

Extrapolations on the Use of *Rhizobium* Inoculants Supplemented with Phosphorus (P) and Potassium (K) on Growth and Nutrition of Legumes

George W. Mmbaga, Kelvin M. Mtei, Patrick A. Ndakidemi*

School of Life Sciences and Bioengineering, the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania
Email: mmbagag@nm-aist.ac.tz

Received 19 August 2014; revised 17 September 2014; accepted 24 October 2014

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Abstract

Land scarcity and poor farming management practices has resulted in to intensive agriculture which rendered most of the soil in sub Saharan Africa depleted in essential plant nutrients. High prices of chemical fertilizer are crucial bottleneck toward increasing production of legumes and other food crops in most countries found in sub-Saharan Africa. Nitrogen, phosphorus and potassium are among the most limiting nutrients for plant growth as they play different but crucial roles in the plant physiological processes. These macronutrients are fundamental components of cell building blocks including genes and chromosomes. This review intend to show the vital roles played by rhizobial inoculants supplemented with phosphorus and potassium in enhancing growth, yield, photosynthesis, nodulation, nutrient uptake and nitrogen fixation of legumes.

Keywords

Biological Nitrogen Fixation (BNF), Nutrients, Legumes, Photosynthesis and Varieties

1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an annual leguminous plant that belongs to the genus, *Phaseolus*, with pinnately compound trifoliolate large leaves. Common bean shows variation in growth habits from determinate bush to indeterminate extreme climbing types [1]. Common bean is an important component of the production

*Corresponding author.

systems and a major source of protein for the poor in Eastern and southern Africa and the crop is grown mainly by women [1]-[3]. Generally, common bean (*Phaseolus vulgaris* L.) is considered a short-season crop with most varieties maturing in a range of 65 to 110 days from emergence to physiological maturity. Maturity period can continue up to 200 days after planting amongst climbers that are used in cooler upland elevations [4]. The climbing bean predominate the highland areas, where population density is high and land is limiting. Climbing bean production in the highlands of Eastern Africa is constraint by various factors ranging from biotic (diseases and pests) to abiotic ones such as soil nutrient availability commonly being limitation of N, P and K [2] [5] [6].

Climbing bean is and is capable of producing a lot of biomass and grain yield in the limited space for planting, commonly grown in highlands where pressure on land is acute.

Climbing beans varieties are sub group of common beans growing vertically and are considered to be more advantageous over the bush type due to high grain yields of up to 5 t/ha, diverse use as human and animal feeds, large biomass production and biological nitrogen fixation which is a basis for improvement of soil fertility [7]. Furthermore, the indeterminate cultivars (climbing beans) play a key role in crop rotation and intercropping systems [8]. The climbing bean cultivars were developed with the purpose of adding more value in yield potentials and improve yield steadiness as compared with bush varieties [9]. Breeding of indeterminate beans was also aimed at combating the land scarce problem in highlands areas of sub Saharan Africa by which a small piece of land can be intensified and be utilized to its full capacity where by providing fully economic land value to the small holder farmers [10]. Thus, in this regards best plant architecture traits and the genes for disease resistance was also enhanced to climbing cultivars which renders them with the potential they hold [11] [12]. Indeterminate beans have been noted to give higher produce than determinate genotype in low plant populations which should therefore benefit subsistence small farmers who frequently plant low plant populations in order to lower seed costs [13] [14].

Dry bean production which is predominantly by small-scale farmers has been on the decline due to various constraints the major ones being diseases, low soil fertility and insect pests [15]. Small-scale farmers do not apply external inputs such as fertilizers, *Rhizobium* inoculants, fungicides and pesticides [16]. Due to these bottlenecks the production per hectare has declined from 800 kg·ha⁻¹ in 1990 to less than 500 kg·ha⁻¹ while the potential yield of climbing beans is estimated to be over 5000 kg·ha⁻¹ under optimal water and nutrients supply and in the absence of other biotic factors such as pest and diseases [17] [18].

Nitrogen, phosphorus and potassium are chief elements that are richly available in the atmosphere (78%) and soil (0.05%) and (0.03) respectively, but in an inaccessible form to crop plants [19]. These elements are often limiting factors for plant growth and development [20]. Intensive farming practices that realize high yield and economic benefits to farmers do require some amount of industrial fertilizers as source of P and K plus rhizobia inoculants to supply nitrogen through process of BNF which is environmental friendly [21] [22].

Rhizobium inoculation increases the availability of nutrients to the plant and in the rhizosphere through process such as biological nitrogen fixation [23]. Being one of the major nutrients needed by the plant, nitrogen is an essential constituent of plant cells at structural, genetic and functional levels, involved in many processes of plant growth and development resulting to gain yield [24]. The extensive use of nitrogenous industrial fertilizers in agriculture is at present under debate due to environmental distress and doubts for consumer health. Consequently, there has recently been a growing level of interest in environmental friendly sustainable agricultural practices [25]. Increasing and extending the role of inoculation with microorganisms (bio-fertilizers) may decrease the need for industrial fertilizers and thereby reduce adverse environmental effects [26]. Thus; soil microorganisms are important living bodies in agriculture as they may play role of supporting the circulation of plant nutrients and thus trim down the need for industrial fertilizers [27].

Phosphorus is vital for development of new tissue and the transfer of the genetic information within the plant from one cell to another during cell formation [28]. It aids in energy transfer in form of ADP/ATP and thus being considered a very essential component in plant bioenergetics of the living cell [29].

Potassium (K) has an important role in regulating the water loss of plants thus help to prevent plant from necrosis [30]. It serves as an activator of enzymes used in photosynthesis and respiration, helps to build cellulose and aids in photosynthesis by the formation of a chlorophyll precursor and finally results in quality fruits [30] [31].

Many climbing beans varieties has been released and used by farmers in the highlands of East and sub Saharan Africa but there is lack of enough information about its agronomical and physiological performance in the field conditions when supplied with *Rhizobium* inoculants, Phosphorus (P) and Potassium (K) fertilizers. New

varieties of climbing bean have been developed and released by various national programs. These have been introduced to farmer's fields in various African countries and are being grown in place of traditionally grown bush beans. However, there is little information on the response of the released climbing bean varieties with higher yielding potential when supplied with *Rhizobium* inoculation and supplementation with different levels of phosphorus and potassium in different agro ecological zones found in Africa. Thus, a need to cover this gap of knowledge by investigating the effect of *Rhizobium* inoculation and supplementation with different levels of phosphorus and potassium on growth, yield, N-fixation, chlorophyll content, nutrients uptake and economic benefits of climbing beans varieties.

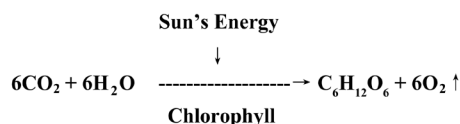
2. *Rhizobium* Inoculation and Supplementation with P and K on Photosynthesis and Chlorophyll Contents of Climbing Beans Varieties (*Phaseolus vulgaris* L.)

Nitrogen is a major constituent of chlorophyll, the most vital pigment needed for photosynthesis and amino acids synthesis [32] [33]. It is a factor in many biological compounds that plays a major role in photosynthetic activity and also acts as part of the enzymes associated with chlorophyll manufacturing which reflect relative crop nitrogen status present in leguminous plants. Nitrogen is a building block of proteins and is highly considered necessary for all enzymatic reactions in a plant [34]. Its supply has considerable effect on leaf growth because it increases the leaf area of plants and consequently it influences photosynthesis function of green plants [35] [36].

Generally, legumes and other leafy vegetables like cabbage, lettuce, amaranthus, Chinese cabbage and some fruit vegetables perform well when supplied with enough nitrogen as it improves the quality and quantity of dry matter and protein contents of the products [37]. However, green colour in the leaf tends to vanish when nitrogen become deficiency and this may cause the reduction in leaf vicinity and intensity of photosynthesis [38]. Nitrogen deficiency is normally associated with symptoms of yellowing, falling of leaves, reduced growth, late flowering and fruiting [37]. Nitrogen constitutes one of the major yield limiting factors for crop production, thus inoculation with appropriate strain of *Rhizobium spp.* can be an effective way of increasing growth and leaf chlorophyll content in legumes [39]-[41].

Inoculation of legumes with appropriate *Rhizobium* and supplementation with some nutrients especially phosphorus has been reported to increase the leaf chlorophyll content of legume crops [37] [41] [42]. For example, Bambara and Ndakidemi [6] reported *Rhizobium* inoculation of legumes to cause an increase in leaf chlorophyll content. Their findings in greenhouse indicated that leaf chlorophyll content significantly increased with rhizobial inoculation by 123% and 178% for the field experiment relative to control (without rhizobial inoculation treatment). Photosynthesis similarly increased significantly with rhizobial inoculation by 140% and 81% for the greenhouse and field experiment respectively, relative to un-inoculated control. Similarly, *B. japonicum* inoculation and phosphorus supplementation was reported to have significantly increased leaf chlorophyll content both in field and glasshouse experiment in soybean and cowpea [41] [42]. The increased chlorophyll content will result in the increased photosynthesis [43] and as a result plant gets enough sugars for its growth and production.

Green plants absorb sun light and convert its energy into usable biomass through the process of photosynthesis and produce diverse products of economic significance such as grain, fibre, tubers, fruits, vegetables, fodder and others [44]-[46]. This is accomplished by the plants provided that there is adequate light, appropriate temperature, and other support gears for plant metabolism such as water, carbon dioxide, oxygen, and a number of nutrients [44] [47].



There are two types of chlorophyll in plants; viz chlorophyll **a** and **b**, both works as photoreceptor in photosynthesis [48] [49]. While chlorophyll b helps in absorbing light and improves photosynthesis, it is reported to be more soluble in polar solvents due to its carboxyl group with a yellow colour and heavy ability to absorb blue light. The opposite is true for chlorophyll a. However, in shade adopted chloroplast, there is low ratio of chlorophyll a to chlorophyll b [50]. Therefore, in legumes, increasing chlorophyll b will increase the range of wavelengths absorbed by the shade chloroplasts and this will enhance photosynthesis for more growth and ultimately yield of legumes.

Human operations such as application of agrochemicals have noticed to have a negative effect in chlorophyll contents of legumes [51]. As an important green pigment responsible to trap sun light, Chlorophyll enhances photosynthesis and creates glucose which refuels the plant metabolic processes and excess carbohydrates are being stored for future use by either plant itself or other organisms including human being [52]-[54]. Thus, supplementing legume plants with nutrients such as rhizobial for nitrogen fixation, phosphorus and potassium will improve the health of legumes and provide the necessary conditions for photosynthesis and ultimately increased yield [55].

Plants require phosphorus for growth throughout their life cycle, especially during the early stages of growth and development [56]. George [57] reported the major factor limiting plant growth and development to be the rate at which phosphorus regulates photosynthesis and carbohydrate metabolism in plant leaves. In photosynthesis and respiration, phosphorus has been reported to play a major role in energy storage and transfer as ADP and ATP (adenosine diphosphate and triphosphate) and DPN and TPN (diphosphopyridine and triphosphopyridine nucleotide) [58]. A deficiency of inorganic phosphate in the chloroplast was reported to reduce photosynthesis [59].

Potassium enhances photosynthesis and cell permeability and increases synthesis of amino acid into protein, it also facilitate cell division in young meristematic tissue, due to its effect on plant cell turgor and permeability, potassium indirectly assists in regulating the water economy of cells [60]. It is very important nutrient for cell organization, salt uptake and permeability into the xylem, the regulation of water uptake and transpiration, and in the metabolism of carbohydrates and nitrogen [61]. It also plays a key function in chlorophyll formation, the control of pH in the cells of the plant, and activates a number of enzymes [62]. Potassium is probably best known for its role in the opening of the stomata in the leaves and hence its role in regulating the rate of transpiration [63] [64]. Large quantities of potassium are normally found in the growing points of the plant, in the leaves and in the cell sap [65]. Furthermore common role of K in agronomy is closely connected with photosynthesis. Potassium enhances photosynthesis process at various levels, such as manufacturing of ATP, activation of the enzymes taking part in photosynthesis, CO₂ uptake, and equilibrium of the electric charges needed for the process of photophosphorylation in chloroplasts, also acting as the counter ion to light-induced H⁺ flux across the thylakoid membranes [57].

Legume crops such as peas, faba beans, and lentils need plenty of potassium during plant growth [66]. It is established that pulse crops regularly take twice as much K as compared with cereals crops. For example, 40 kg crop of peas requires about 110 g of K₂O, while a yield of 40 kg of faba beans needs over 200 g. In contrast, 40 kg of spring wheat only takes up about 90 g of K₂O [67]. Due to its essentiality in facilitating plant growth and its function in photosynthesis, potassium application in legumes based production field is inevitable.

There is very little information about effect of *Rhizobium* inoculants supplemented with P and K on photosynthesis and chlorophyll contents in legumes particularly the new climbing bean cultivars with huge potential in African highlands. Thus, there is need to explore the synergistic effects of supplying inoculants, P and K on enhancing the photosynthesis and chlorophyll contents of different climbing bean varieties recommend in Africa.

3. *Rhizobium* Inoculants Supplemented with Nutrient Elements P and K on Nutrient Uptake in Legumes

Nutrients are essential components required by living plants for normal growth and development. Among the needed 16 nutrients three (Carbon, hydrogen, and oxygen) are derived from the atmosphere and the rest are obtained from the soil, water, soil organic matter and inorganic fertilizers [68]. Shoot and root growth of virtually all legume crops is increased with balanced phosphorus and potassium nutrition [69]. Nutrient uptake by leguminous plants depends mainly in the quantity, concentration and activities in the root zone soil as well as the capacity of soil to replenish the concerned nutrients in the soil solution [70].

Nitrogen being one among the crucial nutrients required by plant for proper growth and development, has been reported to be the most limiting element due to the fact that it is prone to various losses including leaching, volatilization and denitrification [71] [72]. Soil microorganisms such as *Rhizobium* and other plant growth promoting soil bacteria are known to have a constructive influence on the real chemistry of soils nutrients and thus promote nutrients uptake [73]-[75]. In recent decades, microbial inoculants have become promising components for integrated solution to environmental problems due to the fact that inoculants possess the capacity to promote plant growth, enhance nutrient availability and uptake and ultimately support the health of plant for better yield.

Leguminous plants such as *Phaseolus vulgaris* and *Vigna unguiculata* (Walp) have the capacity of forming symbiotic relationship with *Rhizobium* bacteria which transform atmospheric N₂ to soil nitrate or ammonium which can be utilized by leguminous plant for growth and development, in turn the leguminous plant provide housing and carbohydrates to the rhizobia [76]-[78]. Nyoki and Ndakidemi [79] disclosed that *B. japonicum* inoculants supplemented with phosphorus in cowpea improved the uptake of N, P, K, Mg, Ca and Na significantly relative to control. Makoi *et al.*, [41] revealed that rhizobia inoculation significantly increased the uptake of P, K, Ca, and Mg in plant parts such as leaves, shoots, roots and pods of common beans (*Phaseolus vulgaris* L.).

Phosphorus is important in plant bioenergetics. As a component of ATP, phosphorus is needed for the conversion of light energy to chemical energy (ATP) during photosynthesis [80] [81]. Phosphorus can also be used to modify the activity of various enzymes by phosphorylation, and can be used for cell signaling [82]. It aids the plants in root development for smooth nutrients uptake [83], plant stalk and stem vitality, flower and seed creation, crop maturity and resistance to plant pests and diseases. For sustainable nutrient uptake and translocation into developing seeds during reproduction, root life span is important. P increases life span of legume roots and thus aid to provide plant carbon budget and favorable condition for nutrient uptake which finally have an impact in increasing seed yields [84] [85].

Phosphorus application in soil has been reported to influence the uptake and concentration of other nutrients in leaves and seeds [86]. Research shows that Phosphorus application had a positive and significant effect on the nutrients uptake such as Ca, Mg, Zn, Cu, Mn, and Fe [87] [88]. Tairo and Ndakidemi [89] reported that *B. japonicum* inoculants supplemented with phosphorus in cowpea (*Vigna unguiculata* (L.) Walp) significantly improved the uptake of a number of nutrients such as: N, P, K and Na in roots, pods, shoots and whole plant.

Furthermore the application of P and K fertilizers in combination with organic matter improved soil physical and chemical properties by enhancing biological activity and soil organic carbon accumulation thus helping in the uptake of nutrients [90]. Another study reported enhanced nitrate absorption and reduction to amino acids and increased the protein formation in plants following *Rhizobium* inoculation [91].

However, the uptake of nutrients in high yielding legume varieties supplied with *Rhizobium* and P and K supplements is not well established and hence need further investigation.

4. *Rhizobium* Inoculants Supplemented with Nutrient Elements K and P on Growth of Climbing Bean Varieties (*Phaseolus vulgaris* L.)

Rhizobium inoculation in legumes is ascribed to effect stimulation of growth and is therefore suggested to be the alternatives for the expensive inorganic industrial fertilizers [6] [92]. Nitrogen is provided through symbiotic fixation of atmospheric nitrogen by nitrogenase in rhizobial bacteroids [93]. As a macronutrient, nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, amino acids, DNA and RNA, and is therefore important in plant growth [94]. Nitrogen is biologically combined with other building blocks such as C, H, O and S, the combination of various amino acids form proteins. The amino acids are used in forming protoplasm, the site for cell division and thus for plant growth and development [95]. Nitrogen is the critical limiting element for growth of most plants due to its unavailability [96].

Climbing beans need nitrogen more than any other nutrient as nitrogen (N) is a constituent of proteins, enzymes, chlorophyll, and growth regulators [97]. Deficiency of nitrogen causes diminutive growth, leaf yellowing, abridged branching and tiny trifoliolate leaves in legumes [98] [99]. Plants attain nitrogen from two main sources: the soil, through industrial fertilizer, manure, and/or decomposition and mineralization of organic matter; and the atmosphere through symbiotic nitrogen fixation [100] [101]. However, small-scale subsistence farmers who are the principal dry bean producers infrequently apply commercial fertilizers in bean production field; hence the crop is basically dependent on fixed nitrogen [102].

Leguminous crops including climbing beans, possess root nodules, fix and utilize atmospheric nitrogen [103] [104]. They are thus not mostly dependent on industrially fixed nitrogen and may add up to 30kg of nitrogen per hectare to the soil and improve its fertility [105] [106]. Being a leguminous crop, climbing beans (*Phaseolus vulgaris* L.) has unique properties of symbiotically associating with *Rhizobium leguminosarum* and convert atmospheric nitrogen into a usable form to the plants and some may be leaked to the soil and therefore influencing other biological process in the soil [68]. Evidence from the research findings by Rodelas *et al.* [107], suggests that *Rhizobium* inoculation of legumes and the subsequent nitrogen fixation process could have positive effect on plant growth and finally the macro and micronutrients availability and thus improving the nutritional quality

of different plant components [68] [87] [89].

Biological Nitrogen Fixation infrequently covers the full nitrogen requirement of beans [108]. Many researchers have reported the importance of N in beans yields and research evidence revealed that common bean responded very well to nitrogenous fertilization of up to 200 kg·ha⁻¹ [109]-[113]. However, other researchers have shown that they have the ability to use about 50% of its N requirement from the atmospheric N₂ fixation [114] [115]. However, this is in contrast to soybeans and other legumes which are in need of little or no nitrogen as a starter fertilizer to establish efficient symbiosis with nitrogen fixing bacteria and afterward cover most of their nitrogen demand through BNF [104] [116].

Climbing beans undergo symbiotic interaction with rhizobia capable of fixing atmospheric nitrogen (N₂). This consists of formation of nodules on host roots, which harbour the bacterial symbionts, and the N₂ fixation process in the nodules, which occurs thereafter [117]. In recent years, a number of *Rhizobium* inoculants have been developed and are primarily used for supplying nitrogen to plants by enhancing N₂ fixation [79]. Symbiotic nitrogen fixation in soybeans can provide from 65kg to more than 160 kg fixed nitrogen ha⁻¹ [118] [119] representing about 40% - 70% of soybeans nitrogen requirements.

Ravikumar *et al.*; [117] in their study on artificial inoculation of *Rhizobium japonicum* on the production of two pulse grains (*Vigna mungo* and *Vigna radiate*), reported that there was a statistically significance percentage increase in all parameters studied over the non inoculated counterpart. In their results for both varieties studied, the inoculated plants observed significant increases in growth and yield potential over the respective non inoculated control plants (greater height, greater fresh weight, greater number of roots, nodules, greater number of leaves, shoots, pods, greater length of pods and greater seed weight). Thus inoculation of seed beans with appropriate *Rhizobium* strains for superior nitrogen fixation provides an alternative to the application of commercial nitrogenous fertilizers [120] [121]. Other researchers have also reported the increase in legume plant height, number of leaves, and leaf area index and plant girth due to *Rhizobium* inoculation [37] [42] [122].

Unlike Nitrogen, Phosphorus is the element which is frequently found to be most limiting nutrient for plant physiological activities such as growth and maturity in the production soils of the tropics [69]. It is among the critical macro-nutrient needed by plant for its well-being. However, about 40% - 50% of African legumes production areas are affected by moderate to severe phosphorus deficiency [115] [123]. Phosphorus has a major role in carbon, hydrogen, oxygen and fat metabolism, and plays an important role in respiration and photosynthesis [124]. Deficiency symptoms of P include signs of dwarfed plants with thin malformed stems and shortened internodes; small, dark-green upper leaves, and few numbers of pods formed which contains only a tiny number of seeds [115] [125]. Low soil phosphorus status is categorized as a constraint of high significance in 70% (957,000 ha) of legumes production area in Eastern Africa [123].

This nutrient is vital to plant growth and is found in every living plant cell [86] [126]. It plays significant key roles in plant functions, such as energy transfer as ADP/ATP, photosynthesis, transformation of sugars and starches, nutrient movement within the plant and phosphorylation [127] [128]. ATP is very important in the process of nitrogen fixation as it is required to break the triple bond that exists between N atoms in N₂-molecules.

Though, many tropical soils are phosphorus deficient due to various reasons, the element is required by legumes in trace quantities [129]. Despite of being critical nutrient to yields of climbing beans due to its multiple effects on nutrition, all growing plants require phosphorus for growth and development [108] [130]. The low amount of native available phosphorus in Sub Saharan Africa soils is thought to be associated with total soil phosphorus contents of the parent soil forming materials or due to high P-fixing capacity favored by their high Aluminum (Al) and Iron (Fe) oxide contents and their low pH status [131] [132].

Graham [105], Thung and Rao [133] reported significance of P in growth and yield of *Phaseolus vulgaris*, their findings showed that there was about 60% - 70% yield reduction in beans associated with growth retardation of both below and above ground plant organs that resulted from P deficiency. As mentioned earlier, P helps in enhancing Biological Nitrogen Fixation in legumes [96] [121] [134], the reason being high P demand of Biological nitrogen fixation process which creates additional sink of phosphorus to the plant in the nodule thus, deprives the legume plant of the P acquired from the soil [135] [136].

Several workers [1] [37] [69] [102] [133] [137]-[139] have suggested exogenous application of appropriate phosphorus fertilizer as a means of correcting low phosphorus soils, like those frequently encountered in sub Saharan Africa so as to ensure positive effect on growth, Biological nitrogen fixation and yields of targeted legumes. For example, Tairo and Ndakidemi [37] reported phosphorus to have significantly increased plant height,

number of leaves, leaf chlorophyll content, stem girth, leaf area (LA) and leaf area index (LAI) on soybean when inoculated with *B. japonicum* both in the screen house and field experiments. Increased plant height with increased phosphorus application was also reported by [138] [139]. This could be due to the fact that phosphorus being essential constituent of plant tissue significantly influences the plant height of legume crops [69] [137] [139]. Phosphorus (P) deficiency is widespread in most regions where both (Climbing/Bush type) and other legumes are produced in Africa, It is possibly the constraint that mostly hinders nitrogen fixation [2].

Potassium amount found in plant tissue including leguminous plants is close to that of nitrogen thus making potassium the second element needed by plant in large quantity [140] [141]. It increases the efficiency of the leaf in manufacturing sugars and starch, and its action is considered to be complementary to that of nitrogen [142]. While nitrogen increases the size of the leaf, potassium increases its photosynthesis efficiency and thus contributes to the growth of the plant [143] [144].

Rhizobium inoculants, phosphorus and potassium when supplied together have shown a positive outcome on growth and yield of many leguminous plants grown both in the screen house and field [145]-[150]. Therefore, this signifies the importance of using these nutrients in combination in a given plant ecosystem where common bean farming is practiced. Furthermore, the information on the synergetic effect of the package on the growth of newly released bean varieties in African landscape is very limited.

5. Expected Effects of *Rhizobium* Inoculation and Supplementation with P and K on Nodulation and Nitrogen Fixation on Legumes

Nitrogen fixation involving symbiotic association between *Rhizobium* and leguminous plant such as *P. vulgaris* in which the *Rhizobium* bacteria do convert the nitrogen from the air in to the soil and transform it to ammonium (NH₄), which can be direct taken and be used by host plant [103] [104] [151]. The presence of appropriate rhizobial strains in the soil is of paramount importance for N₂ fixation to take place. Studies conducted in East African Highlands by [21] showed that the population of common beans and other selected legume nodule forming *Rhizobium* ranged from zero to very small number [21]. Under such conditions it is necessary to inoculate legumes with rhizobial inoculants [152]. Generally, it is established that under optimum conditions N₂ fixation in legumes is reported to fulfill between 50% - 60% of legume nitrogen requirements [153].

Most grain legumes are capable of fixing atmospheric N₂ through the association with rhizobia [114]. The legume plant supplies sugar for bacterial growth while the bacteria fix atmospheric N₂ into NH₄⁺ or NO₃⁻, to be converted into both essential and non essential amino acids that can be used by the plant to synthesize proteins for its growth and development [154] [155]. The simplified equation for nitrogen fixation is shown in **Figure 1** below.

The process of Biological nitrogen fixation (BNF) shown in **Figure 1** is accomplished by specific strain of nodule forming bacteria for the specific type of legumes, for example *Rhizobium leguminosarum* *bv viciae* induces nitrogen fixing nodules on *Pisum*, *Vicia*, *Lens*, and *Lathyrus spp.*, while *R. leguminosarum* *bv trifolii* do initiates nodules only on species of clover (*Trifolium*) and *Sinorhizobium meliloti* effectively nodulates species of *Medicago*, *Melilotus*, and *Trigonella* [156] [157]. If the *Rhizobium* strains mentioned above are not adequately present in the growth media, or the inefficient strain capable of initiating the nitrogen fixing process dominates, then nodulation and N₂ fixation will be constrained [74] [158]-[160]. The solution to the aforementioned constraints is through seed or soil inoculation with the proper *Rhizobium* bacteria before or at planting [109] [161]-[163].

Several researchers have reported the effects of *Rhizobium* inoculation on nodulation and nitrogen fixation on legumes [164]-[166]. Subba *et al.* [167] performed study on the comparative efficiency of *Rhizobium japoni-*

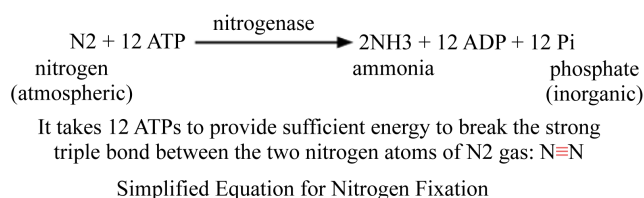


Figure 1. Simplified equation for nitrogen fixation (Source: waynesword.palomar.edu).

cum on soybeans grown in the field, the study findings showed that inoculation with UPAU-culture resulted in significant nodulation. They also reported improved nodulation in *Cajanus cajan*, *Cicer arietinum* and *Lens culinaris* when neutral soils was inoculated with *Rhizobium* inoculants.

Phosphorus, like potassium, is essential for nitrogen fixation in legumes. Several studies have shown that N₂ fixation is reduced when phosphorus is deficient [168]-[170]. With adequate phosphorus, N₂ fixation is improved through increased nitrogen-use efficiency [2] [59] [171]. Phosphorus does not only increases seed yields but also nodulation [172] postulated P concentration in seed to be a source of early nodulation on legumes especially in soils with low phosphorus contents and thus enhances early establishment of symbiosis. Furthermore, phosphorus has stimulating effect on plant nodule development and action of nitrogenase enzyme in nodules of leguminous plants [173]-[176]. Phosphorus application is also reported to significantly enhance number of nodules; nodules dry weight, nitrogen and phosphorus uptake of the cowpea [177]. On the opposite, its deficiency decreased nodule mass [178]-[181] nodule quantity [174] [178] and function of nitrogenase enzyme [175] [182]-[184].

Potassium plays a critical role in enzyme activation, water use efficiency, photosynthesis, transport of sugars, protein synthesis, and starch synthesis in plants [185] [186] is especially important in its interaction with nitrogen throughout the growth cycle. Adequate potassium in the field results in higher crop yields and higher nitrogen-use efficiency as it enhance nodulation and hence N₂ fixation, [181]. Crops respond to higher potassium levels when nitrogen is sufficient, and greater yield response to nitrogen occurs when potassium is sufficient [187], thus a complementary uptake for the plant growth and development. Therefore, contribution of N fixation in K nutrition of legumes is very important.

Abbasi *et al.* [164], Abdelhamid *et al.* [188], Parthipan and Kulasooriya [189] reported that the addition of K in legumes increase dry matter yield of nodule, nodule number, nodule fresh weight per plant and nodule average weight. Also total nitrogen accumulation in the legume plant and enzyme nitrogenase activity increased with increasing K supply. Potassium increases metabolism of nitrogen in legumes plants. For example, Mengel *et al.*, [190] reported high accumulation of ¹⁵N isotope in the soluble amino fraction and in the protein fraction of various leguminous plant organs especially root nodule as a result of high K supply contrary to plants supplied with small amount of potassium. Furthermore, K supply on legumes such as *Vicia faba* resulted into greater sugar supply to the nodules and a higher sugar yield in the nodules thus improving the provision of ATP and decreasing negative charges needed by the nitrogenase enzyme and lastly improved N₂ fixation.

Sangakkara *et al.* [191] reported that, elevated potassium supply had affirmative effect on nitrogen fixation and has a positive effect on shoot and root growth. K amendment improved the nodule activity of legumes, the number of nodules formed, fresh weight of nodules, and amount of N₂- fixed per nodule [191] [192].

The combined influence *Rhizobium* inoculation and supplementation with phosphorus and potassium on symbiotic nitrogen fixation and nodulation in new released leguminous varieties from the breeding programs in Africa has received little attention. So, it is important to study the possible combined role that could be played by P, K and *Rhizobium* inoculants on symbiotic nitrogen fixation in new released leguminous varieties growing in diverse environment such as those found in East African Highlands.

6. Anticipated Effect of *Rhizobium* Inoculation, Phosphorus (P) and Potassium (K) Supply on Yield of Grain Legumes

Limiting factors such as diversity or scarcity of native *Rhizobium* population in soil can affect the legume performance and grain yield [179] [193]-[195]. Supplementing climbing legumes with nutrients has great potential for increasing yield as it is not only promoting growth but also enhance symbiotic establishment for increased nitrogen fixation [153] [196] [197]. In situation where field legumes are exogenously supplied with mineral nutrients they tend to double plant growth, nitrogen fixation and grain yield relatively to their unfertilized counterparts [148] [198].

Nitrogen is a very important macro-nutrient largely involved in metabolic actions and protein synthesis, resulting to increased vegetative and reproductive growth and ultimately leads to yield of the crops [199] [200]. Being the most limiting nutrients to plant physiological process, nitrogen supply to plants has been commonly accomplished by application of industrial fertilizer in various places in Africa [57]. This common solution of nitrogen amendment is extremely expensive and unsustainable to small holder farmers and it is environmental unfriendly [201]-[203].

According to the following researchers [21] [23] [42] solution to nitrogen bottleneck in East and sub Saharan

Africa for the purpose of enhancing legume productivity in the region is through *Rhizobium* inoculation. This will maximize the inherent trait of legume plant species which are capable of solely obtain N required for growth and development from Biological Nitrogen Fixation [204].

Various researchers have performed studies on the effects of *Rhizobium* inoculants on legumes grown in the field and screen house and reported to have positive results in yield increase. For example, Bambara and Ndakidemi [6]; Nyoki and Ndakidemi [79] reported that *Rhizobium* inoculation significantly improved the yield and yield components of legumes such as number of pods per plant, number of seeds per pod, number of seeds per plant, 100-seed weight, and seed yield relative to control. The increase in seed yield could be due to high nodulation which results in high N₂ fixation and hence higher stover yield and seed yield.

Research findings suggests that intensification of rhizobial population through inoculation amplified grain yields by 22.5% in treatments with phosphorus fertilizer (TSP) and by 6.8% in non-TSP fertilized plots, demonstrating that *Rhizobium* augmentation had a positive effect above the naturally occurring *Rhizobium* strains [205]. Moreover Giller [42]; Nyoki and Ndakidemi [78], investigation revealed that the plants which were inoculated with rhizobial inoculants gave significantly higher stover yield and seed yield compared with un-inoculated control, the reason could be high nodulation which result in high nitrogen fixation and consequently higher stover and seed yield [206].

P deficiency is also a chief contributing factor limiting BNF in legumes [105] [133]. Phosphorus is required in large quantities in juvenile cells, such as shoots tips and root tips, where metabolism is high and cell division is quick [59]. Phosphorus aids in root expansion, flower commencement, and seed and fruit development [207]. Yield of beans can be reduced by as much as 60% - 75% in soils that are unable to release sufficient P levels during the growing season.

Studies have shown that phosphorus availability in soils was found to have a positive influence in survival and efficiency of *Rhizobium* bacteria which are responsible for fixing atmospheric nitrogen in legumes. Thus it helps in boosting roots proliferation, shoot growth, flower initiation, seeds and fruits expansion and ultimately total yield of legumes [208]. Many researchers have reported a significant performance of legumes when supplied with phosphorus [102] [209]-[211]. In the study conducted by Turuko and Mohammed [212] in Ambo University, Ethiopia to investigate effect of different fertilizer rate on growth, dry matter yield and yield components of common bean, it was revealed that phosphorus significantly increased dry matter yield, yield components and growth parameters such as leaf area and number of branches per plant and finally the seed yield.

K⁺ is highly mobile and can aid in balancing the anion charges within the plant. Potassium regulates the opening and closing of the plant stomata, thus helping to prevent water loss through transpiration and hence affecting growth and yield [131]. Research findings indicates that potassium application in corn and soyabean increased average yields of both crop when applied at the rate of 120 kg·K·ha⁻¹ based on soil potassium test recommendations as compared with lower rates. The importance of potassium on increasing yields was also revealed in other crops rather than legumes. For example, Borges and Mallarino [213]; Turuko and Mohammed [212] reported K removal in tubers ranged from 203 to 397 kg/ha and was direct proportional to tuber yield, that is, the more the plant absorbs K⁺ the more the yield it produce. Like nitrogen, the importance of potassium in legume production has to be considered and taken into action for proper yield.

The very low nitrogen, phosphorus and potassium status of many soils in East African highlands suggests that availability of these elements will be an emerging limitation to crop productivity in the near future. Therefore, *Rhizobium* inoculation, potassium and phosphorus supply may play a crucial role in enhancing legume productivity in poorly depleted soils of East Africa. Therefore, the synergetic effect of inoculation and nutrient supplementation (K and P) in single or in combination and their influence on the yield of new released legume varieties such as those involving *P. vulgaris* needs to be investigated.

7. Promising Economic Benefits of *Rhizobium* Inoculants and Supplementation with P and K in Production of Climbing Beans Varieties (*Phaseolus vulgaris*)

Various types of pulses are important source of income to farmers in African region, and the efforts at improving their yield has increasing over the year [110]. Despite the attractive market of pulses, the yield of grain legumes has remained very low under farmer's condition in Africa. Studies revealed that the low yields of legumes in Africa is sought to be associated with low soil fertility, low native *Rhizobium* and thus reduced N₂ fixation as a results of various abiotic factors such as N and P [204] [214]. Most farmers in highlands of East Afri-

ca are aware of the aforesaid soil constraint thus, N, P, K fertilizers are applied in major food security crops such as *Zea mays* and *Oryza sativa* [215] and very little is used in legumes.

Nitrogen deficiency problem in legumes may be ameliorated by rhizobial inoculants through seed dressing at planting. Several workers have stipulated that *Rhizobium* inoculation in legumes can fix atmospheric nitrogen between 15 and 210 kg-N-ha⁻¹ [41] [216] [217]. The maximum amount fixed N is approximately equivalent to nine bags of UREA (46%N), the cost of one bag of urea is estimated at T.shs. 65,000/= (40. US\$) thus a total input cost of T.shs. 585,000/= (355. US\$) for fertilizing one hectre with the equivalent nitrogen which can be covered by 100g *Rhizobium* inoculant (Bio fix) sold at only T.shs, 1600/= (1.US\$) Therefore the use of rhizobial inoculants is less expensive than industrial fertilizer for small scale agriculture which is practiced in most sub-Saharan Africa and cover majority of the people of which, chemical fertilizers are unaffordable because of increasing prices in each year. Thus, to these farmers Biological Nitrogen Fixation which is enhanced by inoculation to the compatible host legume is recommended as it is considered to have ecological and high economic benefits.

Studies by Ndakidemi *et al.* [37]; Nyoki and Ndakidemi [79]; Tairo and Ndakidemi [102] worked out economic analysis of rhizobial inoculation and phosphorus supplementation on cowpea, soybean and common beans grown under field conditions in Kilimanjaro region in Tanzania. These studies revealed greater financial benefits from rhizobial inoculation compared with un-inoculated control. For example, inoculation on cowpea have resulted into greater income of 495.2 US\$ as profit relative to uninoculated treatments which offered a profit of 307.4 US\$ and ultimately resulted into higher percentage increase over control and marginal rate of return. However phosphorus supplementation at the rate of (40 kg-P/ha) gave greater revenue as profit over all other treatments [42].

According to Ndakidemi *et al.* [37] on yield and economic payback of legume inoculation under farmer conditions, in Rombo and Moshi districts—Kilimanjro Tanzania, Rhizobial inoculation technology was proved to be capable of giving good results like industrial nitrogenous fertilizer. In this study biofertilizer applied in soybean and common bean produced bumper harvest on field conditions. Relative to control treatments, the economic analysis performed on common beans revealed 66% and 92% profit margin increase in Moshi and Rombo districts respectively. Furthermore when inoculation was supplemented with P at the rate of 26 kg/ha the recorded profit margin increased to 84% and 102% respectively. However with soybeans the reported figure was 140% and 153% for Rombo and Moshi district respectively when rhizobial alone was used and with P supplementation at the same rate as above the profit margin increased up to 224% and 250% for Rombo and Moshi respectively.

Thus, inoculation of newly release legume varieties such as climbing beans recommended for growth in African highlands is essential for obtaining profitable and sustainable yield. Furthermore, inoculation with effective *Rhizobium* strains and supplementation with phosphorus and potassium will supply enough nitrogen, phosphorus and potassium to the legumes and hence enhancing grain yield and finally resulting into higher economic benefits to the growers.

8. Conclusion

Rhizobium inoculation based Agriculture for new released legume varieties such as those involving climbing bean production are of paramount importance especially for resource limited farmers in areas where land is scarce and productivity is low due to nutrients depletion. Thus, rhizobial inoculation and supplementation with phosphorus and potassium has constructive effect in improving photosynthesis, nutrient uptake, nodulation, growth, yield and economic benefits in legumes. Thus the use of *Rhizobium* inoculation for the resource poor farmers to enhance biological nitrogen fixation and supplementation with optimum amounts of phosphorus and potassium for small and large scale production of legumes is strongly recommended in Africa.

Acknowledgements

This study was funded by Tanzania Commission for Science and Technology (COSTECH) and The Nelson Mandela African Institution of Science and Technology (NM-AIST).

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