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Effects of *Bradyrhizobium japonicum* and Phosphorus Supplementation on the Productivity of Legumes

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Authors' contributions

This work was carried out in collaboration between all authors. Author DN managed the literature searches and wrote the first draft of the manuscript. Author PAN read and edited the manuscript. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Most soils in sub-Saharan Africa are depleted and lack important nutrients for proper plant growth and development. The declining trend of soil fertility pronounced in different parts of Africa is attributed to different factors such as continuous cropping without soil replenishment and land degradation leading to poor grain yield of legumes. Nitrogen and phosphorus are among the most limiting nutrients for plant growth as they play different roles in the biochemical processes of plants. Phosphorus is a fundamental component of substances that are building blocks of genes and chromosomes. Adequate supply of phosphorus is essential for development of new cells and the transfer of the genetic code from one cell to another during cell formation. Nitrogen is an essential constituent of plant cells at structural, genetic and metabolic levels, involved in many processes of plant growth and development leading to yield and quality of harvested organs. Traditionally, small-scale farmers use little or no farm-yard manure and chemical fertilizers to improve soil nutrition. However, these fertilizers are expensive to be afforded by small-scale farmers. The alternative to this is the use of cheap and easily applied biofertilizer such as *Rhizobium* bacteria that can fix atmospheric nitrogen to the form that can be taken by plants. The potential role of rhizobia inoculants and P application with respect to growth, nitrogen fixation, nutrient uptake, total leaf chlorophyll content, and grain yield of legumes

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are given attention in this review. The results from different researchers showed that *Rhizobium* inoculation and supplementation of phosphorus independently or in combination had positive effects on growth, leaf chlorophyll content, nutrients uptake, grain yield, and nitrogen fixation. Therefore, reducing N and P deficiencies in the soil through the use of biofertilizers such as *Rhizobium* inoculants and phosphorus supplementation is a good option in promoting legume yield.

Keywords: Biofertilizers, Biological nitrogen fixation, chlorophyll, Legumes growth, Grain yield.

1. INTRODUCTION

All growing plants require P for their growth and development. Loss of soil fertility especially P and N has been mentioned as the most important constraint to yield and food security in sub-Saharan Africa, Tanzania inclusive [1–3]. About the last two decades, Africa was reported to loose 4.4 million t N, 0.5 million t P, and 3 million t K annually from its arable cultivated land [4]. These rates were several times higher than Africa's annual fertilizer consumption, excluding South Africa 0.8 million t N, 0.26 million t P, and 0.2 million t K [4,5]. The major plant nutrients are nitrogen (N), phosphorus (P) and potassium (K). Of these, N is abundant in the air, and deposits of K are plenty, but the phosphate reserves are increasingly becoming scarce [6]. The scarcity of this essential element is reflected on poor crop production as P deficient leads to plant growth and development. The low yields are pronounced in grain legumes and are often associated with low P levels in the soil and reduced N_2-fixation. Although legume yields can potentially improve from the application of moderate levels of chemical fertilizers, these inputs are rarely used either because of the high cost [7,8], lack of awareness of the economic returns from such cultural practice, or both. Supplementing legumes with N and P has great potential for increasing yields, as they do not only promotes plant growth but also enhances symbiotic establishment for increased N_2 fixation [9] and hence high yield. Traditionally, in Africa, this constraint is managed through addition of farmyard manure to the soil prior to planting. Alternatively, farmers are advised to use mineral fertilizer such as NPK, Diamonium phosphate, Triple Super Phosphate, and Urea to improve availability of Nitrogen, Phosphorus, Potassium and other elements in the soil. Unfortunately, the majority of African small farmers are now unable to afford the high mineral fertilizer prices [2].

It is believed that plants supplied with phosphorus at different stress levels and inoculated with *Bradyrhizobium japonicum* may respond differently in their growth, yield, nutrient uptake and N_2 fixation. To date, there is little information on the response of legumes such cowpea to the application of different levels of phosphorus and rhizobia inoculants. Therefore, this review identifies the agronomical and biochemical effects of mineral nutrient supplementation with P and inoculation with *Rhizobium* on growth, yield, N-fixation, other nutrient uptake and chlorophyll content in food grain legumes.

2. AIM OF THIS REVIEW

This review was aimed at getting an overview of the effects of *Bradyrhizobium japonicum* inoculation and phosphorus supplementation on growth, chlorophyll content, economic yield, nutrient uptake and nitrogen fixation in legume crops.

2.1 Effects of Phosphorus and *Bradyrhizobium japonicum* on Growth and Chlorophyll Content of Legumes

Low levels of soil P and N are the major constraints to crop growth on nutrients depleted soils of sub sub-Saharan Africa [1,3]. The application of soluble P fertilizers and locally available rock phosphate can enhance organic carbon fixation in the agro-ecosystem and improve the availability of the nutrients for crop yields [1].

All form of life requires essential elements such as P and N. In plants, they play a critical role in growth, development and finally determines the final yield of the crops. Nitrogen is an important nutrient to plant growth and development as it is a building blocks of protein [10]. Protein is an important substance which provides the plant with resources such as carbon, nitrogen and sulfur which are critical to plant growth and development [11]. Phosphorus is a fundamental component of the substances that are building blocks of genes and chromosomes. So, it is an essential part of the process of carrying the genetic code from one generation to the next, providing the "blueprint" for all aspects of plant growth and development [12]. An adequate supply of P is therefore fundamental to both development of new cells and to the transfer of the genetic code from one cell to another as new cells are formed.

2.1.1 Effects of phosphorus on growth of legumes

Phosphorus is essential for plant growth as it stimulates growth of young plants, giving them a good and vigorous start [13]. It is among the important elements needed for crop growth and production in many tropical soils. However, many tropical soils are P-deficient [1,3]. Phosphorus is vital to plant growth and is found in every living plant cell. It is involved in several key plant functions, including energy transfer, photosynthesis, transformation of sugars and starches, nutrient movement within the plant [14,15]. It is a component of important substances such as nucleic acids, phospholipids, and ATP, and as a result, plants cannot grow properly without an adequate supply of this important nutrient [12]. It is an important plant macronutrient, making up about 0.2% of a plant's dry weight [16]. In addition, P is involved in controlling key enzyme reactions and in the regulation of metabolic pathways [17]. Phosphorus also plays an important role in the buildup and maintenance of soil productivity by legumes through its effect on host plant growth and through its specific effect on *Rhizobium* growth, survival, and nodulation capability [18].

However, irrespective of the fact that the effect of P is closely associated with the stimulation of host plant growth, P is a vital requirement for N_2 fixation. Successful production systems based on legumes therefore require P inputs either from soil reserves or from added fertilizer [9]. Generally, application of phosphorus fertilizers has positive effects on shoot, grain, and root dry weights [19]. According to Singh et al. [19] phosphorus is an element that is not required in large quantities, but it is critical to cowpea yield because of its multiple effects on nutrition as all growing plants require P for proper growth and development [20]. For example, it was observed that the plant height and leaf area of legumes increased significantly with P application [9, 15]. This could be due to the fact that P being essential constituent of plant tissue significantly influences the plant height of crop [15,21]. 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 N_2-fixation [9]. Other researchers reported that application of phosphorus stimulate root development and plant growth, initiates nodule formation as well as influences the general efficiency of the *Rhizobium* – legume symbiosis [22,23] and hence optimize the biological nitrogen fixation (BFN) system of the legumes leading to vigorous growth of plants (Fig. 1).

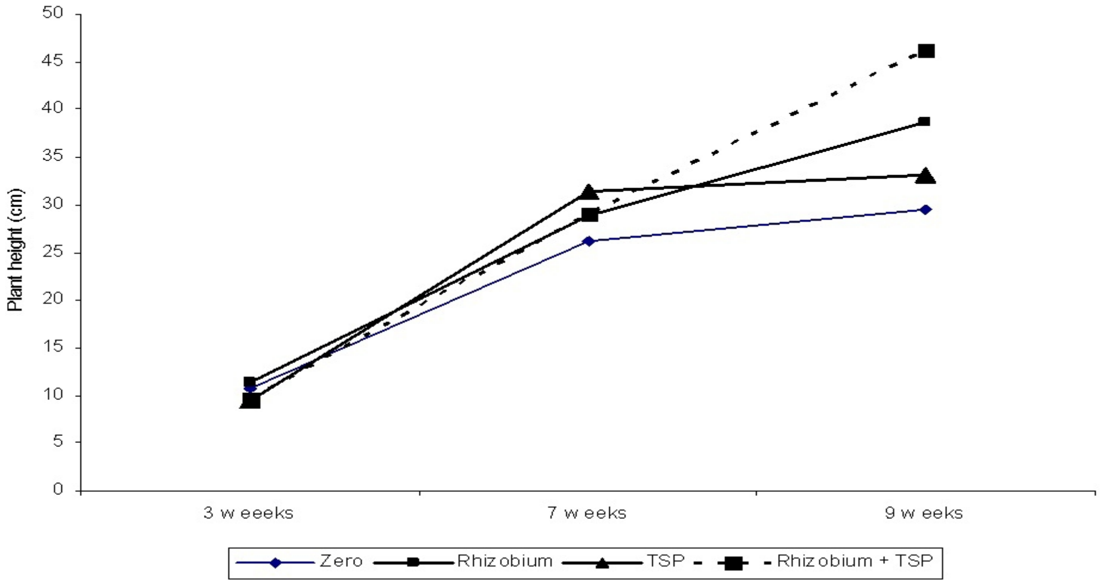

Fig. 1. Height of cowpeas under different treatments over the growing season (Source Onduru et al. [24])

The studies done by Rahman et al. [25] shows that phosphorus had the significant effect on stover yield of mungbean which was also significantly influenced at harvest. The highest stover yield of (26.67 g) per plant was obtained with P applied at 40 kg/ha, which was significantly higher than other treatments (Table 1). However, stover yield decreased significantly ($p \leq 0.05$) with increase in P rate above 40 kg/ha. The lowest stover yield per plant was recorded as 20.75 g (control). Research efforts at improving legume yield in Africa have increased over the past few decades with focus on improving nitrogen and phosphorus nutrition [26-28].

Table 1. Effect of phosphorus, molybdenum and *Rhizobium* inoculation on yield of mungbean

Treatments	Stover yield/plant(g)	Stover yield increase over control (%)	Seed yield/ plant (g)	Seed yield increase over control (%)
Control (T1)	20.75 e	-	5.56 h	-
Rhizobium(*R*)(T2)	25.79 d	24.29	9.53 g	71.40
P_0+$Mo_{1.0}$+*R* (T3)	25.77 d	24.19	10.54 f	89.57
P_{20}+$Mo_{1.0}$+*R* (T4)	26.05 bc	25.54	11.43 e	105.58
P_{40}+$Mo_{1.0}$+*R* (T5)	26.67 a	28.53	14.61 a	162.77
P_{60}+$Mo_{1.0}$+*R* (T6)	26.18 b	26.17	12.77 c	129.68
P_0+Mo1.5+*R* (T7)	25.79 d	24.29	9.74 g	75.18
P_{20}+Mo1.5+*R* (T8)	25.97 c	25.16	10.68 f	92.08
P_{40}+Mo1.5+*R* (T9)	26.09 bc	25.74	13.40 b	141.00
P_{60}+Mo1.5+*R* (T10)	26.08 bc	25.69	12.48 d	124.46
LSD (0.05)	0.146	-	0.264	-
CV%	0.4	-	1.6	-

The figures in a column having common letter(s) do not differ significantly at 5% level of probability. (source: Rahman et al. [25])

2.1.2 Effects of rhizobial inoculants on growth of legumes

Rhizobium inoculation in legumes is an alternative to the expensive inorganic nitrogen fertilizers and is accredited for stimulating plant growth [29]. Nitrogen is provided through symbiotic fixation of atmospheric N_2 by nitrogenase in rhizobial bacteroids [30]. Nitrogen is required for cellular synthesis of enzymes, proteins, chlorophyll, DNA and RNA, and is therefore important in plant growth [30,31]. According to Uchida [31], N is biologically combined with C, H, O, and S to create amino acids, which are the building blocks of proteins, and the amino acids which are used in forming protoplasm, the site for cell division and thus for plant growth and development.

The element is an important constituent of plant cells at the structural, genetic and metabolic levels, getting involved in many processes of plant growth and development which lastly lead to yield as well as the quality of harvested organs (seeds or shoot biomass) [32]. The growth performance of a crop plants is measured in terms of plant height [15]. Research evidence shows that plant height of inoculated seed was significantly higher when compared with un-inoculated seeds, nitrogen fixation by microorganisms such as *Rhizobium* and applied phosphorus was also reported to balance the nutrition of plants and growth of the plants was accelerated [15]. Other researchers reported that increased levels of phosphorus had positive effects on plant height [33,34]. Some studies showed that there was increased plant height due to phosphorus and *Rhizobium* inoculation [33-36]. Bambara and Ndakidemi [29] reported that poor *Rhizobium* - legume symbiosis reduce the amount of nitrogen fixed in legumes and hence resulted to reduced plant growth due to inadequate nitrogen which is required as building blocks of proteins in plants [10]. Poor growth of legumes might be attributed to the absence of specific *Rhizobium* strains [37]. Researchers have indicated significant achievements in legume growth and yield in many parts of the world following inoculation with the appropriate inoculants [9, 29,38-46].

Because both phosphorus and *Bradyrhizobium japonicum* have great role in crop growth, it is therefore important to study the effects of their interaction on growth of legumes commonly grown in Africa such as cowpeas (*Vigna unguiculata* (L.) Walp).

2.1.3 Effects of phosphorus on chlorophyll content in legumes

Chlorophyll is a green molecule in plant cells which plays an important role in photosynthesis. It absorbs sunlight and uses its energy to synthesize carbohydrates from CO_2 and water. There are two types of chlorophyll in plants, chlorophyll a and b, both works as photoreceptor in photosynthesis [47]. Chlorophyll is a key biochemical component in the molecular apparatus of the plant that is responsible for the critical process of photosynthesis, in which the energy from sunlight is used to produce life-sustaining oxygen. Plants convert light energy into biomass through photosynthesis which in turn produces various products of economic value (grain, fibre, tubers, fruits, vegetables and fodder) among others. To do this, plants need sufficient light, suitable temperature, substances such as water, CO_2, oxygen, and a number of nutrients [6].

$$6CO_2 + 6H_2O \xrightarrow[\text{Chlorophyll}]{\text{sun's energy}} C_6H_{12}O_6 + 6O_2$$

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 [31].

Much of the phosphorus found in the plant is in inorganic form and has many roles in cell metabolism [48]. In particular, the coenzyme ATP, which acts as an intermediate energy transfer compound in such cell functions as photosynthesis, respiration, biosynthesis, stomatal opening, and the transfer of organic solutes across membranes, requires inorganic phosphate in its formation from ADP. It is therefore, expected, that deficiency of phosphorus would have wide range effects on cell function. Phosphorus deficiency has been shown to diminish protein synthesis, increase carbohydrate content and decrease moisture content [49].

2.1.4 Effects of rhizobial inoculants on chlorophyll formation in legumes

Rhizobium inoculation of legumes seeds have been reported to increase the leaf Chlorophyll content of legume plants. The glasshouse and field experiments done by Bambara and Ndakidemi [29] showed that, leaf chlorophyll content increased significantly with rhizobial inoculation by 123% for the glasshouse experiment and 178% for the field experiment relative to un-inoculated (control). The photosynthesis similarly increased significantly with rhizobial inoculation as a result of increased chlorophyll content in the plant leaves due to *Rhizobium* inoculation when compared with un-inoculated (control). Beside of the studies above, there is little literature about the effects of phosphorus, *Bradyrhizobium japonicum* and their combined application on the chlorophyll formation in legumes grown in Africa such as cowpeas. Therefore, it is important to establish and quantify the influence of phosphorus and *Bradyrhizobium japonicum* on photosynthetic activities in such legumes.

2.2 Effects of Phosphorus and *Bradyrhizobium japonicum* on Economic Yield of Legumes

The declining soil fertility in smallholder farms is one of the limiting factors in legume production and has been attributed to unsustainable production systems. This is caused by continuous cropping, removal of field crop residues, overgrazing between cropping seasons without sufficient external inputs for soil fertility replenishment [50].

2.2.1 Effects of phosphorus and rhizobial inoculants on yield and yield components of legumes

Phosphorus deficiency is the most limiting soil fertility factor for grain legume production [19]. Phosphorus is responsible for nodulation in legumes such as bean, soybean and cowpea. Thus higher nodulation resulted in higher nitrogen fixation by such legumes and eventually the number of pods and seeds achieved per plant [19]. Seeds have the highest concentration of P in a mature plant, and P is required in large quantities in young cells, such as shoots and root tips, where metabolism is high and cell division is rapid. P aids in root development, flower initiation, and seed and fruit development [51]. Grain and stover yield was significantly recorded higher in plots supplied with P when compared with the control [9,52]. This could again be attributed to the availability of P that would have increased the intensity of nodulation and thus nitrogen fixation. Higher nitrogen fixation would result in higher yield of the crop [19].

Ndakidemi et al. [53] reported significant ($p \leq 0.05$) increases in common bean yields in un-inoculated plots following P application, and attributed it to the presence of effective native rhizobia in the soil. Different researchers [26,28,54,55] have shown that with adequate P application, symbiotic performance can be improved, resulting to greater grain yields. For farmers who can afford P fertilizers, their combined use with rhizobial inoculants can further increase grain yield and reduce the declining state of soil fertility [9]. The study by Stamford et al. [56] on the response of cowpea to the application of biofertilisers produced using phosphate rock plus elemental sulphur inoculated with *Acidithiobacillus* depicted that shoot dry matter and grain yields of cowpea in the field experiment at all treatments with P fertilization were significantly higher than control. Phosphorus application was also reported to significantly enhance shoot and root dry weight, total biomass, number of nodules, nodules dry weight, N and P uptake of the cowpea. Singh et al. [19] in their study concluded that 60 kg P_2O_5 ha^{-1} was not the optimum as further application of P may or may not increase the yield of cowpea suggesting for further investigations.

Rhizobia are special bacteria that can live in the soil or in nodules formed on the roots of legumes. In root, nodules form a symbiotic association with the legume by obtaining nutrients from the plant and producing nitrogen in a process called biological nitrogen fixation (BNF). By fixing atmospheric N_2, legumes offer the most effective way of increasing the productivity of poor soils either in monoculture, crop rotations, or mixed cropping systems. Where access to inorganic N fertilizers is limited by availability or cost, N_2 fixation provides the main pathway through which soil fertility can be improved [18].

The augmentation of Rhizobial population through inoculation increased grain yields by 22.5% in treatments with TSP and by 6.8% in non-TSP fertilized plots, demonstrating that *Rhizobium* augmentation had higher positive effect to naturally occurring *Rhizobium* strains [24]. *Rhizobium* inoculation had significant effects on yield and all the other yield components assessed in the study done by [46]. All parameters measured (number of pods $plant^{-1}$, number of seeds $plant^{-1}$, 100-seed weight, and seed yield) were significantly increased with *Rhizobium* inoculation. The study showed that the number of pods $plant^{-1}$ for both glasshouse and field experiments were increased significantly with *Rhizobium* inoculation relative to the un-inoculated treatment. In field experiment, the number of seeds pod^{-1} in the inoculated treatment was also higher when compared with un-inoculated control. The 100-seed weight (g) was recorded higher in the treatments supplied with *Rhizobium* when compared with control. The grain yield (kg ha^{-1}) of *P. vulgaris* L. were also significantly greater in plots inoculated with *Rhizobium* compared with the un-inoculated control [46].

The research done by Bhuiyan et al. [57] clearly demonstrated that the rhizobial inoculated plants gave significantly higher stover yield and seed yield compared with un-inoculated (control). This could be due to high nodulation which result in high N_2 fixation and hence higher stover yield and seed yield. Inoculation of *Bradyrhizobium japonicum* and application of phosphorus increases nitrogen and phosphorus in the soil. *Rhizobium* inoculation also increased nodules number and nodules dry weight in the field experiment conducted to investigate the performance of three soybean cultivars with five foreign *Bradyrhizobia* strains in different regions in Nigeria [58]. However, Okereke et al. [58] in their experiment observed the presence of nodules in the uninoculated plot (control) which signifies that the soybean cultivars were promiscuous, as they were nodulated by indigenous *Bradyrhizobia* strains present in the soil.

The study by Elsheikh et al. [59] indicated that inoculation with *Bradyrhizobium* strain TAL 377 or Isolate-2 significantly ($p \leq 0.05$) increased 100-seed weight for both monocropped and intercropped soybean seeds (Table 2). The two strains of *Bradyrhizobium* did not show significant differences from each other on 100-seed weight. Application of chicken manure to uninoculated plants had no significant effect on 100-seed weight of soybean seeds in both cropping systems. However, application of chicken manure and inoculation significantly increased 100-seed weight of soybean seeds in both cropping systems over the control.

Table 2. Effect of intercropping (sorghum/soybean), *Bradyrhizobium* inoculation and chicken manure on 100-seed weight of soybean seeds

Treatment	100-Seed weight
Monocropping system	
Control	9.41 (±0.50)a
TAL 377	12.6 (±0.15)bcd
Isolate-2	13.0 (±0.90)bcd
7 t/ha manure	10.6 (±0.74)abc
7 t/ha manure + TAL 377	13.3 (±0.65)cd
7 t/ha manure + Isolate-2	13.8 (±0.29)cd
Mean	12.1
Intercropping system	
Control	8.77 (±0.60)a
TAL 377	12.8 (±0.43)bcd
Isolate-2	13.2 (±0.31)cd
7 t/ha manure	9.81 (±0.71)ab
7 t/ha manure + TAL 377	13.6 (±1.08)cd
7t/ha manure + Isolate-2	13.9 (±0.29)cd
Mean	12.1
LSD (5%) for means	1.35

TAL 377: inoculated with Bradyrhizobium strain TAL 377; Isolate-2: inoculated with Bradyrhizobium strain Isolate-2; 7 t/ha manure: 7 t/ha chicken manure.
Values are means (±SD). Means sharing similar letter(s) in a column are not significantly different at the 0.05 level of probability, according to Duncan's Multiple Range Test.
(Derived from Elsheikh et al. [59])

The *Bradyrhizobium japonicum* inoculation and phosphorus may play a crucial role in food grain legumes grown in poorly depleted soils in Africa. Therefore their influence on the yield and yield components needs to be investigated.

2.2.2 Economic benefits of supplying phosphorus and *Bradyrhizobium japonicum* on grain legume cultivation

The economic growth in sub Saharan Africa (SSA) was estimated at 5.7% in 2006, and the quality of life depends on the agricultural sector. The sector accounts for about 30% of Gross Domestic Product (GDP), 70-80% of employment and is the major source of food, income and raw materials for industries [24,60,61]. The problem of hunger, poverty and poor health are well known globally and are widespread among the rural population in Africa. These constraints have significantly had a negative impact on development and success for an African Green Revolution, particularly so in sub-Saharan Africa (SSA) region which continues to receive world food aid [3].

Declining soil fertility is a major constraint to crop production in most semi-arid areas of sub Saharan Africa in which soils are usually low in nitrogen (N) and phosphorous (P) [1]. *Rhizobium* inoculation of food grain legumes and application of phosphorus have been reported to contribute in arresting declining soil fertility and improving farm agro-economic performance. The potential effect of inoculating legumes with *Rhizobium* strains is enhanced with the application of phosphorus. This is probably due to the role that phosphorus plays in enhancing nitrogen fixation in grain legumes [24]. Inoculation of legumes with *Rhizobium* and application of triple superphosphate fertilizer resulted in high agro-economic performance and positive soil phosphorus balance than planting the same cowpeas variety with either TSP alone or *Rhizobium* alone. The performance of the treatments in terms of major economic indicators (gross margins, net cash income and return to labour) were in the order *Rhizobium* + TSP > TSP >*Rhizobium* > control [24].

Ndakidemi et al. [9] reported that the combined application of bacterial inoculants and P fertilizer to field legume plants of soybean and common bean significantly increased biomass production and grain yield compared with the single use of N and P or (brady)rhizobial strains. From economic analysis, the increase in grain yield with inoculation translated into a significantly higher marginal rate of return and dollar profit for soybean and common bean farmers in northern Tanzania.

However, there is little information on the economic performance and role that could be played by application of phosphorus and rhizobia inoculants on grain legumes grown under different management practices and hence the need to study and establish economic benefits of supplying phosphorus and *Rhizobium* in the small scale farmers settings. Because most of the smallholder farmers are not using fertilizer in grain legume production, for the reason that they have low income and cannot afford the high price of mineral fertilizers.

2.3 Effects of phosphorus and *Bradyrhizobium japonicum* on nutrient uptake in legumes

Plants, like all other living things, need nutrients for their growth and development. Plants require 16 essential elements. Carbon, hydrogen, and oxygen are derived from the atmosphere and soil water. The remaining 13 essential elements (nitrogen, phosphorus, potassium, calcium, magnesium, sulfur, iron, zinc, manganese, copper, boron, molybdenum, and chlorine) are supplied either from soil minerals and soil organic matter or by organic or inorganic fertilizers [31,62].

2.3.1 Nutrient uptake in legumes as influenced by phosphorus

Phosphorus 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). This energy (adenosine triphosphate or ATP) is very important in the process of N fixation as is required to break the triple bond that exists between N atoms in N_2. P is component of the RNA and DNA structures, which are the major components of genetic information. P also aids the plants in root development and it increases seed yields [31]. Phosphorus does not only increases seed yields but also nodulation. Other researchers have reported that phosphorus application influences the content of others nutrients in leaves and seeds [19]. Research done in south western Nigeria by Akande [63] revealed that application of the two local phosphate rocks Ogun Phosphate Rock and Crystallizer super tested along with water soluble phosphate, both proved to be effective in enhancing the P and Ca uptake by maize and cowpea. Research evidence shows that Phosphorus application had a positive and significant effect on the nutrients such as Ca, Mg, Zn, Cu, Mn, and Fe uptake [64] and the application of P in combination with organic matter improve soil physical and chemical properties by enhancing biological activity and soil organic carbon accumulation [8]. Accumulation of organic matter in the soil enhances biological activity, which in turn improves soil physical and chemical properties making plant nutrients readily available for uptake.

2.3.2 Effects of rhizobial inoculants on nutrient uptake in legumes

The availability and uptake of micro and macronutrients is very important for plant growth and development especially in the depleted soils of sub Saharan Africa. According to Makoi et al. [65], nutrient uptake by plants depends largely in the amount, concentration and activities in the rhizosphere as well as the capacity of the soil to replenish the nutrients in the soil-solution. Soil microorganisms such as *Bradyrhizobium japonicum* inoculants and other plant growth promoting rhizobacteria are reported to influence the chemistry of soils nutrients in many ways and enhancing nutrients uptake by plants [66]. In the last few years, the numbers of *Rhizobium* inoculants have been developed and primarily used for enhancing N_2-fixation by legume plants [62]. For example *Bradyrhizobium* are reported to establish symbiosis relationship with legume where they fix nitrogen that is important for plant growth and in turn, the plant provide them with carbohydrates as their source of energy [66]. On top of atmospheric nitrogen fixation, rhizobia inoculation have been reported to improve plant nutrient such as P by mobilizing inorganic and organic P from organic and inorganic sources in the soil rhizopshere [66,67]. Makoi et al. [65] in their study conducted at green house and field experiments also reported that *Rhizobium* inoculation significantly increased the uptake of P, K, Ca, and Mg in the plant parts attributed to increased soil pH.

Other studies have also suggested that the available micronutrients such as Fe, Cu, Zn and Mn were increased significantly in the *Rhizobium* inoculated treatments when compared with the un-inoculated treatments [46,68]. The increased availability of nutrients in the soil increases the chance of uptake by plants. Ndakidemi et al. [62] 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.

There is little literature about the role that is played by different legume rhizoba inoculants and phosphorus on the availability of other nutrients in legume crops. Based on this reason, it is therefore important to establish the possible role which could be played by *Bradyrhizobium japonicum* inoculants and phosphorus on the availability of other nutrients in legumes such as cowpea grown in Africa.

2.4 Effects of combined application of phosphorus and *Bradyrhizobium japonicum* on nitrogen fixation in the legumes

Nitrogen fixation is a dynamic process regulated by a suite of stage- dependent factors including P availability and nitrogen fixing bacteria that adds substantial amounts of nitrogen and organic matter into agricultural system [69]. Nitrogen is an important element for living organisms as it is a constituent of many bio molecules, including all proteins and nucleic acids [70]. In plants, nitrogen is the most limiting nutrient for growth [1– 3,9,46]. In the soil, inorganic N forms are produced by soil microorganisms and represent less than 5% of total soil N which is mostly sequestered in soil organic matter.

2.4.1 Effects of phosphorus on nitrogen fixation in legumes

Phosphorus is an essential macronutrient for plant growth and development. Robson et al. [71] reported that P nutrition increased symbiotic N_2-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.

Phosphorus has important effects on photosynthesis, nitrogen fixation, root development, flowering, seed formation, fruiting and improvement of crop quality [14]. The combination of phosphorus and rhizobia inoculation showed maximum positive effects on nitrogen and phosphorus concentration, their uptake by plant and ultimately growth of the plants [55]. It was recommended by Dugje et al. [72] that about 30 kg of P/ha is good for cowpea production, which help the crop to nodulate well and fix its own nitrogen from the air.

P is reported to stimulate root and plant growth, initiate nodule formation, as well as influence the efficiency of the *Rhizobium*-legume symbiosis [44,73]. It is also involved in reactions with energy transfer, more specifically ATP in nitrogenase activity [6,73,74]. The influence of P on symbiotic nitrogen fixation in leguminous plants has received considerable attention, but its role in the process remains still unclear [75]. So, it is important to study the possible role that could be played by phosphorus when *Bradyrhizobium japonicum* inoculants are used on symbiotic nitrogen fixation in cowpea growing in diverse environment such as those involving controlled and uncontrolled cropping systems.

2.4.2 Effects of *Bradyrhizobium japonicum* inoculation on nitrogen fixation in legumes

Biological nitrogen fixation (BNF) enhanced by phosphorus application is absolutely beneficial to agricultural systems used by small-scale farmers in Africa. It is a major source of fixed nitrogen in agricultural soils. Inoculation of soybean seeds with proper bacterial strains has been reported to increase seed production by 70-75% [76]. According to Simanungkalit et al. [77], there have been good responses by soybean to inoculation with *Bradyrhizobium japonicum* expressed in terms of both the proportion of nodules (Fig. 2) formed by the inoculum and seed yield.

Fig. 2. Well nodulated soybean roots (Adopted from Burton, [78])

Nitrogen is acquired in a variety of forms by most plant species but inorganic N forms such as nitrate and ammonium are preferred over organic forms in most agricultural soils [32]. Nitrogen fixation involving symbiotic association between rhizobia and legumes is influenced by several factors including Phosphorus [9]. Gentili et al. [79] found that phosphorus supplied at medium concentration stimulated root growth and nodule formation in Grey Alder (*Alnus incana*).

The study by Hussain et al. [52] showed that inoculation with *Rhizobium* increased nitrogen fixation in mungbean crop. Bambara and Ndakidemi [46] found that *Rhizobium* inoculation of *P. vulgaris* had a significant influence on the amount of fixed N. Relative to the un-inoculated treatments, inoculation significantly increased the N fixed in different tissues of the plant such as roots, shoots, pods and whole plants of *P. vulgaris* grown both in the greenhouse and in the field [46]. These positive results are a result of improved N nutrition thus plant growth and good performance in terms of yield was significantly achieved through the simple symbiotic relationship between the legume plant and the rhizobia.

3. CONCLUSION

This review focused on the potential role of rhizobia inoculants and phosphorus supplementation with respect to growth, nitrogen fixation, nutrient uptake, total leaf chlorophyll content, and grain yield of legumes. The results from different researchers showed that *Rhizobium* inoculation and supplementation of phosphorus had positive significant effects on all parameters measured, suggesting these important bio-fertilizers supplemented with phosphorus in increasing legume production. Therefore, lessening N and P problem through *Rhizobium* inoculants and phosphorus supplementation is the best option in promoting legume yield in highly depleted soils.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1 Buerkert A, Bationo A, Piepho HP. Efficient phosphorus application strategies for increased crop production in sub-Saharan West Africa. Field Crop Res. 2001;72:1-15. doi:10.1016/S0378-4290(01)00166-6.

2 Chianu JN, Ohiokpehai O, Vanlauwe B, Adesina A, De Groote H, Sanginga N. Promoting a versatile but yet minor crop. Journal of Sustainable Development in Africa. 2009;10(4):324-344.

3 Nekesa ECR, Thuita M, Ndungu K, Kifuko M, Bationo A. The potential of underutilized phosphate rocks for soil fertility replenishment in Africa: case studies in Western Kenya. African Crop Science Conference Proceedings. 2007;8:1589-1598.

4 Food and Agricultural Organisation. FAO fertilizer yearbook 1994. Vol. 44. Food and Agric. Organ, of the United Nations, Rome; 1995.

5 Buresh RJ, Sanchez PA, Calhoun F, Hatfield J, Bigham JM, editors . Replenishing soil fertility in Africa. Soil Science Society of America; 1996.

6 Roy R, Finck A, Blair G, Tandon H. Plant nutrition for food security. FAO fertilizer and plant nutrition bulletin 16. Food and Agriculture Organization, Rome. 2006;348.

7 Akinrinde EA, Okeleye KA. Short and long term effects of sparingly soluble phosphates on crop production in two contrasting Alfisols (Danso SKA, and Abekoa MK. (Eds)). West African Journal of Applied Ecology. 2005;8:141-149.

8 Anetor M, Akinrinde E. Response of soybean [*Glycine max* (L.) Merrill] to lime and phosphorus fertilizer treatments on an acidic Alfisol of Nigeria. Pak J Nutr. 2006;5:286-293. DOI:10.3923/pjn.2006.286.293.

9 Ndakidemi P, Dakora F, Nkonya E, Ringo D, Mansoor H. Yield and economic benefits of common bean (*Phaseolus vulgaris*) and soybean (*Glycine max*) inoculation in northern Tanzania. Aust J Exp Agric. 2006;46:571-578. DOI:10.1071/EA03157.

10 Dutta AC. Botany for Degree students 5^{th} edition Macmillan publisher. Oxford University Press India. 2004.

11 Herman EM, Larkins BA. Protein storage bodies and vacuoles. The Plant Cell Online. 1999;11:601-613. DOI:10.2307/3870887, doi:10.1105/tpc.11.4.601.

12 Rubio V, Linhares F, Solano R, Martin AC, Iglesias J, Leyva A, Paz-Ares J. A conserved MYB transcription factor involved in phosphate starvation signaling both in vascular plants and in unicellular algae. Genes Dev. 2001;15:2122-2133. DOI:10.1101/gad.204401. PMid:11511543 PMCid:PMC312755

13 Tucker MR. Essential plant nutrients: their presence in North Carolina soils and role in plant nutrition, Department of Agriculture and Consumer Services, Agronomic Division. North Carolina, United States; 1999.

14 Brady NC. Phosphorus and potassium. In: The nature and properties of soils. Prentice-Hall of India, Delhi; 2002.

15 Shahid MQ, Saleem MF, Khan HZ, Anjum SA. Performance of soybean (*Glycine max* L.) under different phosphorus levels and inoculation. Pak J Agri Sci. 2009;46:237-241.

16 Schachtman DP, Reid RJ. Ayling SM. Phosphorus uptake by plants: from soil to cell. Plant Physiol. 1998;116:447–453. doi:10.1104/pp.116.2.447.

17 Theodorou ME, Plaxton WC. Metabolic adaptations of plant respiration to nutritional phosphate deprivation. Plant Physiol. 1993;101:339–344

18 McLaughlin MJ, Malik KA, Memon KS, Idris M. Phosphorus requirements for sustainable agriculture in Asia and Oceania. Proceedings of a Symposium. International Rice Research Institute. Manila, Philippines; 1990.

19 Singh A, Baoule A, Ahmed H, Dikko A, Aliyu U, Sokoto M, et al. Influence of phosphorus on the performance of cowpea (*Vigna unguiculata* (L) Walp.) varieties in the Sudan savanna of Nigeria. Agricultural Sciences. 2011;2:313-317. DOI:10.4236/as.2011.23042.

20 Muleba N, Ezumah HC. Optimizing cultural practices for cowpea in Africa. Pages 289-295. *In:* Cowpea research production and utilization, edited by S.R. Singh and K.O. Rachie. John Wiley and Sons Ltd., Chichester, UK; 1985.

21 Kumar R, Chandra R. Influence of PGPR and PSB on *Rhizobium leguminosarum* Bv. viciae strain competition and symbiotic performance in lentil. World J Agric Sci. 2008;4:297-301.

22 Abdel-Wahab SM, Hassman ME, Elwarraky K, Safwat MSA. Evaluation of N_2 – fixation of faba bean, chickpea and lentil as affected by phosphorus fertilization. African Crop Science Conference Proceedings. 1994;1:71-73.

23 Luyindula N, Kabinda PP, Mbaya N, Nwange K, Babela K. Effect of soil *bradyrhizobium* populations on nodulation and growth of *Glycin max* (L.) Merrill. African Crop Science Conference Proceedings. 1994;1:68-70.
24 Onduru D, De Jager A, Muchena F, Gachini G, Gachimbi L. Exploring potentials of rhizobium inoculation in enhancing soil fertility and agroeconomic performance of cowpeas in Sub-saharan Africa: A case study in semi-arid Mbeere, Eastern Kenya. Am.-Eurasian J Sustain Agric. 2008;2:185-197.
25 Rahman MM, Bhuiyan MMH, Sutradhar GNC, Paul AK. Effect of phosphorus, molybdenum and *Rhizobium* inoculation on yield and yield attributes of mungbean. Int J Sustain Crop Prod. 2008;3(6):26-33.
26 Ssali S, Keya SO. The effects of phosphorus and nitrogen fertiliser level on nodulation, growth and dinitrogen fixation of three bean cultivars. Trop Agr. 1986; 63:105-109.
27 Smithson JB, Edje OT, Giller KE. Diagnosis and correction of soil nutrient problems of common bean (*Phaseolus vulgaris*) in the Usambara Mountains of Tanzania. J Agr Sci. 1993;120:233-240. DOI:10.1017/S0021859600074281.
28 Giller KE, Amijee F, Brodrick SJ, Edje OT. Environmental constraints to nodulation and nitrogen fixation of *Phaseolus vulgaris* L. in Tanzania II. Response to N and P fertilisers and inoculation with *Rhizobium.* African Crop Science Journal. 1998;6:171-178. doi:10.4314/acsj.v6i2.27813.
29 Bambara S, Ndakidemi PA. Effects of Rhizobium inoculation, lime and molybdenum on photosynthesis and chlorophyll content of *Phaseolus vulgaris* L. Afr J Microbiol Res. 2009;3:791-798.
30 Hayat R, Ali S, Amara U, Khalid R, Ahmed I. Soil beneficial bacteria and their role in plant growth promotion: a review. Ann Microbiol. 2010;60:579-598. doi: 10.1007/s13213-010-0117-1.
31 Uchida R. Essential nutrients for plant growth: Nutrient functions and deficiency symptoms. Plant nutrient management in Hawaii's soils. College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa. 2000;31-55.
32 Salon C, Avice JC, Larmure A, Ourry A, Prudent M, Voisin AS. Plant N fluxes and modulation by nitrogen, heat and water stresses: A review based on comparison of legumes and non legume plants. In: Venkateswarlu ASAB, editor. Abiotic Stress in Plants- Mechanism and Adaptation; 2011.
33 Rani BP. Response of soybean to nitrogen and phosphorus application in the black soils of Krishna-Godavari zone of Andhra Pradesh. Ann Agr Res. 1999;20(3):367-368.
34 Tomar SS, Singh R, Singh PS. Response of phosphorus, sulphur and *Rhizobium* inoculation on growth, yield and quality of soybean. Prog Agric. 2004;4(1):72-73.
35 Hernandez M, Cuevas F. The effect of inoculating with *Arbuscular mycorrhiza* and *Bradyrhizobium* strains on soybean (*Glycine max* (L.) Merrill). Crop Development. Cultivos-Tropicales. 2003;24(2):19-21.
36 Menaria BL, Singh P, Nagar RK. Effect of nutrients and microbial inoculants on growth and yield of soybean *(Glycine max* L.). Journal of Soils and Crops. 2003;13(1):14-17.
37 Vincent JM, Nutman PS, Skinner FA. The identification and classification of *Rhizobium*. In: Shinner FA, Lovelock DW (eds) Methods for microbiologists. (Society for applied bacteriology, technical series no 14) Academic Press, London. 1979.
38 Hardarson G. Methods for enhancing symbiotic nitrogen fixation. Plant Soil. 1993;152(1):1-17. doi:10.1007/BF00016329.
39 Carter JM, Gardner WK, Gibson AH. Improved growth and yield of faba beans (*Vicia faba* cv. Fiord) by inoculation with strains of *Rhizobium leguminosarum* biovar *viciae* in acid soils in south-west Victoria. Aust J Agric Res. 1994;45:613-623. doi: 10.1071/AR9940613.

40 Brockwell J, Bottomley PJ, Thies JE. Manipulation of rhizobia microflora for improving legume productivity and soil fertility: A critical assessment. Plant Soil. 1995;174:143-180. DOI:10.1007/BF00032245.

41 Wani SP, Rupela OP, Lee KK. Sustainable agriculture in the semi-arid tropics through biological nitrogen fixation in grain legumes. Plant Soil. 1995;174:29-49. DOI:10.1007/BF00032240.

42 Dakora FD, Keya SO. Contribution of legume nitrogen fixation to sustainable agriculture in Sub-Saharan Africa. Soil Biol Biochem. 1997;29:809-817. doi:10.1016/S0038-0717(96)00225-8.

43 Popescu A. Contributions and limitations to symbiotic nitrogen fixation in common bean (*Phaseolus vulgaris* L.) in Romania. Plant Soil. 1998;204(1):117-125. DOI:10.1023/A:1004339313310.

44 Zahran HH. Rhizobium-legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. Microbiol Mol Biol Rev. 1999; 63 (4): 968-989.

45 Vargas MAT, Mendes IC, Hungria M. Response of field-grown bean (*Phaseolus vulgaris* L.) to *Rhizobium* inoculation and nitrogen fertilization in two cerrados soils. Biol Fertil Soils. 2000;32:228-233. doi:10.1007/s003740000240.

46 Bambara S, Ndakidemi PA. *Phaseolus vulgaris* response to *Rhizobium* inoculation, lime and molybdenum in selected low pH soil in Western Cape, South Africa. Afr J Agric Res. 2010;5(14):1804-1811.

47 Khaleghi E, Arzani K, Moallemi N, Barzegar M. Evaluation of chlorophyll content and chlorophyll fluorescence parameters and relationships between chlorophyll a, b and chlorophyll content index under water stress in *Olea europaea* cv. Dezful. World Academy of Science, Engineering and Technology. 2012;68:1154-1157.

48 Arnon DI. The physiology and biochemistry of phosphates in green plants. In: W. H. Pierre and A. G. Norman, eds., Soil and Fertilizer Phosphorus in Crop Nutrition, Chap. 4. Academic Press, Inc. New York. 1953;1-42.

49 Norman T, Albert U. Effects of phosphorus deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol. 1973;51:43-47. doi:10.1104/pp.51.1.43.

50 Bationo A, Traore Z, Kimetu J, Bagayoko M, Kihara J, Bado V, et al. Cropping systems in the Sudano-sahelian zone: Implications on soil fertility management. The Tropical Soil Biology and Fertility Institute of CIAT, Nairobi, Kenya. 2003.

51 Ndakidemi PA, Dakora FD. Yield components of nodulated cowpea (*Vigna unguiculata*) and maize (*Zea mays*) plants grown with exogenous phosphorus in different cropping systems. Aust J Exp Agric. 2007; 47:583. doi:10.1071/EA05274.

52 Hussain A, Ali A, Noorka IR. Effect of phosphorus with and without *rhizobium* inoculation in nitrogen and phosphorus concentration and uptake by Mungbean (*Vigna radiata* L). J Agric Res. 2012; 50(1): 49-57.

53 Ndakidemi PA, Nyaky AS, Mkuchu M, Woomer PL. Fertilization and inoculation of *Phaseolus vulgaris* in Arusha, Tanzania. In ‘Proceedings of the 8th congress of the African Association for Biological Nitrogen Fixation’. (Ed. Dakora, F. D.). (University of Cape Town: Cape Town). 1998;166-167.

54 Cassman KG, Whitney AS, Fox RL. Phosphorus requirements of soybean and cowpea as affected by mode of N nutrition. Agron J. 1981; 73:17-22. DOI:10.2134/agronj1981.00021962007300010005x.

55 Israel DW. Investigation of the role of phosphorus in symbiotic dinitrogen fixation. Plant Physiol. 1987;84:835-840. doi:10.1104/pp.84.3.835.

56 Stamford N, Santos C, Dias S. Phosphate rock biofertiliser with Acidithiobacillus and rhizobia improves nodulation and yield of cowpea (*Vigna unguiculata*) in greenhouse and field conditions. Trop Grasslands. 2006;40:222–230.

57 Bhuiyan M, Khanam D, Hossain M, Ahmed M. Effect of *rhizobium* inoculation on nodulation and yield of chickpea in Calcareous soil. Bangladesh J Agric Res. 2009;33:549-554.

58 Okereke GU, Onochie C, Onunkwo A. Effectiveness of foreign *Bradyrhizobium* strain in enhancing nodulation, dry matter and seed yield of soybean (*Glycine max.* L) cultivars in Nigeria. Biol Fert Soils. 2001; 33:3-9. doi:10.1007/s003740000264.

59 Elsheikh EAE, Salih SSM, Elhussein AA, Babiker EE. Effects of intercropping, *Bradyrhizobium* inoculation and chicken manure fertilization on the chemical composition and physical characteristics of soybean seed. Food Chemistry. 2009; 112:690–694.

60 Commission for Africa. Our common interest. Commission for Africa; 2005.

61 Economic Commission for Africa. Economic report on Africa: Accelerating Africa's development through diversification. United Nations Economic Commission for Africa, Addis Ababa, Ethiopia; 2007.

62 Ndakidemi PA, Bambara S, Makoi JHJR. Micronutrient uptake in common bean ('*Phaseolus vulgaris*' L.) as affected by *Rhizobium* inoculation, and the supply of molybdenum and lime. Plant Omics Journal. 2011;4:40-52.

63 Akande M. Effect of phosphate rock on selected chemical properties and nutrient uptake of maize and cowpea grown sequentially on three soil types in south western Nigeria. Int Res J Agric Sci Soil Sci. 2011;1(11):471-480

64 Fageria NK, Zimmermann FJP, Baligar VC. Lime and phosphorus interactions on growth and nutrient uptake by upland rice, wheat, common bean, and corn in an Oxisol. J Plant Nutr. 1995;18(11):2519–2532. doi:10.1080/01904169509365081.

65 Makoi JHJR, Bambara S, Ndakidemi PA. *Rhizobium* inoculation and supply of Molybdenum and lime affect the uptake of microelements in common bean (*P. vulgaris* L.) Plants. Aust J Crop Sci. 2013;7(6):784-793.

66 Saharan B, Nehra V. Plant growth promoting rhizobacteria: A critical review. Life Science and Medicine Research. 2011;21:1-30.

67 Matiru VN, Dakora FD. Potential use of rhizobial bacteria as promoters of plant growth for ncreased yield in landraces of African cereal crops. Afr J Biotechnol. 2004;3(1):1-7.

68 Fatima Z, Zia M, Chaudhary MF. Interactive effect of *Rhizobium* strains and P on soybean yield, nitrogen fixation and soil fertility. Pak J Bot. 2007;39(1):255-264.

69 Howell RK. *Rhizobium* induced mineral uptake in peanut tissues. J Plant Nutr. 1987;10(9-16):1297-1305.

70 Marschner H. Mineral nutrition of higher plants, 2nd Edn. Academic Press, London; 1995.

71 Robson AD, Hara GW, Abbott LK. Involvement of phosphorus in nitrogen fixation by subterranean clover (*Trifolium subterraneum* L.). Aust J Plant Physiol. 1981;8:427–436. doi:10.1071/PP9810427.

72 Dugje IY, Ekeleme F, Kamara AY, Omoigui LO, Tegbaru A, Teli IA, Onyibe JE. Guide to safe and effective use of Pesticides for crop production. Canadian international Development Agency; 2009.

73 Bationo A, Ntare B, Tarawali S, Tabo R. Soil fertility management and cowpea production in the semi-arid tropics. In Challenges and opportunities for enhancing sustainable cowpea production (Fatokun CA, Tarawali SA, Singh BB, Kormawa PM, Tamo M. eds). International Institute of Tropical Agriculture, Ibadan, Nigeria; 2002.

74 Barker AV, Pilbeam DJ. Handbook of plant nutrition CRC press. Boca Raton; 2007.

75 Tsvetkova T, Nevinsky I, Nevinsky V, Paniyshkina A. Experience of working with SSNTDs in Caucasus: a survey. Radiation Measurements. 2003;38:263–269. DOI:10.1016/j.radmeas.2003.10.008.

76 Simanungkalit RDM, Roughley RJ, Hastuti RD, Indrasumunar A, Pratiwi E. Inoculation of soybean with selected strains of B*radyrhizobium japonicum* can increase yield on acid soils in Indonesia. Soil Biol Biochem. 1996;28:257-259. DOI:10.1016/0038-0717(95)00135-2.

77 Simanungkalit RDM, Indrasumunar A, Pratiwi E, Hastuti RD, Roughley RJ. Population dynamics of bradyrhizobia in an latosol soil used for upland and lowland rice/soybean cropping systems in West Java, Indonesia. Soil Biol Biochem. 1995;27:625-628. DOI:10.1016/0038-0717(95)98641-Z.

78 Burton J. Legumes inoculants and their use. University of Hawaii NifTAL Project and FAO; 1984. Accessed 19 March 2014.
Available: http://www.ctahr.hawaii.edu/MauiSoil/c_nutrients01.aspx.

79 Gentili F, Wall LG, Huss-Danell K. Effects of phosphorus and nitrogen on nodulation are seen already at the stage of early cortical cell divisions in *Alnus incana.* Annals of Botany. 2006;98:309–315. DOI:10.1093/aob/mcl109.

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