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Effects of Rhizobium inoculation and Supplementation with P and K, on Growth, Leaf chlorophyll content and Nitrogen Fixation of Bush bean varieties

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Abstract

Low crop productivity is a general problem facing most farming systems in sub-Saharan Africa (SSA). These low yields are pronounced in grain legumes and are often associated with declining soil fertility and reduced N₂-fixation due to biological and environmental factors. Biological nitrogen fixation (BNF), a key source of N for farmers using little fertilizer, constitutes one of the potential solutions and plays a key role in sustainable grain legumes production. Phosphorus is needed in relatively large amounts by legumes for growth and has been reported to promote legumes growth and yield, nodule number and nodule mass in different legumes. Potassium influences the water economy and crop growth through its effects on water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation. Various Studies have shown that *Rhizobium* inoculation, phosphorus and potassium has the potential to improve legumes growth, seed yield, nitrogen fixation and also nutrient up take of legumes. The potential role of rhizobia inoculants and supplementation of P an K with respect to growth, nitrogen fixation, nutrient uptake, total leaf chlorophyll content, and grain yield of legumes are given attention in this review.

Key words: Nodulation, bio fertilizer, yield, nutrient uptake, chlorophyll, content

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1.0 Introduction

Common bean (*Phaseolus vulgaris* L) is one of the principal food and cash crop legume grown in the tropical world, and most of the production takes place in the developing countries (Pachico, 1989). It is an important herbaceous annual grain legume in the world chiefly grown as a cheap source of protein among majority of Sub-Saharan African people (Dzudie et al., 2002) Farmers frequently use it as food crop and a vital component in crop rotation for its ability to fix nitrogen (Krouma et al., 2006; Medvecky et al., 2006).

According to FAOSTAT (FAO, 2008), (Monfreda et al., 2008) estimate for the year 2006, world beans production was 1235 kg ha⁻¹ while that of Africa was 799 kg ha⁻¹. The average beans yield per annum in many African countries is always lower than that of the world. Lack of improved varieties associated with edaphic and biotic factors has been cited as one of the primary sources of lower beans production (Nzungize Rusagara, 2012).

In Tanzania, common bean is an important food and cash crop which is mostly grown by smallholder farmers (Ojiem et al., 2006; Rubyogo et al., 2007). However, common bean production in Tanzania is low and does not meet the increasing demand. The average yield is 741 kg ha⁻¹ which is lower than that found in the developed country (Hillocks et al., 2006). The low beans yield is mostly contributed by the use of unimproved varieties (Bucheyeki and Mmbaga, 2013) and poor soil nutrition (Ndakidemi and Semoka, 2006).

However, to curb the problem of low yield resulting from the use of unimproved varieties in northern Tanzania, since the mid-1980s, the objective on the National bean Program in Tanzania has been to produce improved bean varieties for smallholder farming systems that meet consumer demand (Fivawo and Msolla, 2012). Thus far, six varieties have been released. This includes Lyamungo 85, Lyamungo 90, selian 94, Selian 97, Selian 2006, and Jesca (Hillocks et al., 2006).

Wortman et al. (1998) and Smithson et al. (1995) identified N, P and K as major constraints to common bean production in northern Tanzania. Giller et al. (1998) also reported poor N₂ fixation as a factor of reduced yield of common bean in northern Tanzania. Because farmers in that part of the country are aware of these soil constraints, N and P fertilizer are routinely applied to cereals such as maize, the major crop for household food security (Ndakidemi et al., 2006) and not in beans. For this reason, research efforts must be directed in evaluating the effects of fertilization with N, P and K on the released bush bean varieties in the country.

A commonly known alternative source of nitrogenous fertilizer to legumes is the well documented Rhizobial inoculants (Ndakidemi et al., 2006). These are generally developed by isolating specific bacterial species within the genera *Allorhizobium*, *Azorhizobium*,

Bradyrhizobium, *Mesorhizobium*, *Rhizobium*, and *Sinorhizobium* (commonly referred to as rhizobia). They have the ability to infect nodulate and symbiotically fix N₂ in legumes (Kevin Vessey, 2004). However, legumes express host specificity, meaning that only certain species or subspecies of rhizobia will infect certain species of legume (Table 1). For example, pea is infected by *Rhizobium leguminosarum* biovar *viciae*, while common bean is infected by *Rhizobium leguminosarum* biovar *phaseoli*. Currently, wide varieties of commercial legume inoculants have been produced and have been tested and therefore are available for use in different part of the world.

It has been reported that legumes inoculated with rhizobium species and supplemented with P and K, respond differently in the growth, yield and nitrogen fixation (Yanni et al., 2001). This review is aimed at providing the background information and an overview of the effects of rhizobium inoculation and supplementation with phosphorus and potassium on yield, nitrogen fixation and economic benefit of bush bean varieties.

Table 1. Example of Forage and grain legumes and rhizobia taxa which infect them

Common name of legumes	Latin name	Rhizobia species or biovars (bv.)
Alfalfa	<i>Medicago sativa</i> L.	<i>Sinorhizobium meliloti</i>
Chickpea	<i>Cicer arietinum</i> L.	<i>Mesorhizobium ciceri</i>
Common bean	<i>Phaseolus vulgaris</i> L.	<i>Rhizobium etli</i> , <i>Rhizobium leguminosarum</i> bv. <i>phaseoli</i> , <i>Rhizobium tropici</i>
Faba bean	<i>Vicia faba</i> L.	<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>
Lentil	<i>Lens culinaris</i> Medik.	<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>
Pea	<i>Pisum sativum</i> L.	<i>Rhizobium leguminosarum</i> bv. <i>viciae</i>
Red clover	<i>Trifolium pratense</i> L.	<i>Rhizobium leguminosarum</i> bv. <i>trifolii</i>
Cowpea	<i>Vigna unguiculata</i> (L.) Walp	<i>Bradyrhizobium japonicum</i>
Soybean	<i>Glycine max</i> (L.) Merr.	<i>Bradyrhizobium elkanni</i> <i>Bradyrhizobium japonicum</i> <i>Sinorhizobium fredii</i>
Yellow sweet clover	<i>Melilotus officinalis</i> (L.) Lam.	<i>Sinorhizobium meliloti</i>

(Kevin Vessey, 2004)

2.0 Influence of rhizobium inoculation and supplementation with Phosphorus and Potassium on growth of legumes

2.1 Effect of Rhizobium inoculation on the growth of legumes

Rhizobium species are usually defined as nitrogen-fixing soil bacteria capable of inducing the formation of root or stem nodules on leguminous plants in which atmospheric nitrogen is reduced to ammonia for benefit of the plant (Boivin et al., 1997). Due to their considerable agricultural and environmental significance, these legume symbionts have been extensively studied (Fred et al., 1932; Giller, 2001; Gupta et al., 2007; Karaman et al., 2013; Nyoki and Ndakidemi, 2014a; Simms and Taylor, 2002; Woomeer et al., 1997).

Legume inoculation is a way of assuring that the strain of *Rhizobium* appropriate for the cultivar being seeded is present in the soil at the proper time and in numbers sufficient to assure a quick and effective infection and subsequent nitrogen fixation (Arsac and Cleyet-Marel, 1986). The inoculation of seeds with *Rhizobium* increase nodulation, nitrogen uptake, seed protein (Bejandi et al., 2011; Tahir et al., 2009; Woomeer et al., 1997).

Study by Yahalom et al. (1987) on Effect of *Azospirillum* inoculation on nitrogen fixation and growth found inoculation of naturally nodulated *Vicia sativa* L. (vetch) grown in soil in the greenhouse and in the field for forage inoculation significantly increased dry matter yield, %N, N-content, and nitrogen fixation activity.

Huang and Erickson, (2007) treated pea and lentil seeds with *Rhizobium leguminosarum* and reported that shoot/root growth and nodulation of pea improved as a result of rhizobia inoculation. Moreover, seed yield of pea was significantly improved. Similarly, inoculation with *Rhizobium leguminosarum*, increased seedling height, nodule mass and shoot biomass of lentil. Likewise, nodulation was improved in peanut by inoculating seeds with a variety of *Rhizobium* species (Dey et al., 2004). Elsewhere, studies by Zahran, (1999) and Rudresh et al. (2005) on chickpea in field and in the glass house reported significantly increase in plant height, branches per plant, number of nodules and dry weight of root per plant by inoculating seeds with *Rhizobium*.

Also studies by Ali et al. (2000) and Malik et al. (2006) on Mungbean (*Vigna radiata* L.) and soybean (*Glycine max* L.) respectively found that *Rhizobium* culture significantly affected the growth and yield components like number of pod bearing branches per plant, number of pods per plant, number of seeds per pod and 1000-seedweight were significantly affected by inoculation.

Other study by Ravikumar, (2012) on the effects of *Rhizobium* inoculation in *Vigna mungo* and *Vigna radiata* found that both *V. mungo* and *V. radiata* varieties inoculated plants possessed greater height, greater fresh weight, greater number of roots, nodules, greater number of leaves,

shoots, pods, greater length of pods, greater seed weight, over their respective controls. The study by Tairo and Ndakidemi (2013) on soybean found that plant height for field experiment increased with *Rhizobium* inoculation for the entire interval of the soybean growth. Also it was found that inoculation significantly increased the stem girth in the glasshouse and field experiments respectively. In another study by Nyoki and Ndakidemi (2014 a), rhizobia inoculation in cowpea significantly improved the plant height measured at four, six and eight weeks after planting (WAP) in both screen house and field experiments relative to the control treatment. (Nyoki and Ndakidemi, 2014a).

Generally, *Rhizobium* inoculation in legumes is accredited for stimulating growth and is an alternative to the expensive inorganic nitrogen fertilizers (Ndakidemi et al., 2006; Abbasi et al., 2010; Mahmoodi et al., 2013). The use of appropriate strains of inoculants in nitrogen deficient soils may offer an excellent opportunity for improving legume growth and development. However, given the modest cost of inoculation compared with the potential agronomic benefits, producers are advised to seriously consider inoculation of their legume crops in all circumstances (Kevin Vessey, 2004).

Thus far, there is a little information on the performance of rhizobia inoculants and the synergistic effect of *Rhizobium* inoculation, P and K on growth of released bean varieties and hence a need for research to exploit the potential of these microorganisms.

2.2 Effect of Phosphorus on the growth of legumes

Phosphorus (P) is essential macronutrient for plant growth and function. The requirements of host plants for optimal growth and symbiotic dinitrogen fixation processes for P have been assessed by determination of nodule development and functioning (Almeida et al., 2000; Chaudhary et al., 2008; Sa and Israel, 1991; Tsvetkova and Georgiev, 2003).

Among the leguminous species, the optimum P requirement varies (Bekele et al., 1983) and this characteristic depends on the efficiency of the species to absorb and utilize P in biomass production. The variations in response to low P and the capacity to store and utilize P by rhizobial species and strains have been examined (Beck and Munns, 1984; Cassman et al., 1981)

Phosphorus is needed in relatively large amounts by legumes for growth and has been reported to promote leaf area, biomass, yield, nodule number and nodule mass in different legumes (Berg Jr and Lynd, 1985; Kasturikrishna and Ahlawat, 1999; Pacovsky, 1988). The application of P in the west Usambara Mountains led to strong increases in shoot biomass but induced a strong inter-veinal chlorosis (Ronner and Giller, 2012).

Buerkert et al., (2001); Kisinyo et al., (2012); NEKESA et al., (2007) have also found that Phosphorous is among the important element needed for growth and production of legumes in many tropical soils. However, many tropical soils are P deficient. Olivera et al., (2004), in Spain

studied the effect of different level of P on growth of *Phaseolus vulgaris* and found that the highest P application increased total plant growth and leaf area. However, concluded that the different behavior shown by shoot and root growth indicates that root growth is less sensitive to P deficiency than is shoot growth.

Studies with several legumes have consistently shown a positive response to P application; whole-plant N concentration and plant dry matter were found to increase in response to phosphate in the growth media (Pereira and Bliss 1987). In Rio De Janeiro Brazil the higher P supply stimulated the vegetative growth of common bean cultivars, increasing Shoot dry weight from 5.34 to 7.10gplant⁻¹, root dry weight from 0.41 to 0.5gplant⁻¹) nodule dry weight from 5.0 to 16.6mgplant⁻¹ and number of nodules from 8.0 to 16.9gramplant⁻¹ (ARAÚJO et al., 2000).

Other Study conducted in Hiroshima Japan on Three legume species: mashbean (*Vigna aconitifolia* cv. Mash-88), mungbean (*Vigna radiata* cv. Moong-6601), and soybean (*Glycine max* L. cv. tamahomare) were grown at different levels of P applied in 1993 and 1994. Dry matter production increased in all the species with the increasing P levels. At low P levels up to 25 kg P ha⁻¹ soybean displayed a relatively higher biomass production than mashbean and mungbean, which was mainly caused by the promotion of root growth and leaf area expansion (Chaudhary and Fujita, 1998).

In northern Tanzania for example, studies in Rombo and Moshi districts observed that the plant height and leaf area of common bean and soybean respectively increased significantly with P application (Ndakidemi et al., 2006). This could be due to the fact that P being essential constituent sugar metabolism, energy storage of plant tissue significantly influences the plant height of crop (Kumar and Chandra, 2008).

Supplementing legumes with nutrients P has great potential for increasing yields, as it not only promotes plant growth but also enhances symbiotic establishment for increased N₂ fixation (Ndakidemi et al., 2006). Nodule number, as well as total and specific nitrogenase activity, increases with the addition of P, implying more efficient nitrogen fixation (Israel, 1987). The study done by Olivera et al., (2004) on common bean (*Phaseolus vulgaris* L.) showed that P application, nodule biomass (4-fold), and shoot and root P content (4- and 6-fold, respectively) in plant harvested at the onset of flowering (28-days-old).

Giller et al., (1998) showed that root nodulation of common bean in the northern highlands of Tanzania was strongly determined by availability of P. On-farm trials with Rhizobial inoculation and P fertilizer in Tanzania showed that yields of common bean increased with inoculation and application of 26 kg P/ha (Ndakidemi et al., 2006).

Currently there is a little information on the effect of phosphorus, when mixed with rhizobium inoculant and potassium on growth of bush bean varieties *Phaseolus vulgaris* L. released in

different agro ecological zones and thus farther investigation to quantify their potential to growth and other growth parameters of new bean line is required.

2.3 Effect of Potassium on the growth of legumes

Potassium is the main osmotic solute in plants and its accumulation in the cell favors water uptake, thus generating the cell turgor pressure required for growth (Mengel and Arneke, 1982). In addition it helps stomatal opening (Fischer and Hsiao 1968). Moreover, it is involved in activating a wide range of enzyme systems regulating photosynthesis, water use efficiency and movement, nitrogen uptake and protein building (Nguyen et al., 2002).

It is documented that potassium may “activate” at least 60 different enzymes involved in plant growth (Marschner and Marschner, 2012). Potassium functions by changes the physical shape of the enzyme molecule, exposing the appropriate chemical active sites for reaction (Ball, 2008). Potassium also neutralizes various organic anions and other compounds within the plant, helping to stabilize pH between 7 and 8 which is considered to be optimum for most enzyme reactions (Wakeel et al., 2011). The amount of K present in the cell determines how many of the enzymes can be activated and the rates at which chemical reactions can proceed. Thus, the rate of a given reaction is controlled by the rate at which K enters the cell (Munson, 1985c).

Potassium plays significant roles in enhancing crop quality. High levels of available K improve the physical quality, disease resistance, and shelf-life of fruits and vegetables used for human consumption and the feeding value of grain and forage crops (Sangakkara et al., 1996). Quality can also be affected in the field before harvesting such as when K reduces lodging of grains or enhances winter hardiness of many crops (Kalavat P, 2012). Potassium influences the water economy and crop growth through its effects on water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation (Khurana and Sharma, 2000; Singh and Kataria, 2012).

Patil (2011) concluded that potassium is required for every major step of protein synthesis. The “reading” of the genetic code in plant cells to produce proteins and enzymes that regulate all growth processes would be impossible without adequate K. When plants are deficient in K, proteins are not synthesized despite an abundance of available nitrogen (N). Instead, protein “raw materials” (precursors) such as amino acids, amides and nitrate accumulates in the tissue. The enzyme nitrate reductase catalyzes the formation of proteins and K is likely responsible for its activation and synthesis (Chandok et al., 2003).

However, K has other important roles in major plant processes such as photosynthesis, respiration, osmoregulation, growth and yield of plant (Li et al., 1989; Sharma et al., 1992; Zaidi et al., 1994, Singh et al., 1997). However does not enter into the composition of any product unlike nitrogen and phosphorus (Chanway et al., 1989) and (Khurana and Sharma, 2000).

Akhtar et al., (2003) studied the effect of various levels of phosphorus and potassium on the growth and green pod yield of pea cv. Samrina zard concluded that mean values for potassium revealed that maximum number of pods was obtained from the plants received K_2O at 150 kg ha^{-1} . Minimum number of pods was recorded in those plants, which received no potassium. Kanaujia et al., (1999) had reported that when 0, 30, 60 or $90 \text{ kg K}_2\text{O ha}^{-1}$ was applied to French bean, number of pods per plant increased with increasing K_2O rates up to 60 kg ha^{-1} . The height, number of branches per pod, pod length, pod girth, number of pods per plant and protein content of *Phaseolus vulgaris* in Himachal Pradesh in India were significantly increased by the increasing level of potassium up to $60 \text{ kg K}_2\text{O ha}^{-1}$ and consequently leading to more green pod yield. The increase in green pod yield with 30, 60, and $90 \text{ kg K}_2\text{O ha}^{-1}$ were 24.19, 42.69 and 33.41% respectively over control (Kanaujia et al., 1999).

In west Usambara Tanzania, a combination of soil analysis, plant analysis and multi-factorial nutrient addition experiments by Smithson et al. (1993) revealed that the leaf symptoms in common bean *Phaseolus vulgaris* were due to K deficiency. The addition of only 25 kg ha^{-1} led to spectacular increases in yield from 0.6 t/ha in the control plot to 1.3 t/ha in one smallholder farmer's field. In other similar related studies, application of potassium also enhanced vegetative growth and increased pod yield in pea (Kanaujia et al., 1997 & 1998), in cowpea (Jamadagni and Birari, 1994) and in French bean (Kanaujia et al., 1999).

Slaton et al., (2007) studied full-season irrigated soy bean response to potassium fertilization. Results showed that soil test K is an excellent means of characterizing the need for K fertilization of soybeans. With soil test K ranging from 46 to 167 ppm, significant yield increases were recorded with K fertilization. Also tissue analyses results indicated 1.8% K may be needed in soybean leaves to achieve 90% of maximum yield.

Smithson et al., (1993); Kurdali et al., (2002) Johnston et al., (2007); Slaton et al., 2007) studied the effects of different rates of K fertilizer on nodulation, dry matter production, and N_2 fixation by chickpea and faba bean subjected to different soil moisture levels at the beginning of flower bud initiation stage. They found that plant species differed in their response to K fertilizer as a means of enhancing growth and overcoming the stress conditions. The higher level of K fertilizer increased both dry matter production and total N_2 fixed in faba bean, but did not have any impact on chickpea.

From the above background, potassium is an important element in legume nutrition. Therefore there is need to study the effects of K and their synergistic effects with N and P on growth of bush bean varieties.

3.0 Effect of rhizobium inoculation and supplementation with phosphorus and potassium on photosynthesis and leaf chlorophyll (Chl) content legumes

3.1 Effect of rhizobium inoculation on photosynthesis and chlorophyll (Chl) content of Legumes

Nitrogen is the critical limiting element for growth of most plants due to its unavailability (Graham and Vance, 2000; Bambara and Ndakidemi, 2010a). Beans need nitrogen more than any other nutrient (Wortmann, 1998). Nitrogen (N) is a constituent of proteins, enzymes, chlorophyll, and growth regulators. Deficiency causes reduced growth, leaf yellowing, reduced branching and small trifoliolate leaves in beans (CIAT, 1989). Nitrogen is a building block of proteins and is highly needed for all enzymatic reactions in a plant (Ayoola, 2010). It is a major part of the chlorophyll molecules and plays a necessary role in photosynthesis and also is a major component of several vitamins (Tairo and Ndakidemi, 2013; Morad et al., 2013).

Concentration of chlorophyll dyes is a reliable index of physiological plant condition (Swędrzyńska and Sawicka, 2000) though frequently it is not duly appreciated in agricultural sciences. Studies by (Bejandi et al., 2011) on the effects of seed inoculation and microelement supplementation Chickpea (*Cicer arietinum* L.) found that plants from combinations inoculated with *Rhizobium cicerea* were characterized by a higher chlorophyll concentration. However, plants treated with *Rhizobium* and microelements scored the highest chlorophyll content. Other studies done by Gwata et al. (2003) reported that chlorotic plants with yellow leaves were visually distinguishable from the vigorous plants with dark green leaves.

In a study involving common bean done by Bambara and Ndakidemi, (2009) in glasshouse and field experiment showed that for the glasshouse experiment leaf chlorophyll (Chl) content increased significantly with rhizobial inoculation. For example, it was found that the leaf Chl content for the glasshouse experiment increased significantly with rhizobial inoculation by 123% and 178% for the field experiment relative to the un-inoculated control. The photosynthesis similarly increased significantly with rhizobial inoculation by 140% for the glasshouse experiment and by 81% in the field experiment compared with control treatment.

Biswas et al., (2000b); Bambara and Ndakidemi, (2010a,) found that soybean inoculated with *Bradyrhizobial japonicum* had great positive response in leaf chlorophyll content and growth parameters measured such as plant height, number of leaves per plant, stem girth (mm), LA (cm²) and LAI (cm²) number of days to 50% flowering and number of days to 50% pod formation as compared with the control (Tairo and Ndakidemi, 2013).

Nyoki and Ndakidemi, (2014a) studied the effect of Effects of *Bradyrhizobium japonicum* inoculation and supplementation with phosphorus on macronutrients uptake in Cowpea (*Vigna unguiculata* (L.) Walp) found that the total leaf chlorophyll content of cowpea measured at five

and seven weeks after planting were also significantly increased with inoculation of *B. japonicum* in the field experiment.

Beside of the studies above, there is little literature about the effects of phosphorus, potassium and *Rhizobium* inoculant and their interaction on the chlorophyll formation in new released bush bean *Phaseolus vulgaris* in Tanzania. Therefore, it is important to establish and quantify the influence of phosphorus, potassium and *Rhizobium* inoculant on leaf chlorophyll content in such legumes.

3.2 Effect of phosphorus on photosynthesis and chlorophyll content of legumes

Phosphorus (P) is frequently one of the most limiting nutrients for plant growth in the tropics, and it is estimated that over 50% of common bean production in tropical soils is limited by phosphate deficiency (Olivera et al., 2004). Phosphorus (P) has a major role in fat, carbon, hydrogen, and oxygen metabolism, and in respiration and photosynthesis. Deficiency symptoms include dwarfed plants with thin stems and shortened internodes; small, dark-green upper leaves, and few pods form and contain only a small number of seeds (Schwartz and Corrales, 1989).

In photosynthesis and respiration, phosphorus have been reported to plays a major role in energy storage and transfer as ADP and ATP (adenosine di- and triphosphate) and DPN and TPN (di- and triphosphopyridine nucleotide) and a shortage of inorganic phosphate in the chloroplast reduces photosynthesis (Uchida, 2000).

Mairura et al., (2007) in central Kenya found that the P-fertilizer application resulted in more vigorous plants with more intense green colour indicating higher chlorophyll content and greater photosynthetic ability. Consequently the fertilized crop when compared with the control produced more pods per plant which were better filled with heavier seeds and this translated to higher grain yield.

Study by Tairo and Ndakidemi, (2013) on effect of P supply on leaf chlorophyll content in soybean (*Glycine max* L.) concluded that chlorophyll content was uniform in all treatments and was numerically but not significantly decreasing with increasing phosphorus. However, in the field study, the leaf chlorophyll content was significantly influenced by P at 6 WAP. The highest chlorophyll content was recorded by supplying 40 kgP.ha⁻¹.

Another study by Nyoki and Ndakidemi, (2014a) on effect of P on cowpea both in screen house and field condition asserted that in the screen house, P supplementation at all levels did not show significant effect on the chlorophyll content of the cowpeas measured at 3, 5 and 7 WAP. However the results show that supplementation of phosphorus at all levels significantly increased the chlorophyll content of cowpea measured at 5 and 7 WAP compared with the control.

Supplementation of P at 80 kg/ha (5 WAP) and 40 kg/ha (7 WAP) resulted in greater values of chlorophyll content compared with other treatments.

Currently there is limited literature explaining the interactive effects of rhizobium inoculation and supplementation with potassium and phosphorus on chlorophyll formation in the released bean varieties in different agro ecological zone. Therefore, it is important to establish and quantify the influence of phosphorus and its interaction with rhizobium inoculation and potassium on leaf chlorophyll content for the released bush beans line in Tanzania.

3.3 Effect of potassium on chlorophyll content of legumes.

The role of K in agricultural production is intimately connected with photosynthesis (Atkin and Macherel, 2009). Potassium influences the process of photosynthesis at many levels, namely synthesis of ATP, activation of the enzymes involved in photosynthesis, CO₂ uptake, balance of the electric charges required for photophosphorylation in chloroplasts, and acting as the counter ion to light-induced H⁺ flux across the thylakoid membranes (Marschner et al., 1996). Stomatal closure in response to K deficiency is often considered a major factor that contributes to decreased net photosynthesis (Jin et al., 2011).

It has been reported that adequate supply of potassium nutrient increase chlorophyll content of plants (Fletcher et al., 1982; Maples et al., 1988; Stromberg, 1960; Zhao et al., 2001). If potassium supply is inadequate, the stomata become sluggish – slow to respond – and water vapor is lost. Closure may take hours rather than minutes and is incomplete. As a result, plants with an insufficient supply of K are much more susceptible to water stress. Accumulation of K in plant roots produces a gradient of osmotic pressure that draws water into the roots. Plants deficient in K are thus less able to absorb water and are more subject to stress when water is in short supply (Kurdali et al., 2002).

The role of K in photosynthesis is complex. The activation of enzymes by K and its involvement in adenosine triphosphate (ATP) production is probably more important in regulating the rate of photosynthesis than is the role of K in stomatal activity (Munson, 1985b). When the sun's energy is used to combine CO₂ and water to form sugars, the initial high-energy product is ATP. The ATP is then used as the energy source for many other chemical reactions. The electrical charge balance at the site of ATP production is maintained with K ions. When plants are K deficient, the rate of photosynthesis and the rate of ATP production are reduced, and all of the processes dependent on ATP are slowed down (Lawlor and Tezara, 2009; Mengel et al., 2001).

Conversely, plant respiration increases which also contributes to slower growth and development. In some plants, leaf blades re-orient toward light sources to increase light interception or away to avoid damage by excess light, in effect assisting to regulate the rate of photosynthesis (Lawlor and Cornic, 2002). These movements of leaves are brought about by

reversible changes in turgor pressure through movement of K into and out of specialized tissues similar to that described above for stomata (Van Brunt and Sultenfuss, 1998).

Studies done by Onanuga, (2013) found that low K and high P and K cotton treated plants enhanced chlorophyll a, b and a & b production at 83 and 91(days after planting) DAT. The result was the confirmation of the report stated earlier that high levels of K nutrition promoted formation of chlorophyll a and b in cucumber (*Cucumis sativus cv Brunex*) leaves (Onanuga et al., 2011; Lamrani et al., 1996).

Collins and Duke, (1981) studied the influence of potassium-fertilization rate and form on photosynthesis and N₂ fixation of Alfalfa concluded that chlorophyll concentration increased linearly in response to K application and net carbon exchange rate (CER) on a per plant basis was increased by average of 28% over the control. Duli et al. (2001) also confirmed that K deficient in legumes is associated with low chlorophyll content.

However, little is known of the effect of potassium on chlorophyll synthesis in new bush bean varieties (*Phaseolus vulgaris L.*) released in different agro-ecological systems found in Africa. Hence, further research is needed to identify the effect of potassium and its interaction with rhizobium inoculant and phosphorus on leaf chlorophyll content.

4.0 The effects of rhizobium inoculation and supplementation with Phosphorus and potassium on nutrient uptake of legumes

4.1 The effects of rhizobium inoculation on nutrient uptake of legumes

Bioavailability and uptake of primary and secondary macro elements such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S) is very important for plant growth and development in Africa where different cropping systems involving legumes such as common beans (*Phaseolus vulgaris L.*) is practiced (Soetan et al., 2010).

Their uptake by plants depends largely on the amount, concentration and activities in the rhizosphere soil as well as the capacity of the soil to replenish them in the soil solution. Specifically, P is essential for plant growth and function including symbiotic N₂-fixation processes (Sa and Israel, 1991).

Mineral nutrient deficiencies are the major constraints limiting legume N fixation and yield (O'Hara *et al.*, 1988). In leguminous crops, this deficiency can be overcome by applying *Rhizobium* strain and providing essential nutrients in the soil (Fatima et al., 2007). However, the deficiencies limit nitrogen fixation by the legume-*Rhizobium* symbiosis and decrease yield of legumes. Nutrient limitations in legume production result from deficiencies of not only major nutrients but also micronutrients such as molybdenum (Mo), zinc (Zn), boron (B) and iron (Fe) (Graham, 1981).

Study by Makoi et al., (2013) on the effect of *Rhizobium* inoculation on *Phaseolus vulgaris* on nutrient uptake concluded that *Rhizobium* inoculation in *P. vulgaris* significantly increased the uptake of P, K, Ca, Mg and S in roots, shoots, pods and whole plant during glasshouse and field experimentation. They asserted that although the amount of P and K increased in the root as a result of *Rhizobium* inoculation, these values were only significant in the glasshouse experiment but not in the field experiment. Singh, (1973) also reported that the significant increase the amount of K uptake was attributed to *Rhizobium* inoculation in Pigeon pea (*Cajanus cajan* L. Millsp) during harvest.

The increased availability of nutrients in the soil increases the chance of uptake by plants (Chapin III, 1980). Ndakidemi et al. (2011) reported that *Rhizobium* inoculation significantly enhanced uptake of micronutrients such as Mn, Fe, Cu, Zn, B and Mo in all organs (roots, shoots, pods and whole plants) except the Mo uptake in roots. *Rhizobium* inoculation was also reported to show significant increase in the soil pH, Ca and Na (Fatima et al., 2007; Kisinyo et al., 2012).

Studies by Nyoki and Ndakidemi, (2014b) on Cowpea in northern Tanzania discovered that there was a significant difference in the uptake of Zn, Cu, Fe, and Mn in the shoots following inoculation with *B. japonicum* and phosphorus application in both the screen house and field experiments. Over the control, inoculation significantly increased the uptake of micronutrients in the shoots from both the screen house and field experiments. Another study by Tairo and Ndakidemi, (2014) showed that *Bradyrhizobium japonicum* inoculation in soybean *G. max* L. plants significantly increased the uptake of Zn, Cu, Fe and Mn in glasshouse experiments, whereas the uptake of Cu, Fe and Mn were significantly increased and that of Zn reduced in the field experiment.

However there is little literature about the role that is played by different legume rhizobia inoculants on the availability of other nutrients in bean varieties released for different use in agro-ecological systems found in Africa. Also the interactive effect of rhizobium inoculant, P and K has not been studied extensively in such systems. Thus, it is therefore imperative to establish the possible role which could be played by *Rhizobium* inoculant phosphorus and potassium on the availability of other nutrients in legumes such as *P. vulgaris* grown in each specific agro-ecological system found in Africa.

4.2 The effects of phosphorus on nutrient uptake of legumes

Phosphorus plays a vital role in physiological and developmental process in plant life and favorable effect of this important nutrient might have accelerated the growth process that increases N uptake in plants (Alam and Pessarakli, 1999). Phosphorus is also an essential ingredient for *Rhizobium* bacteria to convert atmospheric N (N_2) into an ammonium (NH_4) form

usable by plants. Inadequate P restricts root growth, the process of photosynthesis, translocation of sugars and other such functions which directly influenced N fixation by legume plants (Fatima et al., 2007).

Studies have indicated that phosphorus enhanced root system which provides greater root-soil contact and eventually higher uptake of phosphorus and other important and low mobility nutrients and absorption of higher concentration of mineral nutrients (Zafar *et al.*, 2013). Biswas et al., (2000) reported that rhizobial inoculants may also induce an increased number of roots hairs and lateral roots thereby favoring nutrient uptake by exploration of a greater soil volume.

The study by Olivera et al., (2004) reported that phosphorus application to leguminous crops increase plant biomass including nodule biomass and shoot phosphorus content increased by the rate of nitrogen fixation. Phosphorus also increases organic matter (36%) in the soil when applied alone (Palm et al., 1997). Soil organic matter effects on plant growth and referred to as the “glue” that hold soil particles together that also promotes development of soil tilth and texture (Zarrin et al., 2007). Rodelas et al. (1999) also observed increased root development and water and mineral uptake by root with the application of mix *Rhizobium* strain and phosphorus on faba beans.

Recent study by Tairo and Ndakidemi, (2014) on the effect of P on nutrient uptake (Table 2) found that P supply significantly affected the uptake of Zn, Cu and Mn in glasshouse experiment. The highest uptake in Zn was observed by supplying 20 and 40 kg P.ha⁻¹, whereas, for Cu, the best uptake was recorded by supplying between 40-80 kg P.ha⁻¹ in the glasshouse experiment relative to the control treatment. The uptake of Mn was significantly higher by applying 80 kg P.ha⁻¹ in the glasshouse experiment relative to all other treatments. In field experiment, P supply significantly increased the uptake of Cu and Mn only and numerically increased the uptake of Zn and Fe in the field experiment, but the increase was not statistically significant.

Another study by Nyoki and Ndakidemi, (2014b) on effect of Phosphorus supplementation in cowpea (Table 3) and they found that P supply resulted in a significant increase in shoot uptake of Cu, Fe and Mn in the screen house and the shoot uptake of Fe and Mn in the field experiment over the control. In the field experiment, there was a significant decrease in the shoot uptake of Fe by 18.2% and the shoots uptake of Mn was increased by 162.2% at 80 kg P/ha relative to control.

It has been reported that phosphorus may play a crucial role in increasing nutrient uptake in legumes grown in different soils (Nyoki and Ndakidemi 2014; Tairo and Ndakidemi, 2014). Therefore, its influence in nutrient uptake and when supplemented with *Rhizobium* and K grown in different agro-ecological zones on the nutrient uptake in legume varieties of different growth habits need further investigations.

Table 2: Effect of *B. japonicum* and P supplementation on the micronutrient uptake in the whole plant of *G.max.* L

Treatment	Glass house				Field			
	Zn (mg.plant ⁻¹)	Fe (mg.plant ⁻¹)	Mn (mg.plant ⁻¹)	Cu (mg.plant ⁻¹)	Zn (mg.plant ⁻¹)	Fe (mg.plant ⁻¹)	Mn (mg.plant ⁻¹)	Cu (mg.plant ⁻¹)
<i>B.japonica</i>								
R ⁻	268.26±19.80b	63.15±3.04b	576.68±17.79b	143.23±7.54b	888.53±21.27a	582.85±85.74b	612.10±24.19b	495.74±26.45b
R ⁺	398.73±12.32a	77.41±3.83a	736.44±17.63a	172.21±8.97a	523.03±29.77b	884.52±152.57a	701.02±25.05a	644.31±22.58a
Phosphorus kg.ha⁻¹								
0	321.85±28.4ab	56.89±3.68c	650.79±28.17a	113.90±5.18d	690.02±98.79a	387.21±168.24b	650.05±45.62a	509.48±33.24b
20	358.87±33.82a	68.16±4.74bc	678.74±45.40a	153.20±8.86c	743.35±80.78a	397.82±130.41b	654.16±40.96a	583.01±58.0ab
40	362.81±39.37a	74.85±5.80ab	681.13±46.20a	172.79±8.85b	716.80±69.84a	1079.26±139.8a	626.75±33.81a	563.67±27.1ab
80	290.45±27.62b	81.23±3.99a	615.57±30.71a	190.99±7.54a	672.96±57.02a	1070±279.22a	695.27±32.93a	623.92±46.52a
2Way ANOVA (F-statistic)								
B.j	32.69***	12.94**	47.44***	25.51***	102.42***	5.14*	6.15*	19.92***
P	2.24ns	6.88**	1.74ns	33.15***	0.73ns	8.76***	0.63ns	2.04ns
B.j*P	0.20ns	0.38ns	1.92ns	0.54ns	1.53ns	0.53ns	0.82ns	0.88ns

-R: Without *Bradyrhizobium*: +R: With *Bradyrhizobium*: Values presented are means ± SE. *, *** =significant at P≤0.05, P≤0.001 respectively, ns = not significant. Means followed by similar letter in a given column are not significantly difference from each other at p=0.05. *B. j*=*Bradyrhizobium japonicum*, P=phosphorus

(Tairo and Ndakidemi, 2014).

Table 3: Effect of *B. japonicum* and P supplementation on the micronutrient uptake in the whole cowpea plant

Treatment	Glass house				Field			
	Zn (mg.plant ⁻¹)	Fe (mg.plant ⁻¹)	Mn (mg.plant ⁻¹)	Cu (mg.plant ⁻¹)	Zn (mg.plant ⁻¹)	Fe (mg.plant ⁻¹)	Mn (mg.plant ⁻¹)	Cu (mg.plant ⁻¹)
<i>B.japonica</i>								
R ⁻	67.15 ± 2.91b	27.79 ± 2.48b	156.39± 13.65b	24.39 ± 2.76b	319.31 ±11.01b	84.54 ± 4.97b	1036.27 ± 24.36b	341.09 ± 25.06b
R ⁺	90.60 ± 2.81a	39.82 ± 3.21a	235.46 ±18.81a	34.14 ± 2.79a	460.44 ± 12.7a	112.97 ± 4.3a	1422.47 ± 33.89a	455.44 ± 29.73a
Phosphorus kg.ha⁻¹								
0	78.02 ± 6.82ab	20.99 ± 3.27c	109.98 ±10.33c	14.55 ± 2.29c	407.93± 33.7ab	79.28 ± 7.9b	1181.49 ± 77.6b	239.001 ± 16.5c
20	85.91 ± 6.60a	32.19 ± 3.27b	192.47 ±15.03b	26.29 ± 2.43b	423.25 ± 30.1a	102.03 ± 7.4a	1316.04 ± 90.4a	405.32 ± 29.7b
40	82.15 ± 4.96a	41.27 ± 3.42a	238.14 ±21.93a	37.07 ± 2.25a	374.10±30.81bc	107.79 ± 8.6a	1253.79 ± 91.5ab	462.49 ± 27.86a
80	69.43 ± 3.54b	40.74 ±3.40ab	233.12 ±20.65a	36.17 ± 2.89a	105.92 ± 5.91a	354.2± 25.4c	1166.15 ± 64.41b	486.25 ± 32.98a
2Way ANOVA (F-statistic)								
B.j	45.07***	15.64***	38.66***	30.34***	92.95***	25.82***	100.10***	41.79***
P	4.09*	9.76***	32.52***	40.98***	4.60**	5.57**	3.22*	39.71***
B.j*P	1.29 ns	0.01 ns	1.89 ns	0.26 ns	0.62 ns	0.37 ns	0.47 ns	0.59 ns

-R: Without *Bradyrhizobium*: +R: With *Bradyrhizobium*: Values presented are means ± SE. *, *** =significant at P≤0.05, P≤0.001 respectively, ns = not significant. Means followed by similar letter in a given column are not significantly difference from each other at p=0.05. B. j=*Bradyrhizobium japonicum*, P=phosphorus

(Nyoki and Ndakidemi 2014)

4.3 The effects of Potassium on nutrient uptake

Potassium is an essential element for all living organism. In plants, it is an important cation involved in physiological pathways (Beringer et al 1983; Duke and Collins 1985). In particular, the ability of ATPase in membranes to maintain active transport is highly dependent on adequate potassium supply. Deficiency in potassium is on the increase and hence potassium management is of importance specifically because potassium influences the uptake of other major nutrients and influences crop quality (Daliparthi et al., 1994).

Potassium influences the water economy and crop growth through its effects on water uptake, root growth, maintenance of turgor, transpiration and stomatal regulation (Nelson, 1982). Thus, efficient cell development and growth of plant tissues, translocation and storage of assimilate and other internal functions which are based up on many physiological, bio chemical and bio physical interaction require adequate K in the cell sap (Marschner and Marschner, 2012).

Uptake efficiency of soil-grown plants may consist of increased capacity to mobilize/solubilize non-available nutrient forms into plant-available ones, and/or increased capacity to transport nutrients across the plasma membrane of root cells and into other plant parts. For K, it appears likely that the increased capacity to convert non-available into available forms is of greater importance for efficient uptake because K is transported to roots by diffusion, which is a relatively slow process that frequently limits nutrient uptake (Makoi et al., 2013).

Raza et al., (2013) studied the possible role of K applied on wheat foliage at different growth stages under drought and nutrients uptake. The results revealed that application of K at any critical crop growth stage significantly increased wheat N, P, K and Ca uptakes and reduced Na uptake. However, comparing the efficiency of K spray at different growth stages studies indicated that nutrient uptake was affected maximum when K was applied under water stress at grain filling stage.

Other researchers (Leigh and Wyn Jones, 1984; Ruiz and Romero 2002; and Pervez et al., 2006) reported that adequate K is needed for the efficient use and metabolism of N. Generally, it has been established that K has an impact on the uptake of other cationic species and thus may affect the crop yield and crop quality (Mengel et al., 2001).

Despite of the above background information, little is documented about the role of potassium and the interactive effect of P and rhizobium in nutrient uptake in legumes particularly bean varieties *Phaseolus vulgaris* L. of different growth habits grown in different agro-ecological zones found in Africa. Based on this, it is therefore imperative to establish the potential role which could be played by potassium when supplemented with phosphorus and *Rhizobium* inoculants on the availability of other nutrients in legumes grown in different agro-ecological zones found in Africa.

5.0 Effect of rhizobium inoculants and supplementation with Phosphorus and Potassium on nodulation and nitrogen fixation of grain legumes

5.1 Effect of rhizobium inoculants on nodulation and nitrogen fixation of legumes

Rhizobia are soil bacteria inducing root (or stem) nodules on leguminous plants, in which the process of symbiotic N₂ fixation (SNF) occurs. (Giller, 2001) found that major benefits of the legume-rhizobia symbiotic interaction are diminished nitrogen fertilizer requirements and improving plant growth and health. Symbiotic systems such as that of legumes and rhizobia can be a major source of N in most cropping systems with an average of 80% of the required nitrogen coming from biological N₂ fixation (Niehaus and Becker, 1998; Vance et al., 2000) Beck and Munns, 1984; Jakobsen, 1985; Pereira and Bliss, 1987; Chaudhary and Fujita, 1998; Bekele et al., 1983; Cassman et al., 1981).

The inoculation of seeds with rhizobia increase nodulation, nitrogen uptake and seed protein in soybeans (Fehr et al., 1971; Rudresh et al., 2005; Solaiman et al., 1999). The combined inoculation of *Rhizobium* and phosphorus has increased nodulation, plant height, seed protein and yield parameters in chickpea (Khurana et al., 2000; Namvar et al., 2011).

Nitrogen provided in the form of inoculant is not only cheap but also does not impart other undesirable aspects such as pollution hazards due to heavy use of inorganic nitrogen fertilizers (Bashan, 1998; Khonje, 1989). In addition, inoculation of seeds, plants and soil with *Rhizobium* is even simpler than applying correct doses of inorganic nitrogen fertilizers such as urea or di ammonium phosphate (Rao, 1982).

Inoculation of legumes with species-specific *Rhizobium* may increase the success of their establishment, root nodulation, biomass and biomass nitrogen yields (Chianu et al., 2010; Zahran, 2001). Inoculation may be necessary where; 1. Compatible native rhizobia are lacking, 2. Current population compatible rhizobia are insufficient to initiate rapid population, and 3. Indigenous populations of *rhizobium* are ineffective or less effective than elite inoculants strains. (Bekunda et al., 2010).

The impact of rhizobia inoculation in improving nitrogen fixation in legumes is widely documented (Bejandi et al., 2011; Tu, 1981; Keyser and Li, 1992; Elkoca et al., 2007; Ogutcu et al., 2008; Nyoki and Ndakidemi, 2014a). For example, a study by Bejandi et al. (2011) reported that seed inoculation with *Rhizobium cicerea* produced significantly highest nodule number, nodule fresh weight, nodule dry weight and active nodule per plant than control (Bejandi et al., 2011). Favorable effects of inoculation with *Rhizobium* spp. and significant increase in nodulation and yield of legume crops have been reported by many researchers (Elkoca et al., 2007; Keyser and Li, 1992; Ogutcu et al., 2008; Tu, 1981).

Rhizobium inoculant has the potential to increase N in legumes plant and percentage nitrogen derived from the atmosphere (Ndfa) (Bambara and Ndakidemi, 2010; Beck, 1992; Clayton et al., 2004; Kucey et al., 1988; Peoples and Baldock, 2001; Rennie and Kemp, 1983a, b; Rice et al., 2000; Sanginga et al., 1990). Studies in *Phaseolus vulgaris* have shown that N-fixation from Rhizobium inoculation contributed an N equivalence of 20-60 kg N ha⁻¹ in Brazil (Da Silva et al., 1993). Elsewhere (Clayton et al., 2004) has found *Rhizobium* inoculant increased plant nitrogen content from 19- 42 mg plant⁻¹ while increasing percentage Ndfa from 10 to 61% on pea plant.

In another study by Bambara and Ndakidemi, (2010) found that the percentage of Ndfa increased significantly with rhizobium inoculation in roots, shoots, pods, and whole plant both in the glass house and field experiment compared with un inoculated control treatment. For example the estimation per ha basis of the amount of N fixed amounted from 8.6 to 33 kg ha⁻¹ in the un inoculation control and Rhizobium inoculation respectively.

The *Rhizobium* inoculation may play a crucial role in food grain legumes grown in poorly depleted soils in Africa. Therefore, their potential in improving N₂ fixation in the newly released bean varieties in Africa needs to be investigated.

5.2 Effect of phosphorus on nodulation and nitrogen fixation of legumes

Phosphorus (P) is second only to nitrogen as an essential mineral fertilizer for crop production. At any given time, a substantial component of soil P is in the form of poorly soluble mineral phosphates which is not readily available to plants (Marschner and Marschner, 2012). Scientific evidence suggests that a high phosphorus supply is needed for nodulation (Elkoca et al., 2007; Hoque and Haq, 1994) and nitrogen fixation in legumes (Abdul-Jabbar and Saud, 2012; Israel, 1987; Robson et al., 1981). When legumes depend on symbiotic nitrogen and receive an inadequate supply of phosphorus, they may suffer from nitrogen deficiency (Weisany et al., 2013) as a result of poor N₂ fixation.

Generally the influence of P on symbiotic nitrogen fixation in leguminous plants has received considerable attention (Al-Falih, 2002; Israel, 1987; Tsvetkova and Georgiev, 2003). Phosphorus is known it promotes early root formation and the formation of lateral, fibrous and healthy roots, which play an important role in N₂ fixation, nutrient and water uptake (Bhuiyan et al., 2008; Niu et al., 2012). Several studies have reported that application of P along with rhizobium inoculant influenced nodulation and N fixation of legume crops (Lal and Sanchez, 1992; Saini et al., 2004; Solaiman and Habibullah, 1990; Thies et al., 1991).

It has been reported that nodulated legumes require high levels of P for optimal symbiotic performance (Bohloul et al., 1992; Bordeleau and Prévost, 1994; Deng et al., 1998; Zahran,

1999). P contributes in improving the activity of rhizobial bacteroids inside the root nodules (Bildirici and Yilmaz, 2005) and often results in large amounts of N₂ fixed, in most of tropical grain legumes (Dakora and Keya, 1997; Dakora, 2003). Studies by Tsvetkova and Georgiev, (2003) concluded that P nutrition increased symbiotic dinitrogen fixation in subterranean clover (*Trifolium subterraneum* L.) by stimulating host plant growth rather than by exerting specific effects on rhizobial growth or on nodule formation and function. An earlier study conducted in the Usambara Mountains in northern Tanzania also showed a strong response to 26 kg/ha of P fertilizer by increasing the percentage of N₂ fixed by biological N₂-fixation from 25-27% to 48-51% (Giller et al., 1998).

Establishing the optimum level of P to new released bean varieties is important as this may influence nodulation and nitrogen fixation and hence the growth and final seed yield.

5.3 Effect of potassium on nodulation and nitrogen fixation in legumes

Adequate supply of potassium (K) is important for the symbiotic relationship that enables bacteria to fix nitrogen (N) from the air for use by legumes (Parthipan and Kulasooriya, 1989; Premaratne and Oertli, 1994; Weisany et al., 2013). (Sangakkara et al., 1996) has found that, high potassium supply had positive effect on nitrogen fixation on shoot and root growth.

Studies have revealed that increasing the level of K fertilizer increased dry matter production and total N₂ fixed in faba bean (Zahran, 1999; Al-Falih, 2002). Generally, high potassium supply was required in symbiotic system to ensure an optimal growth and N₂ fixation (Kurdali et al., 2002). For example, Imas and Magen, (2007) studied the effect of K on Soybean and concluded that plants growing at higher K level have better development of nodules and consequently higher N₂-fixation. Potassium application increased the nodule activity of soybean, the number of nodules formed, fresh weight of nodules, and amount of N₂- fixed per nodule.

Studies by Mengel et al. (1974) on the effect of K on the N₂- fixation by root nodules of faba bean *Vicia faba* showed that plants supplied well with potassium had better carbohydrate supply to the nodules, and a higher carbohydrate turnover in the nodules thus improving the provision of ATP and reducing electrons required by the nitrogenase enzyme and finally enhanced the N₂ fixation.

The influence of potassium supply on nodulation was studied on soybean grown in nutrient solution. The dry matter yield, nodule parameters (nodule number and fresh weight of nodule per plant, average weight of nodule) and total nitrogen accumulation in the plant increased with increasing K supply (Premaratne and Oertli, 1994). In another study by (Parthipan and Kulasooriya, 1989) on common beans *Phaseolus vulgaris* L., it was found that nitrogenase activity increased due to K fertilization.

The effect of potassium supply of *Vicia faba* on N metabolism was studied by using ^{15}N -labeled molecular nitrogen. Plants well supplied with potassium showed higher contents of ^{15}N in the soluble amino fraction and in the protein fraction of various plant organs as compared with plants of a lower potassium status. This effect was evident particularly in the root nodules (Mengel et al., 1974).

Although several bean varieties have been developed for smallholder production in Africa, the effectiveness of supplying optimum levels of K to enhance N_2 fixation is not widely documented in Africa.

6.0 Effect of rhizobium inoculation and supplementation of P and K on yield and yield components of legumes

Soil micro-organisms play a significant role in regulating the dynamics of organic matter decomposition and the availability of plant nutrients such as N, P and S (Place et al., 2003). It is well-recognized that microbial inoculants constitute an important component of integrated nutrient management that leads to sustainable agriculture. In addition, microbial inoculants can be used as an economic input to increase crop productivity; fertilizer doses can be lowered and more nutrients can be harvested from the soil (Chen, 2006; Rosen and Allan, 2007).

Nitrogen is one of the most limiting nutrients to plant growth. Its supply to plants is mostly done through the application of mineral fertilizer (Marschner and Marschner, 2012). The practice is not only expensive, but also unsustainable to small scale poor farmers such as those found in Africa who cannot afford to purchase them (Altieri, 2009; Marenja and Barrett, 2009; Vanlauwe and Giller, 2006). Alleviation of N problem through rhizobium inoculants is the best alternative in promoting legume productivity in Africa (Sanchez et al., 1997; Bambara and Ndakidemi, 2010a).

The fact that producers inoculate their legume crops to increase yields and decrease the input costs of N fertilizer have reported by several researchers (Dobbelaere et al., 2001; Kennedy et al., 2004; Matiru and Dakora, 2004; Ndakidemi et al., 2006; Sanginga et al., 2003; Zahran, 1999), while improving soil conditions (Dobbelaere et al., 2001; Javaid, 2009; Naeem et al., 2006; Sarig et al., 1988).

Yield responses to inoculation, even in fields with soil rhizobial populations sufficient to infect the particular legume host, are common in field trials in the northern Great Plains of Canada. However, even where yield responses are not evident, inoculation may still have benefits by increasing seed N levels and N levels in plant residues (Kevin Vessey, 2004).

Various studies (Cassman et al., 1981; Giller et al., 1998; Israel, 1987; Pereira and Bliss, 1987; Ssali and Keya, 1986), have found that with adequate P supply, symbiotic performance can be increased, leading to greater grain yield. However, studies by (Da Silva et al., 1993; Dakora, 1994; Ndakidemi and Semoka, 2006) have found that, for farmer who can afford P fertilizers their combined use with rhizobial inoculants can further increase grain yield, enhances symbiotic establishment for increased N₂ fixation, and reduce the declining state of soil fertility in Africa.

Studies by Amjad et al. (2004) on the effect of phosphorus on Pea cv. *Samrina Zard* found that vine length, number of pods plant⁻¹, pod length, number of grains pod⁻¹ and green pod yield were significantly affected by the levels of P₂O₅. Number of grains pod⁻¹ and green pod yield were maximum at the highest dose of P₂O₅ (69kg ha⁻¹). However, vine length, number of pods plant⁻¹ and pod length increased up to the level of 46 kg P₂O₅ ha⁻¹.

Potassium (K) is the most important factor influencing crop metabolism, growth, development and yield. Potassium deficiency in plants resulted in the decrease in net photosynthetic rate (P_N) (Onanuga et al., 2011). (Zhao et al., 2001) stressed the role of K⁺ as the dominant counter ion to light-induced H⁺ flux across the thylakoid membrane. In addition there was a pronounced difference among species in sensitivity of crops to K deficiency (Munson, 1985a).

The effects of K deficiency can cause reduced yield potential and quality long before visible symptoms appear. This “hidden hunger” robs profits from the farmer who fails to keep soil K levels in the range high enough to supply adequate K at all times during the growing season. Even short periods of deficiency, especially during critical developmental stages, can cause serious losses (Kalavat P, 2012).

Studies by Kurdali et al. (2002) on nodulation, dry matter production, and N₂ fixation by faba bean and chickpea as affected by soil moisture and potassium fertilizer concluded that the higher level of K fertilizer increased both dry matter production and total N₂ fixed in faba bean.

In many studies, it has been evidenced that potassium amendments can substantially reduce the severity of several soybean diseases such as purple seed stain (*Cercospora kikuchii*), seed rot (*Phomopsis sp.*), stem and crown blight (*Rhizoctonia solani*) and pod and stem blight (*Diaporthe phaseolorum*) (Basseto et al., 2007; Perrenoud, 1993) which eventually increase yield and hence more profit to farmers. During a long-term International Potash Institute (IPI) experiment in India with soybean, the attacks of insects like blue beetle, grey semi looper, girdle beetle and stem fly were clearly reduced with K applications, and this increased yield (Bansal et al., 2001).

Thus, basing on the fact that inoculation of rhizobia and supplementation of phosphorus and potassium increases yield and yield components of different crop plants, their influence on legume varieties released for different agro ecological zones need to be investigated and documented.

6.1 Economic benefit of rhizobial inoculant and supplementing with P and K on economic benefit on legumes

The economic importance of biological N₂ fixation cannot be overemphasized as its annual benefit is estimated at USD 47.0 million for South African economy and USD 1067.0 million for the USA (Dakora, 1994). In Zimbabwe, studies have shown that, depending on soil fertility and rainfall, inoculant use can be more cost-effective than mineral fertilizer application (Brenner, 1996). However Kipkoech et al. (2007) compared the efficacy of *Rhizobium* (in groundnut cropping systems) with other soil fertility-enhancing technologies (Di-ammonium phosphate, DAP, NPK and Farm yard manure, FYM) and the control in western Kenya. Results show that groundnut yield under *Rhizobium* inoculation (1,362.9 kg/ha) ranked third after yield under DAP (1,800 kg/ha) and NPK (1,646 kg/ha). It was, however, better than the yield under FYM (1,218.5 kg/ha) and the control (1,208.7 kg/ha). The benefit cost ratio (BCR) followed the same trend, with *Rhizobium* inoculation coming third (with a BCR of 2.5:1) after DAP (with a BCR of 3.0:1) and NPK (with a BCR of 2.8:1). With a BCR of 2.2:1, FYM even trailed behind the control (BCR of 2.4:1) (Kipkoech et al., 2007). These results show the importance of promoting inoculant use in African agriculture, especially among resource-poor farmers who cannot afford expensive mineral fertilizers (Ndakidemi et al., 2006).

A great advantage of rhizobial inoculation is that it is much cheaper than mineral nitrogen fertilizer (Chianu et al., 2010). According to (Odame, 1997), field trials in Kabete and Embu showed rhizobium strains fix more nitrogen as compared with applying a recommended 90 kg of mineral nitrogen fertilizer per ha in common beans. From this comparison; the price of KShs 295 for a 100 g packet of Bio fix (a form of *Rhizobium* inoculant sufficient to inoculate 15 kg of common bean or pea seed); and the price of 100 kg of inorganic CAN (Calcium Ammonium Nitrate) fertilizer at KShs 4,341 follows that inoculation may be up to 15 times cheaper than commercially produced nitrogen fertilizers.

Economic analysis by (Ndakidemi et al., 2006) on soybean and common bean in northern Tanzania showed that the increase in grain yield with inoculation translated into higher marginal rate of return (MRR) and profitability for soybean and common bean. With common bean, relative to the control, there was 66% increase in profit with inoculation in the Moshi district and 92% in the Rombo district; with provision of supplemental P (26 kg P/ha), these profit margins rose to 84% (Moshi) and 102% (Rombo). With soybean, however, the increase in profit with inoculation was much larger, about 140% (Rombo) and 153% (Moshi). With P supplementation, these rose to 224% (Rombo) and 250% (Moshi).

Nodulated legumes have the potential to fulfill their demand for nitrogen by fixation and, as a result, can influence the nitrogen balance of the soil (Hardarson and Atkins, 2003). In Africa, they seasonally fix between 15 and 210 kg N ha⁻¹ (Bekunda et al., 2010). Bambara and (Bambara and Ndakidemi, 2010) in glasshouse and field experiment showed that the amount of N-fixed per hectare were significantly increased with *Rhizobium* inoculation and estimated to 32.7

kg N.ha⁻¹. This value is within that reported amounts (20-60 kg N.ha⁻¹) of N-fixation in *Phaseolus vulgaris* from *Rhizobium inoculation* in Brazil (Da Silva et al., 1993).

Small-scale farmers who are the major dry bean producers rarely apply fertilizers in bean production hence the crop is largely dependent on soil and fixed nitrogen. Inoculation of legume seeds with appropriate *Rhizobium* strains for enhanced nitrogen fixation provides an alternative to the application of nitrogenous fertilizers (Mungai and Karubiu, 2011)

Despite of the existence of substantial evidence on the influence of *Rhizobium* inoculation in yield and economic benefit of legumes yet their synergetic effects with P and K supplement in yield and economic benefits of new bean varieties *Phaseolus vulgaris* L. released in different agro ecological zones is not well investigated and documented.

Conclusion

Rhizobial inoculation and supplementation with phosphorus and potassium has potential in improving growth, yield, economic benefits, and photosynthesis in legumes. It is recommended to adopt and incorporate these technologies in legumes production. However, the information available demands more research to be done so as to establish the contribution of these technologies in attaining optimal production of legumes grown in different agro ecological zones in Africa.

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