.

At deficient P level, the number of pods was low, compared to medium or adequate P levels. There was no significant difference between genotypes at low P level in this yield component although NPP ranged from 4 in genotype BAT 477 to 2 in ANT 22 (Appendix 2). Genotypes BAT 477, A 785, MILENIO and VEF 88(40) had above average performance at low P level. Genotypes AFR 708, DOR 714 and ANT 22 had an average of less than six pods per pot, which was the mean for all genotypes at low P. NPP was positively correlated with grain yield at deficient P level ($r = 0.855^{**}$, n=9) (Appendix 5).

At medium P level, NPP was positively correlated with grain yield ($r = 0.142$ ns) (Appendix 7). Genotypes BAT 477 and A785 were more responsive to adequate P (Figure 6). NPP was positively correlated with grain yield at adequate P level ($r = 0.759^{*}$, n=9).

It is evident from the results that the number of pods per plant generally increased significantly with increased P levels. This conforms to the results by Yan *et al.* (1995b). The lack of phosphorus x genotype interaction in this component might suggest that the response of genotypes was similar across the P levels. The effects of P deficiency on the process of pod formation and filling (Marschner, 1997) was reduced as P level was increased. Therefore it is likely that at low P, more pods aborted prematurely in all genotypes, while at adequate P, few or no pod aborted (no data).

4.2.2 Number of seeds per pod (NSP)

There was no significant difference between P levels with respect to the number of seed/pod (Table 11). Though not statistically different, genotypes in 40mgP/kg soil treatment had slightly higher mean number of seed per pod, followed by the high P treatment and the last one was low P treatment. There was significant variability among bean genotypes regarding this yield component (Fig. 2). Genotype MILENIO had the highest average seed number per pod (4 seed/pod), while genotype ANT 22 had the lowest (2 seeds/pod). The effect of P x G interaction was not significant ($P < 0.05$) (Table 11).

At low P, NSP was negatively correlated with 100 seed weight ($r = -0.916$, $n=9$) (Appendix 5) indicating that genotypes that had large seed number per plant had low seed mass at deficient P level.

NSP was not correlated with any yield component at medium P. At high P, this yield component again was negatively correlated with 100 seed weight ($r = -0.737$, $n=9$) indicating that genotypes with large NSP had low 100 seed mass.

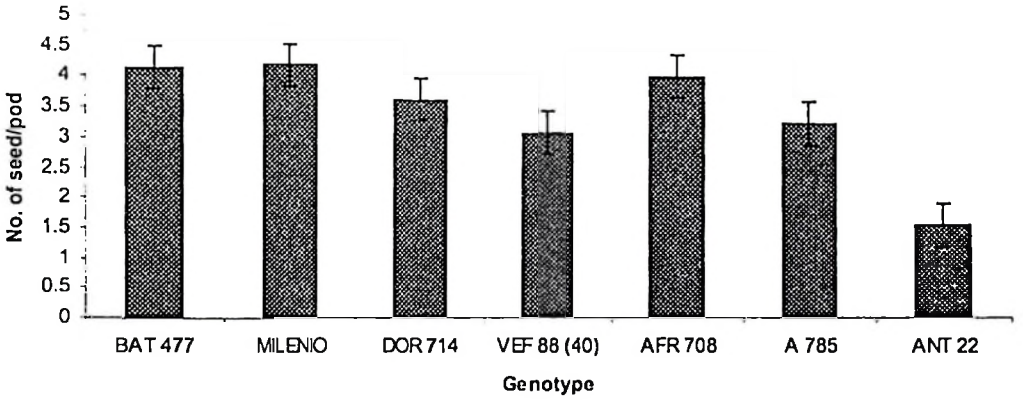

Figure 2: Variation among bean genotypes in number of seed per pod

The results indicate that NSP was not affected by P treatments. Bolland *et al.* (1999) obtained the similar results where NSP in faba beans (*Vicia faba* L) was not affected by P and Zn addition. Yan *et al.* (1995b) reported opposite results, where seed number per pod was increased by P additions. NSP seems to have no any particular importance at low P. A possible reason for higher increase in NSP in genotypes BAT 477, VEF 88 (40) and AFR 708 at medium P than at adequate P is not clear, for it seemed that at adequate P level, all genotypes faced nitrogen deficiency, which resulted in leaf yellowing at time when pod setting had started. Therefore N deficiency might have been the cause for decline in number of seeds per pod in these genotypes. The increase in NSP for genotype ANT 22 with increase in P levels might have been caused by higher rate of individual seed abortion at deficient level of P than at high P level. Tariq *et al.* (2001) reported narrow differences between NSP in mung bean (*Vigna radiata* L.) at varying P and K levels, suggesting that this parameter is controlled more genetically than the environment. This also may be the case in this study, evidenced by lack of P levels and P x G interaction effect on NSP.

4.2.3 Hundred-seed weight (100 seed weight) (g)

Only genotypes affected hundred seed weight (P ≤0.05) (Table 11), this may mean that the genotypes maintained their original seed size as before, irrespective of P treatments. Genotype ANT 22 had significant 100-seed weight due to of its large seed size. This component had significant negative correlation with number of seeds/pod in low and adequate P levels (r = -0.916** and -0.737** respectively) (n=9) (Appendix 5 and 7).

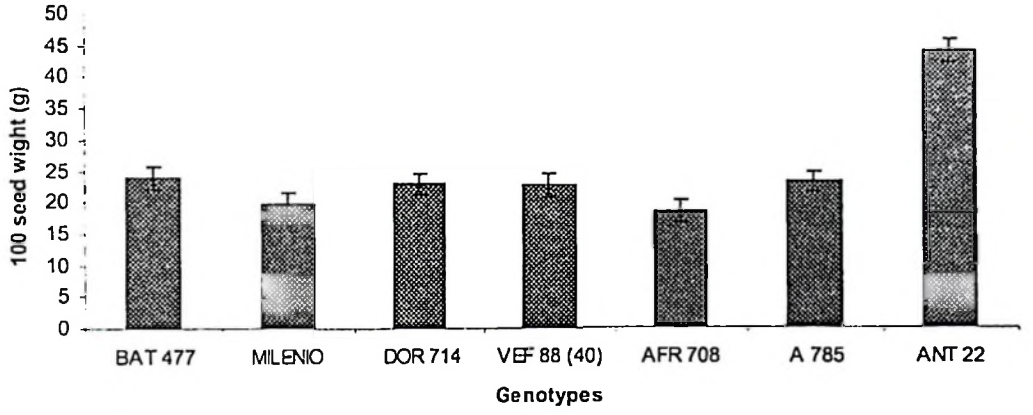

Figure 3: Hundred-seed weight for seven common bean genotypes

Although there was no significant P x G interaction, highest 100-seed weight was produces in genotypes at low P treatment (Appendix 4). Decrease in seed mass at adequate P may be associated with increased number of seeds per pod (r = -0.737*, n=9) and probably N deficiency reported in all genotypes but BAT 477 at adequate P, which could impair metabolic pathways, leading to less assimilation and/or accumulation of photosynthates to the seeds. A sub-optimal supply of N due to failure of symbiotic N fixation might have limited the expression of the seed weight potential of bean genotypes at adequate P supply.

4.2.3 Grain yield (g/plant)

P levels and genotypes but not P x G interaction affected grain yield (GY) significantly (Table 11). GY increased significantly with increase in P levels by 68% and 118% at medium and high respectively (Fig.4). The grain yield ranged from 5.1 g/plant for BAT 477 to 2.75 g/plant for ANT 22 irrespective of P levels (Fig. 6).

Although there was no significant P x G interaction ($P \leq 0.05$) genotypes AFR 708 had highest yield response (188%) at medium P followed by ANT 22 (165%) relative to low P treatment (Fig. 5). The least yield response at medium P level was for genotype A785 (22.6%) while in the adequate P treatment the least yield response was for DOR 714 (78%).

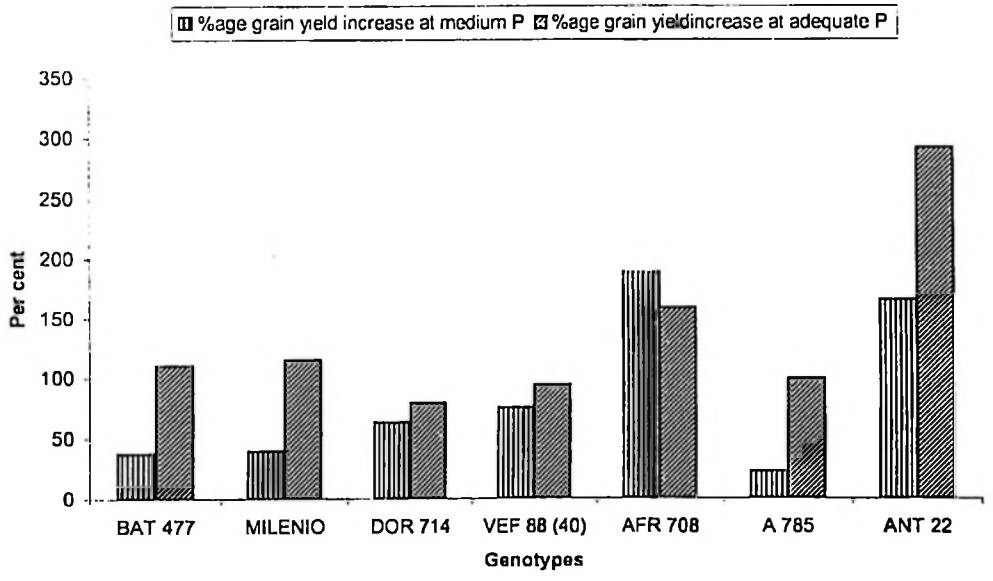

Figure 4: Percentage grain yield increase at medium and high P levels compared to low P level

The relationship between yield and its yield components at different P levels was determined by simple correlation analysis (Appendices 5, 6, and 7). The grain yield at low and high P treatments was positively correlated to number pods per plant ($r = 0.855^{**}$ and 0.759^{*} respectively) ($P < 0.05$) whereas at medium P level, GY was only correlated to number of seed/pod ($r = 0.914^{**}$).

The results for grain yield per pot indicates that P was deficient in this soil, and that addition of P by fertilization is very important in order to maximize yield. Araujo *et al.* (2000) reported the lack of P x G interaction, agreeing with the results from this experiment. This means that genotypes exhibited similar response pattern with P increase, thus implying low genotypic variability for P efficiency.

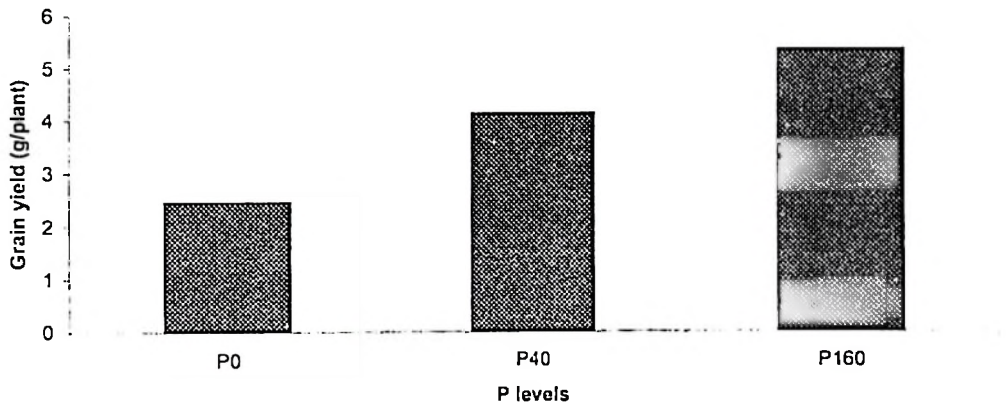

Figure 5: Effect of P levels on grain yield (g/plant

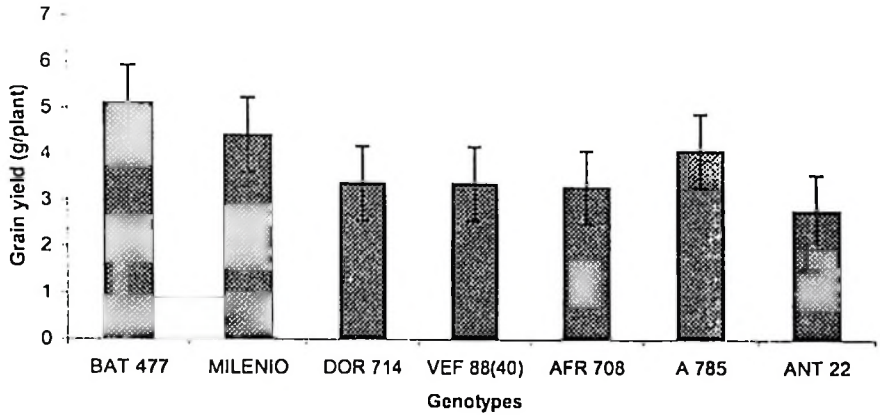

Figure 6: Effect of genotypes on grain yield (g/plant).

Genotypes BAT 477, A785 MILENIO and DOR 714 were able to produce relatively higher grain yield than others and their high performance could be attributed to their high number of pods/pot at P deficiency treatment. Although genotype VEF 88(40) had significantly higher shoot biomass than BAT 477, DOR 714 and MILENIO at low P in experiment one, it has not featured in experiment two in terms of grain yield. This could be associated with the fact that yield is affected by many genes, whose expression depend upon the interaction of environmental and physiological processes.

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

This study was carried out based on assumptions that common bean genotypes differ in phosphorus efficiency. It was further assumed that P efficiency is manifested plants ability to produce large root and shoot biomass, to absorb higher amounts of P from low P soil and finally produce high grain yield. This study revealed that common bean genotypes differed in production of root and shoot biomass; P uptake and final grain yield production. Root and shoot biomass varied significantly ($P < 0.05$) among bean genotypes, increasing with P additions.

Phosphorus uptake was an important screening criterion as it combined shoot-P concentration and the shoot biomass and plants that had high P uptake at low P were regarded as efficient in P acquisition. These were G92, A785, VEF 88(40), BAT-477, ANT-22, MILENIO, IMC-4A, PRETO-143, AFR-708 and DOR-714.

On further evaluation of seven of the above bean genotypes under P stress, all genotypes responded to P addition from 23% grain yield at medium P for genotype A785 up to 291% at high P for ANT 22. This confirms that P was a major limiting factor in less fertile soil as the one used for this study. Grain yield was related to number of pods per plant at low and high P, confirming that when bean plants are grown under P deficiency conditions, yield is negatively affected due to pod abortion. The differences between bean genotypes in producing grain yield imply that genotypes differed in their ability of acquisition or utilisation when P was limiting. Therefore, those genotypes with higher grain yield than

others under the same P level can be said to be P efficient and stood the chance of being favoured in the selection process.

Genotypes BAT 477, A785, MILENIO and DOR 714, produced relatively high grain yield under P deficiency conditions. Also these genotypes responded vigorously with P addition. This implies that resource poor farmers who may not afford P fertilisation can grow these genotypes. Also for a well-of farmer who prefers maximisation of grain yield these genotypes may be suitable because of their high response to added P. For a farmer who can afford little amount of P like the one used in medium P treatment, genotypes VEF 88(40), AFR 708 and ANT 22 are relevant due to their high response to medium P, but are not good yielders at low P availability. Although genotypes produced some grain yield at low P, soil fertility improvement by applying medium P has the chance of improving yields and raising productivity per unit area.

5.2 Recommendations

From the study, common bean adaptation to low P conditions is important because of high costs of fertiliser materials, and vulnerability of P to fixation in acidic soils. When P is applied to the soil, there is a need of addition of nitrogen fertilizer in situations where there is little possibility of symbiotic nitrogen fixation. In order to maximize yield, P addition is necessary in soils with very little available P. In situations where P fertiliser is not obtainable, genotypes BAT 477, MILENIO, A785, and DOR 714, may be used as they have exhibited some response to low P when compared to others. However further investigation is warranted, given that this study was carried under soil medium, which hindered effective recovery of the root system, the result of which was failure to establish the allocation of assimilates between shoot and roots at different P levels.

It is important to investigate the performance of these genotypes under severe nitrogen deficiency to find their ability to nodulate naturally because under poor farmer's circumstances, no fertilizer input or rhizobium inoculation is undertaken. Farmers may opt to apply organic manure before planting as this is will supply starter nitrogen dose and some P, that are important in common bean production.

Traits such as adventitious roots, root length and root volume (which were not investigated in this study) are worthy investigating in order to understand the mechanisms with which these genotypes BAT 477, MILENIO, A785, and DOR 714, were able to excel over others in a soil deficient in phosphorus.

Soil pH might have had a negative effect on the overall performance such as nodulation and P absorption. Farmers may use lime to rise pH so that P fixation by Al and Fe ions and oxides is reduced, thus increasing more available P in soil solution.

Genotype A785 has a black seed coat colour, which is not desired among the producers and consumers in the east Africa region. Therefore it is important that seed coat colour is modified by conventional breeding or by genetic engineering, while maintaining its low P adaptability so as to attract consumers in this part of the continent.

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APPENDICES

Appendix 1: Skeletal ANOVA table for split-plot design

Source of variation	Degree of freedom
Factor A	a-1
Error(a)	a (r-1)
Factor B	b-1
AB	(a-1) (b-1)
Error (b)	a (r-1) (b-1)
Total	

Appendix 2: Effect of phosphorus levels on number of pods/plant for seven bean genotypes

Genotype	P=0 mg P/kg soil	P = 40 mgP/kg soil	P = 160 mg P/kg soil
BAT 477	4.16 a	4.0 a	9.3 a
MILENIO	3.50 a	5.0 a	7.6 a
DOR 714	2.50 a	5.56 a	5.58 ab
VEF 88 (40)	3.33 a	5.50 a	7.16 ab
AFR 708	3.00 a	5.8 a	8.16 ab
A 785	3.65 a	5.50 a	8.58 b
ANT 22	2.00a	5.00 a	5.68 b
MEAN	**3.15**	**5.0**	**7.5**
SE ± 1.689			

Appendix 3: Effect of P levels on number of seed per pod for 7 bean genotypes

Genotype	P= 0 mgP/kg soil	P = 40 mg P/kg soil	P = 160 mg P/kg soil
BAT 477	3.881 a	4.722 ab	3.833 a
MILENIO	3.911 a	4.318 ab	4.304 a
DOR 714	4.15 a	3.163 b	3.597 a
VEF 88 (40)	3 a	3.42 ab	2.811 a
AFR 708	3.282 a	5.331 a	3.463 a
A 785	3.149 a	3.314 d	3.34 a
ANT 22	1.078 b	1.233 c	2.361 a
MEAN	**3.207**	**3.643**	**3.387**
SE ± 0.6183			

Appendix 4: Hundred-seed weight for 7 bean genotypes at three P levels

Genotype	P=0 mg P/kg soil	P = 40 mgP/kg soil	P = 160 mg P/kg soil
BAT 477	21.87 b	28.21 b	21.95 b
MILENIO	21.23 b	18.95 bc	19.04 b
DOR 714	22.52 b	23.87 bc	22.42 b
VEF 88 (40)	24.83 b	22.59 bc	21.06 b
AFR 708	18.88 b	17.9 2c	18.6 b
A 785	23.97 b	22.22 bc	23.56 b
ANT 22	50.41 a	49.22 a	33.4a
Mean	**26.24**	**26.14**	**22.86**
SE ±3.024			

Appendix 5: Simple correlation matrix of yield and yield components at P =0 mg P/kg soil

	Grain yield /pot	# Of pods/pot	# Seed/pod	100 seed wt
100 seed mass	-0.699	0.670	-0.916**	
# Seed/pod	0.801*	0.591		
# Pod/plant	0.855**			
Grain yield/plant				

* , ** Significant at, 0.01, 0.001 respectively

Appendix 6: Simple correlation matrix of yield and yield components at P=40 mg P/kg soil

	Grain yield /pot	No. of pods/pot	# Seed/pod	100 seed wt.
100 seed wt	-0.710	0.670	-0.310	
# Seed/pod	0.914**	-0.021		
# Pod/plant	0.142			
Grain yield/plant				

** Significant at, 0.01 respectively

Appendix 7: Simple correlation matrix of yield and yield components at P = 160 mg P/kg soil

	Grain yield /pot	**No. Of pods/pot**	**# Seed/pod**	**100 seed wt**
100 seed wt	0.327	-0.541	-0.737*	
# Seed/pod	0.676	0.506		
# Pod/pot	0.759*			
Grain yield/pot				

*, **, *** Significant, 0.01 and 0.001 respectively

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